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## RESEARCH ARTICLE | *Translational Human Pathophysiology*

# Effect of different bariatric surgeries on dietary protein bioavailability in rats

Romain Tessier,<sup>1,2</sup> Lara Ribeiro-Parenti,<sup>2,3</sup> Ouafa Bruneau,<sup>1,2</sup> Nadezda Khodorova,<sup>1</sup> Jean-Baptiste Cavin,<sup>2</sup> André Bado,<sup>2</sup> Dalila Azzout-Marniche,<sup>1</sup> Juliane Calvez,<sup>1</sup>  Maude Le Gall,<sup>2,\*</sup> and Claire Gaudichon<sup>1\*</sup>

<sup>1</sup>UMR Physiology of Nutrition and Ingestive Behavior (PNCA), AgroParisTech, INRA, Université Paris-Saclay, Paris, France; <sup>2</sup>INSERM UMRS 1149, UFR de Médecine Paris Diderot, Université de Paris, Assistance Publique–Hôpitaux de Paris, Paris, France; and <sup>3</sup>Department of General and Digestive Surgery, Bichat Hospital, Assistance Publique–Hôpitaux de Paris, Paris, France

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**Tessier R, Ribeiro-Parenti L, Bruneau O, Khodorova N, Cavin JB, Bado A, Azzout-Marniche D, Calvez J, Le Gall M, Gaudichon C.** Effect of different bariatric surgeries on dietary protein bioavailability in rats. *Am J Physiol Gastrointest Liver Physiol* 317: G592–G601, 2019. First published August 28, 2019; doi:10.1152/ajpgi.00142.2019.—Bariatric surgery may induce protein malabsorption, although data are scarce. This study aims at evaluating dietary protein bioavailability after different bariatric surgeries in rats. Diet-induced obese Wistar rats were operated for vertical sleeve gastrectomy (VSG) or Roux-en-Y gastric bypass (RYGB). The control group was composed of pair-fed, sham-operated rats (Sham). Two weeks after surgery, rats were fed a <sup>15</sup>N protein meal. Protein bioavailability was assessed by determination of <sup>15</sup>N recovery in the gastrointestinal tract and organs 6 h after the meal. Fractional protein synthesis rate (FSR) was assessed using a flooding dose of <sup>13</sup>C valine. Weight loss was the highest in RYGB rats and the lowest in Sham rats. Surprisingly, RYGB (95.6 ± 0.7%) improved protein digestibility ( $P = 0.045$ ) compared with Sham (93.5 ± 0.5%) and VSG (93.8 ± 0.6%). In contrast, <sup>15</sup>N retained in the liver ( $P = 0.001$ ) and plasma protein ( $P = 0.037$ ) was lower than in Sham, with a similar trend in muscle ( $P = 0.052$ ). FSR was little altered by bariatric surgery, except for a decrease in the kidney of RYGB ( $P = 0.02$ ). The <sup>15</sup>N distribution along the small intestinal tissue suggests that dietary nitrogen was considerably retained in the remodeled mucosa of RYGB compared with Sham. This study revealed that in contrast to VSG, RYGB slightly improved protein digestibility but altered peripheral protein bioavailability. This effect may be ascribed to a higher uptake of dietary amino acids by the remodeled intestine.

**NEW & NOTEWORTHY** Using a sensitive <sup>15</sup>N meal test, we found that gastric bypass slightly improved protein digestibility compared with sleeve gastrectomy or control but, in contrast, lowered protein retention in the liver and muscles. This paradox can be due to a higher uptake of dietary nitrogen by the intestinal mucosa that was hyper-trophied. This study provides new insight on the digestive and metabolic fate of dietary protein in different models of bariatric surgery in rats.

gastric bypass; <sup>15</sup>N test meal; protein digestibility; protein retention; sleeve gastrectomy

## INTRODUCTION

Bariatric surgery is one of the most efficient treatments of morbid obesity, inducing sustainable weight loss (11, 28, 47) and improvement of associated comorbidities (19). Historically, literature distinguished two kinds of bariatric surgeries: purely restrictive versus restrictive and malabsorptive. Food intake restriction was supposed to be induced by a reduction of stomach size, whereas malabsorption may result from the derivation of the proximal small intestine from the alimentary tract, therefore theoretically reducing nutrient absorption. The purely restrictive vertical sleeve gastrectomy (VSG) and the restrictive and malabsorptive Roux-en-Y gastric bypass (RYGB) are the most performed bariatric surgeries worldwide (4). VSG and RYGB procedures limit the gastric volume and thereby reduce food intake during a single meal, but this mechanical restriction cannot fully explain the reported reduction of energy intake, as subjects could compensate by calorie-dense food or multiple meals. The stomach is also a main source of hormones and peptides such as the orexigenic hormone ghrelin. The latter has reported to be reduced after VSG (3, 35), which can contribute to the anorexigenic effects of this procedure. RYGB has also been associated with reduced ghrelin (20), but this remains disputed (7). Moreover, RYGB was associated with increases in glucagon-like peptide-1 and peptide YY (PYY 3–36), which contribute to reduction of food intake (50). Additionally, as the stomach secretes hydrochloric acid and pepsinogen, which are implicated in the early steps of protein digestion, VSG and RYGB could also lead to a protein mal-digestion.

RYGB has been shown to induce nutrient malabsorption (33, 48) associated with the length of the bypassed limb. Whereas carbohydrate and lipid malabsorption may contribute to the positive effects of bariatric surgery, protein malabsorption (6, 25) can be the cause of a large range of clinical consequences, from severe undernutrition leading to parenteral nutrition to death (30). Low albumin levels and excessive weight loss are signs of protein wasting after bariatric surgery (26). It has been shown that severe protein malnutrition occurred in 4.7% of patients who underwent RYGB surgery (25).

The degree of protein malabsorption after bariatric surgery has not been well established. Using intrinsically [<sup>13</sup>C]casein, Bosjen-Møller et al. (9) showed accelerated blood appearance of dietary amino acids in patients operated for RYGB. In the absence of any digesta collection, it is not known whether this paradoxical effect is only due to a faster digestion kinetic or

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also to a higher protein bioavailability. In contrast, other studies reported a higher fecal output of nitrogen in rats after RYGB and biliopancreatic diversion (14, 42) and in humans after RYGB (48). Overall, none of these studies determined the real digestibility of dietary proteins and their postprandial retention in organs.

This study aimed to characterize digestive and metabolic protein bioavailability by using a test meal containing  $^{15}\text{N}$ -labeled proteins. For this purpose, VSG and RYGB rats were compared with pair-fed Sham rats for weight loss, energy intake, body composition, protein real digestibility, dietary protein retention in tissues, and protein synthesis rate.

## MATERIALS AND METHODS

### Animals and Diets

All animal studies comply with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. They were conducted in compliance with European Union directive 2010/63/EU for animal experiments and approved by the Institutional Animal Care and Use Committee (Comité d'éthique en expérimentation animale no. 121) and the Ministry of Higher Education and Research (Reference no. 02285.03). Male Wistar rats (Janvier Laboratories, Le Genest-St-Isle, France) ( $n = 42$ ) weighing 275–280 g were housed in standard environmental conditions (temperature 21°C–22°C, 12/12-h light/dark cycle with tap water and food ad libitum). Before surgery, they were fed a high-fat diet (Altromin, Genestil, Royancourt, France) for 4 mo. The composition and experimental design of the study are detailed in Supplemental Table S1 (available online at <https://doi.org/10.6084/m9.figshare.8964152>).

### Surgical Procedures

After 4 mo on a high-fat diet, animals were randomly divided into 3 groups: RYGB ( $n = 18$ ), VSG ( $n = 7$ ), and sham-operated (Sham,  $n = 16$ ). Initially, two different Sham procedures were performed to match with sleeve and gastric bypasses, but rats were then pooled together without any differences in principle criteria. The rats were fasted the night before surgery. Anesthesia was induced and maintained by gaseous inhalation of isoflurane (Vetflurane, Virbac, France). Procedures were carried out as previously described (5, 13, 14, 24, 52). After laparotomy, the stomach was isolated outside of the abdominal cavity. Gastric connections to the spleen and liver were severed along the greater curvature, and the suspensory ligament

supporting the upper fundus was dissociated. The surgical procedures are depicted in Fig. 1.

### Vertical Sleeve Gastrectomy

The nonglandular part of the stomach was removed by application of an ETS-Flex 35-mm staple line (Ethicon, Issy les Moulineaux, France). Then, the procedure was performed to resect 80% of gastric fundus stomach by application of a second ETS-Flex 35-mm staple line, leaving a thin gastric tube in continuity with the esophagus.

### Roux-en-Y Gastric Bypass

The nonglandular part of the stomach (forestomach) was resected using an ETS-Flex 35-mm staple line (Ethicon). A second staple line (Proximate TX30V 30 mm, Ethicon), parallel to the first one, was applied to delimit a gastric pouch that represented 20% of the initial stomach size while preserving the arterial and venous supply. The jejunum was transected 20 cm after the pylorus. The alimentary limb was anastomosed to the gastric pouch, and the biliopancreatic limb (BPL) was anastomosed 15 cm distally to gastrojejunal anastomosis. Sutures were made by a 7–0 Prolene wire (Ethicon).

### Sham

To simulate surgery, the stomach was pinched with an unarmed staple gun, and for bypass sham-operated rats, the jejunum was incised and stitched immediately after.

All operations ended with 4–0 vicryl and 3–0 vicryl (Ethicon) sutures to sew up the abdominal wall and the skin, respectively.

### Postsurgery Care

Animals received an analgesic (xylocaine, Astra, 10 mg/kg) and an antibiotic (penicillin, 20,000 U/kg, PanPharma, Boulogne Billancourt, France) after surgery. They were placed in individual cages with a reversed light/dark cycle with lights on from 8:00 PM to 8:00 AM. For 2 days after the surgery, rats were injected subcutaneously with 12 mL/day of an isotonic polyionic solution (Bionolyte G5, Baxter, Guyancourt, France). Between *day 3* and *day 10*, they were refed a hydrated standard diet ad libitum (Supplemental Table S1). Sham animals were pair-fed with operated animals.

### Habituation to Consumption of a Single Test Meal

From *day 11* to *day 19*, rats were accustomed to rapid consumption of a single meal in the morning. For this purpose, they received 4 g of

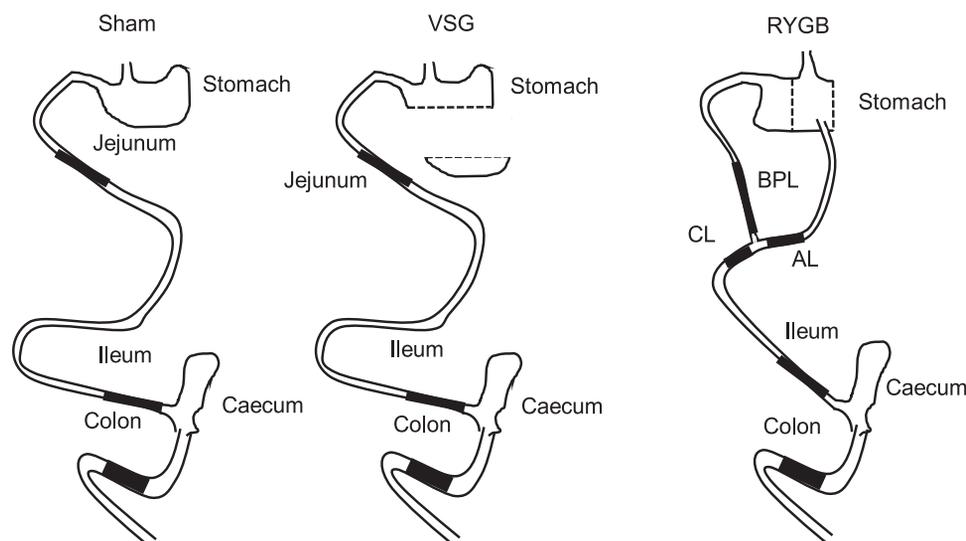


Fig. 1. Schematic representations of normal (Sham) and remodeled gastrointestinal tract after VSG and RYGB procedures. AL, alimentary limb; BPL, biliopancreatic limb; CL, common limb; RYGB, Roux-en-Y gastric bypass; VSG, vertical sleeve gastrectomy.

standard diet between 8:30 AM and 9:00 AM, followed by free access to the diet between 12:00 PM and 6:30 PM. Between those two periods, rats were fasted with free access to water. Sham rats were pair-fed with operated rats. On *day 19*, rats were fed a single test meal of the same composition as the standard diet but in which proteins were intrinsically labeled with  $^{15}\text{N}$ , as described previously (36). Before being euthanized (30 min prior), rats were injected in the lateral tail vein with 150  $\mu\text{mol}/100\text{ g}$  of body weight of [ $1\text{-}^{13}\text{C}$ ]valine (Eurisotop, Saint Aubin, France) under gaseous anesthesia. After the meal (6 h), rats were injected intraperitoneally with a lethal dose of pentobarbital sodium (100 mg/kg) and exsanguinated. Whole blood was collected, and plasma was isolated and stored at  $-20^\circ\text{C}$  after centrifugation. Different intestinal segments were identified depending on the surgery procedure: stomach, jejunum, ileum, cecum, and colon. The feces emitted during the postprandial period were pooled with colon content. For RYGB, the biliopancreatic, alimentary, and common limbs were collected (Fig. 1). The ileum was defined as the 10 cm before the cecum to recover a sufficient number of samples, although ileum might be shorter, according to Vdoviaková et al. (58). Segments were rinsed with NaCl solution (9 g/1,000 ml), and the contents were collected in their entirety, weighed, and stored at  $-20^\circ\text{C}$ . A sample of each intestinal wall was collected and immediately frozen in liquid nitrogen. Liver, kidney, extensor digitorum longus muscle, and skin were also sampled, weighed, frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$ .

#### Measurement of Food Intake, Body Weight, and Whole Body Composition

Body weight and food intake were assessed daily. Whole body composition was measured 7 days before surgery and 15 days after surgery with an Echo Medical Systems EchoMRI 900 (Whole Body Composition Analyzers, Houston, TX). For technical reasons related to the device, body composition was assessed before surgery in only 18 animals.

#### Analytical Methods

Protein bioavailability was assessed by following the  $^{15}\text{N}$  recovery in digestive contents and organs. Digestive contents were freeze-dried. Intestine, kidney, liver, muscle, and skin were cold-grinded with liquid nitrogen and freeze-dried. Plasma protein extraction was performed by precipitation with 5-sulfosalicylic acid (100%).

Nitrogen percentage and  $^{15}\text{N}$  enrichment in digestive samples, organs, and plasma proteins were determined by an elemental analyzer (Vario Micro Cube, Elementar, Lyon, France) coupled with isotopic ratio mass spectrometry (Isoprime, GV Instrument, Manchester, UK). Atropine (Thermo Electron, Milano, Italy) and L-glutamic acid (USGS41, Sigma-Aldrich, St. Louis, MO) were used as elemental and isotopic standards, respectively.

In vivo protein synthesis rate in liver, kidney, skin, and muscle was assessed using the [ $^{13}\text{C}$ ]valine flooding dose method, as described previously (18, 45). After protein precipitation of the different tissues, amino acids and protein-bound fractions were separated. Free amino acids were derivatized with *N*-tert-butyltrimethylsilyl-*N*-methyltrifluoroacetamide mixed with 1% *tert*-butyldimethylchlorosilane and acetonitrile (Sigma-Aldrich). [ $^{13}\text{C}$ ]valine enrichment in free amino acids was analyzed by gas chromatography (GC 6890N, Agilent Technologies, Les Ulis, France) coupled to mass spectrometry (MS 5973N, Agilent Technologies) (GC-MS) with electron impact ionization and selected ion monitoring (ions mass-to-charge ratio 288 and 289). After hydrolysis, protein-bound amino acids were derivatized with propanol and acetyl chloride (Sigma-Aldrich). [ $^{13}\text{C}$ ]valine enrichment in proteins was analyzed by GC-combustion-isotopic ratio mass spectrometry (Isoprime, GV Instrument).

#### Histology

Intestinal segments were fixed overnight in formalin and embedded in paraffin. Three-micrometer blank slides were cut from each block to perform hematoxylin phloxine saffron staining. Each slide was scanned with an Aperio ScanScope CS System (Leica Microsystems SAS, Nanterre, France).

#### Calculations

The exogenous nitrogen in each tissue was determined as follows:

$$N_{\text{exo}} = N_{\text{tot}} \times \frac{\text{APE}_s}{\text{APE}_m},$$

where  $N_{\text{exo}}$  is exogenous nitrogen (mmol),  $N_{\text{tot}}$  is amount of nitrogen in the sample (mmol), and  $\text{APE}_{s/m}$  is enrichment excess in the sample (s) or the meal (m) ( $\text{APE}_{s/m} = \text{AP}_{s/m} - \text{natural enrichment}$ ). Natural enrichment values were determined in previous studies for digesta (26) and tissues (49) in rats after adaptation to a milk protein diet.

Recovery of dietary nitrogen in plasma protein was related to the plasma volume in the rat (3.5% of body weight) (35a).

As amino acids and peptides are absorbed massively in the duodenum and jejunum, dietary N losses were determined from the N recovery in the distal parts of the intestine (ileum and large intestine, including feces). As digestion was almost complete after 6 h according to this study and previous ones (36, 46), the losses because of residual N in the stomach and intestine were considered as negligible. Accordingly, the real fecal protein digestibility (RFD, % of nitrogen ingested) was calculated as follows:

$$\text{RFD} = \frac{N_{\text{ing}} - (N_{\text{exo ileum}} + N_{\text{exo caecum}} + N_{\text{exo fecal}})}{N_{\text{ing}}} \times 100,$$

where  $N_{\text{ing}}$  is the amount of nitrogen ingested (mmol).

The fractional protein synthesis rate (FSR, in %/day) in each organ was calculated as follows:

$$\text{FSR} = \frac{E_{\text{protein-bound valine}} - E_{\text{basal}}}{E_{\text{free valine}} \times t_{\text{inc}}} \times 24 \times 60 \times 100,$$

where  $E_{\text{free valine}}$  and  $E_{\text{protein-bound valine}}$  are the [ $^{13}\text{C}$ ]valine enrichments in the free and protein-bound amino acids, respectively.  $E_{\text{basal}}$  is the value of the nonenriched leucine in the same sample, as a surrogate of basal valine enrichment (10).  $t_{\text{inc}}$  is the time of incorporation of [ $^{13}\text{C}$ ] valine (in min) between the injection and euthanasia (30 min on average). The factors 24 (h) and 60 (min) were used to calculate the value in % per day (18).

The absolute synthesis rate (ASR, g/d) was calculated as:

$$\text{ASR} = \text{FSR} \times P_{\text{content}},$$

where  $P_{\text{content}}$  is the protein content of the tissue ( $P_{\text{content}} = \%N \times 6.25$ ).

#### Statistical Analysis

All results are expressed as means  $\pm$  SE. Follow-up data were analyzed in a mixed model with group as a fixed factor and time as a repeated factor, using SAS 9.1 (SAS Institute, Cary, NC). Otherwise, differences between groups were tested using a one-way ANOVA with the group as a factor and post hoc Bonferroni tests for pairwise comparisons. Differences were considered statistically significant for a value of  $P < 0.05$ .

## RESULTS

### Follow-Up

**Survival rate.** Out of 42 rats, 1 died before surgery, without any explanation. For Sham, the survival rate was 81.3% (13/

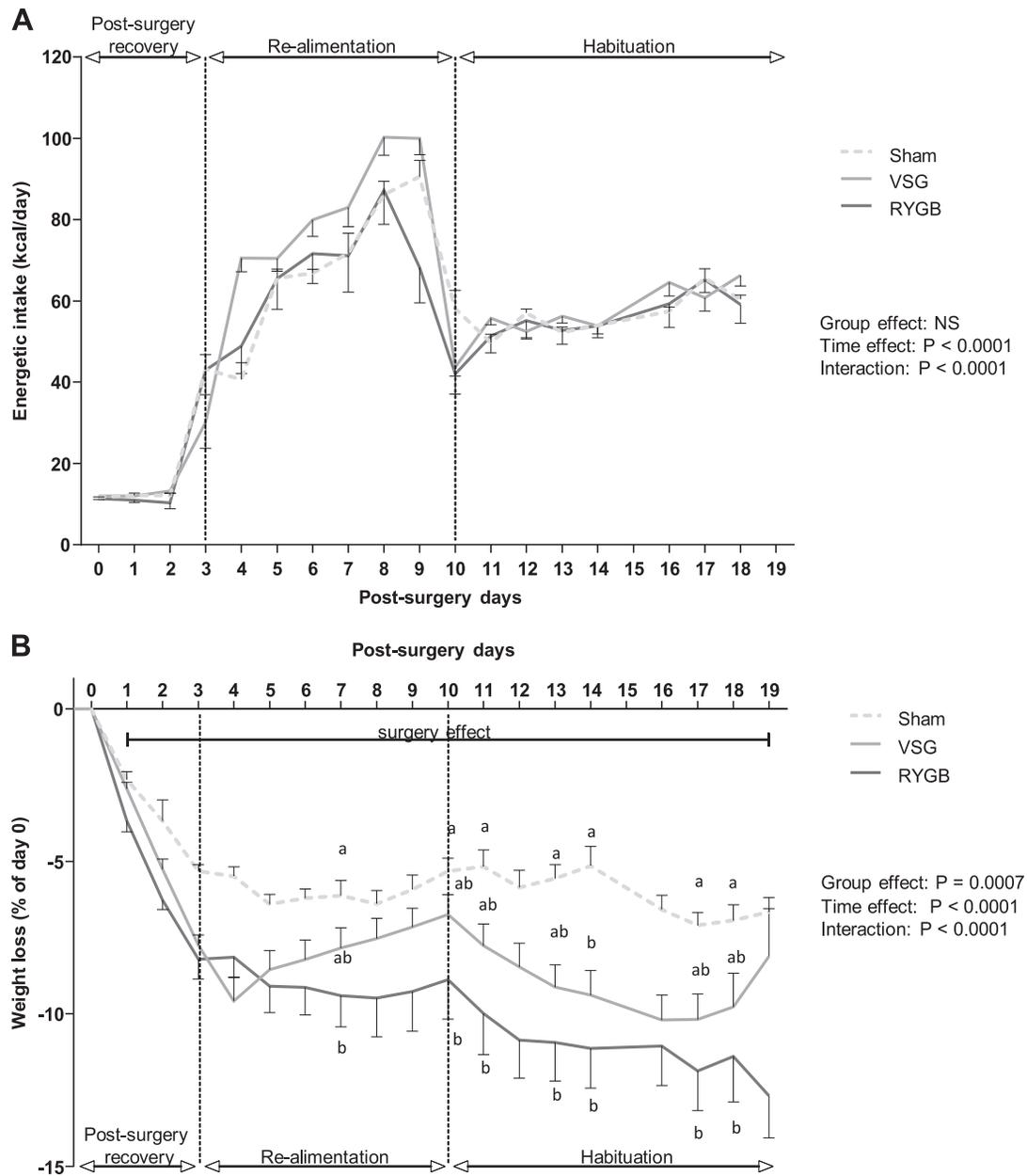


Fig. 2. Energy intake (A) and weight loss (B) after bariatric surgery. Refeeding began 3 days after surgery. Habituation to a discontinuous food pattern began at day 10. Sham rats were pair-fed with operated rats. Values are means  $\pm$  SE.  $n = 13$  rats for Sham,  $n = 7$  rats for VSG, and  $n = 9$  rats for RYGB. Surgery and time effects were tested by a mixed model. Values with different letters at a given day are statistically different (post hoc Bonferroni's test). NS, not significant; RYGB, Roux-en-Y gastric bypass; VSG, vertical sleeve gastrectomy.

16). Deaths occurred during anesthesia, sham surgery, or the first hours following surgery. The survival rate for RYGB was 50% (9/18) because of the complexity of surgical procedures in the context of the learning curve of a new surgeon. All deaths occurred during the early postsurgery period. The survival rate of VSG rats was 100% (7/7).

**Food intake, weight, and body composition.** During the first 2 days, animals did not receive any solid food (Fig. 2A), and they all lost weight (Fig. 2B). When refeeding began on day 3, rats rapidly increased their energy intake. After the beginning of refeeding, rats continued to lose weight for 2 days. Operated rats lost between 7% and 9% of their preoperative weight. Sham rats tended to lose less weight than operated animals

during this period ( $5.3 \pm 0.2\%$ ). From day 5, all groups stopped losing weight; VSG regained weight, whereas RYGB and pair-fed Sham did not. When habituation to consumption of a single test meal in the morning started at day 10, energy intake dropped for all groups. Subsequently, food consumption increased between day 11 and day 18, from  $196.6 \pm 4.2$  kJ/day ( $47.4 \pm 1.8$  kcal/day) to  $255.2 \pm 12.5$  kJ/day ( $61.0 \pm 3.2$  kcal/day). Whereas all operated rats lost weight after the beginning of the protocol, Sham rats maintained their weight ( $\sim 1\%$  loss in 9 days). The total mass loss was  $44 \pm 3$  g in Sham,  $64 \pm 5$  g in VSG, and  $73 \pm 8$  g in RYGB, with a significant effect of the group ( $P = 0.002$ ), a significant difference between Sham and RYGB ( $P = 0.002$ ), and a trend for a difference between Sham

Table 1. Dietary nitrogen recovered in gastrointestinal tract contents (stomach, small intestine, ileum, cecum, and colon) depending on the type of bariatric surgery

	% of N Ingested			Group Effect
	Sham	VSG	RYGB	
Stomach	0.52 ± 0.11	0.10 ± 0.34	0.30 ± 0.15	0.066
Small intestine	0.69 ± 0.05	0.81 ± 0.21	0.65 ± 0.16	NS
Ileum	0.32 ± 0.07	0.69 ± 0.32	0.15 ± 0.05	0.078
Cecum	4.46 ± 0.33 <sup>a</sup>	4.45 ± 0.54 <sup>a</sup>	2.42 ± 0.43 <sup>b</sup>	0.002
Colon	1.76 ± 0.38	1.07 ± 0.21	1.85 ± 0.35	NS
RFD, %	93.5 ± 0.5 <sup>a</sup>	93.8 ± 0.6 <sup>ab</sup>	95.6 ± 0.7 <sup>b</sup>	0.045

Values are means ± SE.  $n = 13$  rats for Sham,  $n = 7$  rats for vertical sleeve gastrectomy (VSG), and  $n = 9$  rats for Roux-en-Y gastric bypass (RYGB). The effect of the group was tested with an ANOVA model and post hoc Bonferroni test for pairwise comparisons. Values with different letters within the same row are statistically different. NS, not significant; RFD, real fecal digestibility.

and VSG ( $P = 0.07$ ). When pooled together, operated rats lost more weight than Sham rats ( $P = 0.001$ ).

The longitudinal follow-up of body composition (Supplemental Table S2, available online at <https://doi.org/10.6084/m9.figshare.8964152>) could be carried out in only some rats, leading to an insufficient number of observations. There was significantly ( $P = 0.02$ ) more fat mass loss in RYGB ( $-48 \pm 5$  g) than in Sham rats ( $-27 \pm 3$  g). The fat mass loss observed in VSG ( $-43 \pm 5$  g) did not differ from the 2 other groups.

The average lean mass loss was  $-27 \pm 4$  g, and we could not detect any differences between groups.

### Dietary Protein Bioavailability

**Digestibility.** To assess dietary protein digestibility, dietary nitrogen in the lumen of the gastrointestinal tract was measured 6 h after ingestion of the test meal (Table 1). Most of the unabsorbed exogenous nitrogen was found in the cecum and the colon lumens. In the stomach, the small intestine, the ileum, and the colon lumens there were no differences between groups. In the cecum, significantly less dietary nitrogen was recovered in RYGB rats than in VSG and Sham rats. Consequently, there was a significant effect of the group on RFD ( $P = 0.045$ ), digestibility being higher in RYGB than in Sham ( $P = 0.049$ ).

**Dietary nitrogen retention in tissues.** To evaluate the metabolic bioavailability, we measured  $^{15}\text{N}$  retention in the blood and different tissues (Fig. 3). There was an effect of the group ( $P = 0.037$ ) on dietary nitrogen found in plasma protein (Fig. 3A). It was similar in Sham ( $9.6 \pm 0.4\%$  of N ingested) and VSG ( $10.4 \pm 1.5\%$  of N ingested) but was lower in RYGB ( $6.7 \pm 1.2\%$ ).

There was an effect of the group ( $P = 0.017$ ) on liver weight, which was  $\sim 2$  g higher in Sham than in the bariatric surgery groups (data not shown). As a consequence, the group influenced dietary nitrogen recovery in the liver ( $P = 0.008$ ), with a lower value in the two operated groups than in Sham

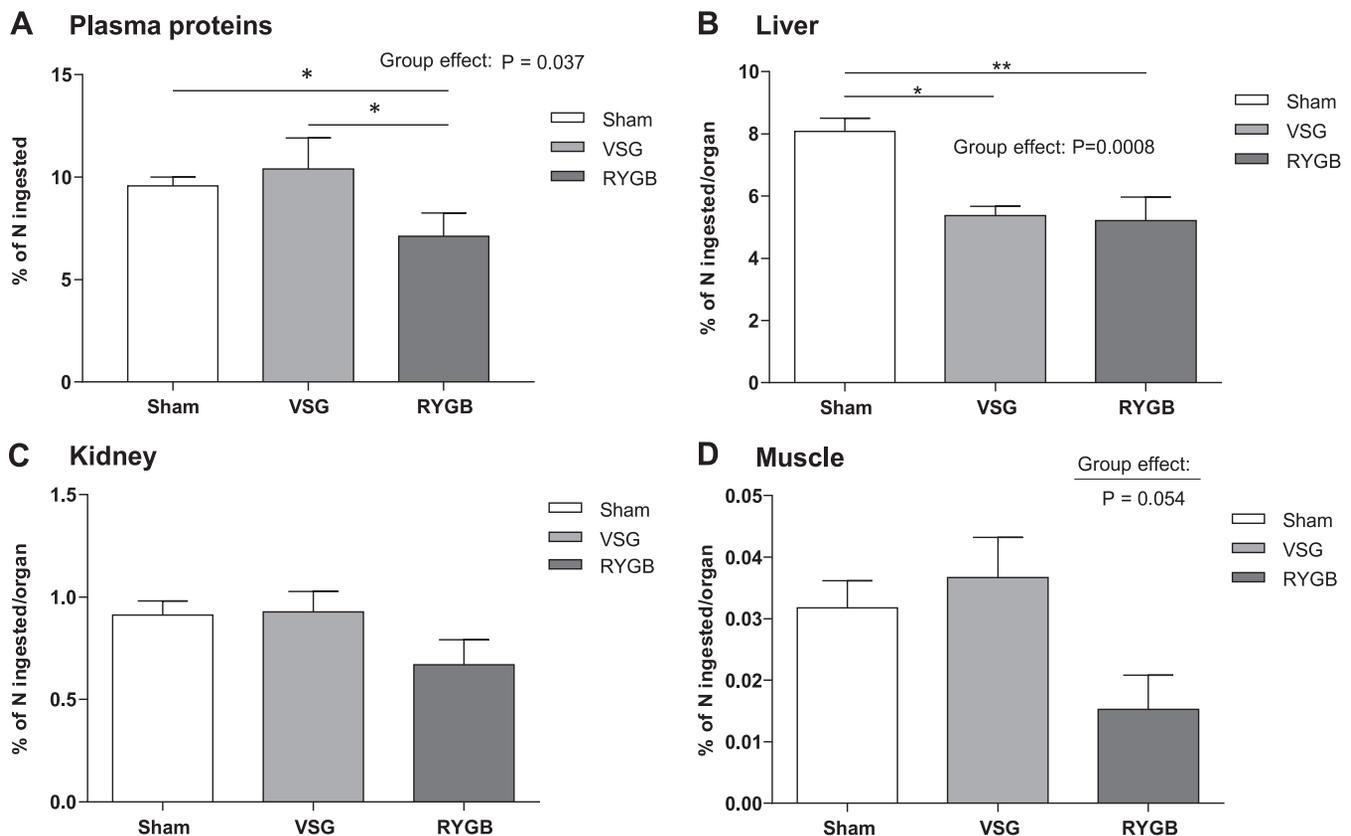


Fig. 3. Exogenous nitrogen recovered in plasma proteins (A), liver (B), kidney (C), and muscle (D) 6 h after the meal, expressed in % nitrogen ingested for the whole organ. Values are means ± SE.  $n = 12$ –13 rats for Sham,  $n = 5$ –7 rats for VSG, and  $n = 8$ –9 rats for RYGB. Surgery effect was tested in each organ by a one-way ANOVA and post hoc Bonferroni's test for multiple comparisons. \* $P < 0.05$ ; \*\* $P < 0.01$ . RYGB, Roux-en-Y gastric bypass; VSG, vertical sleeve gastrectomy.

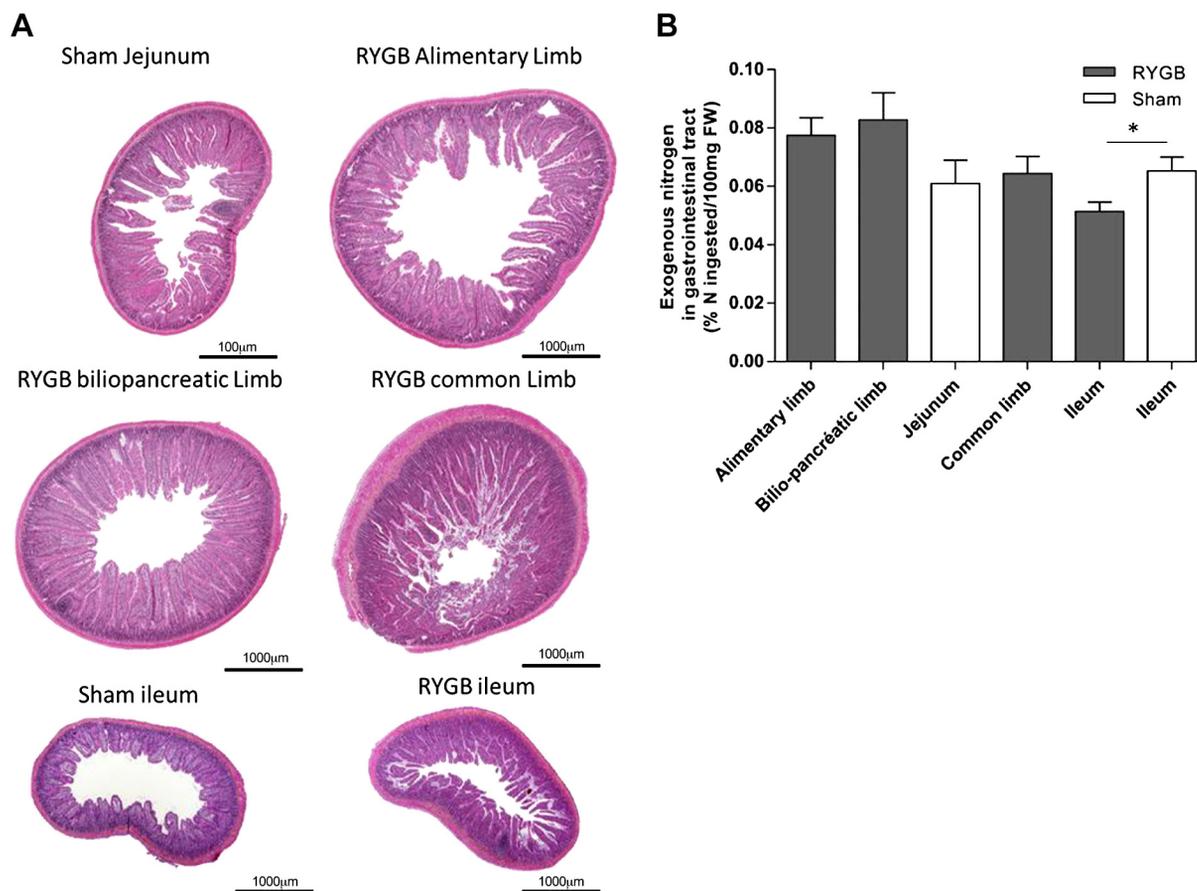


Fig. 4. Histology of intestine (A) and dietary nitrogen recovered in gastrointestinal tract tissues expressed in % of nitrogen ingested per 100 mg of fresh weight (B) of Sham and RYGB rats. Values are means  $\pm$  SE.  $n = 5$  rats for Sham and  $n = 6$  rats for RYGB. The differences between Sham and RYGB were tested in the ileum on one hand and in the jejunum and the common limb on the other hand. \* $P < 0.05$ . RYGB, Roux-en-Y gastric bypass.

(Fig. 3B). Independent of the liver weight, there was also an effect of the group on dietary N retained per 100 mg of liver ( $P = 0.01$ ), which was  $0.057 \pm 0.002\%$  in Sham,  $0.045 \pm 0.009\%$  in VSG, and  $0.039 \pm 0.006\%$  in RYGB, with a significant difference between Sham and RYGB ( $P = 0.001$ , data not shown).

Surgery had no effect on dietary nitrogen found in the kidney (Fig. 3C). In the muscle, there was a trend for an effect of surgery ( $P = 0.054$ ), RYGB rats tending to have less exogenous nitrogen than Sham ( $P = 0.07$ ) (Fig. 3D).

After we observed that the alimentary limb and the ileum of RYGB were hypertrophied compared with the corresponding segments in Sham (jejunum and ileum) (Fig. 4A), the amount of dietary nitrogen in different locations of the intestinal tissue was determined for RYGB and Sham groups (Fig. 4B). Surprisingly, there was as much dietary nitrogen in the BPL ( $0.083 \pm 0.010\%$  of N ingested) than in the alimentary limb ( $0.078 \pm 0.007\%$  of N ingested). There were no differences between the jejunum of Sham rats and the common limb of RYGB rats. In contrast, there was less nitrogen in the RYGB ileum ( $0.052 \pm 0.004\%$  of N ingested) than in Sham ( $0.065 \pm 0.005\%$  of N ingested;  $P = 0.04$ ).

**Protein Anabolism**

The effect of surgery on protein anabolism was assessed by determining the protein synthesis rate in various organs. There

was a group effect ( $P = 0.037$ ) on the FSR in the kidney, with a significant decrease in RYGB compared with Sham and VSG (Table 2). There was also a similar effect on the ASR in the kidney ( $P = 0.017$ ) with a lower value in RYGB than in Sham ( $P = 0.018$ ). There was no effect of surgery on protein

Table 2. Fractional synthesis rate and absolute synthesis rate in liver, kidney, muscle and skin, depending on the type of bariatric surgery

	Sham	VSG	RYGB	Group Effect, $P$
FSR, %/day				
Liver	122.7 $\pm$ 5.6	138.5 $\pm$ 8.9	122.5 $\pm$ 5.4	0.10
Kidney	91.3 $\pm$ 3.5 <sup>a</sup>	79.8 $\pm$ 3.5 <sup>ab</sup>	73.3 $\pm$ 6.0 <sup>b</sup>	0.02
Muscle	15.2 $\pm$ 2.1	15.3 $\pm$ 2.8	17.2 $\pm$ 1.9	0.87
Skin	15.3 $\pm$ 1.1	16.3 $\pm$ 1.8	17.4 $\pm$ 1.6	0.74
ASR, mg·day <sup>-1</sup> , 100 mg <sup>-1</sup>				
Liver	25.1 $\pm$ 1.8	26.2 $\pm$ 1.6	28.0 $\pm$ 3.1	0.41
Kidney	15.9 $\pm$ 0.7	13.3 $\pm$ 0.7	12.8 $\pm$ 1.3	0.04
Muscle	3.1 $\pm$ 0.4	3.2 $\pm$ 0.5	3.6 $\pm$ 0.4	0.72
Skin	4.5 $\pm$ 0.5	4.4 $\pm$ 0.4	5.3 $\pm$ 0.5	0.34

Values are means  $\pm$  SE.  $n = 11-12$  rats for Sham,  $n = 6-7$  rats for vertical sleeve gastrectomy (VSG), and  $n = 8$  rats for Roux-en-Y gastric bypass (RYGB). The effect of the group was tested with an ANOVA model and post hoc Bonferroni test for pairwise comparisons. Values with different letters within the same row are statistically different. ASR, absolute synthesis rate; FSR, fractional protein synthesis rate.

anabolism in the liver, muscle, and skin, regardless of the parameter, FSR or ASR.

## DISCUSSION

In the present study, we assessed the consequences of different types of bariatric surgery on protein bioavailability in diet-induced obese rats. For this purpose, we used a single  $^{15}\text{N}$  test meal to determine dietary protein digestibility and its postprandial retention in tissues. We observed that bypass surgery improved real protein digestibility but that less dietary nitrogen was retained in tissues, except for the hypertrophied intestine. According to our study, intestine remodeling could trigger a preferential use of dietary amino acids by the intestinal mucosa at the expense of other organs.

Concerning weight and food intake, our results are in accordance with other studies on the effectiveness of bariatric surgery on weight loss in rat models (29, 32, 37). During the first days after surgery, all animals experienced rapid weight loss due to substantial food restriction. After refeeding, RYGB continued to lose weight but stabilized around 6 days after surgery, whereas VSG tended to regain weight. Weight regain has been reported in VSG (41) but only after 2 wk.

At day 10, we started a specific feeding schedule to implement the final  $^{15}\text{N}$  test meal. This training implied reduced access time to food. This resulted in a substantial reduction of food intake (almost halved) and therefore a recurrence of weight loss in operated rats, particularly in RYGB. Surprisingly, Sham rats maintained their weight despite their being pair-fed with operated rats. The observation that operated rats lose more weight than pair-fed Sham rats has already been reported (1). This could be the consequence of intestinal remodeling that has been described after bypass surgeries (13, 14, 31), resulting in a higher energy demand. Total energy expenditure has consistently been reported to increase by 13% in RYGB rats compared with body weight-matched Sham rats (12). Thereby, food intake during the habituation protocol was enough for Sham to limit their weight loss in contrast to operated rats. As previously described (12a), malabsorptive bariatric surgery leads to a notable loss of fat as well as lean mass. This loss of lean mass is an indication of protein imbalance (34). In human subjects, protein deficiency after bariatric surgery has been reported to be more or less prevalent, depending on the judgment criteria (albumin, lean mass loss) (6, 8, 25, 39, 59).

Using the  $^{15}\text{N}$  test meal, we aimed at clarifying whether an alteration of protein bioavailability could contribute to the reported protein wasting and loss of lean mass. Nevertheless, because of technical problems in the Eco-MRI follow-up, we could not evidence a lean mass loss after surgery, although it has been shown in rats (55) and humans (12a). Digestive bioavailability was established using a standardized procedure to determine orofecal digestibility 6 h after the test meal ingestion (10, 36, 44, 45) in the absence of any standardized method to determine ileal digestibility in rats. We measured dietary nitrogen in all the distal segments, including cecum, colon, and feces. Amino acids that reach the cecum and colon are metabolized by colonic bacteria, leading to the production of metabolites such as ammonia and indole, which can be absorbed in the colon (23, 62). This results in a systematic overestimation of protein nitrogen digestibility (22, 53). How-

ever, this error is acceptable, especially in the case of highly digestible protein, for which the differences between ileal and fecal digestibility have been shown to be of limited extent (53).

As expected, most of the dietary nitrogen was found in the cecum because of a mostly complete digestion. Surprisingly, there was less dietary nitrogen in the cecum of RYGB than in VSG and Sham rats. Consequently, RYGB rats showed a significant increase of protein digestibility compared with Sham, whereas there was no difference for VSG rats compared with Sham. Although there is no comparable data in the literature, this result concurs with a study on patients operated with RYGB (9) that showed a faster blood appearance of amino acids from [ $^{13}\text{C}$ ]casein given as a meal, thus suggesting a faster absorption. In contrast, a diminution of the apparent fecal protein digestibility was reported 5 and 14 mo after RYGB in with obesity (48). Of note in this last study, the reduced absorption of protein was mostly due to a reduced protein intake rather than to protein malabsorption. Other studies reported an increase of fecal protein output in a rat model of biliopancreatic diversion (42) or one anastomosis gastric bypass (14). This seems to be in contradiction with our results. However, this nitrogen loss may not be the result of lower digestibility of dietary proteins but could arise from cecal bacterial overgrowth and/or an increase of intestinal cell desquamation. Further characterization of these nitrogen losses would be necessary.

The metabolic bioavailability was assessed by measuring  $^{15}\text{N}$  retention in the blood and different tissues. It was noticeable that in operated rats, the liver weighed less than in Sham rats. A decrease in liver weight has already consistently been shown in rats after VSG (61) or gastric bypass surgery (40). An interesting observation is that the  $^{15}\text{N}$  distribution in the splanchnic tissues was altered differently by surgery. In the liver, there was a clear decrease of dietary N uptake regardless of the surgery. This effect can be partly ascribed to the lower liver weight, but when expressed per unit of tissue weight, the  $^{15}\text{N}$  retention was consistently lower in RYGB. Moreover, incorporation in plasma proteins was altered only in RYGB. As plasma proteins are mainly composed of exported liver proteins, this means that the early postprandial incorporation of dietary amino acids in liver protein synthesis was altered because of a deficit of precursor amino acids. In contrast, we found no difference in the liver protein synthesis rate, as assayed by [ $^{13}\text{C}$ ]valine incorporation. This, though, is not inconsistent, because it was measured 6 h after the meal, i.e., in the postabsorptive phase. One may suppose that FSR would have been decreased in the bypass groups if measured just after the meal.

In the peripheral organs, we also found a trend for a decreased retention of  $^{15}\text{N}$  in the muscle RYGB. Regarding protein anabolism, we evidenced little effect of surgery, except in the kidney where FSR and ASR were lower in RYGB than in Sham. It is known that obesity is associated with kidney disorders (60), and it has already been shown that bariatric surgery and subsequent weight loss result in kidney function improvement (17, 21, 43). Our observation of a lower FSR may have been related to a normalization of some biological parameters such as inflammation proteins.

In the intestinal tissues,  $^{15}\text{N}$  retention could only be measured in RYGB and in Sham animals.  $^{15}\text{N}$  recovery was similar in the common limb of RYGB and the jejunum in Sham but

lower in the ileum of RYGB compared with Sham. The latter could be interpreted either as a lower absorption of dietary amino acids in the distal portion of the ileum or by a decrease of substrate availability. The second interpretation is more probable, considering that protein digestibility did increase in RYGB. Surprisingly, we recovered some dietary nitrogen in the BPL, as much as in the alimentary limb, although stomach chyme is not expected to transit through the BPL. One hypothesis is that  $^{15}\text{N}$  amino acids, coming mainly from the mesenteric arteria, were absorbed by the basolateral side of BPL enterocytes. It is possible that mucosa remodeling enhanced this metabolic pathway. A similar observation has been made for glucose, in which glucose transporter 1 has appeared to be overexpressed at the basolateral membrane in RYGB rats (13, 54). This has been linked to the hyperplasia of jejunal mucosa reported in several studies (12, 13, 57), including the present one, resulting in an increase of villi height in the common and alimentary limbs (56) as well as mucosal volume and weight (12). This trophic effect may be mediated by glucagon-like peptide-2, which has been reported to be overexcreted in animal models and in humans (15, 16). We also observed a hyperplasia in the common limb and even in the ileum (Fig. 4). We did not weigh the intestinal mucosa, but the gut weight has been shown to be twofold heavier in gastric bypass than in sham-operated rats (12, 54). As we found a similar amount of dietary N per g of intestine, we can suppose that the intestine sequestered twice as much dietary N in RYGB than in Sham. If we consider an average  $^{15}\text{N}$  retention of 0.06%/100 mg for the whole intestine and an intestinal weight of 20 g in RYGB and 10 g in Sham as previously reported (12), this would result in an intestinal dietary N sequestration of 12% in RYGB versus 6% in Sham rats. This would consistently explain why dietary amino acids were less present in the peripheral area, whereas digestibility was improved. They were likely preferentially used by the remodeled intestine in RYGB rats.

This study had some limitations, in particular, the number of animals in each group, which was a consequence of the high mortality after RYGB procedure and which resulted in a lack of statistical power. Moreover, we did not provide a complete understanding of dietary protein metabolism after bariatric surgery because of missing measurements, such as initial body composition, and postprandial FSR measurements, as well as FSR in the intestine. The latter was not determined because we have chosen an incorporation time of the tracer (30 min) that allowed us to study several organs at once, but that was too long for a very high turnover tissue such as small intestine mucosa (2, 51). This study addressed the short-term consequences of bariatric surgery; it would be useful to study these parameters in the long term. Indeed, alteration of protein bioavailability could be transitory, considering intestine remodeling and adaptation. It would also be necessary to challenge these results in human studies using convenient and poorly invasive procedures to assess dietary protein bioavailability.

In conclusion, this study provided the first data on dietary protein bioavailability after bariatric surgery in rats. Unexpectedly, we reported a slight improvement of protein digestibility after RYGB but not after VSG. In contrast, RYGB decreased dietary nitrogen retention in the peripheral organs, except in the intestine, because of a deficit of amino acid delivery. This

strongly suggests a higher uptake of amino acids by the remodeled intestine for its own needs.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

J.-B.C., M.L.G., and C.G. conceived and designed research; R.T., L.R.-P., O.B., N.K., J.-B.C., M.L.G., and C.G. performed experiments; R.T., O.B., N.K., J.-B.C., M.L.G., and C.G. analyzed data; R.T., L.R.-P., O.B., N.K., J.-B.C., M.L.G., and C.G. interpreted results of experiments; R.T., M.L.G., and C.G. prepared figures; R.T. and C.G. drafted manuscript; R.T., L.R.-P., O.B., N.K., J.-B.C., A.B., D.A.-M., J.C., M.L.G., and C.G. edited and revised manuscript; R.T., L.R.-P., O.B., N.K., J.-B.C., A.B., D.A.-M., J.C., M.L.G., and C.G. approved final version of manuscript.

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