1	Effect of different bariatric surgeries on dietary protein bioavailability in rats				
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22	test meal				
23	Abbreviations: ASR: absolute synthesis rate; BPL, bilio-pancreatic limb; FSR, fractional				
24	synthesis rate; GC, gas chromatography; HFD, high fat diet; IRMS, isotopic ratio mass				
25	spectrometry; MS, mass spectrometry; NAFLD, non-alcoholic fat liver disease; RFD, real				
26	fecal digestibility; RYGB, Roux-en-Y gastric bypass ; VSG, vertical sleeve gastrectomy;				

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#### 28 Abstract

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), Rouxen-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 and the lowest in Sham. Surprisingly, RYGB (95.6  $\pm$ 0.7%) improved protein digestibility (P=0.045) compared to Sham (93.5  $\pm$  0.5%) and VSG (93.8  $\pm$  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 to 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.

47

#### 48 New and Notheworthy

49 Using a sensitive <sup>15</sup>N meal test, we found that gastric bypass slightly improved protein 50 digestibility compared to sleeve gastrectomy or control but in contrast, lowered protein 51 retention in the liver and muscles. This paradox can be due to a higher uptake of dietary

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- 52 nitrogen by the intestinal mucosa that was hypertrophied. This study provides new insight on
- 53 the digestive and metabolic fate of dietary protein in different models of bariatric surgery in
- 54 rats.

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#### 55 Introduction

Bariatric surgery is one of the most efficient treatments of morbid obesity, inducing 56 57 sustainable weight loss (11, 28, 47) and improvement of associated comorbidities (19). Historically, literature distinguished two kinds of bariatric surgeries: purely restrictive 58 59 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 60 61 proximal small intestine from the alimentary tract, therefore theoretically reducing nutrient 62 absorption. The purely restrictive vertical sleeve gastrectomy (VSG) and the restrictive and 63 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 64 65 intake during a single meal but this mechanical restriction cannot fully explain the reported 66 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 67 68 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 69 70 reduced ghrelin (20) but this remains disputed (7). Moreover, RYGB was associated with increases in glucagon-like peptide-1 (GLP-1) and peptide YY (PYY 3-36) that contribute to 71 72 reduction of food intake (50). Additionally, as the stomach secretes hydrochloric acid and 73 pepsinogen that are implicated in the early steps of protein digestion, VSG and RYGB could 74 also lead to a protein maldigestion.

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).

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82 The degree of protein malabsorption after bariatric surgery has not been well established. Using intrinsically <sup>13</sup>C labeled casein, Bosjen-Møller et al. (9) showed accelerated blood 83 84 appearance of dietary amino acids in patients operated for RYGB. In the absence of any 85 digesta collection, it is not known whether this paradoxical effect is only due to a faster 86 digestion kinetic or 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 87 88 in humans after RYGB (48). Overall, none of these studies determined the real digestibility of 89 dietary proteins and their postprandial retention in organs.

This study aimed to characterize digestive and metabolic protein bioavailability by using a test meal containing <sup>15</sup>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.

94

#### 95 Materials and Methods

#### 96 Animal and diets

97 All animal studies comply with the ARRIVE guidelines. They were conducted in compliance 98 with EU directive 2010/63/EU for animal experiments and approved by the Institutional 99 Animal Care and Use Committee (Comité d'éthique en expérimentation animale N° 121) and 100 the Ministry of Higher Education and Research (Ref #02285.03). Male Wistar rats (Janvier 101 Labs, Le Genest-St-Isle, France) (n = 42) weighing 275-280 g were housed in standard 102 environmental conditions (temperature 21-22 °C, 12/12 h light/dark cycle, tap water and food 103 ad libitum). Before surgery, they were fed a high fat diet (HFD) (Altromin, Genestil, 104 Royancourt, France) for 4 months. The composition is detailed in Supplemental Table 1 105 (https://figshare.com/articles/Bariatric\_surgery/8964152). The experimental design of the study 106 is detailed in Supplemental Fig. 1 (https://figshare.com/articles/Bariatric surgery/8964152).

#### 107 Surgical Procedures

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108 After 4 months of HFD, animals were randomly divided into 3 groups: Roux-en-Y gastric 109 bypass (RYGB, n = 18), vertical sleeve gastrectomy (VSG, n = 7) and sham-operated 110 (Sham, n = 16). Initially, two different Sham procedures were performed to match with sleeve 111 and gastric bypasses but rats were then pooled together without any differences in principal 112 criteria. The rats were fasted the night before surgery. Anesthesia was induced and maintained by gaseous inhalation of isoflurane (Vetflurane, Virbac, France). Procedures 113 114 were carried out as previously described (5, 13, 14, 24, 52). After laparotomy, the stomach 115 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 116 fundus was dissociated. The surgical procedures are depicted in Fig. 1. 117

118 VSG

The non-glandular 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 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.

123 RYGB

124 The non-glandular part of the stomach (forestomach) was resected using an ETS-Flex 35 125 mm staple line (Ethicon, Issy les Moulineaux, France). A second staple line (Proximate TX30V 30 mm, Ethicon, Issy les Moulineaux, France), parallel to the first one, was applied to 126 127 delimit a gastric pouch that represented 20% of the initial stomach size while preserving the 128 arterial and venous supply. The jejunum was transected 20 cm after the pylorus. The 129 alimentary limb was anastomosed to the gastric pouch and the biliopancreatic limb was 130 anastomosed 15 cm distally to gastrojejunal anastomosis. Sutures were made by a 7-0 131 Prolene wire (Ethicon, Issy les Moulineaux, France).

132 Sham

Comment citer ce document : Tessier, R., Ribeiro-Parenti, L., Bruneau, O., Khodorova, N., Cavin, Bado, Azzout-Marniche, D., C. Auteur de Caulteur de C 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, Issy les Moulineaux, France)
sutures to sew up the abdominal wall and the skin, respectively.

137 Post-surgery care

Animals received an analgesic (Xylocaine, Astra, 10 mg/kg) and an antibiotic (Penicillin, 20 000 units/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 P.M. to 8.00 A.M. For two days following 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 1). Sham animals were pair-fed with operated animals.

#### 145 Habituation to consumption of a single test meal

146 From day 11 to day 19, rats were accustomed to rapid consumption of a single meal in the 147 morning. For this purpose they received 4 g of standard diet between 8.30 A.M. and 9.00 148 A.M., followed by free access to the diet between noon and 6.30 P.M. Between those two 149 periods rats were fasted with free access to water. Sham rats were pair-fed with operated 150 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 <sup>15</sup>N, as described previously (36). 151 152 Thirty minutes before being euthanized, rats were injected in the lateral tail vein with 150 153 µmol/100 g of body weight of 1-<sup>13</sup>C-Valine (Eurisotop, Saint Aubin, France) under gaseous anesthesia. Six hours after the meal, rats were injected intraperitoneally with a lethal dose of 154 155 pentobarbital sodium (100 mg/kg) and exsanguinated. Whole blood was collected and 156 plasma was isolated and stored at -20°C after centrifugation. Different intestinal segments were identified depending on the surgery procedure: stomach, jejunum, ileum, caecum, and 157 158 colon. The feces emitted during the postprandial period were pooled with colon content. For

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RYGB the biliopancreatic, alimentary and common limbs were collected (Fig. 1). The ileum was defined as the 10 cm before the caecum to recover sufficient amount of samples, although ileum might be shorter accordingly to Vdoviaková et al. (58). Segments were rinsed with NaCl solution (9‰), and the contents were collected in their entirety, weighed and stored at -20°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°C.

## 166 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 with an Echo Medical systems EchoMRI 900 (Whole Body Composition Analyzers, Houston, USA). For technical reasons related to the device, body composition was assessed before surgery in only 18 animals.

#### 171 Analytical methods

Protein bioavailability was assessed by following the <sup>15</sup>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 <sup>15</sup>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 (IRMS) (Isoprime, GV Instrument, Manchester,
UK). Atropine (Thermo Electron, Milano, Italy) and L-glutamic acid (USGS41, Sigma Aldrich,
Saint-Louis, USA) were used as elemental and isotopic standards, respectively.

*In vivo* protein synthesis rate in liver, kidney, skin and muscle was assessed using the <sup>13</sup>Cvaline 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*-butyldimethylsilyl-*N*-methyltrifluoroacetamide mixed with

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1% *tert*-butyldimethylchlorosilane and acetonitrile (Sigma Aldrich, St Louis, USA). <sup>13</sup>C-valine 185 186 enrichment in free amino acids was analyzed by gas chromatography (GC 6890N, Agilent 187 Technologies, Les Ulis, France) coupled to mass spectrometry (MS 5973N, Agilent 188 Technologies, Les Ulis, France) (GC-MS) with electron impact ionization and selected ion monitoring (ions m/z 288 and 289). After hydrolysis, protein bound amino acids were 189 derivatized with propanol and acetyl chloride (Sigma Aldrich, St Louis, USA). <sup>13</sup>C-valine 190 enrichment in proteins was analyzed by GC-combustion-IRMS (Isoprime, GV Instrument, 191 192 Manchester, UK).

193 Histology

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

#### 198 Calculations

199 The exogenous nitrogen  $(N_{exo})$  in each tissue was determined as follows:

$$N_{exo} = N_{tot} \times \frac{APE_s}{APE_m}$$

where  $N_{exo}$  is exogenous nitrogen (mmol),  $N_{tot}$  is amount of nitrogen in the sample (mmol), and APE<sub>s/m</sub> is enrichment excess in the sample (s) or the meal (m). (APE<sub>s/m</sub> = AP<sub>s/m</sub> – 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.

204 Recovery of dietary nitrogen in plasma protein was related to the plasma volume in the rat205 (3.5% of body weight)(63).

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As amino acids and peptides are absorbed massively in the duodenum and jejunum, dietary

208 N losses were determined from the N recovery in the distal parts of the intestine (ileum and

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large intestine, including feces). As digestion was almost complete after 6 h according to this
study and previous ones (36, 46), the losses due to 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:

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

213 where N<sub>ing</sub> is the amount of nitrogen ingested (mmol).

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

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

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

 $ASR = FSR \times P_{content}$ 

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

222

### 223 Statistical analysis

All results are expressed as means ± standard errors of the mean (SEM). 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, USA). Otherwise, differences between groups were tested using a one-way ANOVA with the group as a factor and post-hoc Bonferroni tests for

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228 pairwise comparisons. Differences were considered statistically significant for a value of P <

229 0.05.

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Gastrointestinal and Liver Physiology, 317, G592-G601, DOL: 10.1152/aipgi.00142.2

230 Results

#### 231 Follow up

#### 232 Survival rate

Out of forty two rats, one died before surgery, without any explanation. For Sham, the survival rate was 81.3% (13/16). Deaths occurred during anesthesia, sham surgery or the first hours following surgery. The survival rate for RYGB was 50 % (9/18), due to the complexity of surgical procedures in the context of the learning curve of a new surgeon. All deaths occurred during the early post-surgery period. The survival rate of VSG rats was 100% (7/7).

#### Food intake, weight and body composition

240 During the first two days, animals did not receive any solid food (Figure 2A), and they all lost 241 weight (Fig. 2B). When refeeding began on day 3, rats rapidly increased their energy intake. 242 After the beginning of refeeding, rats continued to lose weight for two days. Operated rats 243 lost between 7 and 9 % of their pre-operative weight. Sham tended to loose less weight than 244 operated animals during this period (5.3  $\pm$  0.2%). From day 5, all groups stopped losing 245 weight; VSG regained weight whereas RYGB and pair-fed Sham did not. When habituation 246 to consumption of a single test meal in the morning started at day 10, energy intake dropped 247 for all groups. Subsequently, food consumption increased between day 11 and day 18, from 248 196.6 kJ ± 4.2 kJ/d (47.4 ± 1.8 kcal/d) to 255.2 ± 12.5 kJ/d (61.0 ± 3.2 kcal/d). Whereas all 249 operated rats lost weight after the beginning of the protocol, Sham rats maintained their 250 weight (about 1% loss in 9 days). The total mass loss was  $44 \pm 3$  g in Sham,  $64 \pm 5$  g in VSG 251 and 73  $\pm$  8 g in RYGB, with a significant effect of the group (P = 0.002), and a significant 252 difference between Sham and RYGB (P = 0.002) and a trend for a difference between Sham and VSG (P=0.07). When pooled together, operated rats lost more weight than Sham rats (P 253 254 = 0.001).

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255 composition 2, The longitudinal follow up of body (Supplemental Table 256 https://figshare.com/articles/Bariatric surgery/8964152) could be carried out in only some rats, 257 leading to an insufficient number of observations. There was significantly (P = 0.02) more fat 258 mass loss in RYGB (-48  $\pm$  5 g) than in Sham rats (-27  $\pm$  3 g). The fat mass loss observed in 259 VSG (lossed  $43 \pm 5$  g) did not differ from the two other groups. The average lean mass loss 260 was  $-27 \pm 4$  g, and we could not detect any differences between groups.

#### 261 Dietary protein bioavailability

#### 262 **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 caecum and the colon lumens. In the stomach, the small intestine, the ileum and the colon lumens there were no differences between groups. In the caecum, 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).

#### 270

## Dietary nitrogen retention in tissues

To evaluate the metabolic bioavailability, we measured <sup>15</sup>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 (Figure 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 about 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 (Figure 3B). Independently of the liver weight, there was also an effect of the group on dietary N retained per 100 mg of liver (P=0.01), that was 0.057 ±

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280 0.002 % in Sham, 0.045  $\pm$  0.009 % in VSG and 0.039  $\pm$  0.006 % in RYGB, with a significant 281 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).

Following our observation that the alimentary limb and the ileum of RYGB were 285 286 hypertrophied compared to the corresponding segments in Sham (jejunum and ileum) (Fig. 287 4A), the amount of dietary nitrogen in different locations of the intestinal tissue was 288 determined for RYGB and Sham groups (Fig. 4B). Surprisingly, there was as much dietary 289 nitrogen in the bilio-pancreatic limb (BPL) (0.083 ± 0.010% of N ingested) than in the 290 alimentary limb (0.078 ± 0.007% of N ingested). There were no differences between the 291 jejunum of Sham rats and the common limb of RYGB rats. In contrast, there was less 292 nitrogen in the RYGB ileum (0.052 ± 0.004 % of N ingested) than in Sham (0.065 ± 0.005% 293 of N ingested; P = 0.04).

## 294 **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 to 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 anabolism in the liver, muscle and skin, regardless of the parameter, FSR or ASR.

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#### 301 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 <sup>15</sup>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 six days after surgery, whereas VSG tended to regain weight. Weight regain has been reported VSG (41), but only after two weeks.

315 At day 10, we started a specific feeding schedule in order to implement the final <sup>15</sup>N test 316 meal. This training implied reduced access time to food. This resulted in a substantial 317 reduction of food intake - almost halved - and therefore a recurrence of weight loss in 318 operated rats, particularly in RYGB. Surprisingly, Sham rats maintained their weight despite 319 their being pair-fed with operated rats. The observation that operated rats lose more weight 320 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 321 a higher energy demand. Total energy expenditure has consistently been reported to 322 323 increase by 13% in RYGB rats compared to body weight-matched Sham rats (12). Thereby, 324 food intake during the habituation protocol was enough for Sham to limit their weight loss in 325 contrast to operated rats. As previously described (27), malabsorptive bariatric surgery leads 326 to a notable loss of fat as well as lean mass. This loss of lean mass is an indication of protein 327 imbalance (34). In human subjects, protein deficiency after bariatric surgery has been

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reported to be more or less prevalent, depending on the judgment criteria (albumin, lean mass loss) (6, 8, 25, 39, 59).

330 Using the <sup>15</sup>N test meal, we aimed at clarifying whether an alteration of protein bioavailability 331 could contribute to the reported protein wasting and loss of lean mass. Nevertheless, due to 332 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 (27). Digestive bioavailability 333 was established using a standardized procedure to determine oro-fecal digestibility 6 h after 334 335 the test meal ingestion (10, 36, 44, 45), in the absence of any standardized method to 336 determine ileal digestibility in rats. We measured dietary nitrogen in all the distal segments, including caecum, colon and feces. Amino acids that reach the caecum and colon are 337 338 metabolized by colonic bacteria, leading to the production of metabolites such as ammonia 339 and indole, which can be absorbed in the colon (23, 62). This results in a systematic 340 overestimation of protein nitrogen digestibility (22, 53). However, this error is acceptable, 341 especially in the case of highly digestible protein, for which the differences between ileal and 342 fecal digestibility have been shown to be of limited extent (53).

343 As expected, most of the dietary nitrogen was found in the caecum due to a mostly complete 344 digestion. Surprisingly, there was less dietary nitrogen in the caecum of RYGB than in VSG 345 and Sham rats. Consequently, RYGB rats showed a significant increase of protein 346 digestibility compared to Sham, while there was no difference for VSG rats compared to 347 Sham. Although there is no comparable data in the literature, this result concurs with a study 348 on patients operated with RYGB (9) that showed a faster blood appearance of amino acids from <sup>13</sup>C casein given as a meal, thus suggesting a faster absorption. In contrast, a 349 350 diminution of the apparent fecal protein digestibility was reported 5 and 14 months after 351 RYGB in obese patients (48). Of note in this last study, the reduced absorption of protein 352 was mostly due to a reduced protein intake rather than to protein malabsorption. Other 353 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 354

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results. However, this nitrogen loss may not be the result of lower digestibility of dietary proteins, but could arise from caecal 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 <sup>15</sup>N retention in the blood and 358 359 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 360 gastric bypass surgery (40). An interesting observation is that the <sup>15</sup>N distribution in the 361 362 splanchnic tissues was altered differently by surgery. In the liver, there was a clear decrease 363 of dietary N uptake regardless of the surgery. This effect can be partly ascribed to the lower liver weight but when expression per unit of tissue weight, the <sup>15</sup>N retention was consistently 364 365 lower in RYGB. Moreover, incorporation in plasma proteins was altered only in RYGB. As 366 plasma proteins are mainly composed of exported liver proteins, this means that the early 367 postprandial incorporation of dietary amino acids in liver protein synthesis was altered due to 368 a deficit of precursor amino acids. In contrast, we found no difference in the liver protein synthesis rate, as assayed by <sup>13</sup>C-valine incorporation. This, though, is not inconsistent 369 370 because it was measured 6 h after the meal, i.e. in the post-absorptive phase. One may 371 suppose that FSR would have been decreased in the bypass groups if measured just after 372 the meal.

In the peripheral organs, we also found a trend for a decreased retention of <sup>15</sup>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, <sup>15</sup>N retention could only be measured in RYGB and in Sham animals.
 <sup>15</sup>N recovery was similar in the common limb of RYGB and the jejunum in Sham, but lower in

Tessier, R., Ribeiro-Parenti, L., Bruneau, O., Khodorova, N., Cavin, Bado, Azzout-Marniche, D.,

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382 the ileum of RYGB compared to Sham. The latter could be interpreted either as a lower 383 absorption of dietary AA in the distal portion of the ileum, or by a decrease of substrate 384 availability. The second interpretation is more probable considering that protein digestibility 385 did increase in RYGB. Surprisingly, we recovered some dietary nitrogen in the bilio-386 pancreatic limb, as much as in the alimentary limb, although stomach chyme is not expected to transit through the BPL. One hypothesis is that <sup>15</sup>N amino acids, coming mainly from the 387 388 mesenteric arteria, were absorbed by the basolateral side of BPL enterocytes. It is possible 389 that mucosa remodeling enhanced this metabolic pathway. A similar observation has been 390 made for glucose, where GLUT1 has appeared to be over expressed at the basolateral 391 membrane in RYGB rats (13, 54). This has been linked to the hyperplasia of jejunal mucosa, 392 reported in several studies (12, 13, 57) including the present one, resulting in an increase of 393 villi height in the common and alimentary limbs (56) as well as mucosal volume and weight 394 (12). This trophic effect may be mediated by GLP-2 that has been reported to be over 395 excreted in animal models and in humans (15, 16). We also observed a hyperplasia in the 396 common limb and even in the ileum (Figure 4). We did not weigh the intestinal mucosa, but 397 the gut weight has been shown to be twofold heavier in gastric bypass than in Sham 398 operated rats (12, 54). As we found a similar amount of dietary N per g of intestine, we can suppose that the intestine sequestrated twice more dietary N in RYGB than in Sham. If we 399 consider an average <sup>15</sup>N retention of 0.06%/100 mg for the whole intestine and an intestinal 400 401 weight of 20 g in RYGB and 10 g in Sham as previously reported (12), this would result in an 402 intestinal dietary N sequestration of 12% in RYGB vs. 6% in Sham rats. This would 403 consistently explain why dietary AA were less present in the peripheral area, whereas digestibility was improved. They were likely preferentially used by the remodeled intestine in 404 405 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

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409 metabolism after bariatric surgery due to missing measurements, such as initial body 410 composition, postprandial FSR measurements as well as FSR in the intestine. The latter was 411 not determined because we have chosen an incorporation time of the tracer (30 min) that 412 allowed to study several organs at once but that was too long for a very high turnover tissue 413 such as small intestine mucosa (2, 51). This study addressed the short-term consequences 414 of bariatric surgery; it would be useful to study these parameters in the long-term. Indeed, 415 alteration of protein bioavailability could be transitory, considering intestine remodeling and 416 adaptation. It would also be necessary to challenge these results in human studies using 417 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, due to a deficit of amino acid delivery. This strongly suggests a higher uptake of amino acids by the remodeled intestine for its own needs.

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427 **Conflict of interest statement**: The authors declare no conflict of interest.

428

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Tessier, R., Ribeiro-Parenti, L., Bruneau, O., Khodorova, N., Cavin, Bado, Azzout-Marniche, D.,

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Figure 1: Schematic representations of normal (Sham) and remodeled gastrointestinal tract
 after VSG and RYGB procedures. BPL: biliopancreatic limb, AL: alimentary limb CL:
 common limb.

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Figure 2: Energy intake (A) and weight loss (B) after bariatric surgery. Refeeding begun 3 days after surgery. Habituation to a discontinuous food pattern begun at d10. Sham rats were pair-fed with operated rats. Values are means  $\pm$  SEM, n = 13 for Sham, n = 7 for VSG, n = 9 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).

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Figure 3: Exogenous nitrogen recovered in plasma proteins (A), liver (B), kidney (C) and muscle (D) six hours after the meal, expressed in % nitrogen ingested for the whole organ. Values are means  $\pm$  SEM, n = 12-13 for Sham, n = 5-7 for VSG and n = 8-9 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.

642

**Figure 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  $\pm$  SEM, n = 5 for Sham and n = 6 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

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

	Sham	VSG	RYGB	Group Effect	
% of N ingested					
Stomach	0.52 ± 0.11	0.10 ± 0.34	0.30 ± 0.15	0.066	
Small intestine	$0.69 \pm 0.05$	0.81 ± 0.21	0.65 ± 0.16	NS	
lleum	$0.32 \pm 0.07$	0.69 ± 0.32	0.15 ± 0.05	0.078	
Caecum	$4.46 \pm 0.33^{a}$	$4.45 \pm 0.54^{a}$	$2.42 \pm 0.43^{b}$	0.002	
Colon	1.76 ± 0.38	1.07 ± 0.21	1.85 ± 0.35	NS	
RFD (%)	$93.5 \pm 0.5^{a}$	$93.8 \pm 0.6^{ab}$	$95.6 \pm 0.7^{b}$	0.045	

Values are means ± SEM, n = 13 for Sham, n = 7 for VSG and n = 9 for 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. RFD: real fecal digestibility.

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**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		
FSR (%/d)						
Liver	122.7 ± 5.6	138.5 ± 8.9	122.5 ± 5.4	P = 0.10		
Kidney	91.3 ± 3.5 <sup>a</sup>	$79.8 \pm 3.5^{ab}$	$73.3 \pm 6.0^{b}$	P = 0.02		
Muscle	15.2 ± 2.1	15.3 ± 2.8	17.2 ± 1.9	P = 0.87		
Skin	15.3 ± 1.1	16.3 ± 1.8	17.4 ± 1.6	P = 0.74		
	A	SR (mg/d/100 mg)				
Liver	25.1 ± 1.8	26.2 ± 1.6	28.0 ± 3.1	P = 0.41		
Kidney	15.9 ± 0.7	13.3 ± 0.7	12.8 ± 1.3	P = 0.04		
Muscle	$3.1 \pm 0.4$	$3.2 \pm 0.5$	$3.6 \pm 0.4$	P = 0.72		
Skin	$4.5 \pm 0.5$	$4.4 \pm 0.4$	$5.3 \pm 0.5$	P = 0.34		

Values are means  $\pm$  SEM, n = 11-12 for Sham, n = 6-7 for VSG and n = 8 for 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.

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# Figure 2







## **B:** Liver



C: Kidney





## Figure 4

