




# Gut Microbial Predictors of Type 2 Diabetes Remission Following Bariatric Surgery

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## Abstract

**Purpose** Distinct anatomical rearrangements of the gastrointestinal tract achieved by various types of bariatric surgery cause changes in nutrient intake and gut microbiota. The contribution of such gut microbiota changes to remission of type 2 diabetes (T2D) remains unclear.

**Aim** We examined gut microbiota changes following banded Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy (SG) in a randomised study, in relation to T2D remission.

**Materials and Methods** Whole-metagenome shotgun sequencing was carried out on paired stool samples at pre- and 1-year post-surgery collected from 44 participants with T2D randomised to banded Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy (SG). Taxonomic composition and predicted functional potential of the gut bacteria were identified using HUMAnN2, and annotated using MetaCyc. Five-day dietary records (analysed using FoodWorks v8.0), body weight and diabetes status were recorded at both time points.

**Results** RYGB participants had higher percentage excess weight loss than SG ( $p = 0.01$ ), even though dietary intake was similar at 1-year post-surgery. Similar proportions achieved diabetes remission (HbA1c < 48 mmol/mol without medications) after either RYGB (68%) or SG (59%). RYGB resulted in increased abundances of Firmicutes and Proteobacteria, while SG resulted in increased Bacteroidetes. Pre-surgery, an increased abundance of Eubacteriaceae ( $p = 0.01$ ) and *Alistipes putredinis* ( $p = 0.01$ ) was observed in those who went on to remit from T2D post-surgery. Following surgery, Lachnospiraceae ( $p = 0.04$ ) and *Roseburia* ( $p = 0.01$ ) species were more abundant in those who had achieved T2D remission.

**Conclusions** Specific stool bacterial taxa may signal likelihood of T2D remission after bariatric surgery which is potentially mediated by increases in *Lachnospiraceae* and *Roseburia*.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11695-020-04684-0>) contains supplementary material, which is available to authorized users.

**Keywords** Bariatric surgery · Fecal microbiota · Bacteria · Bypass · Diabetes · Obesity

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## Abbreviations

ANOVA	Analysis of variance
BMI	Body mass index
GI	Gastrointestinal
LDA	Linear discriminant analysis
LEfSe	Linear discriminant analysis effect size
PERMANOVA	Permutational multivariate analysis of variance
RCT	Randomised controlled trial
RYGB	Roux-en-Y gastric bypass
SCFA	Short chain fatty acids
SG	Sleeve gastrectomy
T2D	Type 2 diabetes
VLCD	Very low calorie diet

## Introduction

Bariatric surgery is currently the only therapy that achieves long-term weight reduction, and has dramatic effects on both remission and prevention of type 2 diabetes (T2D) among those with severe obesity [1]. Both Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) achieve rapid and sustained weight loss of up to 70%, and remission of T2D in 70–85% of patients [2–4], with very few complications [3, 5]. While there are significant differences in the anatomical rearrangements with RYGB and SG [6, 7], the contribution of the associated changes in gut microbiota to the metabolic benefits arising after bariatric surgery is unclear [8].

Obesity and T2D have been reported to hold distinct gut microbial ‘signatures’ [9–13] that are very different to those of a healthy, non-obese population. Several studies to date have also described dramatic alterations to the gut microbiota following bariatric surgery [14–27]. However, due to the heterogeneity of these studies (in terms of surgery type, sample number, methods used and follow-up periods), exactly which of the many reported changes in the gut microbiota are causally linked to the metabolic benefits (e.g. diabetes remission) remains unclear.

Diet [28] and medication use [29] are major contributing factors to the composition of the gut microbiota [30, 31]. Following a change to low calorie diet, large and immediate alterations to the gut bacterial community have been observed [28, 32]. Given that bariatric surgery results in a significant reduction in caloric intake as well as alterations in dietary macronutrient composition, this may represent a confounding factor. Further to this, many glucose-lowering and anti-acid medications (metformin, proton pump inhibitors) have also been shown to alter the gut bacterial community [33–38]. Therefore, it is vital to address diet and medication use before and after surgery, in order to accurately tease out surgery-specific changes to the gut microbiota.

In this study, we wished to identify whether there were surgery-specific changes in gut microbiota among obese people with T2D randomised to either SG or banded RYGB and whether there were common taxa and gut microbiota functional capacity changes among those who achieved T2D remission, irrespective of surgery type. We also wished to identify whether there were any baseline gut microbial predictors of T2D remission following surgery.

## Methods

### Ethics

The trial ‘Sleeve gastrectomy versus Roux-en-Y gastric bypass for type 2 diabetes and morbid obesity: double-blind randomised clinical trial’ [39] is registered with the Australian New Zealand Clinical Trials Registry

(ACTRN12611000751976) and received approval from the New Zealand regional ethics committee (NTY/11/07/082). All participants gave written informed consent. This RCT commenced in September 2011 and recruitment completed in October 2014. Five-year data collection is currently ongoing.

### Randomised Controlled Trial

One hundred and fourteen obese participants with T2D were randomised to either RYGB or SG as part of a double-blind RCT with the primary outcome of diabetes remission, defined as HbA1c < 6.5% (< 48 mmol/mol) without the use of diabetes medication at 5 years [39].

Inclusion criteria for patients were: age between 20 and 56 years, a body mass index (BMI) of 35–65 kg/m<sup>2</sup> and T2D diagnosis duration at least 6 months. Patients were excluded from participating in the trial if they had a BMI greater than 65 kg/m<sup>2</sup>, type 1 diabetes, were pregnant, or were previously diagnosed with cancer, severe lung, kidney, liver, or heart disease, due to increased risk of complications during bariatric surgery in these particular situations. Patients were assessed 2 days prior to surgery and at 12 months following surgery. A detailed protocol has been published previously [39].

### Very Low Calorie Diet and Surgery

Prior to surgery, participants were prescribed a very low calorie diet (VLCD) for 2 weeks to reduce hepatic steatosis and the risks associated with laparoscopic abdominal surgery. The diet consisted of non-starch vegetables and three daily servings of Optifast (Nestle, Vevey, Switzerland), each of which contained approximately 200 cal.

SG was performed starting 2 cm proximal to the pylorus using serial applications of a stapler device over a 36Fr orogastric bougie. RYGB was created with a gastric pouch formed around a 32Fr orogastric tube and a 100-cm Roux limb anastomosed to this pouch (gastrojejunostomy). A 50-cm biliopancreatic limb was then anastomosed in a side-to-side stapled fashion to the distal Roux limb. A 6.5-cm silastic ring was then secured around the gastric pouch approximately 2 cm above the gastrojejunostomy, representing a banded RYGB.

### Post-operative Assessments for T2D Status

All pharmacological agents for diabetes were stopped at the time of surgery. Post-surgery, pharmacological treatment for diabetes was restarted if mean post-operative capillary glucose exceeded 12 mmol/L [39]. Postoperatively, all participants were prescribed a twice daily multivitamin: either Band Buddies (NutriChew, Brisbane, Australia; each tablet containing 300-mg elemental calcium and 500-IU vitamin D3) or

Centrum 50+ (Pfizer New Zealand, Auckland, New Zealand; each tablet containing 200-mg elemental calcium and 600-IU vitamin D3).

Diabetes remission at 1 year was defined as HbA1c < 6.5% (48 mmol/mol) without glucose lowering therapies. Percent excess weight loss (%EWL) was calculated as the difference between initial BMI and final BMI, divided by the difference between the initial BMI and a target BMI of 25 kg/m<sup>2</sup>. Dietary intake data was collected from food diaries in which participants were instructed to record everything they ate and drank (including supplements) for 5 days immediately prior to the pre-surgery and 1-year study assessment visits. Dietary nutrient analysis was conducted using FoodWorks version 8.0 (Xyris software, Highgate Hill, Melbourne, Australia). Items were coded into protein, total fat, saturated fat, carbohydrate, fibre and estimated total caloric intake in kilocalories.

### Faecal Samples and DNA Isolation

Two days prior to each assessment (pre-surgery and 1 year following surgery) faecal samples were self-collected as follows: a plastic collection tray was placed in the toilet bowl; participants produced their sample into the collection container (without urinating); using gloves, a small piece of stool was scooped into the faecal collection tube using the scoop on the lid of the faecal collection tube, sealed and placed into a larger container filled with water, sealed again and frozen immediately in the home freezer (−20 °C). On the day of the appointment, samples were transported (frozen) to the laboratory where they were stored (−80 °C) until DNA extraction. DNA was extracted using the Qiagen QIamp DNA stool mini kit (according to the manufacturer's instructions).

### DNA Sequencing and Library Construction

Whole genome shotgun sequencing was performed on extracted genomic DNA using an Illumina HiSeq 2000 platform. Paired-end reads were trimmed using trimmomatic (v0.33, min length 50 bp), and human contaminants were removed using python scripts (remove\_contaminants.py). Microbial taxa and microbiota functional profiles were identified using HUMAnN2, which incorporates both MetaPhlan 2.0 for species and taxonomic identification, and HUMAnN for metabolic pathway identification using the MetaCyc database (<https://metacyc.org>) [40]. All taxa and pathway data were reported as relative abundances in each sample. Processed sequence data (to remove human sequences) is available on NCBI Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) project number PRJNA588805.

### Statistical Analyses

Alpha diversity was calculated using both Shannon and Simpson indices. One-way ANOVA and Student's *t* test were used to identify significant differences between alpha diversity in each group (surgery group or remitters vs non-remitters).

Permutational multivariate analysis of variance (PERMANOVA) was undertaken to identify differences in the variables of time point, sex, surgery type, BMI (at baseline) and HbA1c (both as a continuous variable and with a categorical variable of 'remitted' or 'not remitted' defined as outlined above).

Linear discriminant analysis (LDA) effect size (LEfSe) is a computational method used specifically for biomarker discovery within high-dimensional class (group) comparisons. LEfSe identifies features (bacterial species and genetic pathways) that most likely explain the differences observed between groups (surgery type, before or after surgery and T2D status) by coupling standard tests for statistical significance with additional tests encoding biological consistency. Finally, the effect size generated by LEfSe provides an estimation of the magnitude of the observed change due to each of the groups specified [41].

Here we used LEfSe to identify statistically significant differences in relative abundances of gut microbiota and metabolic pathways between pre- and post-operative (1 year) RYGB or SG patients, as well as between those who remitted from T2D and those who did not, 1-year post-surgery. A default cutoff value of LDA > 2.0 was used in all tests. Mann-Whitney tests were used to confirm significant differences between taxa or pathways identified by LEfSe. A *p* value < 0.05 was considered statistically significant.

## Results

### Participant Characteristics

Of the 114 participants in the randomised controlled trial who received either RYGB or SG surgery, 44 participants (including 14 whose samples were analysed previously [21]) provided faecal samples at both baseline and 1 year (Supplementary Fig. 1). Age, BMI, HbA1c, T2D medications and T2D status did not differ significantly by treatment group at baseline (Table 1). There was significantly greater %EWL 1-year post-operatively after RYGB compared with SG (*p* = 0.01). T2D remission was achieved at 1 year in 68% of those in the RYGB group and 59% in the SG group. The number of participants on insulin therapy and/or metformin treatment decreased at 1 year following both types of surgery (Table 1). Nutrient intake in kcal, protein, carbohydrate, total fat, saturated fat and fibre is outlined in Table 1. The total caloric intake increased 1 year after

surgery following both RYGB and SG, when compared with the VLCD consumed 4 weeks prior to surgery. There were no significant differences between calorie intake and diet macronutrient composition between the surgery groups at baseline or 1 year following surgery.

### Surgery-Specific Changes to the Gut Microbiota Composition and Functional Capacity

We observed no difference in alpha diversity from baseline to 1 year post-operatively for all 44 samples, or at 1 year following either RYGB ( $n = 22$ ), or SG ( $n = 22$ ).

We identified both time point (pre- vs post-surgery) and sex as significantly associated with microbiome taxonomic composition using PERMANOVA ( $p = 0.023$  for time point and  $p = 0.041$  for sex). We therefore adjusted for time point and sex, where possible, in subsequent analyses.

Using LEfSe [41], we observed small phylum level differences from baseline to 1 year following each type of surgery (Fig. 1). RYGB surgery was associated with an increased abundance of the phyla Firmicutes and Proteobacteria at 1-year post-surgery (Fig. 1a), whereas, SG surgery was associated with an increased abundance of Bacteroidetes post-surgery (Fig. 1b).

To determine if the predicted functional capacity of the gut microbial composition changed as a result of either RYGB and or SG, we used LEfSe to identify changes from baseline to 1 year within each surgery type. LEfSe identified no predicted metabolic functions to be differentially abundant for either surgery type from baseline to 1 year post-surgery.

In a cross-sectional analysis at 1 year post-surgery, LEfSe analysis identified only a single order—Enterobacteriales (phylum = Proteobacteria) that was significantly more abundant (LDA = 4.21,  $p = 0.0084$ ) in patients within the RYGB surgery group (Fig. 2) when compared with the SG group. We did not identify any differentially abundant functional capacity features of the gut microbiome for the same cross sectional analysis of RYGB vs SG at 1-year post-surgery.

### Baseline Gut Microbial Composition and Functional Capacity Differences Among those with Subsequent Diabetes Remission Vs Non-diabetes Remission

LEfSe identified four bacterial taxa that were more abundant in the pre-surgery faeces of those who remitted from T2D at 1 year (Fig. 3a). Both the family Eubacteriaceae (phylum Firmicutes) and the species *Alistipes putredinis* (phylum Bacteroidetes) were significantly more abundant

**Table 1** Characteristics at baseline and 1 year after surgery

Characteristic		RYGB ( $n = 22$ )	SG ( $n = 22$ )	$p$ value (RYGB vs SG)
Age (y)		48.5 ± 5.5	47.7 ± 6.9	0.66
Gender (M:F)		7:15	14:8	0.07
BMI (kg/m <sup>2</sup> )	Baseline	38.2 ± 5.7	40.0 ± 5.9	0.30
	1 year	27.5 ± 5.8	30.4 ± 3.6	0.06
	Δ BMI	− 11.4 ± 2.9	− 9.5 ± 3.6	0.10
EWL%		84.4 ± 30.8	65.3 ± 14.9	0.01
HbA1c (mmol/mol)	Baseline	8.5 ± 1.7	8.0 ± 1.1	0.28
	1 year	6.3 ± 0.8	6.3 ± 0.9	0.89
Insulin, n (%)	Baseline	7 (31.8)	5 (22.7)	0.52
	1 year	1 (4.5)	2 (9.1)	
Metformin, n (%)	Baseline	19 (86.4)	19 (86.4)	1.00
	1 year	2 (9.1)	2 (9.1)	
T2D Remission, n (%)	1 year	15 (68.2)	13 (59.1)	0.76
Caloric intake (kcal/d)	Baseline	774.5 (281.6)	814.5 (179.4)	0.63
	1 year	1209 (495)	1087 (406)	0.50
Protein intake (g/d)	Baseline	58.7 (23.6)	61.3 (10.6)	0.68
	1 year	62.1 (22.6)	67.5 (18.0)	0.50
Total fat intake (g/d)	Baseline	19.6 (9.7)	19.8 (10.5)	0.97
	1 year	47.6 (23.1)	38.5 (19.6)	0.29
Saturated fat intake (g/d)	Baseline	5.3 (3.0)	6.5 (5.9)	0.49
	1 year	20.5 (9.5)	14.3 (7.8)	0.08
Carbohydrate intake (g/d)	Baseline	79.6 (30.8)	89.3 (20.8)	0.29
	1 year	127.7 (62.2)	113.2 (50.4)	0.52
Fibre intake (g/d)	Baseline	15.3 (7.4)	14.1 (7.2)	0.66
	1 year	8.4 (3.2)	10.3 (5.0)	0.28

Data are mean ± standard deviation.  $P$  value determined using  $t$  tests and Fisher's exact test between surgery type

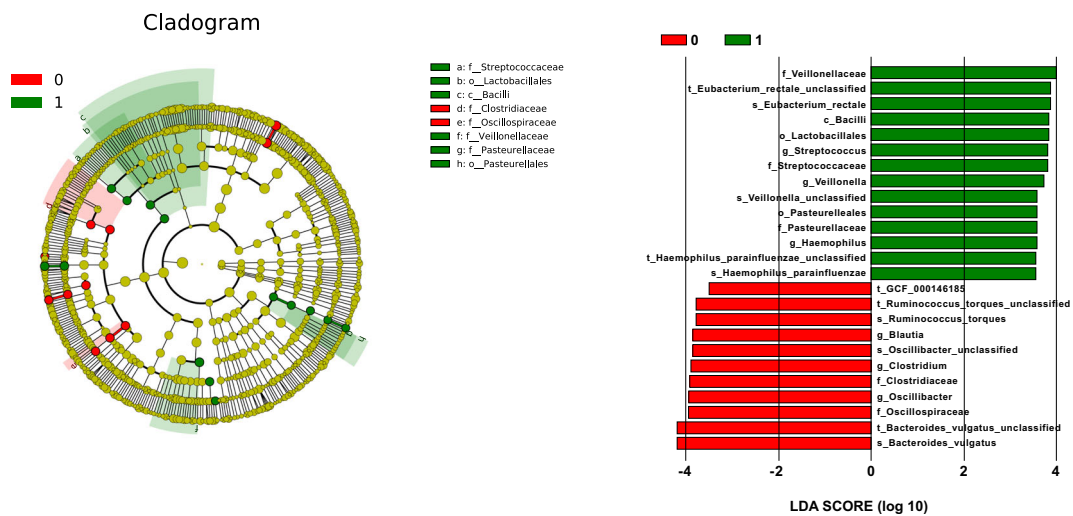
( $p = 0.01$  (Eubacteriaceae),  $p = 0.009$  (*Alistipes putredinis*)) in the pre-surgery gut only in those who went on to remit from T2D following surgery (Fig. 3b). This was true irrespective of surgery type. Importantly, the abundance of these Eubacteriaceae and *Alistipes putredinis* taxa did not correlate with duration of T2D pre-surgery (Supplementary Fig. 2).

LefSe was used to identify predicted metabolic pathways that were significantly different between T2D remitters and non-remitters. At baseline, those who went on to remit at 1 year had significantly increased guanosine nucleotide degradation and n10-formyl-tetrahydrofolate biosynthesis, a component of purine base synthesis (Fig. 5).

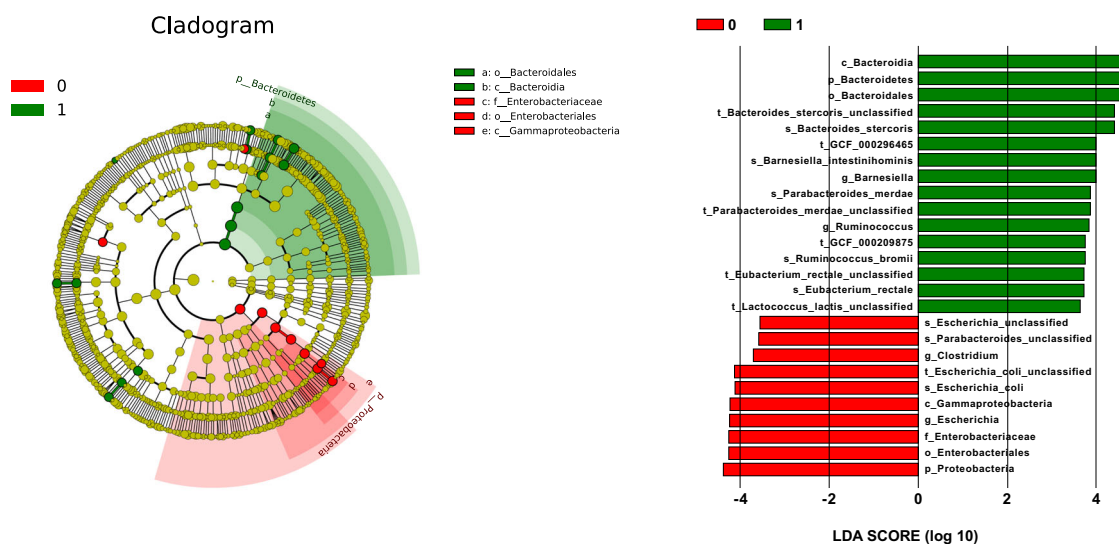
## Gut Microbial Composition and Functional Capacity Among T2D Remitters Vs Non-remitters 1 Year Following Surgery

LefSe analysis identified the family Lachnospiraceae (phylum Firmicutes) ( $p = 0.036$ ) and the genus *Roseburia* (phylum Firmicutes) ( $p = 0.0047$ ) as being more abundant 1-year post-surgery in diabetes remitters, regardless of surgery type (Figs. 4 and 5). At 1 year post-surgery, increased purine biosynthesis was observed only in those with persistent T2D (Fig. 6). However, this was not significant after post hoc analyses (Mann-Whitney,  $p = 0.93$ ).

### (a) RYGB

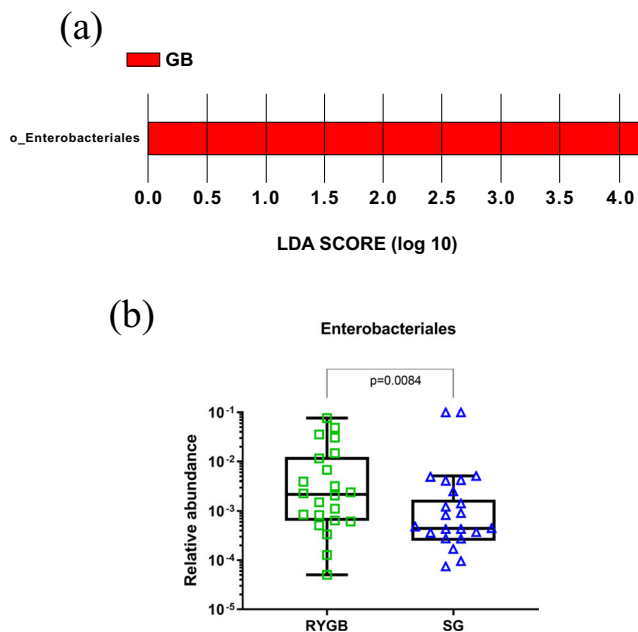


### (b) SG



**Fig. 1** Surgery specific differences in the gut bacteria from pre-surgery (0, red) to 1 year post surgery (1, green) following RYGB (a) and SG (b). Red areas/bars indicate those taxa that were most abundant pre-surgery. Green areas/bars indicate those taxa that were more abundant at 1 year





**Fig. 2** Differentially expressed taxa at 1 year after RYGB versus SG. **a** Red bar indicates the single differentially abundant taxa between RYGB and SG 1 year after surgery. **b** Individual box plot of Enterobacteriales

## Discussion

We have demonstrated that the gut microbiota of those receiving RYGB became enriched in Firmicutes, while the gut microbiota of those receiving SG surgery became enriched in Bacteroidetes, as compared with the baseline gut microbiota. However, these taxonomic changes did not result in any significant difference in overall predicted functional capacity changes of the gut microbiota by type of surgery. Importantly, irrespective of the type of surgery, we identified that *Lachnospiraceae* and *Roseburia* taxa increased among those who achieved diabetes remission. We also identified higher levels of *Alistipes putredinis* species and Eubacteriaceae (family) in baseline stool samples of those who subsequently achieved diabetes remission after either type of bariatric surgery.

Given the comparable T2D remission observed between these two types of bariatric surgery, it is unlikely that surgery-specific changes in gut microbiota will be causal to the common metabolic benefits observed. Indeed, decreases in the relative abundance of Firmicutes after SG and increases in Bacteroidetes and Proteobacteria after RYGB have been reported in two systematic reviews of the changes to the gut microbiota following bariatric surgery [8, 42]. At 1 year after surgery, patients who had banded RYGB surgery had higher Enterobacteriales (Proteobacteria) levels than SG patients, consistent with earlier reports of changes following non-banded RYGB at

**Fig. 3** Differentially abundant taxa in the baseline gut separated by T2D status **a** identified by LEfSe, and **b** Individual box plots of Eubacteriaceae and *Alistipes putredinis* by surgery type and T2D status. Red bars indicate taxa that were more abundant pre-surgery in those with persisting T2D at 1-year, and green bars indicate taxa that were more abundant pre-surgery in those who remitted from T2D at 1 year. R = remitted (green bars), NR = non-remitted (red bars). Squares indicate whole cohort, triangles indicate SG and circles indicate RYGB

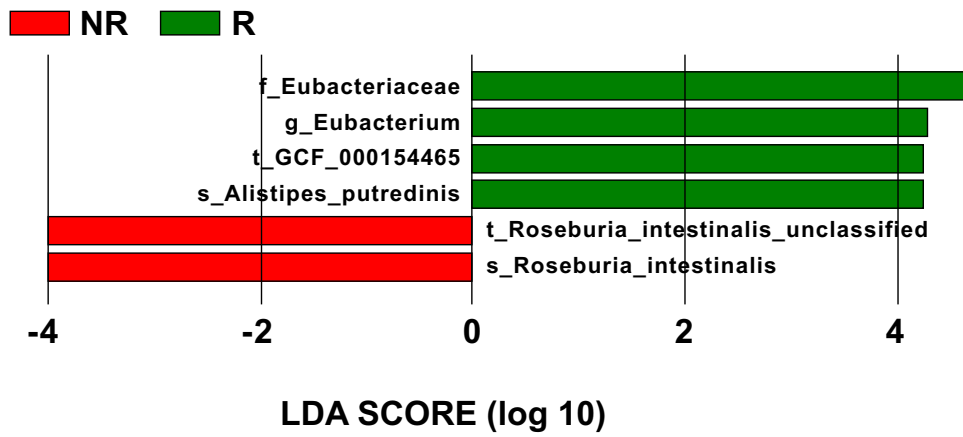
time points ranging from 3 months to 9.4 years [14, 16–18, 20, 26].

We did not see the expected increase in alpha diversity of the gut microbiota from baseline to 1-year post-surgery, in either surgery type, nor was increased alpha diversity associated with T2D remission status. However, the baseline gut microbiota was analysed while patients had been using VLCD in preparation for surgery in which all had markedly reduced their daily caloric intake, which is a lower energy but more nutritionally balanced than a typical obesogenic diet. It has been documented that low caloric intake significantly increases the diversity and richness of the gut bacteria [19, 32]. Therefore, this reduced caloric intake at baseline could have ameliorated possible increases in diversity which may have been seen if we had compared gut microbiota changes after surgery to baseline gut microbiota while on usual diet (prior to VLCD).

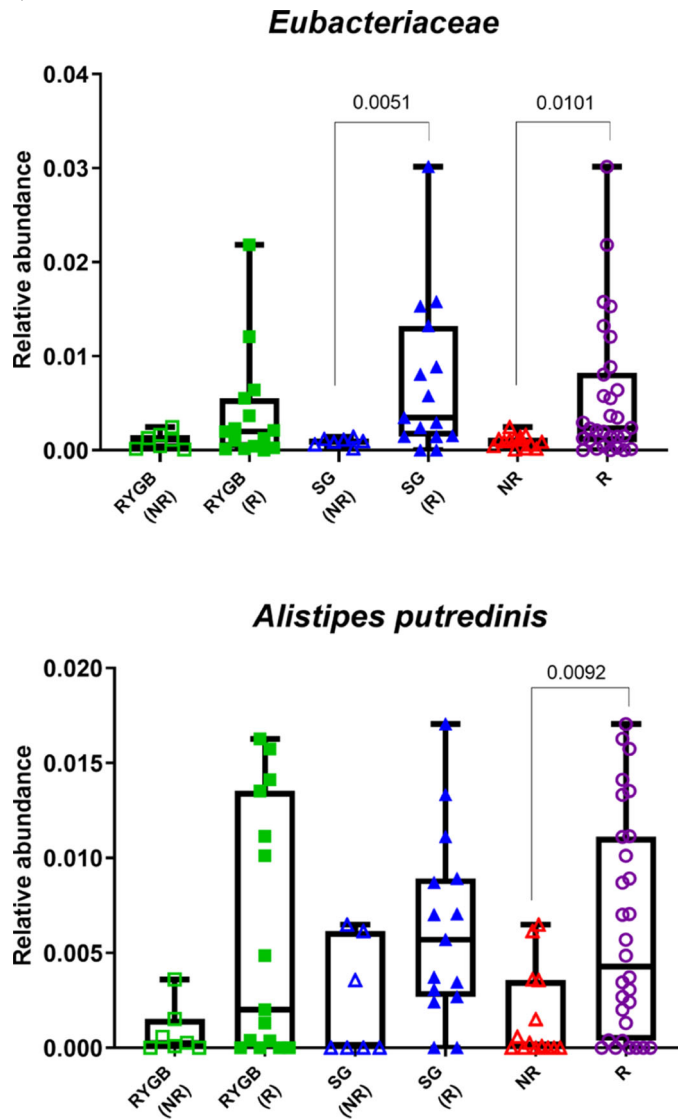
The baseline gut microbiota from people who remitted from T2D 1-year post-surgery had greater abundances of *Alistipes putredinis* species and Eubacteriaceae (family), detected while on VLCD. *Alistipes species* of the phylum Bacteroidetes are commonly ingested via plant sources, and are known butyrate producers [43]. Since participants were on a VLCD diet for 4 weeks before surgery, consisting only of Optifast shakes, accompanied by non-starch vegetables, it is possible that the higher abundance of *Alistipes sp.* among subsequent T2D remitters reflects pre-operative adherence to dietary advice, which in turn may predict post-operative dietary adherence promoting T2D remission. Alternatively, this could be a marker of gut dysbiosis that responds best to bariatric surgery, as *Alistipes putredinis* has also been associated with general gut dysbiosis [44, 45], increased inflammation and irritable bowel syndrome [46]. Some other bariatric studies to date investigating microbiome changes have reported an increased abundance in these species at 6 months [17] and 12 months [20] following RYGB, although they have not looked specifically for gut microbial biomarkers of diabetes remission pre-operatively.

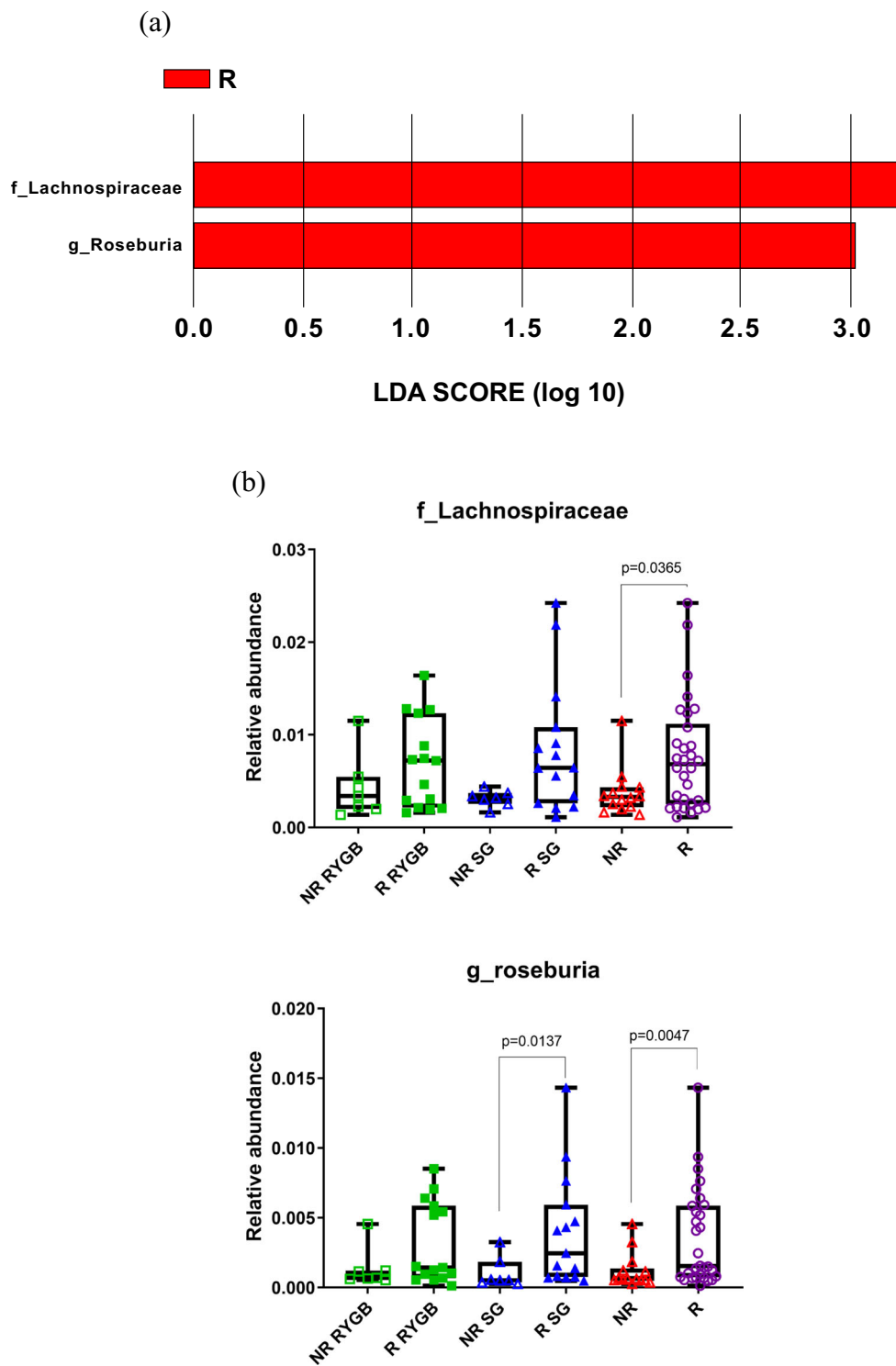
Eubacteriaceae is a core component of a normal healthy gut [47–49], hence, may be enriched at baseline among those more likely to remit from T2D. Eubacteriaceae are more commonly shown to be abundant in healthy populations [47], as compared with those of IBS [50]. Interestingly, when the disease subsides in those patients, levels of Eubacteriaceae are shown to increase [50]. Eubacteriaceae has also been shown to

(a)



(b)





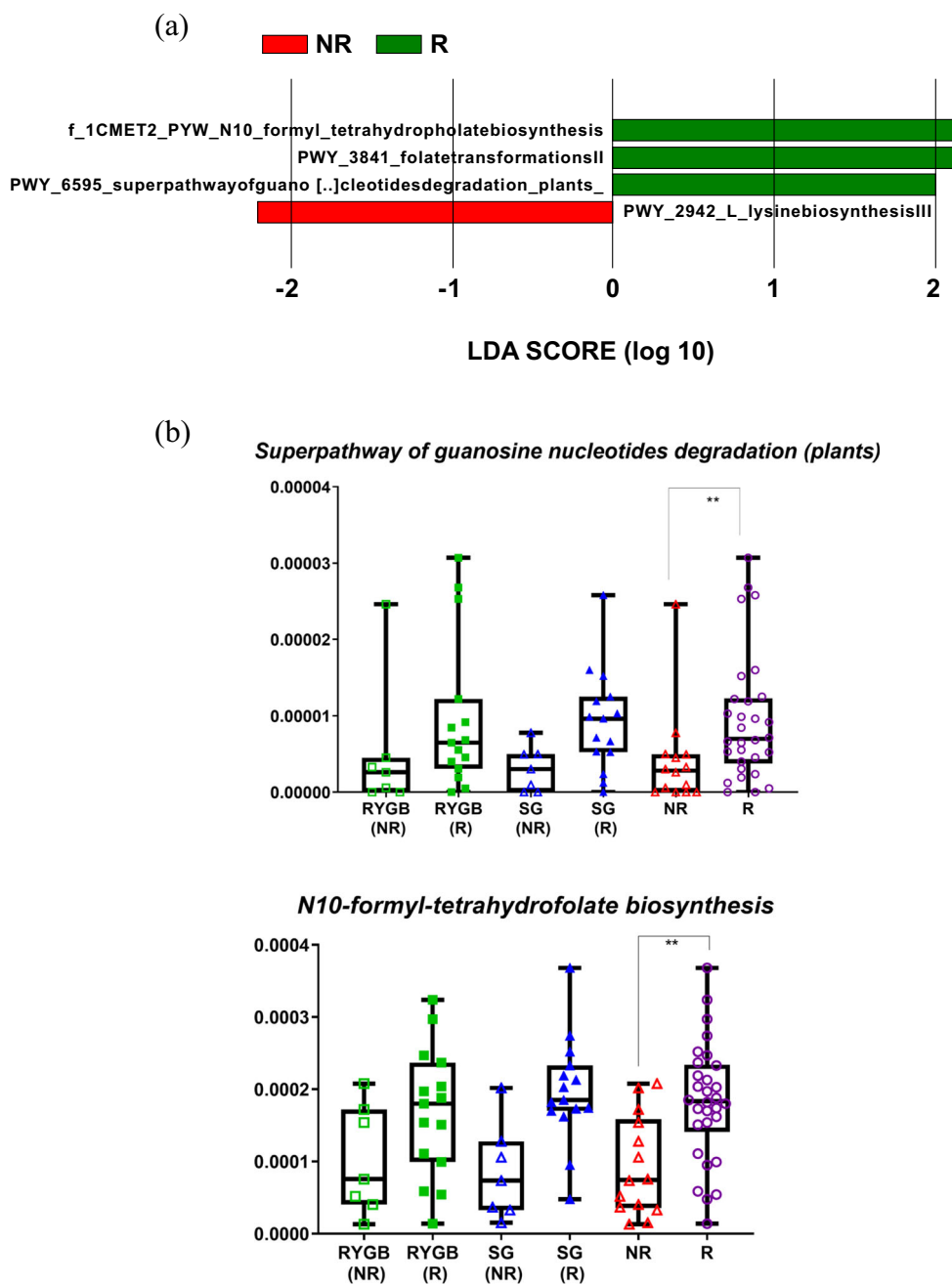
**Fig. 4** Differentially abundant taxa in the gut microbiota 1-year post surgery, separated by T2D status **a** identified by LEfSe, and **b** individual box plots of identified taxa by surgery type and T2D status. Red bars indicate taxa that were more abundant in those with persisting T2D at 1-

year, and green bars indicate taxa that were more abundant in those who remitted from T2D at 1 year. R = remitted, NR = non-remitted. Squares indicate whole cohort, triangles indicate SG and circles indicate RYGB

be low in abundance in children with gut dysbiosis typically associated with autism spectrum disorder [51, 52], as well as

in adults with type 1 diabetes [53]. Although we cannot explain the reason for the observed increase of Eubacteriaceae in





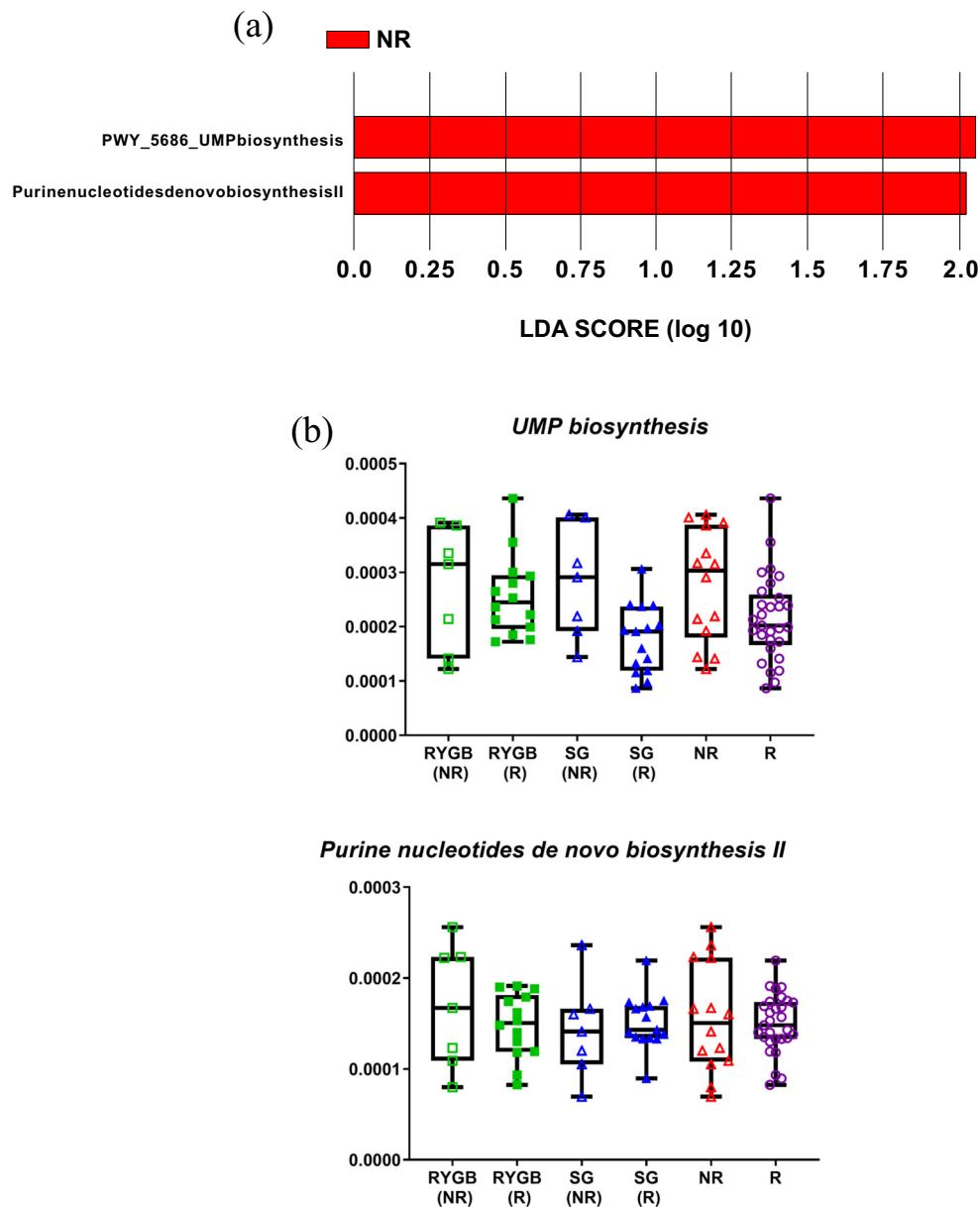
**Fig. 5** Differentially abundant metabolic pathways in the baseline gut microbiota separated by T2D status **a** identified by LEfSe, and **b** individual box plots of identified taxa by surgery type and T2D status. Red bars indicate pathways that were more abundant pre-surgery in those

with persisting T2D at 1-year, and green bars indicate pathways that were more abundant pre-surgery in those who remitted from T2D at 1 year. R = remitted, NR = non-remitted. Squares indicate whole cohort, triangles indicate SG and circles indicate RYGB

the pre-surgery gut among those who went on to remit from T2D after surgery, the observation is consistent with a ‘healthier starting point’ pre-surgery.

Baseline gut microbiota of those who subsequently remitted from T2D had increased capacity for guanosine nucleotide degradation and n10-formyl-tetrahydrofolate biosynthesis. Exogenous purine degradation occurs in the small intestine [54], and includes the conversion of purines (guanine and

adenine) to uric acid. This degradation has been known to be primarily carried out by Firmicutes including *Bacillus* and Enterobacteriaceae [55]. Notably, our data not only showed an increase in the ability to degrade guanine in the pre-surgery gut in those who go on to remit from T2D at 1 year, and also showed an increased abundance of Enterobacteriaceae, indicating this predicted increase in purine degradation in these participants.



**Fig. 6** Differentially abundant pathway in 1 year post surgery, separated by T2D status **a** identified by LEfSe, and **b** Individual box plots of identified taxa by surgery type and T2D status. Red bars indicate

pathways that were more abundant in those with persisting T2D at 1-year. R = remitted, NR = non-remitted. Squares indicate whole cohort, triangles indicate SG and circles indicate RYGB

The composition of the 1-year post-surgery gut microbiota differed by T2D status, with an increased abundance of the family Lachnospiraceae and the genus *Roseburia*, both of the Firmicutes phyla, in patients who remitted from T2D. Increased *Roseburia* levels were also observed only in those achieving T2D remission at 1 year in the earlier pilot analysis ( $n = 14$ ) [21]. A gut bacterial community abundant in Firmicutes, specifically Lachnospiraceae and *Roseburia* species is generally deemed to be ‘a healthy gut signature’ [56]. In fact Lachnospiraceae itself is commonly used as a probiotic to promote gut and intestinal health [57]. Inflammatory

conditions including Crohn’s disease, ulcerative colitis, IBS and T2D have all been found to show low abundance of both *Roseburia* and Lachnospiraceae [56, 58], as compared with higher abundances in non-disease or ‘healthy’ people. These specific taxa (Lachnospiraceae and *Roseburia*) changes have not been observed following dietary weight loss alone [19] but increased Firmicutes following VLCD for 12 weeks show an inverse correlation to resulting body weight. This suggests that the changes we observed among those remitting from T2D after bariatric surgery are similar to those seen after weight loss achieved by dietary means. While we cannot

ascertain causality for these taxa in the remission of T2D remission following surgery, evaluating their metabolic benefits as part of probiotic supplementation would be worth testing.

It is important to note that cessation of metformin or other diabetes medications were not related to the changes to the taxonomic composition and functional potential of the gut bacteria seen. Previous studies [33, 34] have shown that metformin use (for as little as 4 weeks) for the treatment of T2D is associated with an increase in genes and pathways related to bacterial environmental response, drug resistance and carbohydrate metabolism, especially enhanced ability to produce short chain fatty acids (SCFAs). Explicitly determining how these gut microbial changes occurring after bariatric surgery contribute to T2D remission requires further work including the application of other mixed-omics approaches [59, 60] that are tailored to analyse the functionality of the gut microbiome, as well as faecal microbial transplant studies and cell-based and animal studies.

## Limitations

Firstly, we acknowledge that our sample size was limited. However, it is similar to other bariatric cohorts that have been analysed using shotgun sequencing [16, 18, 19]. Secondly, baseline gut microbiota analysis was conducted while all participants were on the same VLCD at the pre-surgery assessment, so we cannot be sure that the baseline microbial predictors of T2D remission will still hold true if conducted while on usual diet. Nonetheless, such a gut microbial predictor taken while on VLCD, if validated, could still be useful for prognostic purposes before a final decision for surgery prioritisation is made. Finally, as with other studies evaluating changes to gut microbiota following bariatric surgery, we cannot be certain as to which of these are directly due to surgical rearrangement of the gut and which are due to accompanying changes in diet, medication and comorbidities.

## Conclusions

In this work, we have identified family, genus and species level differences in the gut microbiota, specifically related to T2D remission status after two types of commonly used bariatric surgery. With further validation of the predictive capacity of certain faecal taxa linked with subsequent T2D remission, in larger cohorts and by using germ-free mice faecal transfer experiments to examine the diabetes remission potential of other taxa, these could be valuable in developing probiotics targeting diabetes remission.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval Statement** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent Statement** Informed consent was obtained from all individual participants included in the study.

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