Surgery for Obesity and Related Diseases

Gastrointestinal manifestations after Roux-En-Y gastric bypass surgery in individuals with and without type 2 diabetes
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Corresponding Author:	Janne Hukkanen, MD, PhD Oulun Yliopisto FINLAND
First Author:	Mari-Anne Härma, MSc
Order of Authors:	Mari-Anne Härma, MSc
	Krishna Adeshara, Ph.D.
	Natalie Istomin, MSc
	Markku Lehto, PhD
	Michael Blaut, MD, PhD
	Markku J. Savolainen, MD, PhD
	Sohvi Hörkkö, MD, PhD
	Per-Henrik Groop, MD, DMSc
	Vesa Koivukangas, MD, PhD
	Janne Hukkanen, MD, PhD
Abstract:	Background: Roux-en-Y gastric bypass (RYGB) surgery is an effective treatment for obesity, which improves cardiovascular health and reduces risk of premature mortality. However, some reports have suggested that RYGB may predispose to adverse health outcomes such as increased risk of inflammatory bowel disease (IBD) and colorectal cancer. Objectives: The present prospective study aimed to evaluate the impact of RYGB surgery on cardiovascular risk factors and gastrointestinal inflammation in individuals with and without type 2 diabetes mellitus (T2DM). Setting: The study was performed at a university hospital setting in Finland. Methods : Blood and fecal samples were collected at baseline and six months after surgery from 30 individuals, of which sixteen had T2DM and fourteen were non-diabetics. There were also single study visits for six healthy reference subjects. Changes in cardiovascular risk factors, serum cholesterol, and triglycerides were investigated before and after surgery. Fecal samples were analyzed for calprotectin, anti-saccharomyces IgA antibodies (ASCA), active lipopolysaccharide (LPS) concentration, short-chain fatty acids (SCFAs), intestinal alkaline phosphatase (IAP) activity, and methylglyoxal-hydro-imidazolone (MG-H1) protein adducts formation. Results : After RYGB, body weight decreased on average -21.5% (-27.2±7.8 kg), excess weight loss (EWL) averaged 51% and there were improvements in cardiovascular risk factors. Fecal calprotectin levels (p<0.001), active LPS concentration (p<0.002), ASCA (p<0.02), and MG-H1 (p<0.02) values increased significantly, whereas fecal SCFAs, especially acetate (p<0.002) and butyrate (p<0.03) levels, were significantly lower. Conclusion. The intestinal homeostasis is altered after RYGB with several fecal markers suggesting increased inflammation, however, clinical significance of the detected changes is currently uncertain. As chronic inflammation may predispose to adverse health effects, our findings may have relevance for the suggested association between RYGB and increased risk of incident IBD and colorectal cancer.

Abstract

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Conclusion. The intestinal homeostasis is altered after RYGB with several fecal markers suggesting increased inflammation, however, clinical significance of the detected changes is currently uncertain. As chronic inflammation may predispose to adverse health effects, our findings may have relevance for the suggested association between RYGB and increased risk of incident IBD and colorectal cancer.

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1. Introduction

Roux-en-Y gastric bypass (RYGB) surgery is one of the most effective treatment options for obesity [1] with long-term benefits on body weight control, remission and prevention of type 2 diabetes (T2DM), hypertension, dyslipidemia [2], cardiovascular disease (CVD) [3], and reduced risk of mortality [4]. However, the effects of bariatric surgery extend beyond weight loss, and it may be that changes in the gut acid production, luminal pH, bile acid secretion, and nutrient transit time [5] could lead to unpredictable consequences for the gastrointestinal health. Previous studies have shown that individuals who underwent bariatric surgery, present with a wide range of intestinal alterations [6] including elevated levels of inflammation markers [7], increased colorectal epithelial proliferation, and reduced apoptosis [8, 9]. In addition, low gut microbial diversity has been found in obesity [10] and RYGB surgery has been shown to cause significant and proportional changes between mutualistic and pathogenic bacteria [11].

Although the overall benefit of bariatric surgery with reduced risk of CVD and mortality outweighs the possible hazard of intestinal side effects, it is of note that long-term alteration of the intestinal homeostasis may lead to increased risk of inflammatory bowel disease (IBD) [12, 13] or even colorectal cancer [14–16], but convincing evidence is still lacking. Hypothetically, bariatric surgery may lead to colonization of pathogenic bacteria and increase in gut permeability - features that are commonly seen in the active phase of IBD [17]. Since there are no ideal blood-based biomarkers currently available, the screening of gut-related diseases still relies largely on stool-based analytics [18]. In this study, we investigated the impact of RYGB surgery on cardiovascular risk factors and biomarkers of the intestinal homeostasis in a Finnish population.

2. Materials and methods

2.1 Study design. Between 2 September 2010 and 24 April 2014, we recruited for this prospective study at the Oulu University Hospital individuals with a clinically indicated need for bariatric surgery. All eligible participants had been evaluated by the Endocrinology Unit and were referred to the Gastroenterology Unit for preoperative evaluation and gastroscopy. Finally, the bariatric surgeons evaluated the patients and written informed consent was obtained. A study visit to the Research Unit of Internal Medicine was organized for blood and fecal sampling as well as a 2-hour liquid meal test before the RYGB surgery. The participants returned for a study visit six months after the RYGB surgery for a second round of blood and fecal sampling and a liquid meal test. The first study visit prior to the surgery was designed to occur before initiating the preoperative very-low-calorie (VLC) diet. However, six participants (four with T2DM and two non-diabetic) had started the VLC diet already before their first study visit due to scheduling difficulties.

The study protocol was approved by the Ethics Committee of the Northern Ostrobothnia Hospital District, and the study procedures followed the ethical standards of the Helsinki Declaration, as revised in 2000. All patients provided written informed consent before any study-related procedure. The trial was registered at ClinicalTrials.gov as NCT01330251.

Inclusion criteria were medical indication for bariatric surgery and age between 18 and 65 years. Exclusion criteria were need for insulin therapy (type 1 or type 2 diabetes) or oral corticosteroids, history of prolonged antibiotic treatments, chronic inflammatory diseases including IBD and rheumatic diseases, coeliac disease and malignant diseases. Additionally, we recruited non-diabetic healthy individuals with a medical indication for gastroscopy without any suspicion of malignancy as a reference group. The ultimate aim was to enroll 30

non-diabetic individuals, 30 individuals with T2DM, and 30 normal-weight healthy subjects. However due to slow recruitment, the study was terminated when a total of 41 subjects had been enrolled including 34 that underwent RYGB and only seven healthy volunteers for the reference group. Three individuals declined the six-month study visit, one individual declined surgery after the first study visit, and one fecal sample from a healthy subject was lost. We here report the results of the 30 RYGB patients, who completed both the pre- and the postoperative study visits (sixteen with T2DM and fourteen without T2DM). Due to the low number of healthy individuals (n=6), their data were treated only as reference for the normal values. A summary of the recruitment procedure is shown in a flow-chart in Figure 1.

2.2 Clinical measurements. At the study visits, the use of medications and supplements was recorded, and clinical measurements were performed. Body weight was measured with a Seca 861 scale (Seca GmbH & Co., Hamburg, Germany), and body mass index (BMI) was calculated as body weight divided by the height squared (kg/m^2). Blood pressure was measured using an Omron HEM-907 sphygmomanometer (Omron Corporation, Kyoto, Japan). After ten minutes of rest, two consecutive readings were obtained from the non-dominant arm (measured in five-minute intervals) with the mean of the readings used for the analysis.

2.3 Blood and fecal measurements. Information about basic blood measurements (e.g. glycated hemoglobin, blood cell counts, lipids), liquid meal tests, as well as fecal sample determinations (calprotectin, intestinal alkaline phosphatase (IAP) activities, anti-Saccharomyces cerevisiae antibodies (ASCA), endotoxin activities, short-chain fatty acids (SCFA), and methyglyoxal-hydro-imidazolone adducts (MG-H1)) are described in the Supplementary material.

2.4 Statistical analysis. All two-group comparisons were tested with the paired t-test for normally distributed data or the Wilcoxon's signed-rank test for paired, non-normally distributed data. Multiple group comparisons were performed with ANOVA for normally distributed data or Kruskal-Wallis rank-sum test for non-normally distributed data, and post-hoc comparisons were performed using Tukey and Kramer test. Distribution was assessed with the Shapiro-Wilks normality test. The AUC and AUMC during liquid meal tests were calculated using the trapezoidal method. Missing data were handled with the use of list wise deletion of missing data. Continuous variables are reported as means and standard deviations or medians and interquartile ranges and categorical variables as numbers and percentages. P-values less than 0.05 were considered as statistically significant. Statistical analyses were done with the R software.

3. Results

3.1 Clinical characteristics of study subjects before and after the RYGB surgery

The clinical characteristics and the serum biomarkers of the study subjects at baseline (T0) and six months (T6) after the RYGB surgery are shown in Table 1. The study population (n=30) comprised of fourteen non-diabetic individuals and sixteen with T2DM. Those with T2DM were on average four years older than the individuals without T2D (p<0.05). Six months after

115 the surgery, the total weight loss was on average -21.6% of the body weight (-27.2±7.8 kg; range -14.8 to -32.3%),

and the excess weight loss (EWL) was on average -51% (range -28.4 to -96.1%). In the whole study group, bariatric surgery resulted in lower diastolic blood pressure (p<0.001). A similar trend was observed for the fasting plasma glucose concentrations (p<0.001). Overall,

120 significant improvements were also seen in the serum lipid concentrations, blood cell counts, and the active serum LPS concentrations. However, no changes were observed in the levels of MG-H1 protein adducts at the different time points (Table 1).

3.2 Fecal biomarkers

Individuals with T2DM had 189% higher fecal protein levels at baseline compared to the non-

125 diabetic individuals (p<0.0001). In the T2DM group, fecal protein concentrations decreased to the level of non-diabetic subjects after the surgery. This finding was independently replicated by two different laboratories (data not shown). Due to a possible confounding effect, data are presented with and without protein normalization for the following fecal biomarkers: ASCA, IAP, and LPS. The summary of the fecal biomarkers at baseline and six months after the RYGB

130 surgery are shown in Table 2.

The study participants showed increased calprotectin levels six months after RYGB surgery compared to baseline. This trend was similar in the individuals with or without T2DM. Also, 22 out of the 30 (73%) participants had calprotectin levels higher than 200 µg/g (336, [251, 415] µg/g) after RYGB surgery.

135 In the whole study group, the fecal concentrations of IgA ASCAs were twelve percent higher after RYGB surgery compared to baseline. When normalized for protein, this effect was more evident in the T2DM group whereas for the non-normalized values, the effect was statistically significant in the non-diabetic group.

The active fecal LPS concentration had increased 123% six months after the RYGB surgery compared to baseline ($p < 0.002$). Although both groups showed a similar trend of increase
140 after surgery, only the individuals with T2DM showed a statistically significant increase ($p < 0.001$). Notably, the statistically significant result appeared only for the non-normalized values.

There was a rather high inter-individual variation in the IAP values (up to 90-fold difference).
145 Compared to baseline, the IAP activity decreased in fourteen and increased in sixteen individuals at six months after the RYGB surgery. Without protein normalization, the fecal IAP activities showed a decreasing trend after surgery ($p < 0.01$) being decreased in 19 individuals and increased in eleven. When non-normalized IAP values were stratified by baseline IAP activity, the individuals with high baseline IAP activity had lower IAP values after surgery
150 ($p < 0.001$), whereas no significant change was seen in the individuals with low baseline IAP activity (Figure 2).

Compared to baseline, the MG-H1 protein adducts, normalized with total protein, increased after surgery in the individuals with T2DM (p<0.001) as well as in the whole study group (p<0.02) (Table 2).

155   The total short-chain fatty acid (SCFAs) concentrations decreased 19% in the whole study group after RYGB surgery (p<0.005), with the most significant decrease in the acetic acid and butyrate levels (Table 2). Acetic acid and butyrate constituted 86.5% of the total SCFA reduction after surgery. The second most abundant SCFA, propionate, was reduced only in the individuals with T2DM (Table 2).

160   **3.3 Healthy reference group**

Individuals in the reference group had by default lower baseline body weight and lower BMI compared to the individuals undergoing RYGB surgery. Data for the reference group are summarized in Table 3. Due to the small number of reference subjects, no statistical analysis were performed for the differences between the RYGB patients and the reference subjects.

165   There was no difference in the fecal calprotectin levels between the reference subjects and the RYGB group before surgery, but those undergoing RYGB seemed to have higher calprotectin levels after the surgery. The individuals in the reference group tended to have lower non-normalized and protein-normalized IAP activity compared to the RYGB participants. The fecal MG-H1 levels tended to be lower in the reference subjects compared to the two

170   RYGB groups. Reference individuals seemed to have higher protein normalized IgA ASCA values compared to those undergoing surgery at baseline, but not after surgery. There were no obvious differences in the total fecal protein values, the non-protein normalized IgA ASCA

values nor the total or individual SCFA values in the reference group compared to the RYGB

group, before or after surgery (Table 3).

4. Discussion

In the present study, we investigated the impact of RYGB surgery on clinical phenotypes in 30 Finnish subjects with obesity before and six months after surgery. Fourteen non-diabetic and sixteen T2DM undergoing RYGB surgery and six healthy individuals were included in the main analysis. As expected, all individuals undergoing surgery showed significant weight loss after the RYGB operation, which was accompanied by improved lipid metabolism and glycemic control. However, several markers of intestinal inflammation were elevated after the surgery including fecal calprotectin, LPS, ASCAs and MG-H1 while potentially beneficial SCFAs were reduced.

It is of note that many of the study subjects displayed changes in the gut-related biomarkers. A significant proportion of the study cohort exhibited elevated levels of fecal calprotectin, LPS, IgA antibodies binding to *S.cerevisiae*, and MG-H1 protein adducts.

Calprotectin is a cytoplasmic biomarker of nonspecific intestinal inflammation. In the presence of active intestinal inflammation, neutrophils infiltrate the intestinal mucosa resulting in increased leakage of calprotectin. Increased levels of fecal calprotectin are typically seen in patients with Crohn's disease and ulcerative colitis [19, 20], bacterial diarrhea [21] or colorectal cancer [22, 23]. We detected significantly elevated fecal calprotectin levels after the RYGB surgery – nearly 75% of the study subjects had values exceeding the threshold indicating intestinal inflammation (>200 µg/g). Elevated fecal calprotectin concentrations have previously been reported after RYGB in cross-sectional studies [7, 24] but no prior study has shown the effect of RYGB on fecal calprotectin prospectively.

Alterations in microbiota could be one of the reasons responsible for the increased calprotectin levels [25]. Tremaroli et al. investigated the human fecal metagenome in women nine years after RYGB surgery. Analysis of the gut microbiota revealed that subjects after RYGB surgery tended to have increased numbers of proinflammatory proteobacteria compared with non-operated individuals with obesity [26]. This observation was in line with previous gut microbiome analyses after bariatric surgery [10, 11, 27, 28]. Proinflammatory bacteria exert their effect through biologically active LPS. Our study is the first to report that the fecal LPS levels are significantly higher after RYGB surgery. Although the circulating LPS levels were reduced, being in line with previous studies [29, 30], the increased fecal active endotoxin levels could theoretically be interlinked with the reduction in IAP activity levels after the surgery [31, 32]. IAP is highly expressed by the intestinal endothelial cells in response to nutrient availability, and IAP regulates intestinal lipid transport and neutralizes bacterial endotoxins by dephosphorylation [33]. Decreased IAP expression has been implicated in many chronic inflammatory diseases such as IBD, celiac disease and metabolic syndrome [34] and recombinant IAP has been proposed as a potential therapeutic for the inflammatory outcomes in IBD [35]. In addition, higher IAP activity has been proposed to be protective for T2DM individuals with obesity [36]. Although, there was a large inter-individual variation in IAP activities in our study, individuals with higher values showed a decrease in the IAP activity after the surgery. Fecal IAP activity can be modulated by various dietary factors [34] as well as by enteral feeding and gastrointestinal inflammation [37]. Whether the reduction of the IAP activity is an effect of the surgery or rather a physiologic response to decreased nutrient availability remains unknown.

The target of ASCA is oligomannosidic epitopes of Saccharomyces cerevisiae [38], the yeast commonly used in baking and brewing. However, positive ASCAs are not indicative of the presence of this yeast in the colon. Serum ASCA levels have previously been associated with obesity and inflammation [39], and IBD, especially Crohn's disease when a positive serum ASCA is combined with a negative serum p-ANCA result [38]. Fecal ASCA levels have been proposed as a putative biomarker of IBD in pediatric patients [40] but, in general, little is known about fecal ASCAs and no previous study has explored the relationship between fecal ASCAs and bariatric surgery. In our study, fecal IgA ASCAs were elevated in the whole study population after RYGB. However, the post-surgery IgA ASCAs resembled the ASCA levels in the reference group. The fecal IgA ASCA levels are of unknown clinical significance in adults and the current assay was originally not designed for fecal measurements, therefore it is difficult to draw any conclusions based on their modulation by RYGB.

Methylglyoxal (MG) is a highly toxic compound produced by eukaryotic and prokaryotic carbohydrate, lipid, and amino acid metabolism [41]. Increased production of MG promotes rapid modification of proteins to form advanced glycated end-products (AGEs) linked to the development of micro- and macrovascular complications in individuals with diabetes [42]. High levels of MG provokes a pro-inflammatory response, protein cross-linking, tissue injury [43] and might have deleterious effects in other age-related diseases such as cancer, obesity, cardiovascular disease, and neurological disorders [44, 45]. MG production affects intestinal bacterial growth, fermentation of SCFAs, and nutrient absorption leading to an imbalance of intestinal metabolites and gut inflammation [41, 46]. Recent studies have demonstrated that bariatric surgery reduces the circulating MG levels in T2DM individuals with obesity [47, 48]. Notably, we did not observe any significant changes in the serum MG-H1 levels six months

after the RYGB surgery, which might be explained by the short follow-up time [47]. However, the fecal MG-H1 levels were significantly increased after surgery, which could be interconnected to unfavorable changes in the microbiota composition or gut physiology.

The total fecal SCFAs were decreased by the RYGB surgery. This is explained by the significant reduction of acetic acid, the most abundant of the SCFAs, with a minor contribution from the decrease of butyrate. The production of SCFAs is mainly interlinked with the fermentation of dietary fibers by gut bacteria. Gut dysbiosis could have a significant impact on the rate of biosynthesis and degradation of bacteria-derived metabolites. SCFAs, such as acetate and butyrate, are important metabolites in maintaining the intestinal homeostasis and have been shown to be downregulated in IBD, but also after biliointestinal surgery in the feces [49] and after RYGB surgery in the serum [50]. Decreased SCFAs may be a direct indication of a reduction in available nutrients due to caloric restriction after RYGB or a secondary consequence of dysbiosis. Nevertheless, SCFAs, especially acetate, butyrate, and propionate, are known for their colon-protective and anti-inflammatory properties [51, 52], and could be important contributors in preventing gut inflammation and carcinogenesis.

In addition to the negative pro-inflammatory and the expected positive metabolic effects, we noticed that fecal protein concentrations were significantly higher in the T2DM group before the surgery when compared with the non-diabetic group as well as with the non-surgery group. After RYGB the fecal protein levels were normalized to the level of the non-diabetic group and the non-surgery group. These findings are surprising and replication in future studies is warranted.

Taken together, we here present evidence of an inflammatory state in the intestine of individuals six months after RYGB surgery. Changes in the metabolism of bile acids, changes

in gastric pH and the level of hormones, incretins, and enzymes due to anatomical changes lead to weight loss and to altered gut microbiota and energy homeostasis. These anatomical and physiological changes could themselves give rise to inflammation and alterations in microbiota in the post-operative state. Furthermore, six months is a too short period to make definitive conclusions on the lifetime health risks. Individuals at six months after RYGB surgery are still adjusting to the digestive changes, which could be responsible for the temporary dysbiosis. However, fecal calprotectin is known to be elevated for at least up to two years after RYGB [7, 24] indicating that the inflammation may persist beyond six months.

The elevated levels of fecal LPS and calprotectin, as well as the reduced levels of fecal SCFAs, may indicate that intestinal and colonic inflammation is altered by RYGB surgery regardless of the well-known positive metabolic and cardiovascular effects. Thus, we present novel data on several indices of inflammation with congruent results indicating increased intestinal inflammation in a prospective setting. However, the clinical significance of the detected changes in the inflammation markers is currently not known. As RYGB surgery has been suggested to be associated with a slightly increased risk of incident IBD [12, 13] and colorectal cancer [14–16], it can be hypothesized that the prolonged inflammatory state after RYGB could be linked to these rare adverse outcomes. There is still controversy regarding the association with colorectal cancer after bariatric surgery – some studies have reported a decreased incidence of all cancers [53, 54], a decrease in short-term risk of colorectal cancer [55, 56], and an increase in longer-term risk of colorectal cancer [14-15, 57]. Clearly, more research is required to explore these putative links.

Our study has some obvious limitations. Due to various obstacles met during the recruitment process, the final size of the study cohort was much smaller than initially expected and an

optimal control group could not be established. Also, some patients started the VLC diet before the first study visit which might have affected the baseline values of the fecal markers. In addition, our study had a single center setting. Although significant weight loss was already achieved six months after the RYGB operation, the final stabilization of the body weight would most likely have taken much longer, and it would have been beneficial to have fecal follow-up samples when the weight stabilization was complete. Furthermore, ideal control groups would have consisted of 1) sham-operated persons and 2) sleeve gastrectomy patients with baseline and follow-up samples.

5. Conclusion

The intestinal inflammation homeostasis is significantly altered in individuals undergoing RYGB surgery, however, the clinical significance of the detected changes is currently uncertain. These findings may have relevance for the suggested association of RYGB with increased risk of incident IBD and colorectal cancer.

Disclosures

P-H.G. has received research grants from Eli Lilly and Roche, lecture fees from Astellas, Astra Zeneca, Boehringer-Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, PeerVoice, Sanofi and SCIARC. He is an advisory board member for AbbVie, Astellas, Boehringer-Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, and Sanofi. All other authors declared that they had no competing interest associated with this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at Surgery for obesity and related diseases website.

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Correspondence

475   Janne Hukkanen, MD, PhD

Research Unit of Internal Medicine,

University of Oulu, Finland.

PO Box 5000, FI-90014

janne.hukkanen@oulu.fi

480   Krishna Adeshara, PhD

krishna.soni@helsinki.fi

Roux-En-Y gastric bypass and gut inflammation

Figure legends

Figure 1 – Flowchart for prospective cohort study.

Figure 2 – Non-protein normalized IAP activity, stratified by baseline values

485   Fecal IAP (intestine alkaline phosphatase) values were stratified by baseline values using a cut-off point of 1000 U/l. Individuals with high baseline values (>1000 U/l, n=14) show a significant decrease in IAP values after the RYGB (Roux-en-Y gastric bypass) surgery, whereas individuals with low baseline values (<1000 U/l, n=16) do not change after surgery. Mo = months. Boxplots represent the median, interquartile range and upper and lower quartile of IAP activity values. Ns= not significant, ** stands for p-value <0.01.

TABLE 1 FECAL BIOMARKERS

Variable	Non-Diabetic Surgery Group			T2D Surgery Group			All patients		
	Baseline	6-months	p-value	Baseline	6-months	p-value	Baseline	6-months	p-value
Total protein (mg/ml)	1.65 [1.27, 2.11]	2.06 [1.77, 2.35]	NS	4.77 [3.84, 6.54]	1.75 [1.32, 2.39]	<0.001	2.99 [1.76, 4.87]	2.00 [1.46, 2.37]	0.007
Calprotectin (µg/g FM)	38.5 [22.75, 110]	311.5 [211, 347.5]	<0.001	63 [43.25, 79]	266.5 [182.75, 434]	<0.001	53 [29, 88.25]	297 [192, 365.75]	<0.001
ASCA (U/mg) #	5.15 [3.68, 6.47]	4.74 [3.48, 6.01]	NS	1.83 [1.36, 2.84]	5.21 [3.81, 6.75]	0.003	3.01 [1.76, 5.09]	4.74 [3.77, 6.55]	0.02
ASCA (U/ml)	8.19 [7.9, 8.83]	9.17 [8.54, 10.08]	<0.001	8.46 [7.79, 11.19]	9.46 [8.83, 10.02]	NS	8.38 [7.82, 9.47]	9.38 [8.54, 10.1]	0.01
LPS (EU/mg) #	813 [591, 1125]	1395 [1081, 1564]	NS	511 [377, 611]	1281 [866, 1546]	0.001	591 [479, 824]	1321 [930, 1580]	0.002
LPS (EU/ml)	1503 [898, 2970]	3181 [2186, 3415]	NS	2686 [1819,2819]	3064 [1950, 3433]	NS	2308 [1186, 2885]	3181 [1956, 3420]	NS
IAP (U/mg) #	717 [311.25, 1525.5]	172 [65.75, 1277]	NS	122.5 [32.25, 843.75]	134.5 [67.25, 474.25]	NS	617 [92, 1033]	155 [65.5, 922]	NS
IAP (U/l)	971 [377, 3072]	421 [130, 2107]	NS	499 [156, 3318]	278 [125, 940]	0.03	790 [184, 3228]	295 [128,1351]	0.015
MG-H1 (µg/mg) #	85±80	75±61	NS	42±48	99±58	<0.001	61±42	88±42	0.02
MG-H1 (µg/ml)	143±80	147±61	NS	184±48	177±58	NS	166±66	164±60	NS

Short-chain fatty acids

Variable	Non-Diabetic Surgery Group			T2D Surgery Group			All patients		
Total SCFA (µmol/g FM)	96 [75, 103]	79 [57, 99]	NS	97 [81, 102]	75 [59, 95]	0.02	96 [78, 103]	78 [58, 96]	0.005
Acetic acid (µmol/g FM)	52 [43, 68]	38 [32, 50]	0.02	52 [45, 59]	40 [34, 48]	0.04	52 [44, 65]	38 [32, 50]	0.002
Propionate (µmol/g FM)	15.7 [11.6, 18.7]	15.9 [10.1, 24.4]	NS	17.7 [14.5, 19.8]	13.8 [9.7, 19.3]	0.03	16.9 [13.7, 19.7]	14.2 [9.9, 20.9]	NS

Isobutyrate (µmol/g FM)	1.3 [0.9, 2.0]	2.4 [1.8, 2.9]	NS	2.1 [1.4, 2.6]	1.7 [1.4, 2.3]	NS	1.8 [1.2, 2.5]	1.9 [1.5, 2.7]	NS
Butyrate (µmol/g FM)	12.5 [9.1, 20.0]	10.7 [7.2, 18.5]	NS	14.0 [11.7, 17.2]	11.5 [7.1, 18.9]	NS	13.6 [10.6, 18.0]	10.8 [7.2, 18.8]	0.03
Isovalerate (µmol/g FM)	3.4 [2.7, 5.6]	4.7 [3.9, 5.7]	NS	4.4 [3.0, 5.5]	3.4 [2.7, 5.7]	NS	3.9 [2.8, 5.6]	3.9 [3.2, 5.7]	NS
Valerate (µmol/g FM)	1.6 [1.22, 2.28]	2.05 [0.46, 3.58]	NS	2.21 [1.64, 2.86]	1.47 [1.1, 2.29]	NS	1.77 [1.26, 2.84]	1.57 [1.09, 2.77]	NS

The data is presented as median [interquartile range]. # normalized with the faecal protein concentrations. P-values <0.05 were considered as statistically significant. Abbreviations: NS = not significant; FM = fecal mass (wet); ASCA = anti-saccharomyces IgA antibody; LPS = lipopolysaccharide; IAP = intestine alkaline phosphatase; MG-H1 = methylglyoxal-hydro-imidazolone; SCFA = short-chain fatty acids.

Table 1 clean

TABLE 1 CLINICAL VARIABLES AND SERUM BIOMARKERS

Variable	Non-Diabetic Surgery Group			T2D Surgery Group			All Patients		
	Baseline	6-months	p-value	Baseline	6-months	p-value	Baseline	6-months	p-value
Number of individuals	14	-		16	-		30	-	
Age (years)	44.5±9.0	-		49.8±8.1	-		47.3±8.8	-	
Female sex – N (%)	11 (79)	-		10 (63)	-		21 (70)	-	
Hypertension – N (%)	8 (57)	-		13 (81)	-		21 (70)	-	
Smoking history – N (%)	6 (43)	-		8 (50)	-		14 (47)	-	
Weight (kg)	133.3±24.6	105.5±23.9	<0.001	120.5 ±18.9	93.9 ±13.2	<0.001	126.5±22.4	99.3±19.5	<0.001
Change from baseline weight – (kg)	-	-27.8±7		-	-26.6±8.6		-	-27.2±7.8	
EWL (%)	-	-51±16		-	-50±13		-	-52±16	
TBWL (%)	-	-21 ± 6		-	-22 ± 5		-	-22 ± 5	
BMI (kg/m^2)	47.5±5.6	37.4±5.9	0.001	41.8±4.4	32.6±3.2	<0.001	44.5±5.7	34.8±5.1	<0.001
Systolic BP (mmHg)	128±11	125±9	NS	130±17.2	126±15	NS	129±14	125±12	NS
Diastolic BP (mmHg)	83±7	78±8	0.03	83±9	77±10	0.002	83±8	77±9	<0.001
Fasting glucose (mmol/l)	6.0±0.5	5.3±0.6	0.001	7.2±1.3	6.3±1.4	0.013	6.6±1.2	5.8±1.2	<0.001
iAUC $_{0-120min}$ (mmol×min/l)	25±3	24±3	NS	31±6	33±8	NS	28±2	29±01	NS
No. with data	*11*	*11*		*13*	*13*		*24*	*24*	
AUMC $_{0-120min}$ (mmol×min^2/l)	82±11	69±12	0.004	106±22	101±27	NS	95±38	86±51	0.010
No. with data	*11*	*11*		*13*	*13*		*24*	*24*	
LDL cholesterol (mmol/l)	3.20±0.88	2.29±0.70	0.004	2.93±0.75	2.50±0.59	NS	3.05±0.80	2.4±0.63	<0.001
No. with data	*9*	*9*		*11*	*11*		*20*	*20*	
HDL cholesterol (mmol/l)	1.42±0.48	1.39±0.44	NS	1.22±0.29	1.17±0.22	NS	1.30±0.38	1.26±0.33	NS
No. with data	*9*	*9*		*14*	*14*		*23*	*23*	
Triglycerides (mmol/l)	1.57±0.68	1.20±0.36	0.039	1.79±0.84	1.20±0.30	0.026	1.70±0.77	1.20±0.31	0.003
No. with data	*9*	*9*		*14*	*14*		*23*	*23*	
Thrombocytes (E9/l)	297±60	270±61	NS	278±66	257±72	0.029	287±63	263±66	0.005
No. with data	*14*	*14*		*15*	*15*		*29*	*29*	
Leukocytes (E9/l)	7.59±1.44	6.38±1.92	0.008	7.35±1.79	6.00±1.82	0.019	7.46±1.60	6.18±1.85	<0.001

No. with data	*14*	*14*		*15*	*15*		*29*	*29*	
LPS (EU/ml)	0.06 [0.03, 0.08]	0.02 [0.01, 0.03]	0.03	0.05 [0.04, 0.09]	0.03 [0.02, 0.05]	NS	0.05 [0.04, 0.09]	0.02 [0.01, 0.05]	0.006
No. with data	*14*	*14*		*15*	*15*		*29*	*29*	
MG-H1(μg/ml)	6.93±1.12	6.69±1.09	NS	7.30±1.84	7.37±1.72	NS	7.12±1.52	7.04±1.47	NS
No. with data	*14*	*14*		*15*	*15*		*29*	*29*	

The data is presented as mean±SD or median [interquartile range]. P-values <0.05 were considered as statistically significant. Abbreviations: NS = not significant; iAUC = incremental area under the curve (glucose tolerance); AUMC = area under the first moment curve; EWL = excess weight loss; TBWL = total body weight loss; BMI = body mass index; BP = blood pressure; LDL = low density lipoprotein; HDL = high density lipoprotein; LPS = lipopolysaccharide; MG-H1 = methyglyoxal-hydro-imidazolone.

Table 3 clean

TABLE 1 NON-SURGERY HEALTHY REFERENCE GROUP

Variable	Non-surgery healthy reference group
Number of individuals	6
Age (years)	49.4±13.8
Female sex – N (%)	4 (66.6%)
Weight (kg)	75±24.9
BMI (kg/m^2)	24.9±4.7
Systolic BP (mmHg)	127±29
Diastolic BP (mmHg)	74±13
Fasting glucose (mmol/l)	5.8±0.6
iAUC $_{0-120min}$ (mmol×min/l)	20±4
AUMC $_{0-120min}$ (mmol×min^2/l)	50±12
LPS (EU/ml)	0.06 [0.04, 0.10]
MG-H1 (µg/ml)	7.04±2.48
Fecal biomarkers	
Total protein (mg/ml)	1.65 [1.12, 2.33]
Calprotectin (µg/g FM)	32 [21.2, 40.8]
ASCA (U/mg) #	6.37 [4.41, 7.41]
ASCA (U/ml)	8.39 [8.15, 9.44]
LPS (EU/mg) #	NA
LPS, (EU/ml)	NA
IAP, (U/mg) #	60.2 [44.6, 62.04]
IAP, (U/l)	81.7 [63.9, 120.6]
MG-H1 (µg/mg) #	56±48
MG-H1 (µg/ml)	90±68
Total SCFA (µmol/g FM)	70.3 [61.1, 79.6]
Acetic acid (µmol/g FM)	38.5 [35.1, 47.02]
Propionate (µmol/g FM)	12.8 [9.8, 15.3]
Isobutyrate (µmol/g FM)	1.38 [1.19, 1.62]
Butyrate (µmol/g FM)	10.6 [8.6, 11.4]
Isovalerate (µmol/g FM)	2.48 [2.06, 3.49]
Valerate (µmol/g FM)	1.61 [1.31, 2.02]

The data is presented as mean±SD or median [interquartile range]. Abbreviations: iAUC = incremental area under the curve (glucose tolerance); AUMC = area under the first moment curve; BMI = body mass index; BP = blood pressure; LDL = low density lipoprotein; HDL = high density lipoprotein; LPS = lipopolysaccharide; FM = fecal mass (wet); ASCA = anti-saccharomyces IgA

antibody; IAP = intestine alkaline phosphatase; MG-H1 = methylglyoxal-hydro-imidazolone; SCFA =
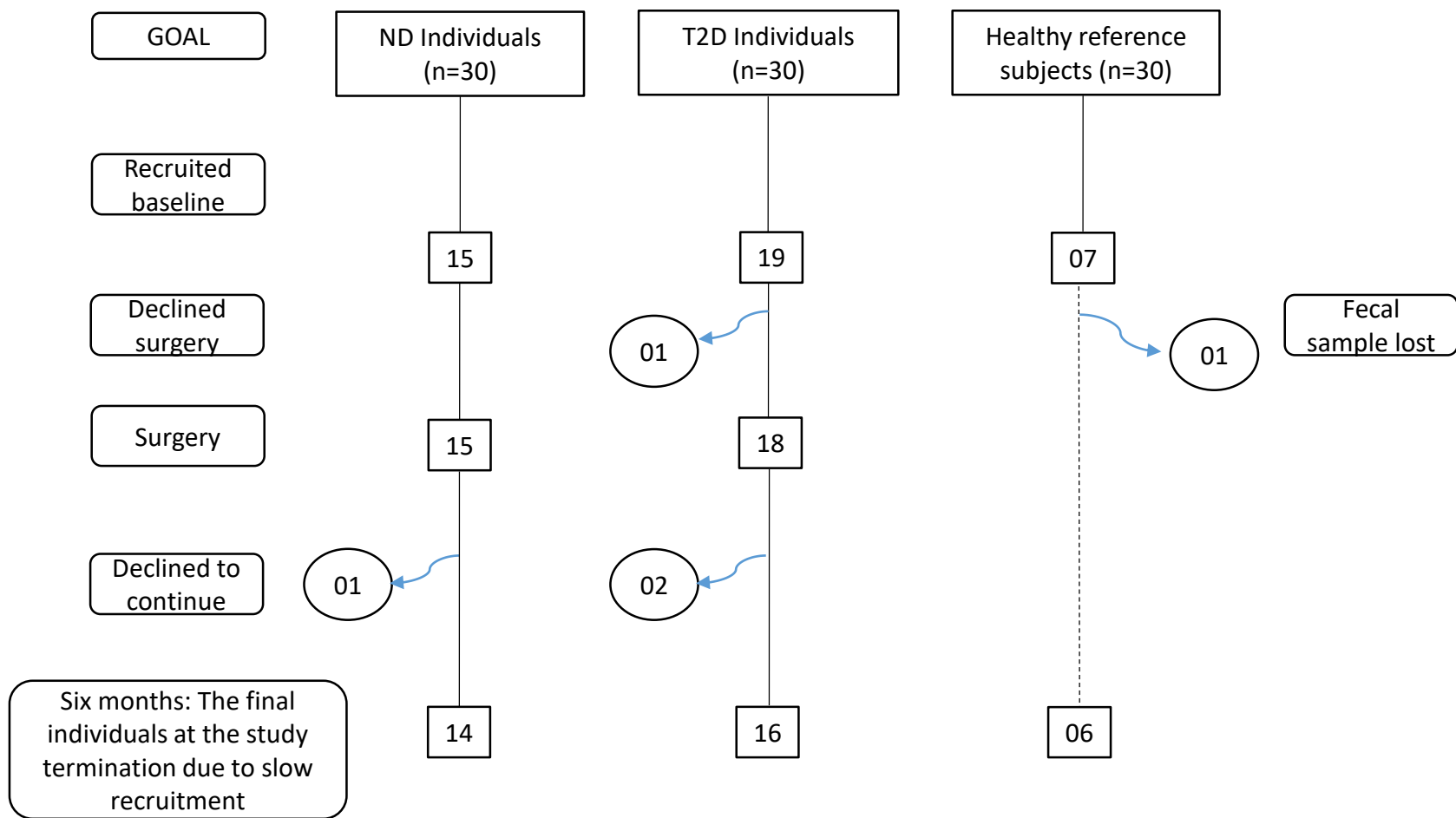
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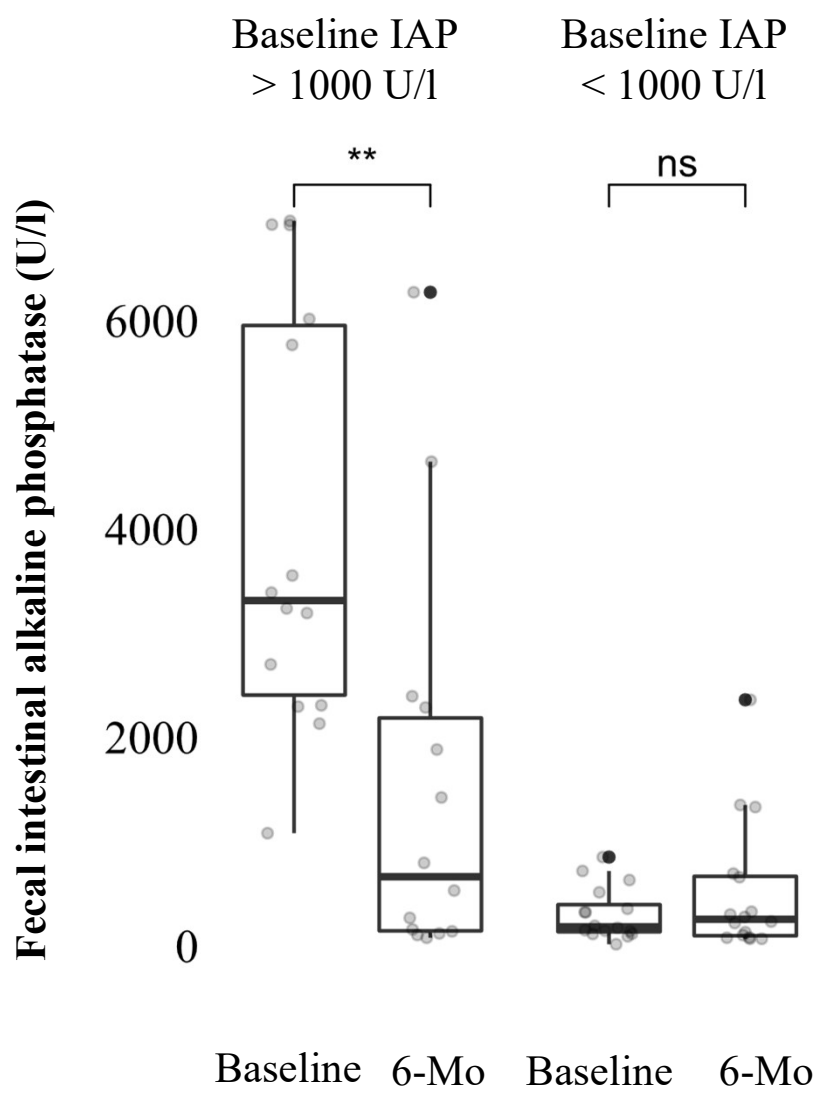
Figure 1: Flowchart for prospective cohort study.

Gastrointestinal manifestations after Roux-En-Y gastric bypass surgery in individuals with and without type 2 diabetes

Highlights

- RYGB surgery elevates fecal inflammation markers calprotectin, LPS, ASCA and MG-H1

- The fecal levels of potentially beneficial SCFAs are reduced by the surgery

- The abnormally high fecal protein levels in T2D patients are normalized by RYGB