Title: Insulin resistance modulates gut microbiota and incretin response remodeling after bariatric surgery in severe obesity

Authors: Rocío Puig[1,2]†, M-Mar Rodríguez-Peña[3,4]†, Laura Hernández-Montoliu[5], Brenno Astiarraga[3,4], Eva Martínez[6], Jose M. Balibrea[2,7,8], Gemma Llauradó[4,9], Jordi Tarascó[7], Albert Caballero[7], Clara Joaquín[10], Manel Puig-Domingo[2,10], Nuria Vilarrasa[4,5], Sonia Fernández-Veledo[3,4,11], Joan Vendrell[3,4,11] ‡, Silvia Pellitero[10]‡.

†These authors have contributed equally to this work and share the first authorship

‡These authors have contributed equally to this work and share the last authorship

Rocío Puig Piña

[1]Department of Endocrinology and Nutrition, Hospital de la Santa Creu i Sant Pau, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

[2]Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

Mail: rpuig@santpau.cat

ORCID number: 0000-0001-9184-5583

M-Mar Rodríguez-Peña

[3]Department of Endocrinology and Nutrition, Hospital Universitari Joan XXIII de Tarragona, Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain

[4]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Mail: mar.rodriguez@iispv.cat

ORCID number: 0000-0003-2553-9769

Laura Hernández-Montoliu

[5]Department of Endocrinology and Nutrition, Hospital Universitari de Bellvitge-Institut d'Investigació Biomèdica de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain

Mail: laura.hermont@gmail.com

ORCID number: 0000-0003-3935-3922

Brenno Astiarraga

[3]Department of Endocrinology and Nutrition, Hospital Universitari Joan XXIII de Tarragona, Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain

[4]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Mail: brenno.dominguez@iispv.cat

ORCID number: 0000-0003-2216-8974

Eva Martínez-López

[6]Clinical Nutrition Unit. Hospital Germans Trias i Pujol, Badalona, Spain

Mail: emartinezl.germanstrias@gencat.cat

Jose M. Balibrea

[2]Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

iVascular®-UAB Surgical Research Chair

[7]Department of General and Gastrointestinal Surgery. Health Sciences Research Institute and University Hospital Germans Trias i Pujol, Badalona, Spain

[8]CIBER Pathophysiology of Obesity and Nutrition (CIBEROBN)

Mail: jmbalibrea.germanstrias@gencat.cat

ORCID number: orcid.org/0000-0002-0764-7179

Gemma Llauradó

[4]Department of Endocrinology and Nutrition, Hospital del Mar, Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

[9]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Mail: gllauradoc@gmail.com

ORCID number: 0000-0002-3237-6366

Jordi Tarascó

[7]Department of General and Gastrointestinal Surgery. Health Sciences Research Institute and University Hospital Germans Trias i Pujol, Badalona, Spain

Mail: jorditarasco@gmail.com

ORCID number: 0000-0001-6820-7946

Albert Caballero

[7]Department of General and Gastrointestinal Surgery. Health Sciences Research Institute and University Hospital Germans Trias i Pujol, Badalona, Spain

Mail: acaballeroboza@gmail.com

ORCID number: 0000-0003-2666-4967

Clara Joaquín

[10]Department of Endocrinology and Nutrition. Health Sciences Research Institute and University Hospital Germans Trias i Pujol, Badalona, Spain

Mail: clara.joaquim@gmail.com

ORCID number: 0000-0002-9063-4853

Manel Puig-Domingo

[2]Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

[10]Department of Endocrinology and Nutrition. Health Sciences Research Institute and University Hospital Germans Trias i Pujol, Badalona, Spain

Mail: mpuigd@igtp.cat

ORCID number: 0000-0002-6744-7195

Nuria Vilarrasa

[4]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[5]Department of Endocrinology and Nutrition, Hospital Universitari de Bellvitge-Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain

Mail: nuriavilarrasa@yahoo.es

ORCID number: 000-0003-3188-1990

Sonia Fernández-Veledo

[3]Department of Endocrinology and Nutrition, Hospital Universitari Joan XXIII de Tarragona, Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain

[4]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[11]Universitat Rovira i Virgili (URV), Reus, Spain

Mail: sonia.fernandez@iispv.cat

ORCID number: 0000-0003-2906-3788

Joan Vendrell

[11]Universitat Rovira i Virgili (URV), Tarragona, Spain

[3]Department of Endocrinology and Nutrition, Hospital Universitari Joan XXIII de Tarragona, Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain

[4]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Mail: jvortega@iispv.cat

ORCID number: 0000-0002-6994-6115

Silvia Pellitero

[10]Department of Endocrinology and Nutrition. Health Sciences Research Institute and University Hospital Germans Trias i Pujol, Badalona, Spain

Mail: spellitero.germanstrias@gencat.cat

Corresponding author

Silvia Pellitero, MD. PhD

Department of Endocrinology and Nutrition Universtiy Hospital and Health Sciences Research Institute Germans Trias i Pujol, Badalona, Spain

Carretera del Canyet, s/n, 08916 Badalona, Barcelona, Spain

Telephone: +3493 497 88 60.

E-mail address: spellitero.germanstrias@gencat.cat

Joan Vendrell MD. PhD.

Universitat Rovira i Virgili (URV), Tarragona, Spain

Department of Endocrinology and Nutrition, Hospital Universitari Joan XXIII de Tarragona, Institut d'Investigació Sanitària Pere Virgili (IISPV)

Carrer Dr. Mallafré Guasch, 4, 43005 Tarragona, Spain.

117 Telephone: +34977 29 58 00

118 e-mail: jvortega@iispv.cat

119 **Competing interests**

120 The authors declare that the research was conducted without any commercial or financial

121 relationships that could be construed as a potential conflict of interest.

Abstract:

Background

This study aims to assess the impact of insulin resistance (IR) on gut microbiota (GM) composition, incretin responses, and metabolic outcomes following sleeve gastrectomy (SG) in people with severe obesity who do not have diabetes.

Methods

A prospective single-center study encompassed patients with severe obesity and normal glucose tolerance who underwent SG. Participants were stratified into two cohorts based on the magnitude of their insulin resistance state, as determined by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index: high-IR (Hi-IR; HOMA-IR > 95th percentile) and low-IR (Lo-IR; HOMA-IR < 25th percentile). Body composition measurements, biochemical analyses, and microbiota assessments were performed before and six months post-surgery. Additionally, the responses to a standardized meal tolerance test (MTT) of glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) were evaluated.

Results

The study cohort consisted of 18 patients (9 with Hi-IR and 9 with Lo-IR), with a mean age of 48.8 ± 9.2 years and a mean body mass index (BMI) of 45.03 ± 4.82 kg/m². Six months post-surgery, the mean percentage of total weight loss (WL) was 26.5 ± 6%, with both groups exhibiting enhanced secretion of GLP-1 and GLP-2 following MTT.

At baseline, participants exhibited distinct microbiota profiles; the Hi-IR group showed a higher relative abundance of *Prevotella* species, which are previously associated with adverse metabolic and inflammatory profiles. Post-surgery, both groups exhibited positive incretin responses and significant modifications in GM composition. Notably,

Hi-IR people experienced more pronounced changes in microbial diversity, including increases in *Akkermansia* and *Veillonella* species and decreases in *Prevotella* species. Enhanced GLP-1 and GLP-2 responses were correlated with WL and metabolic improvement, particularly in the Lo-IR population.

Conclusions

These findings underscore the role of GM in metabolic changes and surgical outcomes after SG. Targeting gut microbiota may offer a promising avenue for improving obesity treatment strategies.

KEYWORDS: Insulin resistance; incretin; microbiota; severe obesity; bariatric surgery.

LIST OF ABBREVIATIONS

AUC Area under the receiver operating characteristic curve

BA Bile acids

BMI Body mass index

BS Bariatric surgery

CSS Cumulative sum scaling

CV Coefficient of variation

DXA Dual-energy X-ray absorptiometry

EE Energy expenditure

ELISA Enzyme-linked immunosorbent assay

FC Fold change

FFMI Fat-free mass index

FitZig Zero-inflated Gaussian mixture model

FMI Fat mass index

FLI Fatty liver index

GLP-1 Glucagon-like peptide-1

GLP-2 Glucagon-like peptide-2

GM Gut microbiota

HDLc High-density lipoprotein cholesterol

Hi-IR High insulin resistance

HOMA-IR Homeostatic Model Assessment of Insulin Resistance

IL-6 Interleukin-6

IQR Interquartile range

IR Insulin resistance

KEGG Kyoto Encyclopedia of Genes and Genomes

Lo-IR Low insulin resistance

Log2FC Log2 fold-change

MTT Meal tolerance test

PCA Principal Component Analysis

ROC Receiver operating characteristic

RYGB Roux-en-Y gastric bypass

SD Standard deviation

SG Sleeve gastrectomy

WL Weight loss

INTRODUCTION

Severe obesity is characterized by insulin resistance (IR) and chronic low-grade inflammation. Bariatric surgery (BS) remains the most effective treatment for achieving substantial weight loss (WL), improving glucose metabolism, and reducing comorbidities. However, long-term outcomes are variable (1,2), and some patients fail to reach WL goals or experience weight regain (3). Identifying predictors of long-term success, particularly after sleeve gastrectomy (SG), remains a research priority (4,5).

SG, a less invasive option compared to malabsorptive techniques, induces major metabolic benefits (6). Improved insulin sensitivity after SG is attributed mainly to WL (7), and both SG and Roux-en-Y gastric bypass (RYGB) show comparable improvements in hepatic and peripheral insulin resistance (8,9).

Beyond WL, other mechanisms contribute to BS benefits. Incretin responses, which regulate insulin secretion and appetite, are markedly enhanced after surgery (10,11), while long-term gastrointestinal adaptations may influence incretin secretion and WL durability. Gut microbiota (GM) remodeling also emerges as a determinant of outcomes (12,13), with effects on enteroendocrine signaling (14), short-chain fatty acid production (15), and host metabolism (16). However, GM may return to baseline or reach a new equilibrium, potentially limiting WL durability (17).

Body composition is another determinant: preserving lean mass sustains energy expenditure, while excessive loss may predispose to regain (3,18). Yet its role in long-term WL after BS remains unclear.

Finally, severe obesity encompasses a heterogeneous range of metabolic phenotypes. While most patients display marked IR, some remain insulin sensitive despite severe

adiposity, showing more favorable profiles (19,20). Such baseline differences may affect WL and metabolic responses after surgery (21).

A deeper understanding of how IR, incretin responses, GM, and body composition interact in the context of BS may provide valuable insights into the mechanisms underlying variable postoperative outcomes. We therefore hypothesize that, in people with severe obesity, baseline IR is associated with distinct incretin secretion, GM composition, and body composition, which in turn may influence postoperative WL, metabolic outcomes, and microbiota remodeling after SG.

MATERIAL/SUBJECTS AND METHODS

Study population

This study included a cohort of eighteen patients with severe obesity and normal glucose tolerance, defined as a fasting plasma glucose <100 mg/dL (5.6 mmol/L), and a HbA_{1C} <5.7% according to American Diabetes Association criteria (22). All participants underwent consecutive SG procedures at our facility from September 2014 to January 2015. The participants were divided into two groups based on their status of IR, determined by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). To maximize phenotypic contrast within a relatively homogeneous population (people with severe obesity and without diabetes), we selected individuals at the extremes of the cohort distribution: the Hi-IR group comprised subjects above the 95th percentile of HOMA-IR, and the Lo-IR group comprised subjects below the 25th percentile. Each group included nine participants. The use of cohort-specific percentile cut-offs, rather than fixed thresholds, is consistent with prior metabolic studies, where extreme percentiles (e.g., 75th–95th) have been applied to define IR and metabolic risk groups (23,24). The study was approved by the Ethics Committee of Germans Trias i Pujol Hospital (protocol PI-

14-103). Written informed consent was obtained from all participants before their inclusion in the study.

Methodology section

All patients underwent comprehensive clinical assessments in accordance with our institutional BS protocol, which is aligned with the guidelines of the Spanish Society of Endocrinology and Nutrition (25). Assessments included anthropometry, body composition, and fasting blood sampling, which were conducted preoperatively and at 6 months postoperatively. Demographic and clinical data (age, diabetes history, and hypertension) were collected at both time points. Weight-loss outcomes were documented 5 years post-surgery. Exclusion criteria included type 2 diabetes, inflammatory or gastrointestinal disorders, and recent antibiotic use.

Anthropometric measurements

Body Mass Index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. Waist circumference was measured at the highest point of the iliac crest by the same endocrinologist. The percentage of WL (%WL) was calculated as (baseline weight – follow-up weight) / baseline weight × 100. At 5 years after SG, patients were classified as responders if they achieved ≥20% WL, and as non-responders otherwise.

Body composition assessment

Body composition was assessed by dual-energy X-ray absorptiometry (DXA; Hologic QDR 4500, Hologic Inc., Waltham, MA, USA) at baseline and six months after surgery. Fat-free mass (FFM) and fat-free mass index (FFMI) were derived using standard formulas. Further technical details are provided in the Supplementary Methods (Supplementary File 1).

Surgical procedure

All patients underwent laparoscopic SG performed by the same surgical team using a standardized technique with a 36-Fr bougie for calibration and resection from 4–6 cm proximal to the pylorus up to the angle of His. Staple-line reinforcement and intraoperative leak testing were routinely performed. Detailed operative steps and perioperative care are provided in the Supplementary Methods (Supplementary File 1).

Biochemical measurements

Blood samples were obtained after an overnight fast. Plasma glucose, lipid profile, and HbA1c were determined using standard clinical laboratory methods. Insulin resistance was assessed by HOMA-IR, and fatty liver risk was estimated using the Fatty Liver Index (FLI). Zonulin was measured as a marker of intestinal permeability (see Supplementary Methods for technical details).

Standard meal test

A standardized mixed meal tolerance test (MTT) was performed at baseline and 6 months after SG, following an overnight fast. Participants ingested 200 mL of a liquid meal (320 kcal; 16% protein, 49% carbohydrate, 30% fat) within 5 minutes, and venous blood samples were collected at 0, 15, 30, 60, and 120 minutes. Total GLP-1 and GLP-2 concentrations were measured by ELISA in duplicate using paired pre- and postoperative samples from each participant. Incretin responses were evaluated as percentage change from baseline to peak and by calculating the area under the curve (AUC0–120). Full methodological details are provided in Supplementary Methods (Supplementary File 1).

Statistical analysis

Continuous variables were expressed as mean ± SD or median (interquartile range), depending on the distribution (Shapiro–Wilk test). Wilcoxon matched-pairs test was used for within-group comparisons (pre- vs. post-surgery), and Student's t-test or Wilcoxon rank-sum test for between-group analyses. A p-value <0.05 was considered significant. Analyses were performed using Stata v14 (StataCorp, College Station, TX, USA). Full details of extended regression analyses and multiple testing procedures are available in the Supplementary Methods (Supplementary File 1).

Stool sample collection, DNA extraction, and metagenomic sequencing

Stool samples were obtained at baseline and 6 months post-surgery, immediately frozen at –20 °C, and stored at –80 °C until analysis. DNA was extracted using the QIAamp DNA stool kit (Qiagen) and sequenced on an Illumina NextSeq 500 platform. Extended protocols for library preparation, sequencing, and bioinformatics are provided in the Supplementary Methods (Supplementary File 1).

Bioinformatics analysis

Taxonomic and functional analyses were conducted using established pipelines. Taxonomic assignment was performed with Kaiju (28), and functional annotation was based on KEGG orthology. Alpha diversity (Shannon index, observed species) and beta diversity (Bray–Curtis dissimilarity) were computed, and differential abundance was assessed using zero-inflated Gaussian mixture models. Further details of the workflow, software versions, and parameters are provided in the Supplementary Methods (Supplementary File 1).

Microbiome data analysis

Microbiome data were processed and analyzed in R using standard pipelines (phyloseq, vegan, metagenomeSeq, and ggplot2) (33,34). Alpha diversity (Shannon index and

observed species) and beta diversity (Bray-Curtis dissimilarity with Adonis testing) were calculated using CSS-normalized data. Differential abundance was assessed using the zero-inflated Gaussian mixture model (FitZig), with statistical significance defined as an adjusted p-value < 0.05 after the Benjamini–Hochberg correction to control the false discovery rate (FDR). Group differences and associations with clinical variables were evaluated as described in detail in Supplementary Methods (Supplementary File 1).

Statistical testing and correlation analysis

Normality of clinical variables was assessed with the Shapiro-Wilk test. Group comparisons were performed using parametric or non-parametric tests, as appropriate, and p-values were corrected for multiple testing using the Benjamini-Hochberg method. Associations between microbial abundances and clinical variables were examined by Spearman's rank correlation (details in Supplementary Methods, Supplementary File 1).

Intervention success and WL prediction

An unnested two-step machine learning approach was employed to discover potential microbial species for intervention success (IS) and WL predictions. The first step used all taxa that passed the prevalence filter and involved feature selection using bootstrap sampling with replacement (B=100) of 2/3 of the dataset, followed by cross-validation with glmnet (k=5). Features selected in at least 5% of the bootstraps were retained for the next step.

The second step involved model evaluation and prediction using Random Forest (ntree=100) with cross-validation (k=5) on all samples. For predicting intervention success, the threshold between success and failure was determined using the Youden index. For WL prediction, a regression-based random forest was used, utilizing the same selected variables as in the first step.

Successful WL after BS was defined as WL above 20% at 5 years post-treatment.

RESULTS

Baseline results

Clinical and biochemical characteristics

A total of 18 patients with severe obesity (13 females, 5 males) were enrolled, matched by age and gender. The mean age was 48.83 ± 9.2 years, and the preoperative BMI was 45.03 ± 4.82 kg/m².

Clinical, biochemical, and hormone data for the Hi-IR and Lo-IR groups are shown in Table 1.

Incretin response

Before surgery, fasting GLP-1 levels were significantly higher in the Hi-IR compared to the Lo-IR group, whereas fasting GLP-2 did not differ. During the mixed-meal test, GLP-1 showed a greater relative peak increase in Lo-IR compared with Hi-IR subjects (median % increase: 76.9% vs. 52.0%; $p = 0.030$), while $AUC_{0–120}$ did not differ significantly between groups. For GLP-2, the relative peak increase was comparable between groups, but Hi-IR subjects displayed a higher total AUC ($p = 0.038$) (Table 2, Figure 1).

Correlations

The GLP-1 response to the MMT correlated positively with the GLP-2 response (r = 0.58, p = 0.018) and HDL-c (r = 0.66, p = 0.003), and negatively with HOMA-IR (r = -0.53, p = 0.02). HOMA-IR correlated positively with triglycerides (r = 0.52, p = 0.02) and FLI (r = 0.55, p = 0.02), while FLI also correlated positively with fat mass (r = 0.49, p = 0.04). Zonulin levels were negatively correlated with HDL cholesterol (r = -0.49, p = 0.03).

Six months post-SG outcomes

Clinical and biochemical characteristics

After 6 months, the mean %WL for the entire cohort was 26.5 ± 6%, with no significant differences between groups. HOMA-IR levels decreased significantly in both groups, with no differences between them post-BS (Table 1).

Only the Hi-IR group showed improvements in triglycerides, HDL-c, IL-6, and zonulin. The FLI decreased significantly in both groups (Lo.IR: p = 0.002; Hi-IR: p = 0.008), with a more pronounced reduction in the Lo-IR group (56.21 ± 27.56% vs. 30.70 ± 20.11%, p = 0.05, trend).

Incretin response

Fasting GLP-1 and GLP-2 concentrations decreased in both groups, reaching statistical significance only in Lo-IR subjects. After SG, the relative peak increase in both GLP-1 and GLP-2 was significantly greater in Lo-IR compared with Hi-IR patients ($p = 0.021$ and $p = 0.009$, respectively). In contrast, no differences were observed between groups for total AUC ($p = 0.171$ and $p = 0.354$). Within-group analyses revealed a significant postoperative increase in GLP-2 AUC in Lo-IR subjects ($p = 0.028$) and a trend toward higher GLP-1 AUC ($p = 0.066$) (Table 2, Figure 1). When evaluating relative changes from baseline, GLP-1 responsiveness increased by 478.7 ± 437.1% in Lo-IR versus 250.4 ± 188.5% in Hi-IR, although this difference did not reach statistical significance. By contrast, GLP-2 responsiveness improved significantly more in Lo-IR (732.9 ± 576.1%) than in Hi-IR (80.1 ± 111.1%, $p < 0.001$).

Correlations

The %WL at six months correlated positively with the improvement in GLP-1 response to the MMT ($r = 0.468$, $p = 0.058$, trend only) and strongly with reduction in fat mass ($r = 0.8$, $p < 0.001$).

Five-year follow-up

At five years, the %WL was similar between the Lo-IR and Hi-IR groups (24.96 ± 13.47% vs. 23.08 ± 8.87%, p = ns). At this time point, 66.6% of patients (55.5% of Lo-IR and 83.3% of Hi-IR, p = ns) were classified as responders (≥20% WL). Baseline FFMI was significantly higher in responders compared to non-responders (21.40 ± 1.69 vs. 19.50 ± 1.08, $p = 0.037$). The secretory patterns of GLP-1 and GLP-2 at baseline or at six months did not differ between responders and non-responders. %WL at five years correlated positively with %WL at six months ($r = 0.70$, $p = 0.004$) and with reductions in waist circumference ($r = 0.63$, $p = 0.01$) and fat mass ($r = 0.60$, $p = 0.03$) during the first six months post-surgery.

Microbiota results

Taxonomy basal composition of the study population

Before surgery, we assessed the baseline microbiota abundance levels, distinguishing between Lo-IR and Hi-IR patients. Figure 2 illustrates a distinctive signature when comparing both groups based on their insulin resistance (IR) status. The yellow and purple bars represent the Log2FC values of these species, which showed a greater increase in HI-IR compared to Lo-IR patients. Note that a higher Log2FC value indicates a greater relative change in the abundance of a specific species, not necessarily higher microbial diversity. Hi-IR patients were enriched in taxa such as *Prevotella*, while Lo-IR patients showed higher abundance of *Akkermansia*, *Clostridium*, and *Dialister*.

We conducted a correlation analysis between bacterial species showing significant differences in abundance between the two groups and incretin levels. The *Prevotella* genus, which was more abundant in Hi-IR patients, correlated positively with higher basal incretin levels and a blunted postprandial response. In contrast, Lo-IR patients displayed the opposite pattern (Figure 2). When we examined parameters related to obesity and inflammation, we found that species prevalent in the Lo-IR group, mainly species of *Clostridium*, were negatively correlated with baseline incretin levels, weight, BMI, fat mass, glucose, HbA1c, FLI, triglycerides, and IL-6.

Impact of surgical intervention on microbial ecology

Following surgical intervention, we observed differential effects on microbiota ecology based on insulin resistance status. In Lo-IR patients, surgery did not induce statistically significant alterations in microbial richness ($p = 0.668$) or alpha diversity evenness ($p = 0.841$), indicating a stable microbiota composition. Conversely, Hi-IR patients exhibited significant changes in microbial richness ($p = 0.002$) and beta diversity ($p = 0.006$) post-surgery compared to Lo-IR patients. This indicates a substantial shift in microbiota composition towards a greater diversity, specifically in Hi-IR patients following the intervention. Before intervention, no significant beta diversity differences existed between groups ($p = 0.2$), suggesting that the potentially beneficial effect of the surgery on gut microbiota was primarily observed in the Hi-IR group.

Impact of intervention on microbiota composition and clinical parameters

Post-surgery, significant compositional changes were detected in both groups (31 species in Lo-IR, 84 in Hi-IR; adjusted $p < 0.05$, Supplementary Table S1). As shown in Figure 3, Hi-IR patients primarily drove the reduction in *Prevotella* spp. and *Bacteroides coprocola*, together with increases in *Akkermansia*, *Veillonella*, and *Streptococcus*. Lo-

IR patients showed decreases in *Dialister* and *Megamonas*, and increases in *Methanobrevibacter*, *Alistipes*, *Ruminococcus*, and *Roseburia*. Overall, taxa that decreased post-surgery (mainly *Prevotella*) were associated with higher adiposity, inflammation, and poorer incretin responses, whereas taxa that increased (*Clostridium*, *Ruminococcus*, *Akkermansia*, *Streptococcus*) correlated with improved metabolic and hormonal parameters.

Intervention success and WL prediction

We developed a predictive model of intervention success using baseline microbiota abundances, defining success as >20% body WL five years post-surgery. Figure 4 (Bottom) illustrates the species contributing most to the model's probability of success or failure. The model's predictive performance was evaluated using the area under the receiver operating characteristic curve, yielding an AUC of 0.870. An optimal threshold was determined by the Youden index of 0.645 (Fig. 4, Top-Right). Additionally, we constructed a regression-based model to predict the %WL at five years post-surgery (Fig. 4, Top-Left).

DISCUSSION

This prospective study offers novel insights into the impact of IR on incretin response and alterations in GM composition following SG in people with obesity without diabetes. Our findings reveal significant modifications in GM composition and its associations with metabolic parameters before and after surgical intervention, mainly related to the IR state.

Baseline microbiota and metabolic associations

Before surgery, the Hi-IR group exhibited a higher prevalence of *Prevotella* spp., particularly *Prevotella copri*, previously linked to branched-chain amino acid production

and IR (35,36). Consistent with prior human studies in obesity and IR, *Prevotella* abundance was higher in the Hi-IR group compared to their Lo-IR counterparts (37). In our cohort, Prevotellaceae correlated positively with waist circumference, FLI, and baseline incretin levels (GLP-1, GLP-2), and inversely with meal-induced incretin responses. These associations suggest that *Prevotella* abundance may serve as a microbial marker associated with incretin resistance and adverse metabolic profiles, although causality cannot be inferred.

Conversely, Lo-IR patients exhibited a higher abundance of *Akkermansia muciniphila*, a genus consistently associated with improved metabolic status and gut-barrier function in human and experimental studies (38). *Dialister* species (*D. succinatiphilus and D. invisus*) were also more prevalent in this group, in line with previous reports linking *Dialister* abundance to favorable metabolic states before diabetes onset (39,40). Additionally, *Clostridium* species enriched in Lo-IR patients correlated inversely with adiposity and inflammatory markers, in line with observational data linking members of this group to healthier metabolic outcomes (41, 42).

Post-surgery incretin responses

After SG, incretin responses improved overall, although the magnitude and pattern differed by IR status (Table 2). Lo-IR patients exhibited a significantly greater relative peak increase in both GLP-1 and GLP-2 compared with Hi-IR, despite similar %WL and HOMA-IR values at follow-up. However, when analyzed by AUC, no between-group differences were observed, underscoring the importance of the metric in capturing hormonal dynamics. Within-group analyses showed that Lo-IR patients displayed a significant postoperative increase in GLP-2 AUC and a trend toward higher GLP-1 AUC. Recent work also confirmed pronounced postprandial GLP-1 increases after both SG and

RYGB, reflecting enhanced enteroendocrine stimulation and activation of the ileal brake (11).

The role of GLP-2 in bariatric outcomes remains less well defined. In our cohort, the marked GLP-2 response observed in Lo-IR patients did not correlate with WL or metabolic changes, in line with prior studies indicating that GLP-2 may support intestinal adaptation, glucose homeostasis, and barrier integrity, but without consistent links to body weight regulation (43,44).

Based on our observations, we cautiously propose the concept of "incretin resistance" to describe a state in which incretin hormones are secreted at normal or elevated levels but fail to elicit an adequate metabolic response. This concept, directly analogous to "insulin resistance," does not reflect a defect in hormone secretion per se, but rather an attenuated functional effectiveness. In our cohort, insulin-resistant individuals exhibited higher basal GLP-1 concentrations together with blunted postprandial responses, a dissociation consistent with impaired incretin responsiveness. While preliminary, this framework may help interpret interindividual variability in metabolic and surgical outcomes.

Post-surgical microbiota shifts

The surgical procedure had distinct effects on microbiota ecology in both groups. Still, the impact was more pronounced in Hi-IR patients, who exhibited significant increases in microbial richness and beta diversity. These findings suggest that surgery may confer an additional benefit by enhancing microbial diversity in metabolically unfavorable phenotypes. At the compositional level, Hi-IR patients exhibited marked reductions in *Prevotella* species, accompanied by increases in *Akkermansia*, *Streptococcus*, and *Veillonella*. These shifts—reduced *Prevotella* and increased *Akkermansia*, *Veillonella*, and *Streptococcus*—are consistent with recent bariatric studies reporting associations

with improved metabolic status, gut barrier function, and enhanced incretin responses (12,45–48). Notably, *Streptococcus* enrichment has also been linked to GLP-1 and GLP-2 secretion in patients with type 2 diabetes after RYGB (39,49).

Some of the changes observed in Hi-IR patients, including increases in *Akkermansia*, *Streptococcus*, *Veillonella*, and *Clostridium* species, mirror our previous findings in patients with type 2 diabetes undergoing RYGB (39). This concordance across procedures and metabolic contexts suggests that these microbial shifts represent reproducible features of bariatric surgery rather than surgery-specific phenomena.

In the Lo-IR group, surgery enriched Roseburia, Alistipes, and Ruminococcus, taxa involved in complex substrate fermentation and short-chain fatty acid production, all recognized for supporting gut health (50). *Roseburia*, a major butyrate producer, increased markedly; butyrate promotes barrier integrity and has anti-inflammatory effects, consistent with the inverse correlations we observed with adiposity and inflammation and the positive association with incretin responsiveness (48,51,52). Both groups also showed increased *Methanobrevibacter*, in line with reports linking methanogens to carbohydrate metabolism and long-term weight regulation (53,54). Overall, these shifts suggest that Lo-IR patients may benefit particularly from taxa promoting barrier protection, anti-inflammatory activity, and hormonal responsiveness.

Predictive value of baseline microbiota for weight outcomes

We also examined the relationship between pre-surgery GM composition and WL outcomes. Despite the small sample size (18 participants), our predictive model aligns with previous studies linking baseline microbiota to weight trajectories after BS. These findings suggest that microbial signatures may contribute to long-term weight management, although validation in larger cohorts is required. Beyond microbiota,

genetic background, psychological factors, dietary patterns, and physical activity likely interact to influence outcomes. Notably, we observed reductions in *Prevotella*—previously associated with adverse metabolic profiles and blunted incretin responses—together with enrichment of taxa linked to healthier states, such as *Akkermansia*, *Roseburia*, and *Methanobrevibacter*. These patterns, consistent with prior reports (52,55,56), support the hypothesis that SG may help establish a gut microbial environment associated with improved metabolic profiles, particularly in individuals with baseline IR.

Surgical considerations

All participants in our study underwent SG, which allowed us to minimize variability and focus on the role of baseline IR. It is, however, well recognized that incretin responses are generally more pronounced after RYGB or BPD than after SG, due to stronger stimulation of L-cells through nutrient rerouting (8,57). This may partly explain why the incretin response in Hi-IR patients remained attenuated despite weight loss. Moreover, long-term evidence suggests that SG may be less effective than bypass procedures in individuals with very high BMI or severe IR, raising the possibility that SG might not represent the optimal strategy in all subgroups (6). Future comparative studies including different surgical techniques are needed to clarify whether more potent incretin stimulation could mitigate this phenotype or whether "incretin resistance" persists independently of procedure type.

This study has certain constraints, mainly the small sample size and the unnested design of the machine learning model, which limit the generalizability of our findings and may increase the risk of overfitting to our cohort. Additionally, data on post-surgical physical activity and dietary intake were not collected. These variables are known to strongly

influence both weight trajectories and GM composition, and their absence limits our ability to disentangle the relative contributions of surgery versus lifestyle to the observed outcomes. It is important to note that our findings do not establish causality; instead, they should be viewed as hypothesis-generating. Further research is required to deepen our understanding of the observed changes in microbiota composition following bariatric surgery and their potential links to metabolic outcomes. Finally, the HOMA-IR cut-offs used to define Hi-IR and Lo-IR groups were derived from the cohort distribution. While this strategy enhances internal consistency, external validation in independent cohorts will be necessary to confirm the broader applicability of these thresholds.

CONCLUSIONS

In conclusion, this prospective study reveals distinct gut microbiota patterns between people with obesity and differing degrees of insulin resistance before and after sleeve gastrectomy. Post-surgically, we observed improved incretin responses together with decreases in *Prevotella* and increases in taxa such as *Akkermansia*, *Roseburia*, and *Methanobrevibacter*, all of which were associated with more favorable metabolic markers. Our findings also suggest potential associations between pre-surgical gut microbiota composition and weight-loss outcomes, pointing to the potential relevance of microbial signatures in shaping individual responses. Given the limited sample size, these observations should be regarded as exploratory. Larger studies are required to validate these associations and to clarify their potential application in developing personalized approaches to bariatric surgery.

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564 **Author contributions**

565 RP, M-MR-P, JV, and SP contributed to the conception of the work and wrote the
566 manuscript. LH-M and GLL contributed to the data analysis. BA performed the
567 bioinformatics and statistical analyses. GLL, JB, JT, and AC participated in the study
568 design. Gll, CJ, MP-D, NV, and SFV critically revised the manuscript. All authors
569 contributed to the article and approved the submitted version.

570 **Competing interests**

571 The authors declare that the research was conducted without any commercial or financial
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Data Availability Statement

The microbiome data presented in this study are available in the European Nucleotide Archive (ENA) at https://www.ebi.ac.uk/ena/browser/view/PRJEB48776, accession number PRJEB48776. The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

DECLARATIONS

Ethics approval and consent to participate

All participants provided informed consent, and the study was approved by the ethics committee of Germans Trias I Pujol Hospital (PI-14 103). All methods were performed in accordance with the relevant guidelines and regulations.

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778

779 **FIGURE LEGENDS**

780 **Figure 1. GLP-1 and GLP-2 responses to MMT at baseline and 6 months in Lo-IR**
781 **and Hi-IR.**

782 GLP-1 and GLP-2 responses to the meal tolerance test (MTT) at baseline and 6 months
783 in participants with low (Lo-IR) and high insulin resistance (Hi-IR). Panels A and B
784 represent mean ± standard deviation plasma concentrations of GLP-1 (1A) and GLP-2
785 (1B), respectively, measured at each time point during the MTT. Lo-IR participants are
786 shown in green, and Hi-IR participants are shown in blue. Dashed lines indicate baseline
787 data; solid lines indicate 6-month follow-up. Abbreviations: GLP-1, glucagon-like

peptide-1; GLP-2, glucagon-like peptide-2; Lo-IR, low insulin resistance; Hi-IR, high insulin resistance; MTT, meal tolerance test.

Figure 2. Baseline gut microbiota abundance and correlations with clinical and hormonal variables. Baseline microbiota abundance levels. Log fold change (FC) and Spearman correlation analyses of the relative abundance at the species level of gut microbiota and clinical variables and incretin hormones in all individuals. For FC analysis, a zero-inflated Gaussian mixture model (fitZig) from the metagenomeSeq R package was used. The subject factor in IDPAT is used as a batch effect. The yellow and purple bars represent the Log2FC values of these species. All adjusted p-values <0.05 in FC and + p-value <0.05; *adjusted p-value<0.05 for Spearman correlation analyses. Abbreviations: FC, fold change; Log_2FC, log_2-transformed fold change; BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; IL-6, interleukin-6.

Figure 3. Surgical-induced microbiota changes and associations between species and variables related to metabolic health status. Log fold change (FC) and Spearman correlation analyses of the relative abundance at the species level of gut microbiota and clinical variables and incretin hormones in all individuals. A zero-inflated Gaussian mixture model (fitZig) from the metagenomeSeq R package was used for FC analysis. Contrast 6M/basal. All adjusted p-values <0.05 in FC and + p-value <0.05; *adjusted p-value<0.05 for Spearman correlation analyses. Abbreviations: Log_2FC, log_2-transformed fold change; Lo-IR, low insulin resistance; Hi-IR, high insulin resistance; BMI, body mass index; FMI, fat mass index; FFMI, fat-free mass index; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; IL-6, interleukin-6.

Figure 4. Gut metagenomic signature predicts intervention success at 5-year follow-up. A predictive model (glmnet for feature selection and Random Forest for binary classification) identified potential microbial taxa associated with intervention success. These taxa were subsequently used in a weight loss regression model. Top-left: predicted probabilities of intervention success and predicted values of weight loss; Top-right: classification model performance (AUC=0.870); Bottom: classification model most important taxa, ranked by cumulative importance across folds. Abbreviations: AUC, area under the ROC curve; ROC, receiver operating characteristic; OTU, operational taxonomic unit.

Table 1. Main anthropometric and metabolic variables at baseline and 6 months post-surgery.

	LO-IR (n=9)			HI-IR (n=9)		
	Basal	**6-month**	**P**	**Basal**	**6-month**	**P**
BMI (Kg/m^2)	41.76±3.66	30.58±2.82	0.007	48.30±3.46$^{\#\#}$	35.52±4.45$^{\#}$	0.007
Waist (cm)	116.77±6.89	92.0±7.83	0.007	130.27±9.11$^{\#\#}$	104.16±11.61$^{\#}$	0.007
Fat Mass (%)	53.89±9.92	30.07±7,46	0.007	65.59±7.26$^{\#}$	39.59±11.03	0.007
Lean Mass (%)	51.79±8.28	46.41±7.44	0.007	54.93±8.72	49.64±8.12	0.007
FFM (Kg)	49.52±9.03	55.36±5.93	0.015	42.82±6.93	55.42±9.43	0.007
FFMI (Kg/m2)	18.97±2.69	21.22±1.02	0.020	16.46±2.85	21.12±2.47	0.007
%WL	-	24.7 (22.7–31.1)	-	-	27.1 (20.9–32.5)	-
FPG (mmol/L)	4.96±0.45	4.65±0.32	0.13	5.27±0.55	4.55±0.35	0.007
Insulin (pmol/L)	54.1 (43.4-84.7)	28.3 (22.9-38.7)	0.007	94.6 (83.5-107)	33.4 (26.6-52.7)	0.03
HOMA-IR	2.66±1.14	1.28±0.39	0.007	4.48±1.14$^{\#\#}$	1.47±0.96	0.007
HbA1c (%)	5.35±0.16	5.77±1.56	0.26	5.47±0.37	5.26±0.25	0.15
Total cholesterol (mmol/L)	4.76±0.69	4.52±0.79	0.26	4.70±0.84	5.08±0.78	0.31
HDL-cholesterol (mmol/L)	1.32±0.22	1.30±0.21	0.40	1.06±0.2$^{\#}$	1.18±0.13	0.12

LDL-cholesterol (mmol/L)	2.83±0.66	2.73±0.71	0.63	2.76±0.98	3.34±0.68	0.17
Triglycerides (mmol/L)	1 (0.9-1.2)	1.1 (0.9-1.3)	0.85	1.45 (0.9-1.7)	1.1 (1-1.4)	0.03
FLI	94.65 (90.65-97.59)	39.75 (23.85-59.53)	0.018	99.30## (98.89-99.71)	65.41# (57.45-82.12)	0.007
IL-6 pg/mL	1.84±0.62	1.57±0.78	0.44	2.94±0.91#	1.93±0.69	0.017
Zonulin ng/mL	2.72± 0.35	2.50± 0.43	0.08	2.90±0.36	2.67± 0.35	0.038

# $p < 0.05$; ## $p < 0.01$ (Lo-IR vs. Hi-IR).

Data are expressed as median (25th–75th percentiles) or mean ± SD, as appropriate. Fat, lean, and body mass were measured by DXA. Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; WL, Weight loss; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; FLI, fatty liver index; IL-6, interleukin 6.

Table 2. GLP-1 and GLP-2 fasting levels, peak responses, and AUC before and after sleeve gastrectomy.

(A) Fasting GLP-1 and GLP-2 levels

	Group	Pre-SG	Post-SG	p-value (pre vs. post)	p-value (Lo vs. Hi, pre)	p-value (Lo vs. Hi, post)
GLP-1 (pmol/L)	Lo-IR	29.14 (19.25–29.75)	16.63 (16.00–18.99)	**0.010**	**0.011**	0.057
GLP-1 (pmol/L)	Hi-IR	34.23 (29.17–42.91)	23.00 (21.58–35.12)	0.138		
GLP-2 (ng/mL)	Lo-IR	3.60 (3.44–4.31)	3.02 (2.75–3.13)	**0.007**	0.085	**0.004**
GLP-2 (ng/mL)	Hi-IR	4.39 (3.69–4.99)	3.89 (3.74–4.21)	0.173		

(B) GLP-1 and GLP-2 response (% increase-to-baseline)

	Group	Pre-SG	Post-SG	p-value (pre vs. post)	p-value (Lo vs. Hi, pre)	p-value (Lo vs. Hi, post)
GLP-1 (%)	Lo-IR	76.93 (69.19–140.18)	531.67 (283.94–804.16)	**0.007**	**0.030**	**0.021**
GLP-1 (%)	Hi-IR	51.96 (37.54–67.68)	166.43 (127.93–283.12)	**0.012**		
GLP-2 (%)	Lo-IR	22.41 (12.14–41.95)	195.59 (90.99–267.99)	**0.007**	0.965	**0.009**

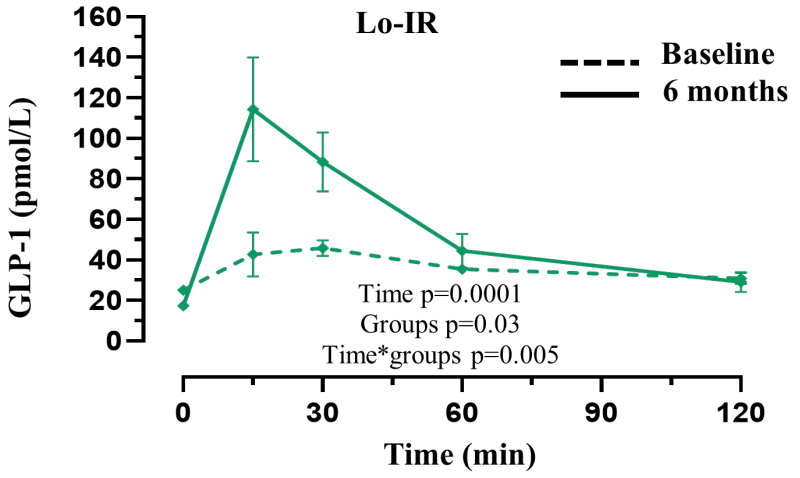
GLP-2 (%)	Hi-IR	27.10 (15.05–33.80)	31.11 (21.86–68.04)	**0.050**		

(C) GLP-1 and GLP-2 responses ($AUC_{0–120}$ min)

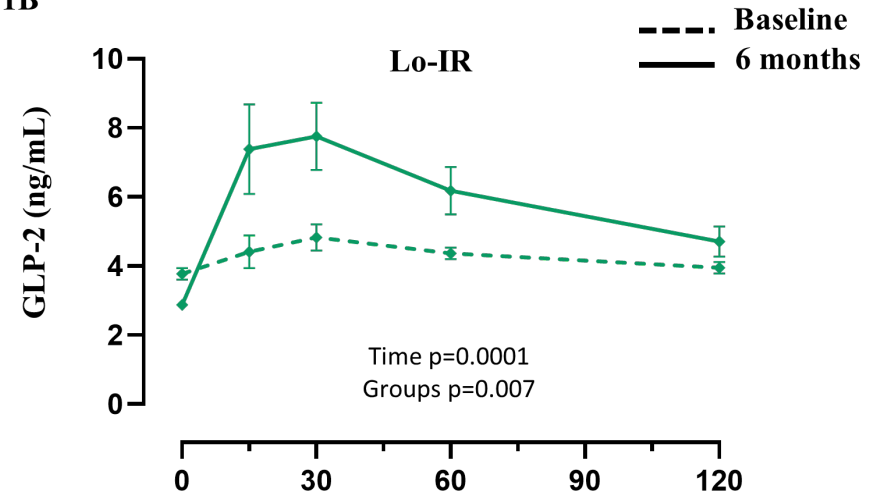
	Group	**Pre-SG**	**Post-SG**	**p-value (pre vs. post)**	**p-value (Lo vs. Hi, pre)**	**p-value (Lo vs. Hi, post)**
AUC GLP-1	Lo-IR	4365.1 (3715.6–4732.2)	6694.6 (4041.9–8927.5)	0.066	0.070	0.171
AUC GLP-1	Hi-IR	5170.6 (4644.0–6230.1)	4399.9 (3490.1–5044.0)	0.110		
AUC GLP-2	Lo-IR	488.8 (470.2–541.1)	729.3 (521.7–960.6)	**0.028**	**0.038**	0.354
AUC GLP-2	Hi-IR	582.2 (549.6–668.3)	597.0 (504.5–637.1)	0.441		

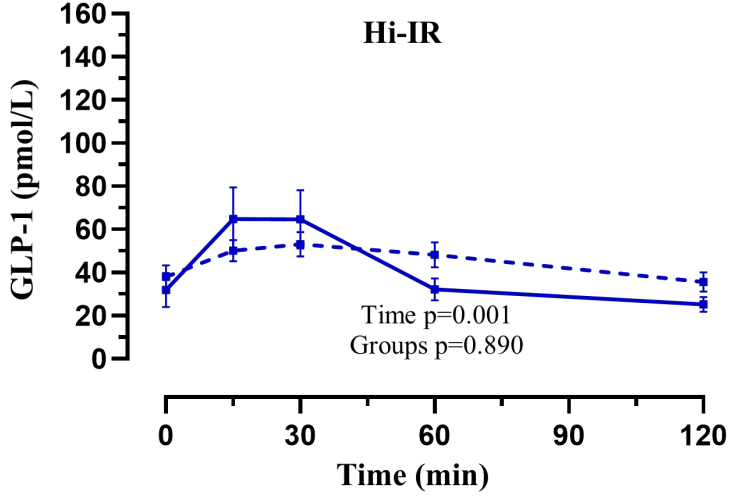
Median fasting concentrations (A), percentage increase from baseline (B), and area under the curve ($AUC_{0–120}$ min) of GLP-1 and GLP-2 (C) measured before and 6 months after sleeve gastrectomy in participants with low (Lo-IR) and high (Hi-IR) insulin resistance. Comparisons were made within each group (pre- vs. post-SG; Wilcoxon signed-rank test) and between Lo-IR and Hi-IR at each time point (Mann–Whitney U test). Values are reported as median (25th–75th percentiles). Abbreviations: GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; Lo-IR, low insulin resistance; Hi-IR, high insulin resistance; SG, sleeve gastrectomy.

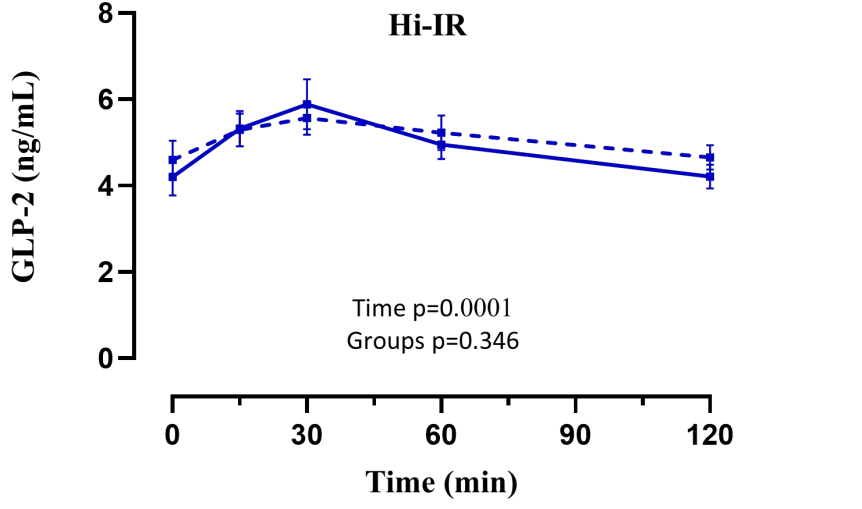
1A

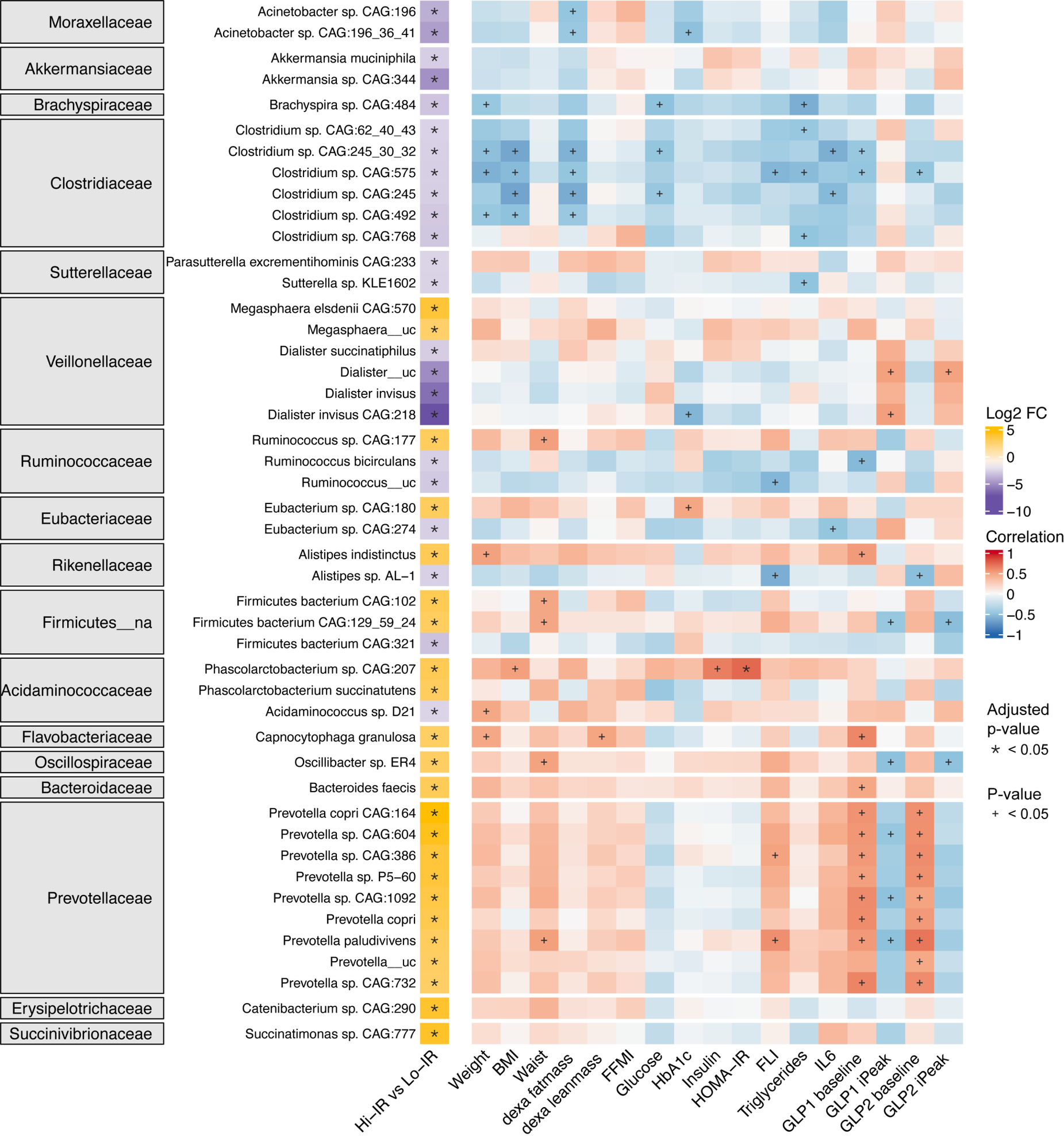

Lo-IR
Baseline
6 months
GLP-1 (pmol/L)
160
140
120
100
80
60
40
20
0
Time p=0.0001
Groups p=0.03
Time*groups p=0.005
0
30
60
90
120
Time (min)

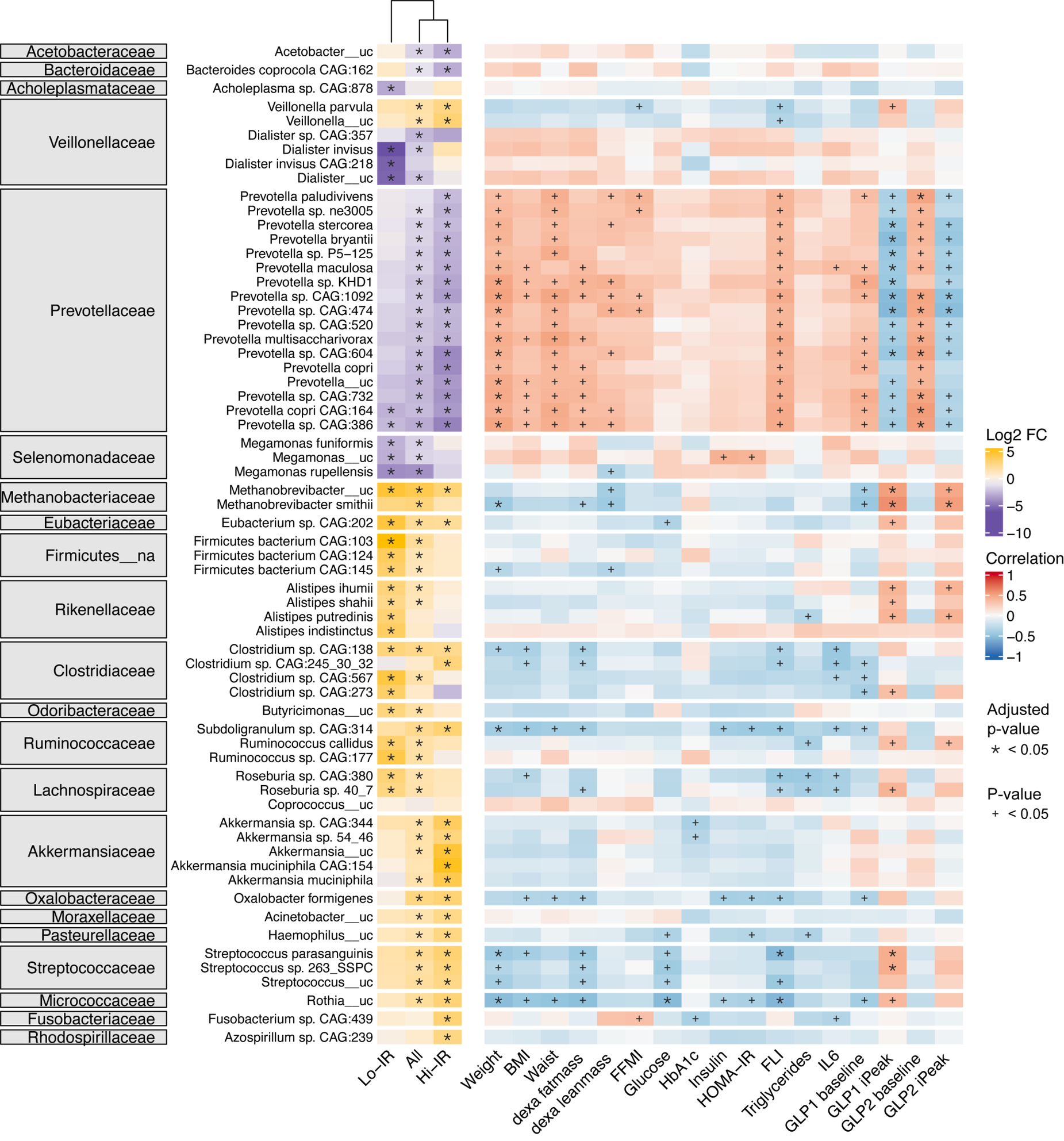
1B

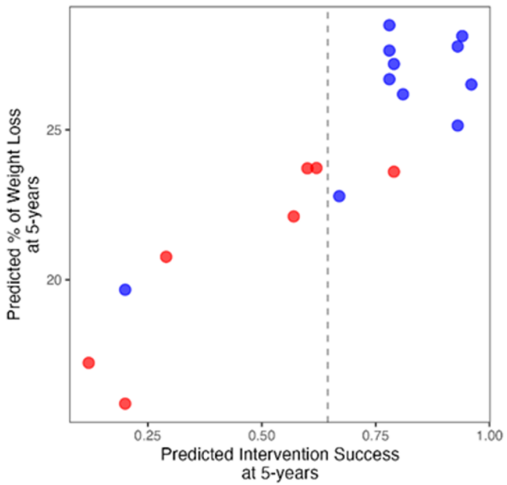

Lo-IR
Baseline
6 months
GLP-2 (ng/mL)
10
8
6
4
2
0
Time p=0.0001
Groups p=0.007
0
30
60
90
120

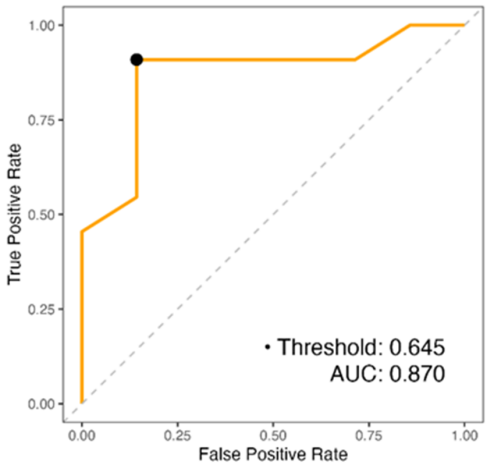

Hi-IR
GLP-1 (pmol/L)
160
140
120
100
80
60
40
20
0
Time p=0.001
Groups p=0.890
0
30
60
90
120
Time (min)

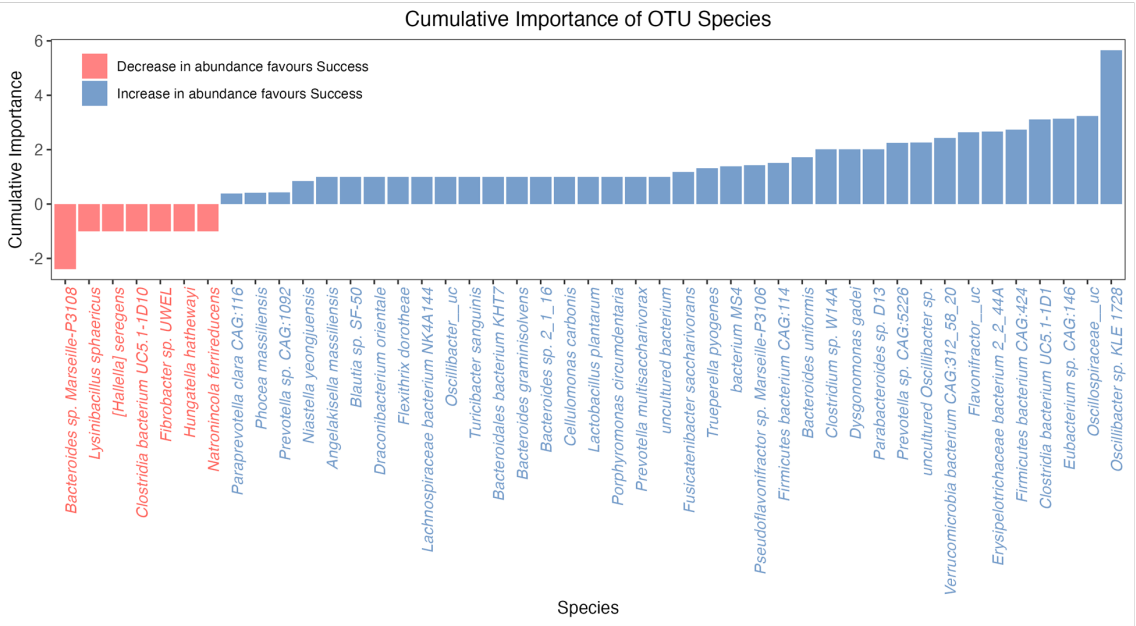

Hi-IR
GLP-2 (ng/mL)
8
6
4
2
0
Time p=0.0001
Groups p=0.346
0
30
60
90
120
Time (min)


Moraxellaceae
Acinetobacter sp. CAG:196
Acinetobacter sp. CAG:196_36_41
Akkermansiaceae
Akkermansia muciniphila
Akkermansia sp. CAG:344
Brachyspiraceae
Brachyspira sp. CAG:484
Clostridiaceae
Clostridium sp. CAG:62_40_43
Clostridium sp. CAG:245_30_32
Clostridium sp. CAG:575
Clostridium sp. CAG:245
Clostridium sp. CAG:492
Clostridium sp. CAG:768
Sutterellaceae
Parasutterella excrementihominis CAG:233
Sutterella sp. KLE1602
Veillonellaceae
Megasphaera elsdenii CAG:570
Megasphaera__uc
Dialister succinatiphilus
Dialister__uc
Dialister invisus
Dialister invisus CAG:218
Ruminococcaceae
Ruminococcus sp. CAG:177
Ruminococcus bicirculans
Ruminococcus__uc
Eubacteriaceae
Eubacterium sp. CAG:180
Eubacterium sp. CAG:274
Rikenellaceae
Alistipes indistinctus
Alistipes sp. AL−1
Firmicutes__na
Firmicutes bacterium CAG:102
Firmicutes bacterium CAG:129_59_24
Firmicutes bacterium CAG:321
Acidaminococcaceae
Phascolarctobacterium sp. CAG:207
Phascolarctobacterium succinatutens
Acidaminococcus sp. D21
Flavobacteriaceae
Capnocytophaga granulosa
Oscillospiraceae
Oscillibacter sp. ER4
Bacteroidaceae
Bacteroides faecis
Prevotellaceae
Prevotella copri CAG:164
Prevotella sp. CAG:604
Prevotella sp. CAG:386
Prevotella sp. P5−60
Prevotella sp. CAG:1092
Prevotella copri
Prevotella paludivivens
Prevotella__uc
Prevotella sp. CAG:732
Erysipelotrichaceae
Catenibacterium sp. CAG:290
Succinivibrionaceae
Succinatimonas sp. CAG:777
Hi−IR vs Lo−IR
Weight
BMI
Waist
dexa fatmass
dexa leanmass
FFMI
Glucose
HbA1c
Insulin
HOMA−IR
FLI
Triglycerides
IL6
GLP1 baseline
GLP1 iPeak
GLP2 baseline
GLP2 iPeak
Log2 FC
5
0
−5
−10
Correlation
1
0.5
0
−0.5
−1
Adjusted p-value
* < 0.05
P-value
+ < 0.05


Acetobacteraceae
Bacteroidaceae
Acholeplasmataceae
Veillonellaceae
Prevotellaceae
Selenomonadaceae
Methanobacteriaceae
Eubacteriaceae
Firmicutes__na
Rikenellaceae
Clostridiaceae
Odoribacteraceae
Ruminococcaceae
Lachnospiraceae
Akkermansiaceae
Oxalobacteraceae
Moraxellaceae
Pasteurellaceae
Streptococcaceae
Micrococcaceae
Fusobacteriaceae
Rhodospirillaceae
Acetobacter__uc
Bacteroides coprocola CAG:162
Acholeplasma sp. CAG:878
Veillonella parvula
Veillonella__uc
Dialister sp. CAG:357
Dialister invisus
Dialister invisus CAG:218
Dialister__uc
Prevotella paludivivens
Prevotella sp. ne3005
Prevotella stercorea
Prevotella bryantii
Prevotella sp. P5−125
Prevotella maculosa
Prevotella sp. KHD1
Prevotella sp. CAG:1092
Prevotella sp. CAG:474
Prevotella sp. CAG:520
Prevotella multisaccharivorax
Prevotella sp. CAG:604
Prevotella copri
Prevotella__uc
Prevotella sp. CAG:732
Prevotella copri CAG:164
Prevotella sp. CAG:386
Megamonas funiformis
Megamonas__uc
Megamonas rupellensis
Methanobrevibacter__uc
Methanobrevibacter smithii
Eubacterium sp. CAG:202
Firmicutes bacterium CAG:103
Firmicutes bacterium CAG:124
Firmicutes bacterium CAG:145
Alistipes ihumii
Alistipes shahii
Alistipes putredinis
Alistipes indistinctus
Clostridium sp. CAG:138
Clostridium sp. CAG:245_30_32
Clostridium sp. CAG:567
Clostridium sp. CAG:273
Butyricimonas__uc
Subdoligranulum sp. CAG:314
Ruminococcus callidus
Ruminococcus sp. CAG:177
Roseburia sp. CAG:380
Roseburia sp. 40_7
Coprococcus__uc
Akkermansia sp. CAG:344
Akkermansia sp. 54_46
Akkermansia__uc
Akkermansia muciniphila CAG:154
Akkermansia muciniphila
Oxalobacter formigenes
Acinetobacter__uc
Haemophilus__uc
Streptococcus parasanguinis
Streptococcus sp. 263_SSPC
Streptococcus__uc
Rothia__uc
Fusobacterium sp. CAG:439
Azospirillum sp. CAG:239
Lo−IR
All
Hi−IR
Weight
BMI
Waist
dexa fatmass
dexa leanmass
FFMI
Glucose
HbA1c
Insulin
HOMA−IR
FLI
Triglycerides
IL6
GLP1 baseline
GLP1 iPeak
GLP2 baseline
GLP2 iPeak
Log2 FC
5
0
−5
−10
Correlation
1
0.5
0
−0.5
−1
Adjusted p-value
* < 0.05
P-value
+ < 0.05


Predicted % of Weight Loss at 5-years
Predicted Intervention Success at 5-years
Failure
Success


True Positive Rate
False Positive Rate
• Threshold: 0.645
AUC: 0.870


Cumulative Importance of OTU Species
Cumulative Importance
Decrease in abundance favours Success
Increase in abundance favours Success
Species
Bacteroides sp. Marseille-P3108
Lysinibacillus sphaericus
[Hallella] seregens
Clostridia bacterium UC5.1-1D10
Fibrobacter sp. UWEL
Hungatella hathewayi
Natroniincola ferrireducens
Paraprevotella clara CAG:116
Phocea massiliensis
Prevotella sp. CAG:1092
Niastella yeongjuensis
Angelakisella massiliensis
Blautia sp. SF-50
Draconibacterium orientale
Flexithrix dorotheae
Lachnospiraceae bacterium NK4A144
Oscillibacter_ uc
Turicibacter sanguinis
Bacteroidales bacterium KHT7
Bacteroides graminisolvens
Bacteroides sp. 2_1_16
Cellulomonas carbonis
Lactobacillus plantarum
Porphyromonas circumdentaria
Prevotella multisaccharivorax
uncultured bacterium
Fusicatenibacter saccharivorans
Trueperella pyogenes
bacterium MS4
Pseudoflavonifractor sp. Marseille-P3106
Firmicutes bacterium CAG:114
Bacteroides uniformis
Clostridium sp. W14A
Dysgonomonas gadei
Parabacteroides sp. D13
Prevotella sp. CAG:5226
uncultured Oscillibacter sp.
Verrucomicrobia bacterium CAG:312_58_20
Flavonifractor_ uc
Erysipelotrichaceae bacterium 2_2_44A
Firmicutes bacterium CAG:424
Clostridia bacterium UC5.1-1D1
Eubacterium sp. CAG:146
Oscillospiraceae_ uc
Oscillibacter sp. KLE 1728