Supplemental material and methods

Clinical phenotype.

Patients were defined according to ADA definitions (1). Dyslipidemia was defined with the criteria of the IDF definition of the metabolic syndrome (2). Surgery type decision was based on international BS guidelines (i.e. BMI $\geq$ 40kg/m², or $\geq$35kg/m² with at least one severe obesity-related comorbidity) and on the patient's choice with subsequent validation by a multidisciplinary panel (3).

Gut microbiota analysis by quantitative metagenomics

Primary analysis, from quality cleaning of reads to mapping over reference gene catalog and counting was performed using METEOR Studio[1]. First, reads were quality-filtered and non-prokaryotic reads were excluded by aligning reads against the *H sapiens, B Borus* and *A thaliana* genomes with *bowtie2*. Second, an average of 16.42 million high-quality reads per sample (sd. 8.2M) were mapped and used to construct a raw gene count matrix by mapping over the 3.9 million gene reference catalogue (4) using bowtie2 with a maximum of three mismatches and selection of the best hit. Only unique alignments were counted.

Microbial gene richness was measured by counting the number of genes that are present for a given sample (i.e. at least one read mapped on that gene). To decrease technical bias due to different sequencing depth, 11 million reads were randomly selected for each sample using a draw without replacement to compute gene richness, ten times an average value was retained. When a sample had less than 11M reads, the richness was estimated using

[1] Pons, N. et al. METEOR, a platform for quantitative metagenomic profiling of complex ecosystems. Journées Ouvertes en Biologie, Informatique et Mathématiques http://www.jobim2010.fr/sites/default/files/presentations/27Pons.pdf (2010).

an upsizing approach from lower levels of down-sampling, through a linear regression approach. To be able to compare the present study with the former ones, we used an identical methodology and pipeline, first estimating gene richness, using 11 million uniquely mapped reads and a second gene richness categorical variable computed by applying a threshold of 480 000 bacterial genes, to distinguish low (LGC) from high gene count (HGC) samples (5,6).

Secondary analyses, from gene abundance normalization to MGS projection and statistical analysis, were performed using the *momr* R package[2]. The abundance table was normalized using the RPKM method (normalizing by gene length and abundance). Abundance for each MGS was computed as the mean value of the 50 most connected genes as proposed in the original study (4). Only MGS with more than 500 genes were used in the statistical analyses. MGS taxonomical annotation was performed using all genes by sequence similarity using NCBI blastN; a species-level assignment was given if >50% of the genes matched the same reference genome of the NCBI database at a threshold of 95% of identity and 90% of gene length coverage. The remaining MGS were assigned to a given taxonomical level from genus to superkingdom level, if more than 50% of their genes had the same level of assignment.

Bray-Curtis beta-diversity distance matrix between Microbaria samples was computed using *vegdist* function of *vegan* R package[3] from MGS abundance matrix collapsed at genus level. Principal Coordinate Analysis (PCoA) from Bray-Curtis beta-diversity matrix was carried out with *cmdscale* function of vegan R package.

[2] E Prifti*, E.L. Chatelier*, N Pons, M Almeida, P Leonard, JM Batto and D Ehrlich. MetaOMineR: A fine-tuned pipeline for whole metagenomic data analyses. 13-15 September 2013, 4th International Human Microbiome Congress, Hangzhou, China.

[3] J. Oksanen et al., vegan: Community Ecology Package. R package version 2.2-1 (2015).

Enterotyping of Microbaria cohort was performed following the Dirichlet Multinomial Mixture (DMM) method of Holmes et-al (7) using MGS abundance matrix collapsed at genus level. The DMM approach groups samples if their taxon abundances can be modeled by the same Dirichlet-Multinomial (DM) distribution. Classification at K=4 groups (4 DM distributions) showed the best model fit, in line with results of Holmes et-al (ref) and Vandeputte et-al (reference). The association between enterotype composition and microbiome composition was evaluated through PERMANOVA test with the function *adonis* (*vegan*).

Metabolomics analysis

13C and 15N stable isotope-labeled algal mix of 16 amino acids (internal standards) were purchased from Sigma (Saint Quentin Fallavier, France). LC-MS grade Acetonitrile and Formic acid were also purchased from Sigma (Saint Quentin Fallavier, France). Deionized water comes from a Milli-Q Elix system fitted with a LC-PaK and a MilliPak filter at 0.22µm (Merck Millipore, Guyancourt, France).

Serum samples were extracted using 8 volumes of cold acetonitrile (ACN) containing 13C and 15N stable isotope-labeled mix of 16 amino acids at 12.5 µg/ml. Samples were vortexed, sonicated and incubated at 4°C during 1 hour for slow protein precipitation. Samples were centrifuged at 20.000xg and 4°C. Supernatants were transferred to another series of tubes and dried. Samples were reconstituted with a mix of H2O/ACN (*99:1; v:v*) containing 0.1% of formic acid and transferred to vials before LC-MS analysis. LC-MS analysis was carried out on a UPLC acquity (Waters Corp, Saint-Quentin-en-Yvelines, France) and Orbitrap-based instrument (Q-Exactive™, Thermo Fisher Scientific, Illkirch, France) and was performed using a modified method described to screen microbiota-derived

metabolites (8). Briefly, a 'PFPP column' Discovery HS F5 3 µm, 2.1 × 150 mm (Sigma, Saint Quentin Fallavier, France) kept at 35°C. Injection volume and autosampler temperatures were set to 10 µL and 5°C. Flow rate was set to 0.250 mL/min and mobile phases consisted of 0.1% of formic acid in water (A) and 0.1% of formic acid in acetonitrile (B). pFPP chromatography method starts with an isocratic gradient of 1% B during 4 min, followed by a linear gradient from 1% to 3% B in 2 minutes. This proportion was held for 1 minute before increasing to 10% B in 3 minutes. Gradient was set to 25% B in 5 minutes followed by a linear gradient to 50% in 3 minutes. Then, B proportions were set to 100% in 0.5 minute with an increase of flowrate from 0.25 mL/min to 0.3 mL/min. This step is the column washing step in order to elute residual compounds and avoid sample carry-over. This eluting condition was kept constant during 3.5 minutes before returning to initial conditions in 0.1 minute. Column was thoroughly conditioned during 6 minutes. The chromatographic run time is 28 minutes.

MS data were analyzed based on standard protocols (9,10). Preprocessing steps of MS data, including peak picking, peak grouping, retention time and correction were performed using XCMS R tools (11). Resulted data matrix was filtered, normalized based on a median fold change normalization approach (12) and log10 transformed. Annotation and identification of features were performed using standards proposed by (13). Features were annotated putatively using a local database and then confirmed based on MS/MS experiments. Remaining features were either characterized using available public databases (14,15) or discarded when feature status are still unknowns.

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Supplemental figure titles and legends

Supplemental Figure 1: Enterotype composition of Microbaria cohort

Ordination of the entire MB cohort according to Principal coordinates analysis (PCoA) from Bray-Curtis beta-diversity distance matrix. **A:** Enterotype distribution of MB samples in PCoA space and PERMANOVA pvalue and R^2 for the association test between enterotype classification and genus-level microbiome composition of Microbaria samples. Enterotype composition have major impact in microbiome composition, explaining 40% of variability according with PERMANOVA tests **B:** Abundance distribution of *Bacteroides* genus in Microbaria samples across PCoA ordination space. **C:** Abundance distribution of *Prevotella* genus in MB samples across PCoA ordination space. **D:** Abundance distribution of Ruminococcaceae family in Microbaria samples across PCoA ordination space. Distribution of main drivers of enterotype composition according with Vandeputte et-al (16) fits with enterotype classification in Microbaria cohort.

Supplemental Figure 2: PCoA analysis

A: PCoA analysis to discriminate patients according to their treatment group (brown RYGB; beige AGB; yellow diet). **B**: Same PCoA map depicting the gene richness groups: high gene count (HGC) (blue) or low gene count (LGC) (red). Brown outline denotes RYGB; beige outline denotes AGB and yellow outline denotes the diet group.

Supplemental Figure 3: Validation of MGR-related MGS at baseline:

Heatmap of spearman correlations between gene richness and abundances of 78 richness-related MGSs in Atox and Microbaria cohorts. ***=qvalue <0.001; **=qvalue<0.05; *=pvalue<0.05

Supplemental Figure 4: Validation of associations between clinical variables and MGR-related MGS at baseline:

Heatmap of Spearman pairwise correlation coefficients between MGR-associated-MGS abundance and metabolic variables (body composition and metabolic traits) in Microbaria and Atox cohorts. P-value significance denoted by (*) and FDR significance by (#).

Supplemental Figure 5: Metabolites associated with gene richness

Significant correlations between MGR at baseline and nine serum metabolites (brown RYGB and beige AGB).

Supplemental Figure 6: Target MGS functional module prevalence in relation with the metabolism of target metabolites:

Gene families (KO groups) coding for enzymes involved in the metabolism of target metabolites as obtained from metabolomic data, are projected onto MGR-associated MGS based on reference gene catalog annotations. Greyscale colors represent the number of gene copies for each gene family in each MGS (white = no present genes; max=5 genes (K04486)).

Supplemental Figure 7: Microbiome similarity

A: Similarity (Spearman correlation of the whole 3.9M gene abundance profiles) heatmap with follow-up samples for the three time-points. Left-side colors depict groups (brown

RYGB; beige AGB); top-side colors depict samples from a given patient (4 for each). Ward hierarchical clustering groups samples mostly by patient and by surgery group. **B**: Intra-sample similarity between different time points for each group (brown RYGB; beige AGB); unrelated are depicted in gray and the same in black. BS modifies the gut microbial ecosystem and these alterations occurred all along the follow-up as seen by the trajectories from M1 to M12, which were significantly different between AGB and RYGB ($p<0.013$). The similarity remained lower for RYGB even between M3 and M12, though not significant. The impact of both surgeries on the microbiome is significant (rho=0.5 and rho=0.57 for RYGB and AGB, respectively, as compared with similarity between same sample s (rho=1) and unrelated samples (rho=0.36, $p<6.7e-05$).

Supplemental Figure 8: Significant MGS changes after bariatric surgery

Boxplots of baseline and 5Y abundance of the 11 MGS significantly differing between baseline and M12 in Microbaria RYGB patients in Atox validation cohort (RYGB surgery; 10 patients on follow-up cohort). 6 of the 11 MGS shows significant abundance differences 5 years after bariatric surgery, displaying the same pattern direction as those observed in Microbaria cohort one year after bariatric surgery ($p<0.05$; Non-parametric Wilcoxon tests). The dynamics of the non-significant ones are the same as those observed in Microbaria cohort. Microbaria data at baseline and M12 is included for comparison. Blue for Atox cohort and Yellow and brown for Microbaria.

Supplemental Figure 9: Serum metabolite abundance evolution post-BS

Evolution of the 30 metabolites that changes significantly over time after both surgery (brown RYBG, beige AGB). *Unk_107.0486* (highlighted in blue) is the only one associated with MGR at baseline) that changes significantly from baseline to T12M.

Supplemental Figure 10: Serum metabolites evolution post-BS in validation cohort

Evolution, in EROIC cohort one year post-RYGB, of 18 of the 30 metabolites that changes significantly over time at M12 in Microbaria. These 18 metabolites correspond to annotated compounds for which matching between metabolomics datasets of both cohorts were possible. 16 of the 18 metabolites showed significant changes one year post-RYGB in EROIC cohort (FDR<0.05; Non-parametric Wilcoxon test) independently of diabetic status, following the same dynamics as in Microbaria patients.

Supplemental Figure 11: Metabolite changes in relation with changes in bioclinical parameters and Bariatric surgery induced MGS-significant changes:

A: Heatmap of Spearman pairwise correlation coefficients between changes in metabolites and changes in bio-clinical variables (changes represent delta between T12M and baseline). (*) depicts significant p-value; and (#) significant FDR. **B:** Heatmap of Spearman pairwise correlation coefficients between changes in metabolites and changes in the abundance of the 12-significanty altered MGS post-BS._Delta-change of a variable (metabolites, clinical variables, MGS) is computed as the difference between the value of the variable in baseline and T12M divided by the value of the variable at baseline. (*) depicts significant p-value; and (#) significant FDR

Supplemental Figure 12: Validation of metabolite changes in relation with changes in bioclinical parameters

Heatmap of Spearman pairwise correlation coefficients between delta changes in metabolites and changes in bio-clinical variables (changes represent delta between T12M and baseline) in Microbaria and Eroic cohorts. (*) depicts significant p-value; and (#) significant FDR. (*) depicts significant p-value; and (#) significant FDR.

Supplemental Table 1: Patients' clinical characteristics before the intervention

Baseline description of patients undergoing RYGB or AGB. Significant differences ($p<0.05$) are indicated as well as adjusted p-values for multiple testing (q) as performed with Benjamini Hochberg (BH) method < 5%.

Supplemental Table 2: Clinical characteristics between MGR groups (LGC and HGC).

Top: Baseline characteristics of low (LGC) and high (HGC) MGR patients in the MicroBaria (MB) study. Significant differences ($p<0.05$) are indicated as well as adjusted p-values for multiple testing (q) as performed with Benjamini Hochberg (BH) method < 5%. Bottom: Same analysis restricted to the follow-up cohort at baseline.

Supplemental Table 3: Richness associated MGS in MB at baseline

MGR-associated MGS (as correlated against the continuous MGR and also the MGR groups represented by LGC/HGC). Values for (Spearman rho and rho^2, p and q-values as well as positive or negative relation) are provided. Also, are provided Mann-Whitney p and q-values along with the group in which they are more abundant when comparing the LGC vs. HGC groups. Next, information is given on the phylogenetic annotation of the MGS as described in (5) as well as the number of genes in the MGS and the percentage of non-annotated genes (NA_pc). We also provide information whether these MGS were found to be associated with gene richness in the MetaHIT study (5) as well as significance p-values when associated with clinical phenotypes of interest and between baseline and M12. Red-conditional formatting depicts different levels of significance.

Supplemental Table 4: Richness associated serum metabolites at baseline in the MB cohort.

MGR-associated metabolites (i.e. continuous variable). Values for (Spearman rho and rho^2, p and q-values as well as positive or negative relation) are provided. Next, information is given on the annotations of these metabolites (peak information and annotation on different databases). Also, are provided Mann-Whitney p and q-values along with the group in which they are more abundant when comparing the LGC vs. HGC groups. We also provide significance p-values when associated with clinical phenotypes of interest and between baseline and M12. Red-conditional formatting depicts different levels of significance. Spearman's rho correlation coefficients are colored in red (negative)-blue (positive) color-scale.

Supplemental Table 5: MGR-associated MGS significantly altered after bariatric surgery

Bypass surgery altered a number of MGS summarized in abundance at each time-point (mean +/- standard deviation). Significance is determined using Mann Whitney tests, where p and q-values between baseline and M12 indicate significance before and after adjusting for multiple testing. The time point when they are more abundant is also provided. Next, information is given on the phylogenetic annotation of the MGS as described in (Le Chatelier et al, 2013) as well as the number of genes in the MGS and the percentage of non-annotated genes (NA_pc). We also provide significance p-values when associated with clinical phenotypes of interest. Red-conditional formatting depicts different levels of significance. Spearman's rho correlation coefficients are colored in red (negative)-blue (positive) color scale.

Supplemental Table 6: Serum metabolites (30) that change significantly after bariatric surgery

Bypass surgery altered serum metabolites summarized in abundance at each time-point (mean +/- standard deviation). Significance is determined using Mann Whitney tests, where p and q-values between baseline and M12 indicate significance before and after adjusting for multiple testing. The time point when they are more abundant is also provided. Next, information is given on the annotations of these metabolites (peak information and annotation on different databases). We also provide significance p-values when associated with clinical phenotypes

of interest. Red-conditional formatting depicts different levels of significance. Spearman's rho correlation coefficients are colored in red (negative)-blue (positive) color scale.