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Running head:

Akkermansia muciniphila in severe obesity

ABSTRACT

The gut bacterial species, *Akkermansia muciniphila* is associated with a healthier clinical profile. The purpose of this study was to determine the association between *A. muciniphila* and glucose homeostasis in patients undergoing bariatric surgery (BS): gastric banding (GB) or Roux-en-Y gastric bypass (RYGB). This non-randomized prospective study included 65 women with severe obesity. Longitudinal analysis included subjects for whom *A. muciniphila* data was available at follow up (1, 3, and 12 months; GB (N=10) or RYGB (N=11)). Glucose homeostasis markers were measured under fasting (glucose, insulin, HbA1c) or during an oral glucose tolerance test. Fecal microbiota was analyzed using shotgun metagenomics, and *A. muciniphila* relative abundance was assessed with 16S rRNA qPCR. *A. muciniphila* relative abundance was significantly lower in severe obesity (BMI mean (SD) 45.7 (5.4) kg/m^2) than moderate obesity (33.2 (3.8) kg/m^2) but not associated with glucose homeostasis markers. A significant increase in *A. muciniphila* relative abundance after RYGB was not correlated with metabolic improvement. Baseline *A. muciniphila* was correlated with bacterial gene richness and was highest in the high-richness Ruminococcaceae enterotype. *A. muciniphila* increased in relative abundance after BS in patients with low baseline *A.* muciniphila, especially those with a Bacteroides 2 enterotype classification. Although decreased in severe obesity, relative abundance of *A. muciniphila* was not associated with glucose homeostasis before or after BS. A certain level of *A. muciniphila* abundance might be required to observe a beneficial link to health. The severity of obesity and gut dysbiosis may partly explain the discrepancy with previous findings in less obese populations.

KEY WORDS

Akkermansia muciniphila, bariatric surgery, dysbiosis, gut microbiota, severe obesity.

INTRODUCTION

Gut microbiota composition and function, including diversity, abundance of microbial groups, and microbe-derived metabolites may participate in the development of obesity-associated diseases such as type 2 diabetes (21, 39, 42). *Akkermansia muciniphila* has been associated with a metabolically healthy status (7, 13, 30, 47). In mice, gavage with *A. muciniphila* or intake of a prebiotic that induced increase in *A. muciniphila* abundance led to protection against fat deposition, endotoxemia, glucose intolerance, inflammation of gut and adipose tissue, and led to the maintenance of gastrointestinal integrity in genetic and high fat diet- (HFD) induced obesity (15). A recently identified membrane protein from *A. muciniphila*, Amuc_1100, has been shown to recapitulate the beneficial effects as the single bacterium in mice, showing potential mechanisms through activation of Toll-like receptor 2 (TLR2) pathways and protection of the integrity of the intestinal epithelium (35).

In overweight and moderate obesity, we showed that relative abundance of fecal *A. muciniphila* is associated with glucose homeostasis and smaller adipocyte size (10), and that higher *A. muciniphila* abundance at baseline was predictive of better glucose homeostasis, blood lipids and body composition after calorie restriction. Other human cross-sectional studies have described similar associations, although the results have not always been consistent (20, 36, 51). Age, degree of obesity, and polypharmacy may influence the relationship between *A. muciniphila* and health. A recent study found that mice receiving HFD and metformin improved their metabolic profile concomitantly with an increase in both *A. muciniphila* and mucin-producing goblet cells, similarly to mice treated with *A. muciniphila* alone (40). In humans, metformin increased *A. muciniphila* abundance in patients with type 2 diabetes (48).

Weight loss interventions in obesity lead to clinical improvement and changes in gut microbiota (11). Bariatric surgery (BS) is currently the most effective way to treat severe obesity,

and Roux-en-Y gastric bypass (RYGB) in particular may have additional benefits pertaining to type 2 diabetes remission (3, 12, 22, 24, 37). BS leads to changes in fecal microbiota (1, 32), but it is not fully demonstrated whether these changes impact metabolic outcomes. In rodent studies, *A. muciniphila* abundance increased after bypass, and this was linked to an improved metabolic profile (27). Human studies with low sample size or with a cross-sectional design have also shown a tendency for *A. muciniphila* abundance to increase with BS (18, 50). However, larger longitudinal studies investigating *A. muciniphila* and health after BS are needed.

In this study, we quantified *A. muciniphila* relative abundance before and up to 1 year after two types of BS in relation to clinical outcomes in women with severe obesity. We hypothesized that *A. muciniphila* abundance would increase after surgery and that higher *A. muciniphila* abundance at baseline would be indicative of a healthier metabolic status and predictive of better metabolic outcomes from surgery. To account for the importance of the gut microbiota ecosystem beyond a single isolated species, we considered the metagenome composition (richness and enterotype classification) as well as the functional potential in relation to *A. muciniphila* abundance.

SUBJECTS AND METHODS

Study population

At baseline, 65 adult women (>18 y) with severe obesity were included in this study (60 of them with shotgun metagenomics data from Aron-Wisnewsky *et al* (2)), 21 of whom were followed at 1, 3, and 12 months after bariatric surgery (**Figure 1**) at the Obesity Unit in the Pitié-Salpêtrière Hospital between 2011 and 2014. The 21 patients were selected based on sample availability for the analysis of fecal *A. muciniphila* with quantitative polymerase chain reaction (qPCR), which is the primary outcome of the reported *post hoc* analysis. This was a non-

randomized prospective study where subjects underwent either a RYGB or gastric banding (GB). The intervention decision was made according to the preferences of the patients and a multidisciplinary healthcare panel following international BS guidelines as described in detail in Aron-Wisnewsky *et al* (2).

At each time point, sample collection included: blood samples after a 12-hour fast for clinical profile assessment, anthropometric measures and body composition analysis with dual X-ray absorptiometry, fecal sample collection as described in Thomas *et al* (41) for microbiota analysis with metagenomics sequencing and 16S rRNA qPCR in the case of *A. muciniphila*. Oral glucose tolerance tests (OGTT) were performed at baseline in 22 patients for measurement of glucose and insulin dynamics. OGTT were not performed post-intervention. Exclusion criteria were antibiotic treatment within three months prior to BS, history of chronic or diagnosis of acute gastrointestinal conditions. Procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. This study has been reviewed and approved by Pitié-Salpêtrière Hospital Research Ethics Committee (CPP Ile-de-France, Paris, France). Informed consents were signed by each participant at study enrollment. This study has been registered at clinicaltrials.gov (NCT01454232). The STROBE cohort reporting guidelines have been used in the preparation of this manuscript (46).

Fecal microbiota analysis

A. muciniphila quantification by qPCR with primers targeting 16S ribosomal DNA and normalization to total 16S rRNA was performed as described in Dao *et al* (10). The baseline relative abundance distribution of *A. muciniphila* qPCR in these patients had a bimodal distribution, and so for some of the analysis *A. muciniphila* qPCR relative abundance was categorized around the median into two groups: Low and High *A. muciniphila* (Akk LO and Akk

HI, respectively). The bimodal distribution was confirmed also with shotgun metagenomics (**Supplemental Fig. S1C** https://doi.org/10.6084/m9.figshare.c.4465919.v1). To improve resolution in quantitative metagenomics analyses, sequencing data from the MicroBaria study (2) were mapped over the 9.9 million integrated gene catalog (IGC) (26) following the same methodology as described in Aron-Wisnewsky *et al* (2). We thus computed gene richness and abundance of metagenomic species (MGS). The threshold to define high and low gene richness (high gene count or HGC and low gene count or LGC, respectively) was fixed at 769,240 genes per sample based on a linear regression analyses of gene richness estimated from 9.9 million gene catalog and gene richness from Aron *et al* quantified on the 3.9 million gene catalog (**Supplemental Fig. S2** https://doi.org/10.6084/m9.figshare.c.4465919.v1). Enterotype classification was performed following the Dirichlet Multinomial Mixture (DMM) method of Holmes *et al* (19) using as input the MGS abundance matrix collapsed at the genus level. The DMM approach groups samples if their taxon abundances can be modeled by the same Dirichlet-Multinomial (DM) distribution. Importantly, to gain more relevant resolution in enterotyping, these analyses were carried out over an extended dataset of 428 samples that includes individuals from the MicroBaria study described in detail in (2), additional patients recruited for BS program at the Pitié-Salpêtrière hospital (12), and individuals from the MICRO-Obes study corresponding to less obese and dysbiotic individuals previously described in (8).

For analyses of the functional potential of shotgun metagenomics data, a Kegg Orthology (KO) abundance matrix was computed by adding the abundances of individual genes belonging to the same KO identifier. Spearman correlation analysis between KO abundances and *A. muciniphila* qPCR abundances in the baseline cohort (N=60 individuals with qPCR and shotgun metagenomics data) was followed by P-value adjustment for multiple comparisons with the Benjamini-Hochberg method (4). To analyze the resulting list of KOs in the context of broader

functional groups, the KO adjusted P-values and Spearman rho were analyzed together with the KO module membership (811 reference KEGG modules on November 2018) using the Reporter Feature algorithm as implemented in the Piano R package (45). The null distribution was used as significance method and P-values were adjusted for multiple comparisons with the Benjamini-Hochberg method (4).

Phylogenetic placement of the *Akkermansia* MGS was carried out with the Evolutionary Placement Algorithm implemented in RAxML (5) over a reference phylogenetic tree of 2,977 reference KEGG genomes built from a concatenated alignment of 40 phylogenetic marker genes defined in Mende *et al* (28).

Statistical analysis

SAS (version 9.4, SAS Institute Inc., Cary NC USA) or R (version 3.5.1) software were used for the analysis presented in this manuscript. Non-parametric statistical tests (Wilcoxon, Kruskal-Wallis, or Spearman correlation) were used for comparison between *Akkermansia* abundance, gene richness, or enterotype groups, and to analyze the association between *A. muciniphila* qPCR, *Akkermansia* MGS, KO abundances, enterotypes and clinical parameters. Statistical differences in the proportions of enterotypes between Akk HI and Akk LO individuals were evaluated with Chi-square tests. Mean and standard error or median and interquartile range (IQR) are shown. Linear regression was used to determine the effect of surgery type and baseline *A. muciniphila* qPCR relative abundance on clinical changes over 12 months, adjusting for baseline value of the clinical outcome. Unless correction for multiple testing is indicated, statistical significance was set at alpha=0.05.

RESULTS

Baseline population characteristics

Population characteristics for this study have been recently described (2). There was no difference in most clinical outcomes between the two surgical groups at baseline, except for HbA1c and triglycerides, which were higher in the RYGB group (**Table 1**). The median (interquartile range, IQR) for baseline HbA1c was 6.0 (0.6) % in RYGB and 5.5 (0.5) % in GB. For baseline triglycerides the median (IQR) was 1.7 (1.0) in RYGB and 1.0 (0.7) for GB. Candidates considered for RYGB tended to have more comorbidities than those considered for GB, including type 2 diabetes and glucose intolerance (P=0.08, Fisher's exact test).

Glucose tolerance, insulin sensitivity markers and *A. muciniphila* relative abundance in severe obesity

In these patients with severe obesity, *A. muciniphila* relative abundance at baseline displayed a bimodal distribution (**Figure 2A, Supplemental Fig. S1 https://doi.org/10.6084/m9.figshare.c.4465919.v1**), consistent with previous observations (10). *A. muciniphila* was measured using the same 16S qPCR methodology as in Dao *et al*. There was a significantly lower relative abundance (approximately a 290 median fold difference) of *A. muciniphila* in severe obesity compared to less obese patients (P=0.0038, **Figure 2B** and Dao *et al* (10)).

In the previous study in overweight/moderate obesity, when categorizing *A. muciniphila* around the median, the group with highest abundance was the most metabolically healthy, having higher glucose tolerance and insulin sensitivity. We therefore compared these markers to high or low *A. muciniphila*, based on categorization around the median in the individuals with severe obesity. Contrary to expectations, there was no association between *A. muciniphila* relative abundance and parameters of glucose tolerance and insulin sensitivity, including fasting markers,

OGTT and HOMA indexes (**Figure 2C and Table 2**). On average, there was a lower *A. muciniphila* relative abundance in the group with type 2 diabetes compared to patients with glucose intolerance and normal glucose tolerance, but this difference was not statistically significant (P = 0.13, **Figure 2D**). Patients with type 2 diabetes were receiving a variety of anti-diabetic combination treatments that mostly included metformin: metformin + insulin (N=2), metformin + GLP1 (N=1), insulin + GLP1 (N=1), metformin + insulin + GLP1 (N=2), metformin alone (N=2), and dietary advice alone (N=1). Consequently, it was not possible to ascertain the effect of metformin on *A. muciniphila* abundance *per se* in this group. Thus, in patients with severe obesity (BMI mean (SD) 45.7 (5.4) kg/m^2), *A. muciniphila* relative abundance was lower and not associated with glucose tolerance and insulin sensitivity markers, contrary to what is observed in less severe obesity (33.2 (3.8) kg/m^2) (10). This finding suggests the existence of a minimal threshold of abundance of *A. muciniphila* in order to observe metabolic improvements, to be confirmed in mechanistic studies and interventions.

A. muciniphila relative abundance increases after RYGB but not after GB

Mean *A. muciniphila* relative abundance (qPCR) increased at follow-up for the 21 patients (**Figure 3A**). There was a significant increase in *A. muciniphila* relative abundance as early as 3 months after surgery in the RYGB group (**Figure 3B**), increasing almost 200-fold with respect to baseline after one year, although always remaining lower than subjects with less extreme obesity (10). On the other hand, *A. muciniphila* did not significantly change in the GB group. Patients with low *A. muciniphila* abundance (Akk LO) at baseline, regardless of surgery type, experienced the greatest increase after the intervention (**Figure 3C**). Baseline *A. muciniphila* relative abundance was inversely correlated with increase in its abundance one year after surgery for both surgical groups, and one month after surgery for the GB patients (**Figure 3D**). However, baseline

A. muciniphila relative abundance was not associated with changes in clinical outcomes either when using a categorical (**Table 3**) or continuous version of this variable (data not shown).

Notably, abundance trajectories at the individual level were quite variable in both surgical groups and according to baseline *A. muciniphila* relative abundance (**Supplemental Fig. S3** https://doi.org/10.6084/m9.figshare.c.4465919.v1). Therefore, while there is a surgery-specific effect on *A. muciniphila* abundance, leading to a significant increase after RYGB but not GB, baseline abundance was a contributing factor to *A. muciniphila* dynamics after surgery.

There was no association between changes in *A. muciniphila* and the changes of clinical variables (**Supplemental Fig. S4** https://doi.org/10.6084/m9.figshare.c.4465919.v1). To determine whether *A. muciniphila* would be predictive of better outcomes after surgery, linear regression analysis was performed with models that included baseline *A. muciniphila* category, surgical group and baseline clinical measurement as predictors. This analysis showed that surgery type alone was predictive of better outcomes one year after surgery (**Table 3**). Patients undergoing RYGB had the greatest increase in percentage of fat-free mass, and a decrease in BMI, percentage of fat mass, fasting insulin, total cholesterol and LDL, as expected.

***Akkermansia* genus phylogeny assessed by shotgun metagenomics in relation to *A. muciniphila* qPCR abundance**

To increase the analytical resolution of *A. muciniphila*, we analyzed the *Akkermansia* genus from shotgun metagenomics data. Out of the 1,072 metagenomic species (MGS) identified in the 9.9 million integrated gene catalog IGC (26), there were 4 MGS of a minimum size of 500 genes annotated within the *Akkermansia* genus and a fifth unclassified MGS (CAG00276) that had best sequence similarity with *Akkermansia* MGS from previous gene catalog (**Table 4**). Of those genus-level *Akkermansia* MGS, 3 had more than 2,000 genes, a size similar to the average

genome size of complete *Akkermansia* genomes, pointing to a high level of completion in the MGS composition. There were no significant correlations between the *Akkermansia* MGS and clinical outcomes at baseline (**Supplemental Fig. S5A** https://doi.org/10.6084/m9.figshare.c.4465919.v1), in agreement with data found with the *A. muciniphila* qPCR measurement).

Furthermore, using the in house "Integromics *phylomgs*" pipeline, we created a phylogenetic tree including 2,960 reference prokaryotic genomes built from a concatenated alignment of 40 phylogenetic markers (28) over which we placed all MGS identified in the IGC catalog in order to determine whether additional MGS could be evolutionarily related to *A. muciniphila* (**Supplemental Fig. S5B** https://doi.org/10.6084/m9.figshare.c.4465919.v1). The phylogenetic tree showed that 5 MGS were taxonomically close to *A. muciniphila,* including the 4 MGS previously described based on reference taxonomic annotation and the additional unclassified MGS (CAG00276). This unclassified MGS showed best hit similarity with *Akkermansia* according to its reference annotation but with an average percentage of *Akkermansia* homologous genes (21%) below the threshold for taxonomic assignment based on sequence similarity alone. Also when focusing on the entire Verrucomicrobia phylum, 10 additional MGS are placed evolutionarily close to *Akkermansia* group (**Supplemental Fig. 5B** https://doi.org/10.6084/m9.figshare.c.4465919.v1). Next, we compared the log-transformed abundances of these 5 MGS evolutionarily close to *A. muciniphila* with *A. muciniphila* qPCR log-transformed profile. We observed significant correlations for four of them (CAG00844, CAG00027_1, CAG00095, and CAG00301) whereas CAG00276 was not quantified by shotgun sequencing in any of the samples analyzed (**Figure 4A**), indicating that absence of this genome in our dataset. A stronger association was observed with the cumulative abundance of the 4 MGS (rho = 0.87, P<0.0001, **Figure 4A**). Similarly, when considering only baseline data points, we

also observe a significant correlation between *A. muciniphila* qPCR and *Akkermansia* genus measured with shotgun metagenomics (**Supplemental Fig. S1A** https://doi.org/10.6084/m9.figshare.c.4465919.v1), together with a significant correspondence between undetectable values with shotgun, and low relative abundance with qPCR (**Supplemental Figure S1B** https://doi.org/10.6084/m9.figshare.c.4465919.v1), and a bimodal distribution in the baseline *Akkermansia* genus abundance (**Supplemental Fig. S1C** https://doi.org/10.6084/m9.figshare.c.4465919.v1) similar to that observed with qPCR (**Figure 2A**). The CAG00301 had the strongest correlation with *A. muciniphila* qPCR abundance (**Figure 4A**, rho = 0.75, P<0.0001) and it was the most prevalent MGS.

Of note, we observed discrepancies between *A. muciniphila* qPCR and some of the 4 quantified *Akkermansia* MGS. For example, the CAG00301 was absent in 58 fecal samples on shotgun metagenomics while still showing a wide range of *A. muciniphila* qPCR abundance in the same sample subset. We observed similar outcomes (even though less pronounced) when pooling the abundance of the 4 *Akkermansia* MGS into the *Akkermansia* genus. This observation indicates that the qPCR approach may eventually capture other microbial DNA in some cases, while *Akkermansia* may not be detectable through metagenomics approach in other cases. We may not rule out that the qPCR primers do not include these MGS and therefore underestimate the levels of other potential *A. muciniphila*-related uncultured MGS. Nevertheless, we confirmed a significant increase in richness of *Akkermansia* MGS throughout the intervention in both surgery groups in agreement with the increase observed with the *A. muciniphila* qPCR results (**Figure 4B**).

The gut ecosystem in severe obesity and *A. muciniphila*

Besides the lower abundance of *A. muciniphila* observed in severe obesity, we examined parameters related to the gut microbiota ecosystem (richness and enterotypes), to determine if severe gut dysbiosis could partially explain the lower levels or the absence of an association between *A. muciniphila* and metabolic health in these patients with severe obesity. Of interest, we did not observe a significant association between *A. muciniphila* relative abundance and stool consistency measured with the Bristol Stool Score (BSS, **Supplemental Fig. S6** **https://doi.org/10.6084/m9.figshare.c.4465919.v1**), although there was a tendency for a higher prevalence of Akk LO subjects with the higher BSS level, which is indicative of softer stools. A higher BSS was also previously associated with inflammation and inversely associated with microbial richness in this population (2).

Fecal microbial richness

In our previous study, we reported that 75% of these patients had severe microbiome alterations as displayed by low microbial richness at baseline, with GB patients having slightly higher richness than RYGB (2). There was a significant correlation between baseline richness and *A. muciniphila* qPCR relative abundance (rho = 0.32, P = 0.013, **Figure 5A**). In line with this positive association, Akk HI baseline individuals showed a significantly higher richness than Akk LO individuals (Akk LO vs. HI, P = 0.028, **Figure 5B**). However, contrary to previous findings in less obese individuals showing a healthier clinical phenotype with both high *A. muciniphila* abundance and microbial richness (Akk HI + HGC) (10), we did not find associations between improved metabolic health and the group with combined Akk HI+HGC. (**Supplemental Fig. S7** **https://doi.org/10.6084/m9.figshare.c.4465919.v1**).

Microbial enterotypes

Patients were subsequently characterized according to microbial enterotype, as described in Holmes *et al* (19). *A. muciniphila* relative abundance was significantly increased in the

Ruminococcaceae enterotype in comparison with other enterotypes in the baseline assessment (**Figure 6A** for qPCR, and data not shown for MGS), in line with its higher richness profile (**Figure 6B**). Categorical analysis of baseline *A. muciniphila* abundance (Akk LO and HI) showed no significantly different distribution of microbial enterotypes (Chi Square test, P = 0.13), although Akk HI had a higher prevalence of the Ruminococcaceae enterotype (absent in Akk LO) and Akk LO had a higher prevalence of low-richness Bacteroides B2 enterotype (**Figure 6C**). At baseline, B2 patients (N=15 out of 61) had a worse clinical profile than non-B2 patients; namely they had a significantly higher Glucose AUC (P = 0.04), higher HbA1c (0.05), and a type 2 diabetes prevalence of 47% compared to 3.8% in non-B2 patients (P = 0.011, **Table 5**).

When studying enterotype composition over time according to baseline categorical classification of *A. muciniphila* abundance (Akk LO / HI) in the 21 individuals for whom data were available at all time points, we observed no significant changes in enterotype composition across time, even if the B2 enterotype increased in prevalence in both Akk LO and HI one month after BS, and decreased at subsequent time points (**Figure 7A**). The Ruminococcaceae enterotype appeared at months 1 and 3, but it was no longer present after 1 year in the 21 patients. When analyzing the evolution of *A. muciniphila* relative abundance after BS according to baseline enterotype classification, its abundance increased significantly for individuals characterized as having Akk LO and B2 at baseline (**Figure 7B**). On the other hand, *A. muciniphila* did not change significantly for individuals with Akk HI at baseline, regardless of baseline enterotype classification. Therefore, it appears that *A. muciniphila* increased after BS for patients with greater gut dysbiosis at baseline.

Functional profile of the metagenome associated with *A. muciniphila* abundance

We examined the functional potential differences between Akk HI and LO individuals at baseline using the approach by Väremo *et al* (45). Higher *A. muciniphila* qPCR relative abundance was associated with a decrease of functional modules associated to the biosynthesis of bacterial lipopolysaccharide (LPS, M00063, M00060), branched-chain amino acids (BCAA, M00019, M00570, M00432, M00535) and metabolic pathways including glycolysis and the tricarboxylic acid cycle (**Figure 8**). Increases in bacterial LPS have been associated to systemic low-grade inflammation in the context metabolic disorders (23), whereas increases in BCAA levels have been proposed as a metabolic signature that differentiates obese and lean humans, and that contributes to insulin resistance (29, 34, 43). Therefore, these inverse associations are in line with the previous associations between *A. muciniphila* abundance and a healthy status.

A. muciniphila relative abundance was also positively associated with a multidrug resistance module (M00643), Microcin C transport system (M00349), vancomycin resistance (M00651) and O-glycan biosynthesis (M00056) (**Figure 8**). From these results, it appears that higher *A. muciniphila* abundance is inversely related with high metabolic output from the gut microbiome, as well as an inflammatory and metabolically unhealthy functional profile (low LPS, BCAA). At the same time, it is positively related with multidrug resistance pathways.

The Amuc_1100 protein is a critical mediator of the biological effect of *A. muciniphila* on metabolic status (35). We therefore conducted a search for Amuc_1100 homologs in the 10 million gene catalog from IGC. There were eight genes identified by BLASTN using the Amuc_1100 gene as a reference (**Supplemental Fig. S8A,** **https://doi.org/10.6084/m9.figshare.c.4465919.v1**). These genes had at least 80% identity with Amuc_1100, and four of them were associated to *A. muciniphila* MGS including two genes (gene IDs 2500343 and 6515199) belonging to CAG00301, which is the most prevalent *Akkermansia* MGS in this study's subjects (**Figure 4A**). These two genes had the strongest identity with the

reference Amuc_1100 (>97% at the nucleotide level), with gene 2500343 being full-length gene and 6515199 being a fragmented gene (partial homology with the 3' region of the reference gene). Both genes had a positive association with *A. muciniphila* qPCR (**Supplemental Fig. S8B** https://doi.org/10.6084/m9.figshare.c.4465919.v1), which was also observed with *Akkermansia* MGS (**Supplemental Fig. S1** https://doi.org/10.6084/m9.figshare.c.4465919.v1), being stronger for the full-length gene (gene ID 2500343). In addition, there was a bimodal distribution of the abundance of the Amuc_1100 homologs in the baseline samples (**Supplemental Fig. S8C** https://doi.org/10.6084/m9.figshare.c.4465919.v1), as observed with *Akkermansia* relative abundance assessed with both methodologies.

DISCUSSION

In this study, we have analyzed the relationship between fecal *A. muciniphila* relative abundance and clinical status in adult women with severe obesity before as well as 1, 3 and 12 months after two types of BS, i.e. RYGB or GB. We found that the relative abundance of fecal *A. muciniphila* is significantly lower in severe obesity compared to moderate obesity. In addition, although we have found that *A. muciniphila* increased significantly after RYGB, there was no association between its baseline abundance nor with its increase after surgery and clinical improvements induced by BS. To date, this study has the largest sample size and follow-up period showing *A. muciniphila* kinetics, and including a thorough analysis of the fecal microbiota composition.

A. muciniphila has been repeatedly associated with a healthier metabolic outcome by our groups and others (10, 14, 15). The mechanisms underlying potential benefits of this bacterium have not been fully identified, although it is possible that *A. muciniphila* may be modulating the host immune system and activating TLR2 to preserve gut barrier integrity (7, 31, 35, 38) through

its membrane protein Amuc_1100. Assessing the abundance of the Amuc_1100 gene by shotgun sequencing confirmed observations about *Akkermansia* made with qPCR and MGS abundance data. However, these observations indicate abundance and not function, which should be measured in future studies. We have previously shown that a higher baseline *A. muciniphila* was predictive of better metabolic outcomes after a dietary weight loss intervention in adults with overweight and obesity. An increase in *A. muciniphila* after RYGB has previously been observed in mice (27) and humans (17, 49). However, the human studies were either cross-sectional or were conducted in small sample sizes and had shorter follow up periods. Metformin treatment has been shown to have an impact on gut microbiome composition (9, 16), leading to the increase of *A. muciniphila* abundance in mice and humans (9, 40). This evidence suggests that the mechanism of action of metformin to induce an antidiabetic effect may at least in part be occurring through this bacterium. However, a study dissecting this potential link did not confirm such direct relationship between *Akkermansia* and metformin (48).

Actually, in our study, only individuals with lower *A. muciniphila* abundance at baseline experienced a significant increase after the bariatric interventions, and this was independent of individual microbial community (i.e. enterotype classification), surgery type, or medication use (metformin was discontinued after surgery for most patients due to diabetes resolution). However, neither *A. muciniphila* assessed by qPCR or by metagenomics was significantly associated with metabolic outcomes before or throughout the intervention period. Importantly, we recently demonstrated that although BS increases gene richness significantly one year post-BS, the surgery was not able to fully restore gut microbiota dysbiosis in individuals with severe obesity (2). Thus, it may not be possible to fully rescue an appropriate level of *Akkermansia* and/or the relationship between the gut microbiome and health outcomes merely through significant weight loss. Dietary intake is known to be associated with changes in gut microbial

composition (33). In this study it was not possible to differentiate the effect of surgery from dietary changes on gut microbial composition. This is a crucial question that should be addressed in future research. Furthermore, our findings call for the study of potential interventions, prior or concomitant to BS, to rescue gut dysbiosis, possibly through prebiotic or probiotic treatment, specialized diets, gut microbiota transfer or even combinations thereof in severe obesity. In fact, *A. muciniphila*, is currently being considered as a next generation probiotic, and its potential metabolic effect needs to be investigated in link with the individual gut microbiota ecosystem (6, 7). Exploring the existence of a link between *A. muciniphila* supplementation on metabolic health and its effect on the gut ecosystem will provide valuable information on the conditions needed for there to be a positive relationship between this bacterium and metabolic health.

There were two distinct MGS within the *Akkermansia* genus that correlated with *A. muciniphila* abundance as measured by qPCR. This indicates that several different species tentatively are being detected through the qPCR approach. While this observation does not impact the utility of *A. muciniphila* measured by qPCR as a biomarker of metabolic health, which has been repeatedly supported thus far, further investigation into the different species within the *Akkermansia* genus is warranted to evaluate and compare their relative metabolic capacities, as well as interaction with the host metabolism.

We studied the fecal microbial ecosystem composition and functional potential in relation to *A. muciniphila* relative abundance. Gut microbiota dysbiosis has been associated to an array of clinical complications, including obesity (8, 25). Dysbiosis may be partially rescued with dietary weight loss interventions, but in severe obesity BS may not be sufficient (2). Our group recently showed that microbial richness increases significantly up to one year and remains stable thereafter when followed 5 years after BS. While there was a significant correlation between *A. muciniphila* abundance and microbial richness at baseline, contrary to findings in Dao *et al* (10),

individuals with severe obesity and with a relatively higher *A. muciniphila* abundance and microbial richness did not have a healthier clinical profile in terms of fasting blood and lipid values and body composition. This can be explained either by the lower abundance of *A. muciniphila* measured in these patients with severe obesity as compared to subjects with overweight and obesity (10), or through the observed dysbiosis. Moreover, the co-abundance of other species may play a role in host health as we previously discovered that *A. muciniphila* correlates with 26 other metagenomic species (10). Thus, it remains to be explored whether emphasis should be made on the abundance of a specific consortium of bacteria and/or their produced metabolites.

Along these lines, there was a lower prevalence of the Bacteroides B2 enterotype in Akk HI. This enterotype was previously associated with lower microbial richness (2), low microbial cell density, and higher prevalence of several pathologies (44). In line also with the results of Vandeputte *et al* (44), the Bacteroides B2 enterotype was more prevalent in RYGB patients at baseline, who had a worse clinical profile compared with GB. We observed no significant association between baseline continuous or categorical *A. muciniphila* relative abundance and BSS, but we observed a tendency towards softer stools associated to Akk LO (**Supplemental Fig. S6 https://doi.org/10.6084/m9.figshare.c.4465919.v1**), which is in line with the higher prevalence of B2 enterotype in Akk LO. Furthermore, functional potential analysis revealed an inverse association between *A. muciniphila* and a wide array of biosynthetic and metabolic pathways. This is consistent with other findings in this study suggesting a dysbiotic ecosystem concurrent with low *A. muciniphila* abundance.

It is important to note that we observed an increase in the prevalence of Bacteroides B2 with respect to the results of Aron-Wisnewsky *et al* (2). This discrepancy between the two studies of the same population is explained by the use of a larger cohort for the enterotyping analyses in

the present study. The larger cohort includes less dysbiotic patients represented by samples of the MICRO-Obes study (8).

A limitation to this study is the relatively small sample size in the follow-up population (N=21 versus 65 at baseline), although it is the largest sample size to date for the assessment of *Akkermansia* short and long-term changes after BS. No other study has collected the comprehensive amount of information presented herein on fecal microbiota composition, functional potential, and patient clinical profiles. Comparison with a weight-stable, severely obese group is warranted in future studies to assess naturally occurring fluctuations of *Akkermansia* abundance, especially in individuals characterized by a low abundance of this bacterium. Larger studies are also warranted to explore the generalizability of these results.

While *A. muciniphila* is considered as a marker and perhaps even to play a role in the maintenance of metabolic health in humans, its association with a healthy status disappears in severe obesity. Although its relative abundance increases after surgery, particularly in patients with greater dysbiosis at baseline, the relationship between this bacterial species and health outcomes was not restored, suggesting that a certain threshold of *Akkermansia* may exist and that further research is needed to understand how a symbiotic gut ecosystem can be refurbished in this population. This remains a hypothetical notion, however, as only relative abundance has been measured in the present study. The lack of association between a post-surgical improvement in metabolic health and increase in *A. muciniphila* abundance could indicate that fecal abundance of *A. muciniphila* does not reflect mucosal abundance in the context of a bariatric intervention. Furthermore, other glucose homeostasis markers not explored here may have a stronger association with this bacterium. Furthermore, other bacterial groups and metabolic potential may be needed in addition to *A. muciniphila* for there to be a relationship between microbial composition and health outcomes. There is a need to explore these alternatives and to investigate

the impact of the administration of *A. muciniphila* in humans to study the direct effect of this bacterium on metabolic health.

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DATA AVAILABILITY

Shotgun metagenomics data described in the manuscript are publically and freely available without restriction at https://www.ebi.ac.uk/ena/data/view/PRJEB23292

Clinical data described in the manuscript will be made available upon request from the corresponding author.

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CONFLICT OF INTEREST

AE and PDC are inventors of patent applications dealing with the use of *A. muciniphila* and its components in the context of obesity and related disorders. PDC is co-founders of A-Mansia Biotech SA. MCD, EB, EP, BK, JLB, JMC, NP, ELC, DE, JD, JAW, JDZ, KC: no conflicts of interest.

AUTHOR CONTRIBUTIONS

MCD, EB, EP, JAW and KC conceptualized the clinical study and analytical approach, MCD and KC drafted the manuscript and have primary responsibility for the final content of the manuscript; MCD, EB, EP, JAW, BK, JDZ: analyzed data, conducted statistical tests, and drafted tables and figures; AE and PC conducted the *A. muciniphila* qPCR analysis and contributed to the study rationale and interpretation of results; JLB and JMC performed the bariatric surgeries; NP, ELC, FL, SDE produced metagenomics data and determined microbiome composition;. All authors: read and approved the final manuscript.

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774 **Table 1. Baseline population characteristics by surgical group.**

BASELINE	Baseline group		GB		RYGB	
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)
Sex, (% Female)	65	100	10	100	11	100
Age (y)	65	37 (16)	10	38 (13)	11	40 (9)
BMI (kg/m^2)	65	44.1 (6.2)	10	43.2 (2.1)	11	46.3 (12.9)
Fat mass (%)	62	50.5 (5.1)	10	50.2 (6.3)	11	51.3 (8.1)
Fat free mass (%)	62	47.2 (4.8)	10	47.2 (6.2)	11	46.7 (7.7)
Android-to-gynoid fat mass ratio	62	1.6 (0.5)	10	1.5 (0.3)	11	1.8 (0.6)
Fasting glucose (mM)	65	5.1 (1.0)	10	5.2 (0.9)	11	5.4 (2.2)
Fasting insulin (mUI/L)	64	17.8 (12.5)	10	18.4 (15.6)	10	18.1 (13.7)
HOMA2-IR	64	2.7 (1.7)	10	2.7 (2.1)	10	2.7 (1.8)
HbA1c (%)	65	5.8 (0.5)	10	5.5 (0.5)	11	6.0 (0.6)*
T2D (N/total) Glucose Intolerance (N/total)	9 / 65 30 / 65		0 / 10 4 / 10		3 / 11 6 / 11	
Total cholesterol (mM)	65	4.6 (1.4)	10	4.5 (0.9)	11	5.7 (2.0)
LDL (mM)	65	2.8 (1.0)	10	3.0 (0.8)	11	3.2 (1.9)
HDL (mM)	65	1.1 (0.4)	10	1.1 (0.4)	11	1.1 (0.8)
Triglycerides (mM)	65	1.1 (0.6)	10	1.0 (0.7)		1.7 (1.0)*
Dyslipidemia (N/total)	53 / 65		8 / 10		11 / 11	
CRP (mg/L)	61	7.3 (6.0)	10	6.0 (7.8)	9	9.0 (4.9)
IL-6 (pg/ml)	62	3.9 (3.3)	8	2.5 (3.6)	11	4.3 (2.5)

775 *A. muciniphila data* were available at all follow up points for 10 subjects in GB and 11 subjects
776 in RYGB. *P<0.05 Wilcoxon test. CRP = C-reactive protein; HbA1c = hemoglobin A1c; HDL =
777 high density lipoprotein; HOMA2-IR = homeostasis model assessment of insulin resistance; IL-6
778 = interleukin 6. IQR = Interquartile range; LDL = low density lipoprotein; T2D = type 2 diabetes;

779 **Table 2. Baseline glucose homeostasis markers by A. muciniphila category (Akk LO/HI) in**

780 **severe obesity**

BASELINE	Akk LO		Akk HI	
	N	Median (IQR)	N	Median (IQR)
Glucose (mM)	32	5.1 (0.9)	33	5.0 (1.0)
Insulin (μIU/mL)	32	17.2 (14.7)	32	18.5 (9.8)
HOMA2-IR	32	2.6 (2.0)	32	2.7 (1.6)
HOMA2-B (%)	32	186.0 (101.2)	32	184.6 (112.4)
HbA1c (%)	32	5.8 (0.7)	33	5.7 (0.5)

781 No significant differences, Wilcoxon test. HbA1c = hemoglobin A1c; HOMA-%B = homeostasis

782 model assessment of beta cell function; HOMA2-IR = homeostasis model assessment of insulin

783 resistance.

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Table 3. Baseline *A. muciniphila* was not predictive of improvements after surgery and RYGB led to greater improvements in clinical outcomes than GB.

	Akk LO (N=9)		Akk HI (N=12)			GB (N=10)		RYGB (N=11)		
	BS	M12	BS	M12	$P_{Akk\ effect}$	BS	M12	BS	M12	$P_{surgery\ effect}$
BMI	42.6 (1.7)	32.6 (2.1)	46.3 (1.5)	34.8 (1.5)	NS	43.0 (0.7)	35.7 (0.7)	46.2 (2.1)	32.2 (2.2)	***
Fat mass (%)	50.3 (1.5)	43.1 (2.1)	48.9 (1.2)	41.5 (1.3)	NS	49.1 (1.3)	44.7 (1.2)	49.9 (1.4)	39.9 (1.7)	***
Fat free mass (%)	47.4 (1.4)	54.0 (2.0)	48.8 (1.2)	55.6 (1.2)	NS	48.5 (1.2)	52.4 (1.1)	47.9 (1.3)	57.1 (1.5)	***
Total cholesterol (mM)	5.1 (0.5)	4.7 (0.2)	4.8 (0.2)	4.4 (0.2)	NS	4.6 (0.2)	4.7 (0.2)	5.3 (0.4)	4.4 (0.2)	*
Triglycerides (mM)	1.3 (0.2)	0.9 (0.2)	1.4 (0.2)	0.9 (0.1)	NS	1.0 (0.1)	0.8 (0.1)	1.6 (0.2)	1.0 (0.1)	NS
HDL (mM)	1.2 (0.1)	1.6 (0.1)	1.1 (0.1)	1.3 (0.1)	NS	1.1 (0.1)	1.4 (0.1)	1.2 (0.2)	1.5 (0.1)	NS
LDL (mM)	3.3 (0.4)	2.7 (0.2)	3.1 (0.2)	2.7 (0.2)	NS	3.0 (0.2)	3.0 (0.2)	3.3 (0.3)	2.4 (0.2)	**
Glucose (mM)	6.2 (0.7)	4.8 (0.4)	5.4 (0.2)	4.6 (0.2)	NS	5.3 (0.2)	4.7 (0.1)	6.1 (0.6)	4.6 (0.3)	NS
Insulin (μIU/mL)	22.2 (3.6)	9.5 (1.5)	26.9 (7.7)	9.0 (1.4)	NS	27.8 (8.5)	11.8 (1.1)	21.7 (3.1)	6.7 (1.1)	*
HOMA2-IR	3.3 (0.5)	0.7 (0.1)	3.8 (1.0)	0.7 (0.0)	NS	3.9 (1.1)	0.7 (0.02)	3.2 (0.4)	0.7 (0.1)	NS
HbA1c (%)	6.2 (0.4)	5.6 (0.3)	5.8 (0.1)	5.4 (0.1)	NS	5.6 (0.1)	5.2 (0.1)	6.3 (0.3)	5.8 (0.2)	NS

Linear regression for surgery type and baseline *A. muciniphila* effect on change in clinical outcome (M12-BS) adjusting for baseline value for effect. *p≤0.05, **p≤0.01, ***p≤0.001 for surgery effect. There was no significant effect of baseline *A. muciniphila.* Mean (SE). BS = Baseline; HDL = High density lipoprotein; LDL = Low density lipoprotein; HbA1c = hemoglobin A1c; HOMA2-%B = homeostasis model assessment of beta cell function; HOMA2-IR = homeostasis model assessment of insulin resistance; NS = not significant.

Table 4. MGS annotated to the *Akkermansia* genus from the 9.9 million gene catalog based on reference annotation and phylogenetic placement

	MGS size	BHit	BHit_pc	Species	Genus	Family	Order	Class	Phylum	Super kingdom
CAG00301	3187	*Akkermansia muciniphila ATCC BAA-835*	99.7	*Akkermansia muciniphila*	*Akkermansia*	Verrucomicrobiaceae	Verrucomicrobiales	Verrucomicrobiae	Verrucomicrobia	Bacteria
CAG00095	2923	*Akkermansia sp. CAG:344*	99.9	*Akkermansia sp. CAG:344*	*Akkermansia*	Verrucomicrobiaceae	Verrucomicrobiales	Verrucomicrobiae	Verrucomicrobia	Bacteria
CAG00027_1	2712	*Akkermansia sp. CAG:344*	70.1	*unclassified Akkermansia*	*Akkermansia*	Verrucomicrobiaceae	Verrucomicrobiales	Verrucomicrobiae	Verrucomicrobia	Bacteria
CAG00276	2206	*Akkermansia sp. CAG:344*	25.7	*unclassified*	*unclassified*	unclassified	Unclassified	unclassified	unclassified	unclassified
CAG00844	1432	*Akkermansia sp. CAG:344*	61.5	*unclassified Akkermansia*	*Akkermansia*	Verrucomicrobiaceae	Verrucomicrobiales	Verrucomicrobiae	Verrucomicrobia	Bacteria

Size=Number of genes; Bhit= Best hit on sequence similarity (nucleotides); Bhit_pc= Percentage of identity; CAG = coabundance group; MGS = metagenomic species.

806 **Table 5. Clinical profile of patients with enterotype B2 vs. patients with other enterotypes at**
807 **baseline.**

Variable	B2 enterotype (N=15)	Other enterotypes (N=46)	P value	Q value
Age (y)	39.9 (2.95)	36.8 (2.08)	0.4	0.73
BMI (kg/m^2)	47.1 (1.8)	46.3 (0.999)	0.69	0.98
Fat mass (kg)	63.8 (3.0)	60.9 (2.1)	0.42	0.73
Fat free mass (kg)	59.0 (1.8)	58.6 (1.39)	0.86	1
Trunk fat mass (kg)	31.3 (1.51)	29.2 (0.9)	0.25	0.7
% Gynoid fat	37.0 (1.7)	37.6 (1.14)	0.78	1
HbA1c (%)	6.5 (0.3)	5.7 (0.1)	**0.05**	0.19
OGTT AUC Glucose	938 (53)	803 (32)	**0.04**	0.19
Glucose intolerance (%)	33.3	53.9	1	1
Diabetes (%)	47.0	3.8	**0.01**	0.11
Dyslipidemia (%)	87.0	88.0	1	1

808 Mean (SE), Wilcoxon test (for continuous variables) or Chi square tests (for categorical
809 variables) are shown. B2 = Bacteroides 2; HbA1c = hemoglobin A1c; OGTT AUC = Oral
810 glucose tolerance test area under the curve. The Q value column represents P-values adjusted for
811 multiple testing by the Benjamini-Hochberg method (4).

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Figure 1. Study flow chart. RYGB = Roux-en-Y gastric bypass; GB = gastric banding.

Figure 2. *A. muciniphila* baseline relative abundance and association with glucose tolerance markers in severe obesity. A) *A. muciniphila* abundance measured with 16S qPCR. The dotted line represents median *A. muciniphila* abundance, dividing the subjects into Akk LO (N=32) and Akk HI (N=33) groups. **B)** Comparison of *A. muciniphila* abundance between two studies: MICRO-Obes (including 49 adults with overweight and obesity, Dao *et al*(10)) and MicroBaria (including 65 women with severe obesity, present study). **C)** Baseline comparison in glucose tolerance by OGTT (N=22) in patients with low and high *A. muciniphila* abundance (Akk LO N=12/HI N=10). No significant differences were observed between groups. **D)** Difference in *A. muciniphila* abundance according to type 2 diabetes status. No significant differences were found between groups (Kruskal-Wallis test). In the group with type 2 diabetes (N=9), patients were undergoing the following treatments: control through diet (N=1), metformin only (N=2), metformin + insulin (N=2), metformin + GLP1 (N=1), metformin + GLP1 + insulin (N=2), insulin + GLP1 (N=1). N = normal glucose tolerance; GI = glucose intolerance; T2D = type 2 diabetes.

Figure 3. *A. muciniphila* change in relative abundance after bariatric surgery. A) Change in *A. muciniphila* relative abundance 1, 3 and 12 months after bariatric surgery (N=21). Categorization by **B)** surgery group (N=10 for GB and N=11 for RYGB) and **C)** baseline *A. muciniphila* relative abundance around the median (N=9 for Akk LO and N=12 for Akk HI). Signed Rank test: * $P \leq 0.05$; ** $P \leq 0.01$ for within surgery group change. Wilcoxon: $^{\&}$ $P \leq 0.05$ for comparison between surgery groups, # P=0.0001 for comparison between baseline Akk

838 categories. **D)** Scatter plot and Spearman correlation between baseline *A. muciniphila* relative
839 abundance and its change 1, 3 and 12 months after bariatric surgery.

840 **Figure 4. Abundance of MGS from the *Akkermansia* genus and correlation with *A.***
841 ***muciniphila* abundance qPCR results. A)** Spearman correlation between *A. muciniphila* qPCR
842 abundance and individual *Akkermansia* MGS abundances, as well as *Akkermansia* genus
843 abundance (sum of abundances of *Akkermansia* MGSs) in all studied samples with shotgun and
844 qPCR data (N=166). **B)** *Akkermansia* MGS richness in fecal samples (number of *Akkermansia*
845 genus MGS detected per sample) of patients at baseline and at follow up (GB=10 patients, 40
846 samples; RYGB=14 patients, 56 samples). * P<0.05 in Wilcoxon tests of *Akkermansia* MGS
847 richness between baseline and 1 year after bariatric surgery.

848

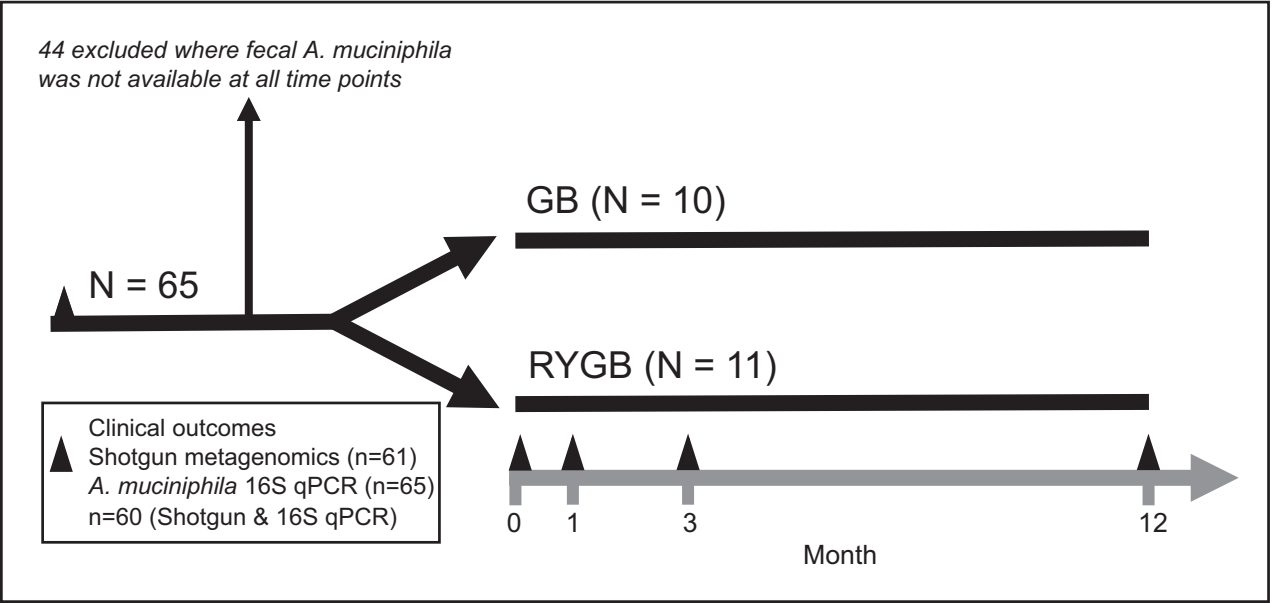
849 **Figure 5. Baseline association between *A. muciniphila* relative abundance and gene richness**
850 **and comparison of clinical outcomes. A)** Spearman correlation analysis showing significant
851 correlation between baseline *A. muciniphila* and metagenomic richness (N=60 patients for whom
852 both richness and *A. muciniphila* abundance data were available). **B)** Baseline metagenomic
853 richness according to baseline *A. muciniphila* category (Akk LO N=32 / HI N=28), Wilcoxon
854 test.

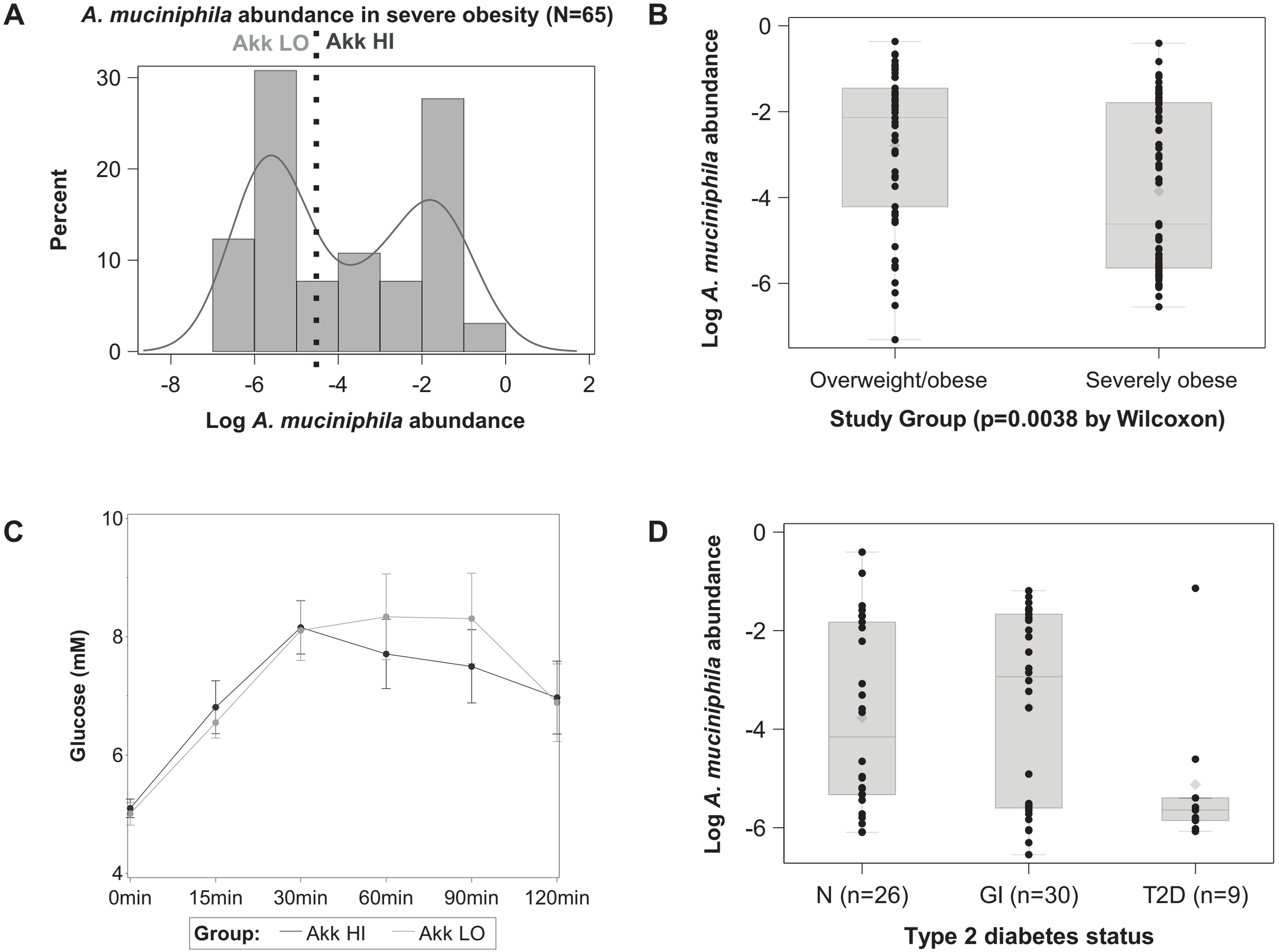
855

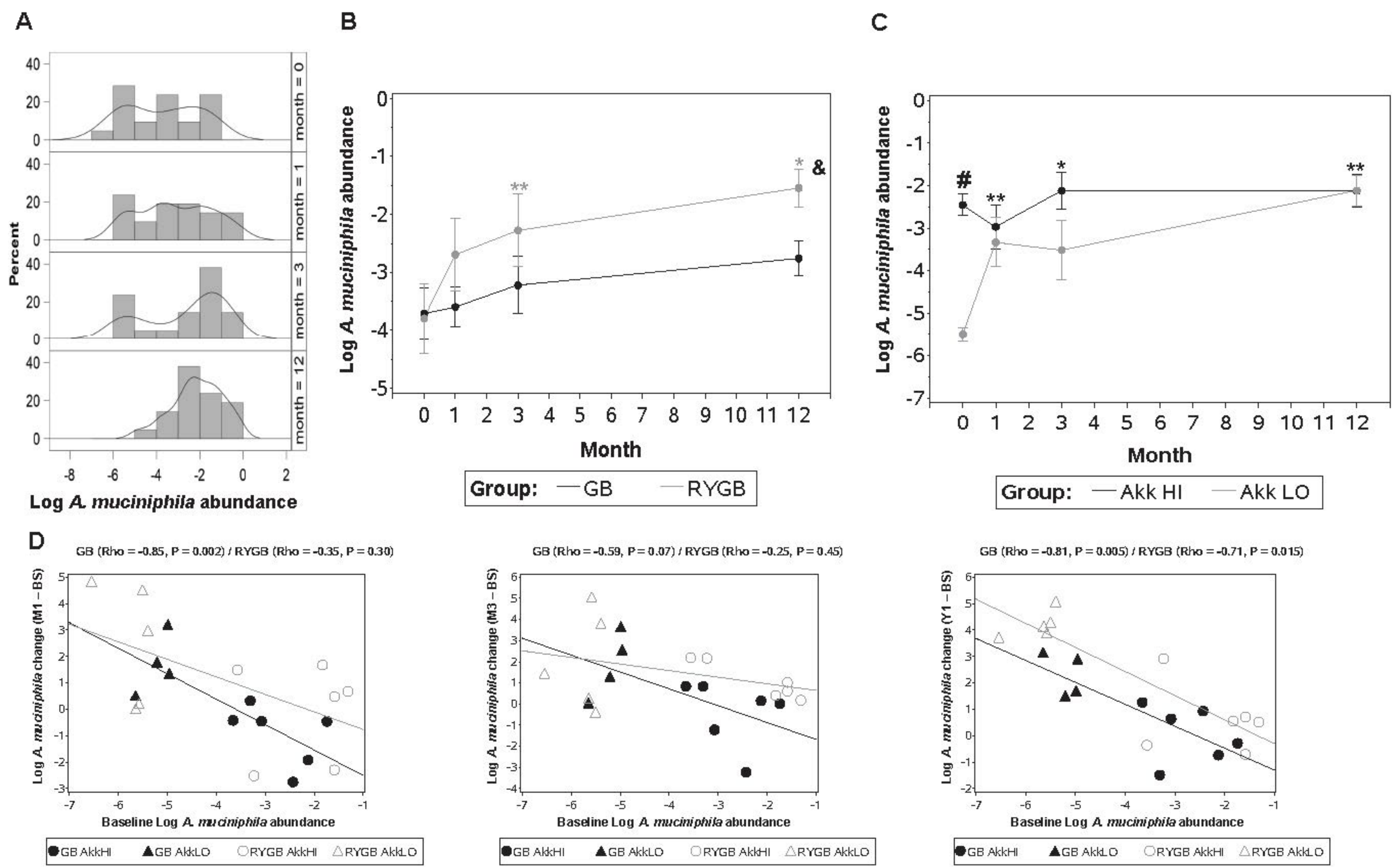
856 **Figure 6. Relationship between baseline *A. muciniphila* relative abundance and microbial**
857 **enterotype. A)** *A. muciniphila* abundance according to enterotype composition in baseline
858 samples with *A. muciniphila* qPCR and metagenomics data (N=60) and **B)** Metagenomic gene
859 richness according to enterotype composition in baseline cohort (n=61). **C)** Enterotype
860 composition according to baseline *A. muciniphila* relative abundance (N=60). Symbols in
861 boxplots represents * P<0.05, ** P < 0.01, *** P<0.001 and **** P<0.0001 in Wilcoxon tests.

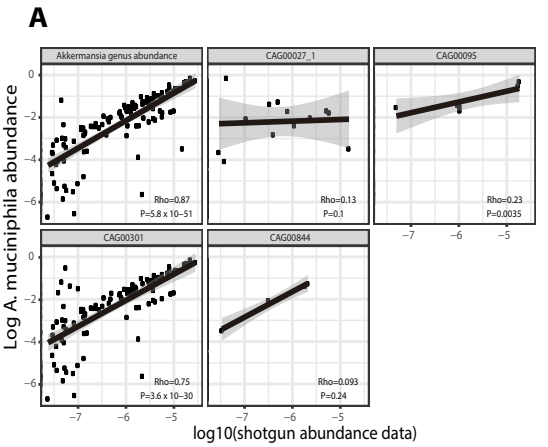
Figure 7. Change in *A. muciniphila* relative abundance over time according to microbial enterotype in 21 patients of follow-up cohort. A) Enterotype classification throughout the intervention according to baseline *A. muciniphila* relative abundance category. **B)** Change in *A. muciniphila* relative abundance (shown in log-10 scale) according to baseline enterotype and *A. muciniphila* classification. Dashed lines connect samples from the same patient at different time points. * P<0.05 in pairwise Wilcoxon tests.

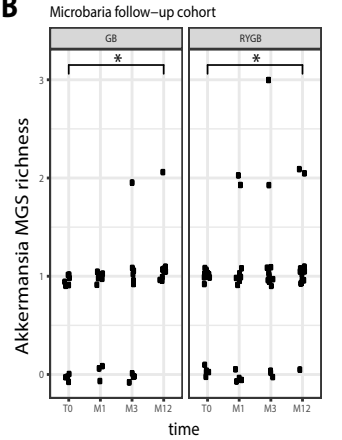
Figure 8. Functional modules associated with baseline *A. muciniphila* relative abundance. KEGG modules significantly enriched in KO groups with significant associations with *A. muciniphila* qPCR abundances from gene set enrichment analyses (FDR <0.05, N=60 individuals with qPCR and shotgun metagenomics data). The weight of the enrichment of KO module members according to *A. muciniphila* qPCR baseline abundances is represented in the x-axis by distinct directional statistic from the Reporter Features algorithm implemented in the Piano R package (45) (black bars = KO abundance modules positively associated with *A. muciniphila* qPCR abundance; gray bars = KO abundance modules negatively associated with *A. muciniphila* qPCR abundance).

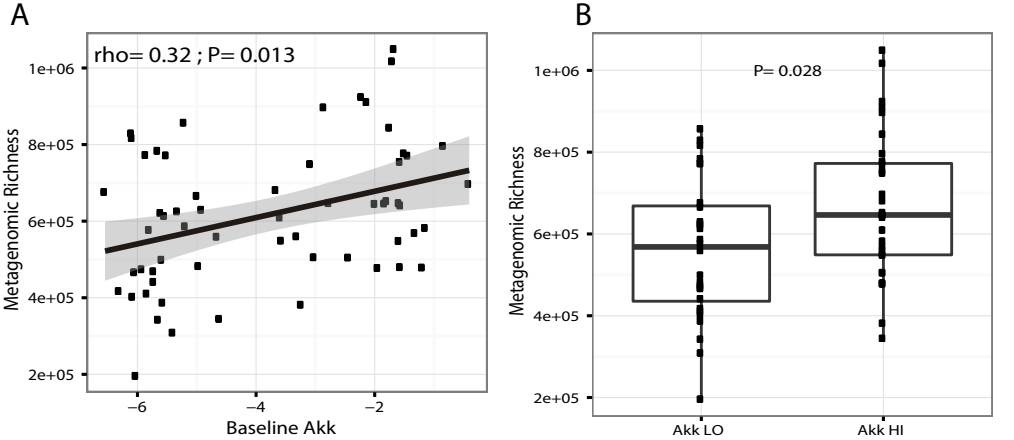

44 excluded where fecal A. muciniphila was not available at all time points
N = 65
GB (N = 10)
RYGB (N = 11)
Clinical outcomes
Shotgun metagenomics (n=61)
A. muciniphila 16S qPCR (n=65)
n=60 (Shotgun & 16S qPCR)
0
1
3
12
Month

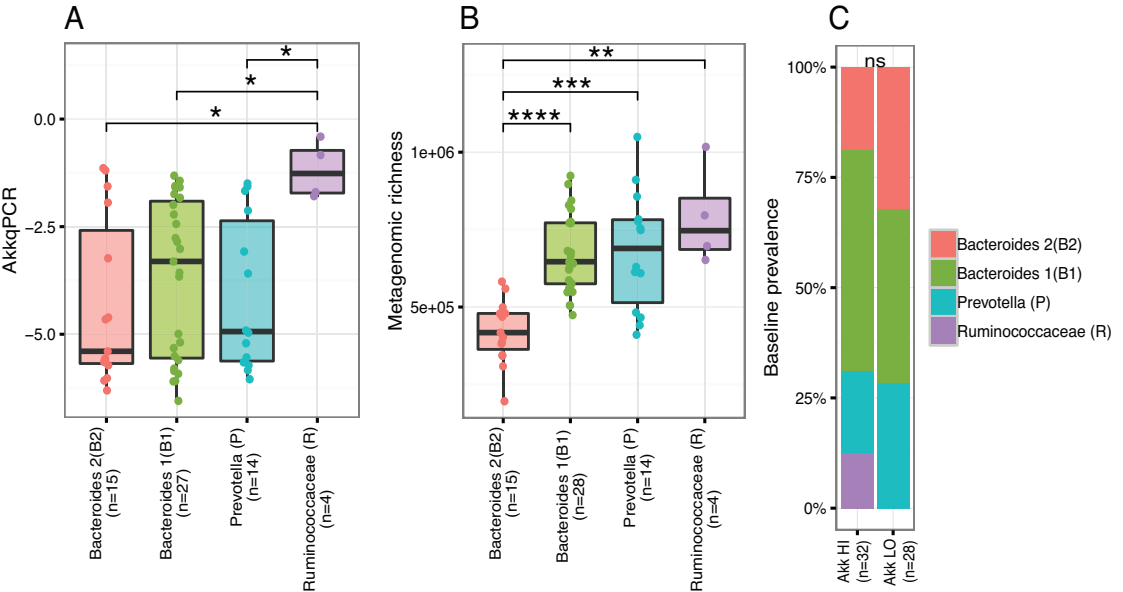

A
A. muciniphila abundance in severe obesity (N=65)
Akk LO
Akk HI
Percent
30
20
10
0
-8
-6
-4
-2
0
2
Log A. muciniphila abundance
B
Log A. muciniphila abundance
0
-2
-4
-6
Overweight/obese
Severely obese
Study Group (p=0.0038 by Wilcoxon)
C
Glucose (mM)
10
8
6
4
0min
15min
30min
60min
90min
120min
Group:
Akk HI
Akk LO
D
Log A. muciniphila abundance
0
-2
-4
-6
N (n=26)
GI (n=30)
T2D (n=9)
Type 2 diabetes status

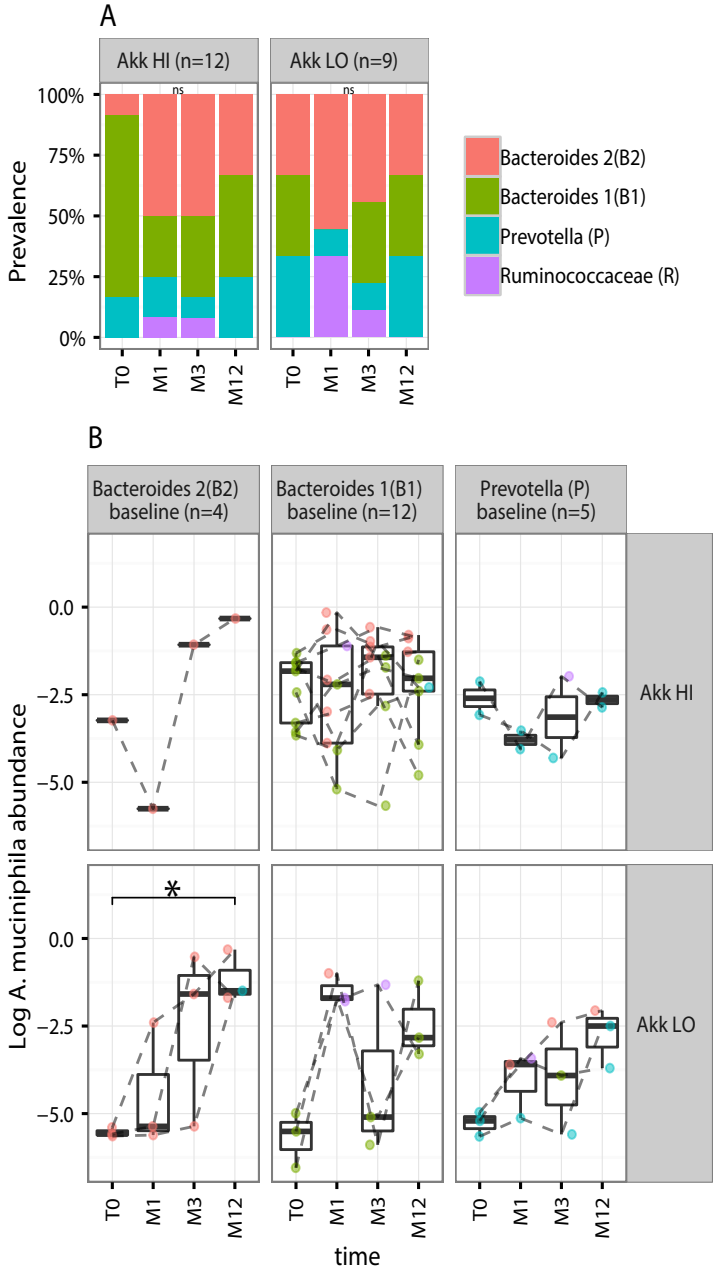

A
Percent
month = 0
month = 1
month = 3
month = 12
Log A. muciniphila abundance
B
Log A. muciniphila abundance
Month
Group: GB RYGB
C
Log A. muciniphila abundance
Month
Group: Akk HI Akk LO
D
GB (Rho = -0.85, P = 0.002) / RYGB (Rho = -0.35, P = 0.30)
Log A. muciniphila change (M1 – BS)
Baseline Log A. muciniphila abundance
GB AkkHI GB AkkLO RYGB AkkHI RYGB AkkLO
GB (Rho = -0.59, P = 0.07) / RYGB (Rho = -0.25, P = 0.45)
Log A. muciniphila change (M3 – BS)
Baseline Log A. muciniphila abundance
GB AkkHI GB AkkLO RYGB AkkHI RYGB AkkLO
GB (Rho = -0.81, P = 0.005) / RYGB (Rho = -0.71, P = 0.015)
Log A. muciniphila change (Y1 – BS)
Baseline Log A. muciniphila abundance
GB AkkHI GB AkkLO RYGB AkkHI RYGB AkkLO

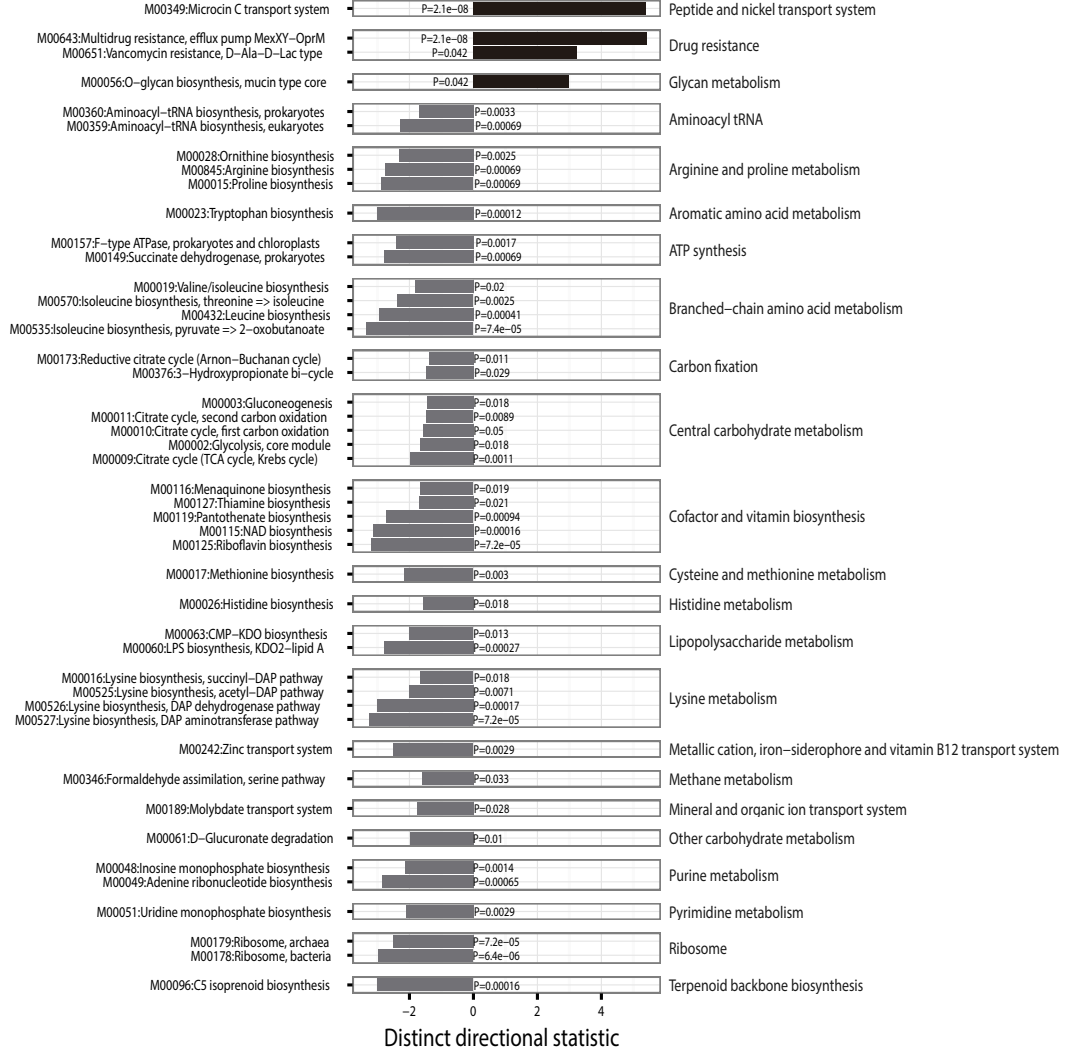

A
Akkermansia genus abundance
CAG00027_1
CAG00095
CAG00301
CAG00844
Rho=0.87
P=5.8 x 10−51
Rho=0.13
P=0.1
Rho=0.23
P=0.0035
Rho=0.75
P=3.6 x 10−30
Rho=0.093
P=0.24
Log A. muciniphila abundance
log10(shotgun abundance data)


B
Microbaria follow−up cohort
GB
RYGB
Akkermansia MGS richness
time
T0
M1
M3
M12


A
rho= 0.32 ; P= 0.013
Metagenomic Richness
1e+06
8e+05
6e+05
4e+05
2e+05
−6
−4
−2
Baseline Akk
B
P= 0.028
Metagenomic Richness
1e+06
8e+05
6e+05
4e+05
2e+05
Akk LO
Akk HI


A
B
C
AkkqPCR
0.0
−2.5
−5.0
*
*
*
Metagenomic richness
1e+06
5e+05
**
***
****
Baseline prevalence
100%
75%
50%
25%
0%
ns
Bacteroides 2(B2) (n=15)
Bacteroides 1(B1) (n=27)
Prevotella (P) (n=14)
Ruminococcaceae (R) (n=4)
Bacteroides 2(B2) (n=15)
Bacteroides 1(B1) (n=28)
Prevotella (P) (n=14)
Ruminococcaceae (R) (n=4)
Akk HI (n=32)
Akk LO (n=28)
Bacteroides 2(B2)
Bacteroides 1(B1)
Prevotella (P)
Ruminococcaceae (R)


A
Akk HI (n=12)
Akk LO (n=9)
ns
ns
100%
75%
50%
25%
0%
Prevalence
T0
M1
M3
M12
Bacteroides 2(B2)
Bacteroides 1(B1)
Prevotella (P)
Ruminococcaceae (R)
B
Bacteroides 2(B2) baseline (n=4)
Bacteroides 1(B1) baseline (n=12)
Prevotella (P) baseline (n=5)
Akk HI
Akk LO
*
0.0
−2.5
−5.0
Log A. muciniphila abundance
time


M00349:Microcin C transport system
P=2.1e−08
Peptide and nickel transport system
M00643:Multidrug resistance, efflux pump MexXY−OprM
M00651:Vancomycin resistance, D−Ala−D−Lac type
P=2.1e−08
P=0.042
Drug resistance
M00056:O−glycan biosynthesis, mucin type core
P=0.042
Glycan metabolism
M00360:Aminoacyl−tRNA biosynthesis, prokaryotes
M00359:Aminoacyl−tRNA biosynthesis, eukaryotes
P=0.0033
P=0.00069
Aminoacyl tRNA
M00028:Ornithine biosynthesis
M00845:Arginine biosynthesis
M00015:Proline biosynthesis
P=0.0025
P=0.00069
P=0.00069
Arginine and proline metabolism
M00023:Tryptophan biosynthesis
P=0.00012
Aromatic amino acid metabolism
M00157:F−type ATPase, prokaryotes and chloroplasts
M00149:Succinate dehydrogenase, prokaryotes
P=0.0017
P=0.00069
ATP synthesis
M00019:Valine/isoleucine biosynthesis
M00570:Isoleucine biosynthesis, threonine => isoleucine
M00432:Leucine biosynthesis
M00535:Isoleucine biosynthesis, pyruvate => 2−oxobutanoate
P=0.02
P=0.0025
P=0.00041
P=7.4e−05
Branched−chain amino acid metabolism
M00173:Reductive citrate cycle (Arnon−Buchanan cycle)
M00376:3−Hydroxypropionate bi−cycle
P=0.011
P=0.029
Carbon fixation
M00003:Gluconeogenesis
M00011:Citrate cycle, second carbon oxidation
M00010:Citrate cycle, first carbon oxidation
M00002:Glycolysis, core module
M00009:Citrate cycle (TCA cycle, Krebs cycle)
P=0.018
P=0.0089
P=0.05
P=0.018
P=0.0011
Central carbohydrate metabolism
M00116:Menaquinone biosynthesis
M00127:Thiamine biosynthesis
M00119:Pantothenate biosynthesis
M00115:NAD biosynthesis
M00125:Riboflavin biosynthesis
P=0.019
P=0.021
P=0.00094
P=0.00016
P=7.2e−05
Cofactor and vitamin biosynthesis
M00017:Methionine biosynthesis
P=0.003
Cysteine and methionine metabolism
M00026:Histidine biosynthesis
P=0.018
Histidine metabolism
M00063:CMP−KDO biosynthesis
M00060:LPS biosynthesis, KDO2−lipid A
P=0.013
P=0.00027
Lipopolysaccharide metabolism
M00016:Lysine biosynthesis, succinyl−DAP pathway
M00525:Lysine biosynthesis, acetyl−DAP pathway
M00526:Lysine biosynthesis, DAP dehydrogenase pathway
M00527:Lysine biosynthesis, DAP aminotransferase pathway
P=0.018
P=0.0071
P=0.00017
P=7.2e−05
Lysine metabolism
M00242:Zinc transport system
P=0.0029
Metallic cation, iron−siderophore and vitamin B12 transport system
M00346:Formaldehyde assimilation, serine pathway
P=0.033
Methane metabolism
M00189:Molybdate transport system
P=0.028
Mineral and organic ion transport system
M00061:D−Glucuronate degradation
P=0.01
Other carbohydrate metabolism
M00048:Inosine monophosphate biosynthesis
M00049:Adenine ribonucleotide biosynthesis
P=0.0014
P=0.00065
Purine metabolism
M00051:Uridine monophosphate biosynthesis
P=0.0029
Pyrimidine metabolism
M00179:Ribosome, archaea
M00178:Ribosome, bacteria
P=7.2e−05
P=6.4e−06
Ribosome
M00096:C5 isoprenoid biosynthesis
P=0.00016
Terpenoid backbone biosynthesis
−2
0
2
4
Distinct directional statistic