Metabolism Open 20 (2023) 100256

Contents lists available at ScienceDirect

Metabolism Open

journal homepage: www.sciencedirect.com/journal/metabolism-open

Microbiome differences related to metformin intolerance among Black individuals with diabetes, a pilot cross-sectional study

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ARTICLE INFO

Keywords:
Microbiome
Metformin intolerance
Type 2 diabetes
Diabetes in minorities

ABSTRACT

Aims: Metformin is the broadly accepted the first-line medication for diabetes. Its use, however, is limited by gastrointestinal side effects present in approximately 25% of patients. This study aimed to better understand the interplay between metformin intolerance and gut microbiota among Black individuals with diabetes.
Methods: We performed a cross-sectional study among 29 Black individuals living with diabetes with or without metformin intolerance. Participants with mean age 59±11, 58% female, were stratified into three groups: 1) intolerant: metformin intolerance in the past, not on metformin; 2)partially intolerant: mild to moderate gastrointestinal symptoms, currently taking metformin 3)tolerant: using metformin without symptoms. We collected and analyzed rectal swabs and analyzed microbiota composition using V3–V4 regions of the 16s rRNA.
Results: Metformin intolerant subjects trended towards having greatest alpha diversity, followed by tolerant and partially tolerant (Intolerant:4.9; Tolerant:4.2; Partially tolerant:3.9). Mean difference in alpha diversity for intolerant versus partially tolerant was 1.0 (95% CI-0.1,2.1) and intolerant versus tolerant were 0.7 (95% CI -0.4,1.8).
Conclusion: This was the first study to evaluate the role of microbiota and metformin intolerance among Black individuals. We report on differences in alpha diversity as well as microbiota composition.

1. Introduction

Metformin is one of the oldest and most widely used medications for the treatment of type 2 diabetes [1]. Owing to its favorable safety profile, low cost, and effectiveness in improving glycemic control with ≥ 1.5% HbA1c reduction, metformin has been considered the first-line therapy for diabetes for many years [1,2]. Metformin lowers blood glucose levels and increases insulin sensitivity through multiple mechanisms, including reduction in hepatic glucose production, greater glucose utilization by the skeletal muscle, and increased fatty acid oxidation. Recently, there has been increasing interest in its role in the gastrointestinal tract, where it is known to promote glucose uptake by intestinal cells and increase the production of the incretin hormone GLP-1, which has a significant role in glucose metabolism [3]. Metformin is largely concentrated in the gut with little systemic absorption. Metformin is known to exert changes in the microbiome and has been reported to reverse some of the dysbiosis associated with diabetes, in part by increasing the abundance of butyrate-producing organisms such as *Akkermansia* [3–5]. Evidence suggests that some of its glucose-lowering effects are mediated by changes in intestinal microbiota [6].

Use of metformin in the clinical setting is limited by significant gastrointestinal side effects in approximately 25% of patients taking it [7–10]. This intolerance necessitates either discontinuation or reduction of the dose, often resulting in reduced maximal drug efficacy or the need to switch to more expensive treatment. Multiple mechanisms likely contribute to metformin intolerance including genetic variation in metformin transport proteins such as OCT1 [11], variable stimulation of

Abbreviations: HbA1c, Hemoglobin A1c; NS, not significant; FDR, False Discovery Rate.

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https://doi.org/10.1016/j.metop.2023.100256
Received 5 June 2023; Received in revised form 6 September 2023; Accepted 13 September 2023
Available online 15 September 2023

serotonin receptors in the gut [12], increased intestinal lactate production [13], and reduced absorption of bile acids [3,14]. Racial minorities, such as Blacks and Latin Americans, have a higher prevalence of diabetes [15] and are disproportionately affected by diabetes-related complications, such as nephropathy and retinopathy [16–20]. Metformin has been shown to have a greater glucose-lowering effect in Black than White individuals; thus, identifying factors leading to intolerance may be of greater impact in this population [21].

Gastrointestinal side effects associated with metformin are similar to those seen in irritable bowel syndrome, another condition that has been linked with microbial dysbiosis in the gut [22–25]. Alterations in the gut microbiota in diarrhea-predominant irritable bowel syndrome are also associated with increased bile acids, providing a potential common mechanism for metformin intolerance [26]. It remains unclear why there is significant variability in the side effects among people treated with metformin, ranging from absence of symptoms to intolerable nausea, vomiting, diarrhea, and bloating. Given the evident relationship between metformin and the microbiome, it is conceivable that underlying baseline differences in individuals' microbial milieu can impact the likelihood of developing gastrointestinal side effects. Accordingly, we performed a pilot cross-sectional analysis of the microbiota of Black individuals with diabetes and variable metformin intolerance.

2. Materials and methods

2.1. Patient population

We conducted a cross-sectional study of Black participants with a history of diabetes with current or previous metformin use comparing those with and without gastrointestinal symptoms related to metformin. We enrolled participants from a hospital-based diabetes center in Atlanta, Georgia between July 2019 and October 2020. Patients meeting the following inclusion criteria for approached for enrollment: age 18–80 years old, self-identifying as Black, HbA1c $\leq$ 9 (minimizing effects of severe hyperglycemia), and on metformin therapy for a least the past 3 months or prior metformin treatment discontinued due to gastrointestinal intolerance. To minimize confounding effects on the microbiota, potential participants were excluded if they had any of the following: antibiotic therapy within the past 12 weeks, clinically significant gastrointestinal, hepatobiliary or pancreatic disease or any condition that in the opinion of the investigator and/or sponsor may interfere with the gut microbiome, significant renal impairment with GFR <45, current drug addiction or current alcohol abuse, or history of substance or alcohol abuse within the last 2 years, treatment with glucagon-like-peptide-1 receptor agonist or proton pump inhibitor (agents known to significantly alter the gut microbiome), diarrheal illness within the past 8 weeks, current use of probiotic supplement, pregnancy or nursing.

2.2. Study procedures

Participants who met the inclusion criteria were approached for participation. After completing the informed consent process, the subjects were asked to complete a brief survey relating to the history of metformin use, and type and severity of gastrointestinal side effects. Additional information obtained included anthropometric data, medical history, and concurrent medication use. The most recent laboratory data (within the past three months) were collected from electronic medical records. Participants were categorized into three groups based on their medical history and survey responses: metformin tolerant: patients currently on metformin without any gastrointestinal side effects and no history of needing to reduce the dose due to intolerance; partially tolerant: participants currently taking metformin and reporting gastrointestinal side effects with use and/or previously needing to reduce the dose due to intolerance; metformin intolerant: patients previously on metformin but had to stop taking it due to gastrointestinal symptoms. These three groups were established to account for the known role of metformin on the microbiome and isolate the effects of intolerance itself. Thirty subjects were enrolled in the study, with a goal of 10 subjects per group. After 10 subjects were enrolled in a given group, enrollment for that category was stopped to ensure equal distribution of subjects. All data was collected during a single study visit, at which time, subjects we also provided stool samples. The participants were provided with swabs and collection kits for the collections. One participant in the tolerant group had sample loss during processing and so was not included in any of the analyses.

2.3. Outcomes measures

The primary outcome of the study was to assess the differences in Shannon alpha diversity index among Black participants with metformin tolerance, intolerance, and partial tolerance. The Shannon alpha diversity index is a measure of microbiota species diversity that factors their total number as well as relative abundance [27]. We hypothesized that those who were metformin tolerant and partially tolerant would have differences in alpha diversity and microbiome distribution representative of the dysbiosis associated with metformin intolerance. We also aimed to evaluate the effects of metformin on the microbiota of Black patients enrolled by comparing those who were and were not currently receiving metformin therapy.

2.4. Microbiota analysis

16S rRNA gene amplification and sequencing were done using the Illumina MiSeq technology following the protocol of Earth Microbiome Project with their modifications to the MOBIO PowerSoil DNA Isolation Kit procedure for extracting DNA (https://press.igsb.anl.gov/earthmicrobiome). Bulk DNA was extracted from frozen feces using a Qiagen PowerSoil Pro kit from Qiagen with mechanical disruption (bead-beating). The 16S rRNA genes, region V4, were PCR amplified from each sample using a composite forward primer and a reverse primer containing a unique 12-base barcode, designed using the Golay error-correcting scheme, which was used to tag PCR products from respective samples). We used the forward primer 515F 5′- *AATGATACGGCGACCACCGAGATCTACACGCT*XXXXXXXXXXXX**TATGGTAATT*GT***GTGYCAGCMGCCGCGGTAA-3': the italicized sequence is the 5′ Illumina adaptor, the 12 X sequence is the golay barcode, the bold sequence is the primer pad, the italicized and bold sequence is the primer linker, and the underlined sequence is the conserved bacterial primer 515F. The reverse primer 806R used was 5′-*CAAGCAGAAGACGGCATACGAGAT***AGTCAGCCAG*CC***GGACTACNVGGGTWTCTAAT-3': the italicized sequence is the 3′ reverse complement sequence of Illumina adaptor, the bold sequence is the primer pad, the italicized and bold sequence is the primer linker and the underlined sequence is the conserved bacterial primer 806R. PCR reactions consisted of Hot Master PCR mix (Quantabio, Beverly, MA, USA), 0.2 mM of each primer, 10–100 ng template, and reaction conditions were 3 min at 95 °C, followed by 30 cycles of 45 s at 95 °C, 60 s at 50 °C and 90 s at 72 °C on a Biorad thermocycler. PCRs products were purified with Ampure magnetic purification beads (Agencourt, Brea, CA, USA), and visualized by gel electrophoresis. Products were then quantified (BIOTEK Fluorescence Spectrophotometer) using Quant-iT PicoGreen dsDNA assay. A master DNA pool was generated from the purified products in equimolar ratios. The pooled products were quantified using Quant-iT PicoGreen dsDNA assay and then sequenced using an Illumina MiSeq sequencer (paired-end reads, 2 x 250 bp) at Cornell University, Ithaca.

2.5. 16S rRNA gene sequence analysis

16S rRNA sequences were analyzed using QIIME2—version 2019. Sequences were demultiplexed and quality filtered using the Dada2

method with QIIME2 default parameters in order to detect and correct Illumina amplicon sequence data, and a table of Qiime 2 artifact was generated. A tree was next generated, using the align-to-tree- mafft-fasttree command, for phylogenetic diversity analyses, and alpha and beta diversity analyses were computed using the core-metrics-phylogenetic command. Principal coordinate analysis (PCoA) plots were used to assess the variation between the experimental group (beta diversity). For taxonomy analysis, features were assigned to operational taxonomic units (OTUs) with a 99% threshold of pairwise identity to the Greengenes reference database 13_8. Unprocessed sequencing data are deposited in the Genome Sequence Archive (GSA) in BIG Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences, publicly accessible at http://bigd.big.ac.cn/gsa.

2.6. Statistical analysis

All continuous variables are reported as mean ± standard deviation, except where noted otherwise. Tukey's multiple comparisons test was performed to compare Shannon diversity between tolerance groups.

To assess species differences among the tolerance groups we implemented the Microbiome Multivariable Associations with Linear Models, with software version 2.0 (MaAsLin-2) R package.

3. Results

We analyzed results of 29 Black participants enrolled in the study, mean age was 59 ± 11, 58% female. Patient characteristics are summarized in Table 1. Mean HbA1c was 7.46 ± 0.80% in the intolerant, 7.62 ± 1.21% in the tolerant, and 7.55 ± 1.12% in the partially tolerant group. Individuals' patterns of metformin use and tolerance are shown in Table 2. Mean duration of metformin use was 17.3, 59.1, and 84.8 months in the intolerant, tolerant, and partially tolerant groups, respectively. Those with metformin intolerance reported diarrhea most frequently (100%) following by nausea, bloating, and abdominal pain (80% for all). Participants with partial tolerance reported nausea most often (90%) following by diarrhea, bloating, and abdominal pain (70% for all).

Shannon alpha diversity indices for each of the treatment groups is shown in Fig. 1. Metformin intolerant individuals showed a trend towards greatest alpha diversity, followed by metformin tolerant and partially tolerant (intolerant: 4.9; tolerant: 4.2; partially tolerant: 3.9, p=NS). The largest difference was seen between the intolerant and partially tolerant groups (mean differences 1.01, adjusted p value=0.083). Among the three groups, use of metformin was associated with significantly reduced microbiota richness (Fig. 3), 4.9 vs 4.1, p=0.03. To assess for the impact of BMI, we analyzed the relationship between BMI and microbiota diversity without our study sample (Appendix 4). We did not find a significant relationship between the Shannon Index for alpha diversity and BMI (p=NS).

Fig. 2 shows the phylum level distribution of microbiota among the three tolerance groups. We did not identify significant differences in the phyla distributions among the tolerance groups. Comparison of relative phyla concentrations of *Actinobacteria, Firmicutes, and Proteobacteria* did not differ significantly between the three tolerance groups (Appendix 2. We further performed targeted analysis of species of interest *Akkermansia muciniphila, Veikkinellaceae megamonas, Veikkinellaceae phascolarctobacterium, Ruminococcus toques,* which also did not show intergroup differences (Appendix 3).

Table 1
Participant characteristics.

	Intolerant (n=10)	Tolerant (n=9)	Partially Tolerant (n=10)
Male Sex, n (%)	4 (40%)	3 (42.86%)	4 (50%)
Age, mean ± SD	55.80 ± 14.40	63.70 ± 6.38	58.90 ± 10.35
HbA1c, % (mean ± SD)	7.46 ± 0.80	7.62 ± 1.21	7.55 ± 1.12
Weight, Kg (mean ± SD)	92.92 ± 17.18	83.96 ± 27.68	89.60 ± 19.24
BMI, Kg/m^2 (mean ± SD)	31.54 ± 7.86	32.11 ± 9.42	31.65 ± 5.59
eGFR, mL/min/1.73 m^2(n,%)			
≥60	9 (90%)	4 (40%)	10 (100%)
45–59	1 (10%)	6 (60%)	0 (0%)

Table 2
Patterns of metformin use and tolerance.

	Intolerant (n=10)	Tolerant (n=9)	Partially Tolerant (n=10)
Dose, mg[a] (mean ± SD)	0 ± 0	1450 ± 497.21	1050 ± 550.25
Using extended release, yes (n, %)	0 (0%)	6 (60%)	7 (70%)
Duration of metformin use, months	17.25	59.10	84.80
Reported Nausea (n, %)	8 (80%)	0 (0%)	9 (90%)
Reported Vomiting (n, %)	4 (40%)	0 (0%)	4 (40%)
Reported Diarrhea, (n, %)	10 (100%)	0 (0%)	7 (70%)
Reported Bloating, (n, %)	8 (80%)	0 (0%)	7 (70%)
Reported abdominal pain, (n, %)	8 (80%)	0 (0%)	7 (70%)

[a] Current dose at time of study visit. Not applicable for those not on metformin.

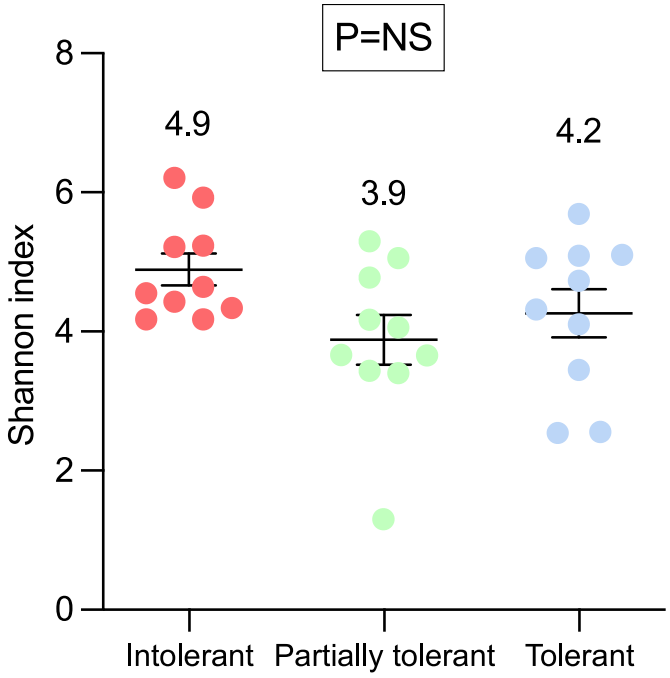


Fig. 1. Alpha (Shannon) diversity by metformin tolerance group. Shannon diversity is listed above each group. Means are indicated on graph, NS=not significant.

4. Discussion

In this study, we report the gut microbiome characteristics among Black individuals with diabetes comparing those with and without metformin intolerance as well as those currently taking and not taking metformin therapy. We found that those with partial tolerance tended to have the lowest Shannon diversity indices, suggesting a greater degree of dysbiosis. This may allude to underlying predisposition for dysbiosis among those who continue to take metformin despite the presence of gastrointestinal side-effects. The effect of metformin therapy was seen in

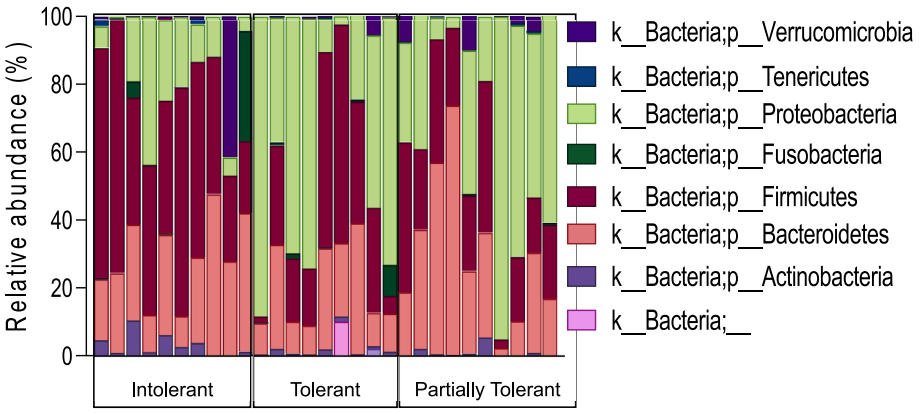


Fig. 2. Phyla distribution by individual.

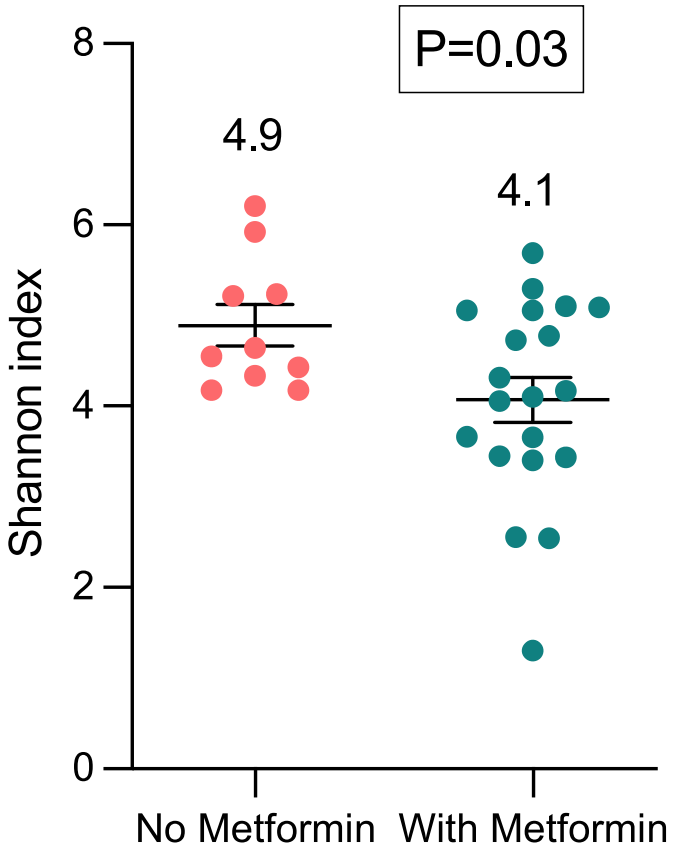


Fig. 3. Effect of metformin intake on microbiota richness. Individuals currently on metformin (combined tolerant and partially tolerant groups) compared to those not currently on metformin (intolerant group). Means are indicated on graph.

reduced microbiota richness in the tolerant and partially tolerant groups, both of whom were on current metformin therapy. The symptoms of individuals with metformin intolerance are similar to those of patients with irritable bowel syndrome, which has been linked to microbial dysbiosis characterized by bacterial overgrowth in the small intestine and low-level intestinal inflammation [25]. Similar mechanisms such as increased bile acid may link microbial changes to symptoms in both conditions [26].

Metformin is known to exert effects on the gut microbiota that mediate some of its beneficial metabolic effects [6,28]. For example, metformin has been shown to increase the production of mucin-degrading *Akkermansia muciniphila* and other microbial changes that lead to increased short-chain fatty acid production [5,29,30]. Metformin's changes in gut microbial composition may also contribute to gastrointestinal side effects and intolerance frequently reported in patients taking metformin [31]. In our study, metformin was associated with an overall decrease in microbial diversity. Similar findings have been reported in both human and animal models [4,5]. Several studies demonstrated differences in enrichment of certain species among patients with and without metformin-associated gastrointestinal side effect. In one study, Eldere *et al.* administered metformin to drug-naïve healthy volunteers and assessed microbiome changes and gastrointestinal symptoms after seven days of therapy. They found that the presence of metformin-associated gastrointestinal symptoms was associated with increased abundance of certain pathogenic organisms including *Escherichia-Shigella* [32]. Diaz-Perdigones *et al.* conducted a prospective study of 35 patients with type two diabetes not on metformin due to suspicion of intolerance. All patients were gradually started on metformin and stratified into groups based on the presence or absence of gastrointestinal symptoms. Those with symptoms were further grouped based on whether the symptoms developed at initiation versus metformin dose increase. Patients tolerant to metformin had enrichment of *Prevotellaceae funiformis* and *Megamonas funiformis,* while those who were not tolerant of higher doses of metformin had higher presence of *Phascolarctobacterium faecium* and *Ruminococcus torques_1* [33]. In our study, when we did not find intergroup differences in these species of interest (Appendix 3), however, this was a pilot study and likely underpowered to detect such changes.

Identifying unique microbial patterns may allow for prediction of side effects prior to initiation of therapy allowing for more targeted treatment. Several studies show promising findings to this end. Bryrup et al., reported pre-treatment microbial differences that were associated with risk of developing gastrointestinal side effects after starting metformin [34]. A few studies have shown that pre-treatment interventions targeting the microbiome may modulate gastrointestinal symptoms in individuals taking metformin therapy, including in those with previous intolerance [22,35]. One recent randomized controlled trial found that altering the gut microbiome with the addition of the GI microbiome modulator (GIMM), which contains beta-glucan, inulin, blueberry anthocyanins and blueberry polyphenols led to improved metformin tolerance and allow for dose titration [35]. They found that addition of the microbiome modulator to metformin led to reduced gastrointestinal symptoms among patients with type 2 diabetes and previous metformin intolerance.

The present work, while providing valuable insights, has several limitations. One factor that may have influenced our results is the relatively small sample size for each tolerance group, likely limiting observed differences in individual species of interest. Additionally, our study design was cross-sectional and did not allow for assessment of the microbiome prior to initiation of therapy, limiting our ability to identify

predisposing patterns that could predict metformin intolerance. Another potential limitation is that participants in the intolerant group were not currently taking metformin, which may have affected their ability to recall certain details. Despite these limitations, our study offers several strengths. By studying a homogeneous population of Black individuals, we were able to reduce heterogeneity and account for racial differences in the microbiome. Furthermore, we included a partial tolerance group to create an internal control for the effect of metformin itself on the microbiome. We obtained detailed participant information through questionnaires that incorporated various aspects of metformin intolerance. Finally, our study was the first to investigate the relationship between metformin intolerance and Black individuals, providing a valuable contribution to the field.

Here, we present the first report of microbiome differences relating to metformin tolerance among Black individuals with diabetes. Blacks and other ethnic minorities are disproportionately impacted by both diabetes and its complications. Some reports suggest that Blackpeople may have greater glycemic benefits from metformin therapy as compared to their white counterparts [36]. Racial and population differences in the gut microbiota are well documented [37]. Thus, it is important that we gain a better understanding of the effects of metformin in this population and explore mitigating methods to reduce intolerance.

5. Conclusion

This was the first study to evaluate differences in the gut microbiota relating to metformin intolerance among Black individuals. We identified differences in alpha diversity between those currently taking and not taking metformin therapy. Prospective studies are needed to assess whether gut microbial patterns predispose to risk of developing metformin intolerance among individuals with type 2 diabetes.

Funding

Research reported in this publication was supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under award number P30DK111024. BC's laboratory is supported by a Starting Grant from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No. ERC-2018-StG- 804135), an award from the Fondation de l'avenir (AP-RM-21-032), ANR grants EMULBIONT (ANR-21-CE15-0042-01) and DREAM (ANR-20-PAMR-0002) and the national program "Microbiote" from INSERM. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CRediT authorship contribution statement

Maya Fayfman: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Andrew T. Gewirtz:** Conceptualization, Methodology, Writing – review & editing. **Clara Delaroque:** Data curation, Validation, Writing – review & editing. **Gerardo Blanco:** Data curation, Writing – review & editing. **Seid Gibanica:** Investigation, Data curation. **Shanthi Srinivasan:** Conceptualization, Writing – review & editing. **Benoit Chassaing:** Conceptualization, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

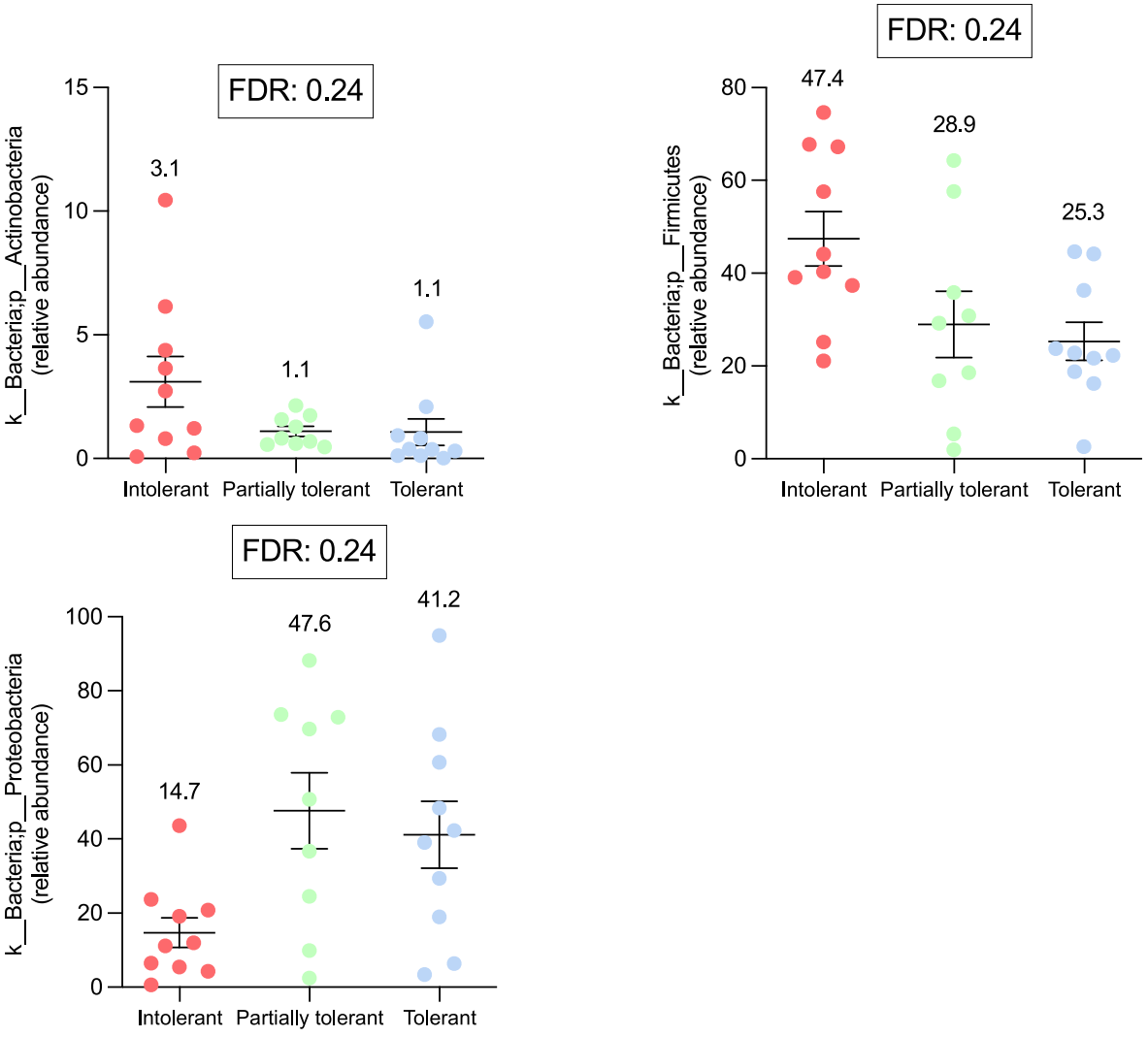
We report no conflict of interests for any authors involved in this manuscript.

Appendix 1. Metformin Tolerance Survey

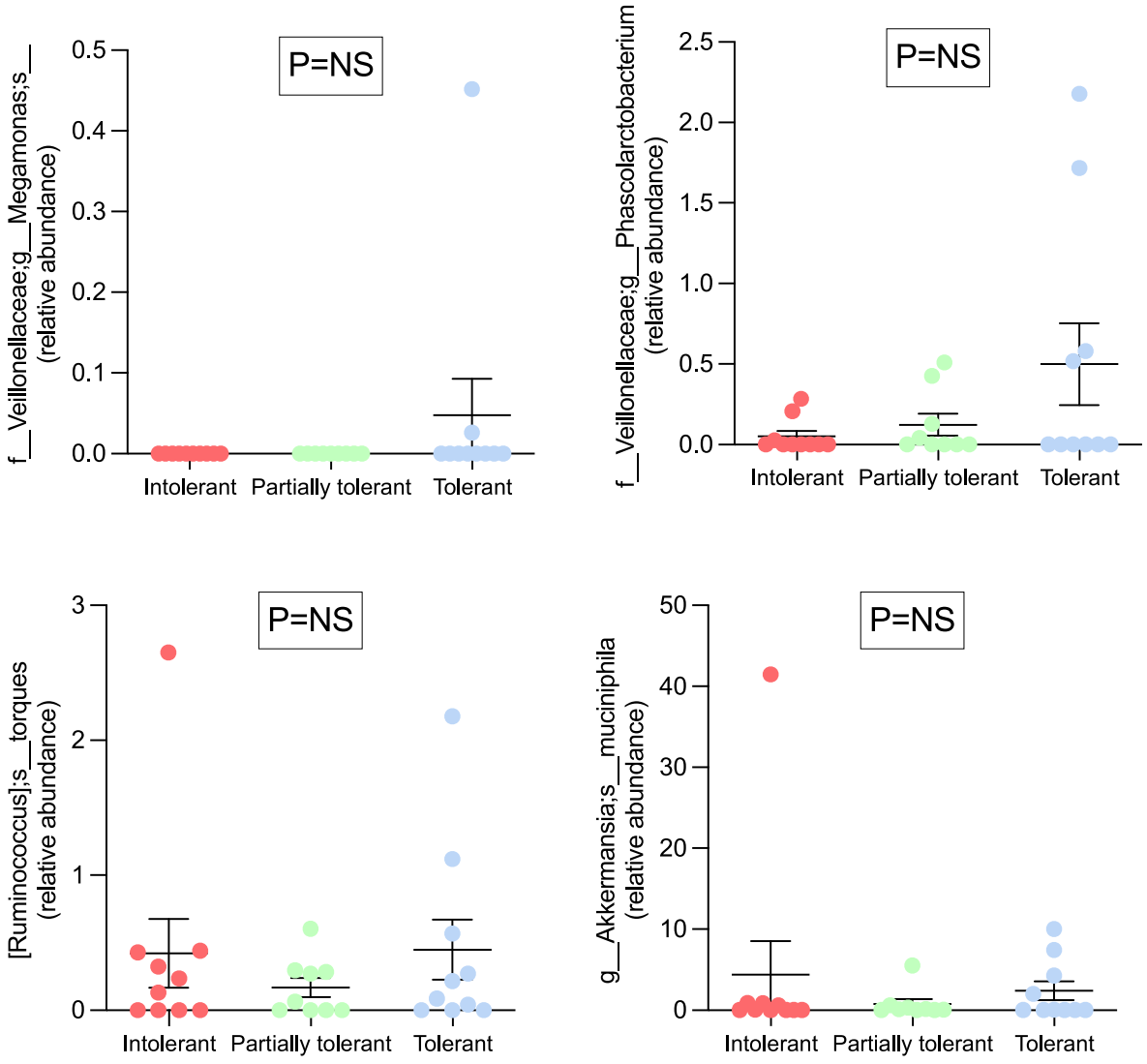
Name: _____
Date: _____
Date of Birth: _____

1. Are you currently taking metformin? yes/no
2. If so, what is your current total daily dose of metformin? _____
3. Except for the first week after you first started taking metformin, did you experience any of the following symptoms that you think were due to the metformin:
   (Rate each symptom on a scale of 0–10, 0 meaning no symptoms and 10 being very severe symptoms)
   a. Nausea: ___
   b. Vomiting: ___
   c. Diarrhea: ___
   d. Bloating: ___
   e. Stomach pain or cramping: ___
4. Have you ever had to lower your dose of metformin because of these symptoms? yes/no
   a. If so, did your symptoms get better after lowering your dose of metformin?
      Pick one: No change/got better/completely went away
5. Did you stop taking metformin because of these symptoms?
   a. If so, did your symptoms get better after stopping metformin? Yes/no
      Pick one: No change/got better/completely went away
6. Do you ever skip you dose of metformin because of any of these symptoms: yes/no
7. If you had symptoms with metformin, which one was most bothersome?_____
8. For how have you been on metformin? ____
9. For how many years have you had diabetes? _____

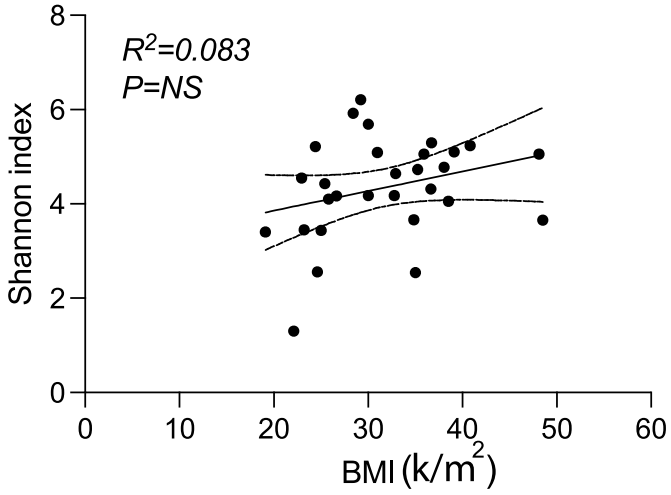
Appendix 2. Select microbiota members relative abundance at the phylum level, by metformin tolerance group. Relative abundance of microbiota members (phylum taxonomy level) with lowest FDR value following Maaslin2 analysis. Means and FDR values are indicated on graphs.



Appendix 3. Targeted microbiota members abundance comparison among metformin tolerance profile groups. NS=not significant.



Appendix 4. Correlation between BMI and Shannon alpha diversity



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