Clinical Research Protocol

National Institute of Diabetes and Digestive and Kidney Diseases

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Title: **Prebiotics and Metformin Influences Gut and Hormones in Type 2 Diabetes Youth (MIGHTY-Fiber)**
Short Title: MIGHTY - Fiber
Identifying Words: Gastrointestinal side effects, Metformin, Type 2 Diabetes, Youth

Principal Investigator: Stephanie T. Chung, M.B.B.S. NIDDK

Estimated Duration of Study: 4 years
Start Date: Summer 2019 **End Date:** Summer 2023
Number and Type of Patients: Accrual Ceiling: 50

	Number	Sex	Age Range	Ethnic Group
Patients	**50**	Male/Female	10-25 years	All

Project Uses Ionizing Radiation:
_X__Research indicated: RSC Approval Number: 2741 Expiration Date: 06/27/2022

Project Uses "Durable Power of Attorney": No **Off-site Project:** No
Multi-site enrollment: No
Multi-institutional project: No

Contents

Precis

Metformin is the most widely prescribed anti-diabetes medication in the world and the first-line therapy for treating type 2 diabetes (T2D) in youth and adults. However, metformin's glucose-lowering ability is variable in clinical practice, and efficacy is further limited by poor medication adherence because of metformin-associated adverse effects. Gastrointestinal (GI) symptoms such as bloating, abdominal discomfort, cramping, and diarrhea are the most common side effects associated with metformin use occurring in up to 80% of individuals at drug initiation and up to 30% in individuals on chronic treatment. In youth with type 2 diabetes, the burden of metformin-associated side effects is high because metformin is the only oral FDA-approved for treatment and there are no other oral alternatives. Therefore, identifying ways to mitigate these GI side effects, especially in youth with type 2 diabetes, is of high clinical significance. New data suggest that metformin-induced changes in the gut and/ or the microbiome may be related to both its beneficial (glucose-lowering) and adverse effects. To address this clinical challenge, prebiotic fibers that are non-digestible food ingredients, may help to improve metformin tolerability by increasing beneficial bacteria and stool metabolites, such as short chain fatty acid (SCFA) stool concentrations. This pilot study will test the hypothesis that a prebiotic microbiome modulator (MM) – containing prebiotic fibers and polyphenols – will reduce GI side effects of metformin at time of initiation and change the stool metabolite profile in youth and young adults with T2D treated with metformin, age 10-25 years who are not on insulin therapy. The 9-week study will have 2 phases and 6 outpatient visits at the NIH Clinical Center. Phase 1 is a 5-week randomized double blind cross-over trial with two 1-week intervention periods (metformin + prebiotic and metformin + placebo) during which subjects will eat a standardized diet. Phase 2 will occur immediately following phase 1 in which participants will start an open-label 4-week intervention with metformin and the prebiotic MM.

1.0 Background

Metformin is the most widely prescribed anti-diabetic agent in the world and is first line for treatment of type 2 diabetes (T2D) in both adults and children [1, 2] . However, metformin non-responsiveness is an important clinical challenge, occurring in 20-50% of youth and adults [3], and reduced treatment adherence is a well-recognized and potentially modifiable risk factor [4]. Metformin related gastrointestinal (GI) side effects (bloating, diarrhea, cramping, nausea and vomiting) are a common barrier to maximal dose escalation and treatment optimization [5]. These side effects are also a frequently reported reason for non-adherence [5]. Side effects are observed in > 80% of individuals newly initiated on metformin and ~10-30% of patients on long-term therapy [6, 7], with estimates of 1 in 4 youth taking metformin having at least one GI side effect [8]. Challenges are magnified in youth with T2D, as metformin is the only oral medication that is approved for use in the 10-17-year age group. Yet, there is a paucity of studies examining the mechanisms of metformin's GI side effects in vivo, and thus limited ability to mitigate these

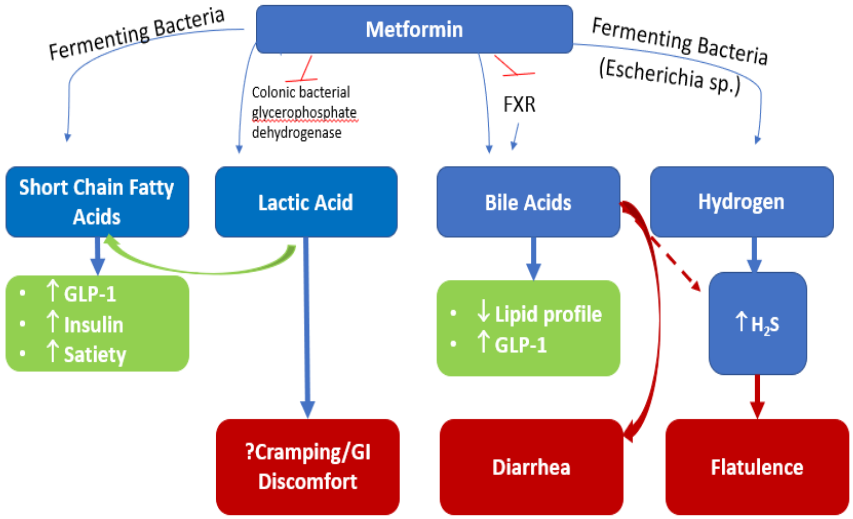

Figure 1Potential mechanisms of metformin-related changes in gut metabolites and lower GI side effects. H2S: hydrogen sulfide, GLP-1: glucagon-like peptide 1,FXR: farnesoid X receptor (References: 14-16)

issues. This pilot study, a randomized double-blind crossover trial, examines the use of a prebiotic as an adjunct to improving metformin tolerability, facilitating short-term dose escalation, and also explores the underlying gut-based mechanisms of both metformin action and intolerance in youth and young adults with type 2 diabetes.

Metformin-induced changes in the microbiome are emerging as an important primary mechanistic target. Initial studies in adults suggest that short-term metformin therapy increases production of short chain fatty acids (SCFAs) and incretins (glucagon-like peptide-1 [GLP-1] and peptide YY [PYY]) [9, 10] by increasing populations of SCFA-producing bacteria, such as *Akkermansia muciniphila* and *Bifidobacterium adolescentis* [11-13]. However, other changes in the gut milieu may also contribute to the adverse GI side effects commonly seen with metformin use, but few studies have resolved the cellular mechanisms that cause of metformin's GI side effects. Figure 1 illustrates three (3) hypothetical mechanisms of metformin-induced changes in the gut microbiome that may contribute to its GI symptoms. For example, metformin-induced inhibition of colonic bacterial glycerophosphate dehydrogenase increases colonic lactate acidosis that may be toxic to the gut mucosa [14]. Alternatively, high concentrations of stool bile acids, secondary to direct farnesoid X receptor (FXR) inhibition by metformin, may cause osmotic diarrhea [15]. Additionally, metformin-treated patients have increased abundance of *Escherichia sp* that was associated with functional shifts with enrichment of virulence factors and gas metabolism genes, specifically for the production of hydrogen sulfide gas [16].

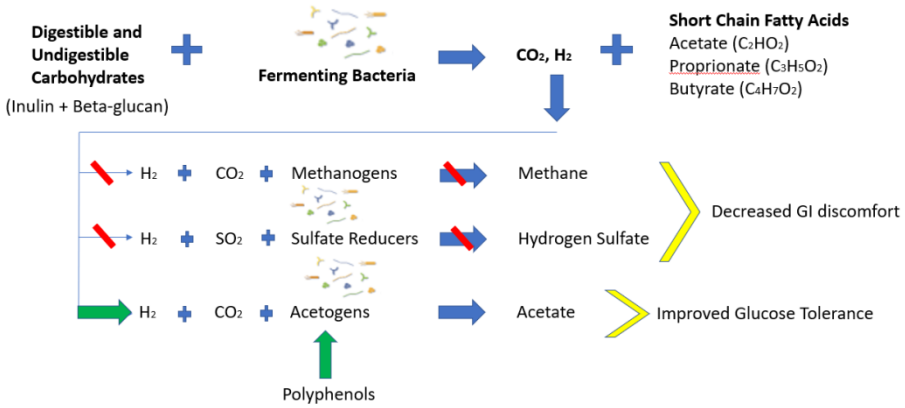

Figure 2- Proposed Mechanism of Prebiotic Microbiome Modulator, adapted from [25]

While the precise mechanism of metformin-induced GI side effects remains to be elucidated, microbiome-influencing supplements may benefit patients with diabetes. Even without other medications, prebiotic fiber has been shown to improve the metabolic profile in patients with and without diabetes [17-19]. Prebiotics are substrates for bacterial fermentation and SCFA production (Figure 2) and are defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health" [20]. These supplements are effective in reducing the severity and duration of infectious diarrhea [21], as well as improving bowel regularity. Prebiotics may also improve intestinal permeability and gut inflammation – two factors which have been linked to obesity and metabolic syndrome [22, 23]. However, prebiotic supplements, when used in isolation, could increase flatulence by producing methane and hydrogen sulfide gas as byproducts via methanogen and sulfate-reducing bacteria, respectively (Figure 2) [24]. To decrease these unwanted side effects, preliminary studies have demonstrated that using polyphenols -- naturally occurring compounds that are metabolized by the SCFA-producing bacteria (acetogens)-- may be helpful in reducing flatulence (Figure 2) [25]. The presence of polyphenols, commonly found in fruits and vegetables, allows acetate-producing bacteria to thrive, while methane- or hydrogen sulfate-producing bacteria have less available hydrogen substrate to convert to their respective gases [25, 26]. In a pilot randomized trial of 10 adults with T2D, a prebiotic microbiome modulator (MM) with inulin and beta glucan plus polyphenols from blueberry pomace improved metformin tolerability and fasting glycemia [27]. This same cocktail improved glucose profiles in healthy adults with overweight and obesity during an oral glucose tolerance test [28]. However, the mechanisms by which prebiotics influence metformin-induced GI side effects are unknown. Importantly, it remains to be established whether using this supplement is feasible in youth and young adults with T2D, whose microbiome signatures may be different from adults [29]. **This study is innovative and clinically significant because it uses a prebiotic intervention to evaluate gut-incretin pathways while identifying whether a relatively safe supplement could**

improve quality of life and glycemia in youth and young adults with type 2 diabetes who are high-risk for diabetes-related complications.

Preliminary studies:

Currently, we are exploring metformin's mechanism of action in African American youth with T2D (NCT02960659), including changes in their microbiome. Our preliminary findings from 4 youth who were evaluated before and after 3-months of metformin therapy showed a decreasing trend for HbA1c (-0.56±0.2%) and increasing trends for stool propionate and butyrate (P=0.1, Figure 3). In collaboration with Dr. Hariom Yadav at Wake Forest, using 16S Ribosomal targeted sequencing and Qiime analysis of Operational Taxonomic Units (OTUs), we also observed modest changes in microbial diversity, a decreasing trend in the Shannon index (an index of complexity, *P*=0.15). This decreased diversity has been seen in other studies of metformin-induced changes in the gut microbiota [14]. Therefore, metformin may change gut microbial diversity in association with changes in SCFA stool concentrations in youth, but larger samples sizes are needed for confirmation. While metformin alone can increase SCFA, prebiotic MM may be able to augment this increase by shifting substrate metabolism away from gas (hydrogen sulfide and methane) to acetate production. Further investigations into these mechanisms will help us determine the feasibility of an oral supplement to mitigate side effects of metformin and improve quality of life, as well as glycemia, in youth with T2D.

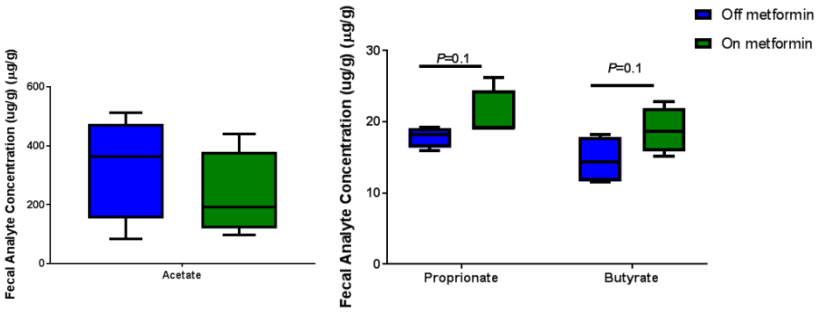

Figure 3. Stool short Chain Fatty Acid Concentrations in feces before and after 3 months of metformin therapy in youth with type 2 diabetes (n=4).

2.0 Specific Objectives/ Aims

The primary objective of this study is to determine the feasibility and efficacy of a prebiotic MM to improve metformin-induced lower GI side effects in youth with T2D and the secondary objective is to measure the change in stool SCFA content after prebiotic MM intervention.

Aim 1. To compare lower-GI side effects and stool consistency (tolerability score) in youth and young adults with T2D at initiation of daily metformin therapy when used with a daily prebiotic microbiome modulator (MM) vs placebo over a 1-week period.

Hypothesis: A prebiotic MM will be associated with higher tolerability scores (a composite score of lower GI-related side effects and stool consistency) compared to placebo.

Rationale: A prebiotic MM, containing both fiber and polyphenols, reduced metformin-related GI side effects in adults with T2D while retaining, and even augmenting, the hypoglycemic effects expected with metformin therapy in a small study (4). Due to the significant potential for clinical benefit, we propose to validate these results in youth, in whom metformin is the only FDA approved anti-diabetic therapy.

Aim 2. To compare the change in SCFA stool content in youth with T2D on metformin therapy when treated with prebiotic MM vs. placebo after 1 week and 4 weeks.

Hypothesis: The prebiotic MM will be associated with higher SCFA stool content compared to placebo.

Rationale: SCFAs are microbial metabolites derived from fermentation of dietary fibers that are likely gut mediators of glucose metabolism. Metformin and prebiotic fiber individually have been demonstrated to increase stool SCFA [13, 16, 30, 31]. We propose to further elucidate the mechanism of metformin and MM, both individually and when taken together.

Exploratory aims: To evaluate the relationship of change in tolerability score with change in stool SCFA, change in gut microbial diversity and SCFA-producing phyla, change in intestinal permeability markers, determine tolerability score for metformin and prebiotic MM over 1 month, and change in measures of glucose, insulin and incretin kinetics after treatment with placebo compared to 4 weeks of continuous prebiotic MM intervention.

2.1 Study Outcomes

Primary Outcome: A composite tolerability score based on 4 GI side effect profile categories over 1 week.
Secondary Outcome: Stool SCFA content (proprionate and butyrate levels) after 1 week and 4 weeks of treatment.
Exploratory Outcomes: Stool microbial diversity indices, stool metabolites (bile acid concentrations, lactic acid, hydrogen sulfide), serum markers of intestinal permeability; glucose, insulin, GLP-1 and PYY concentrations during a mixed meal test.

3.0 Study Design

3.1 Study Design and Timeline

This 9-week pilot study will have 2 phases and 6 outpatient visits at the NIH Clinical Center (Figure 4). Phase 1 is a 5-week randomized double-blind randomized cross-over trial with two 1-week intervention periods (metformin + prebiotic and metformin + placebo). Phase 2 will occur immediately following phase 1 in which participants will start an open-label 4-week intervention with metformin and the prebiotic MM.

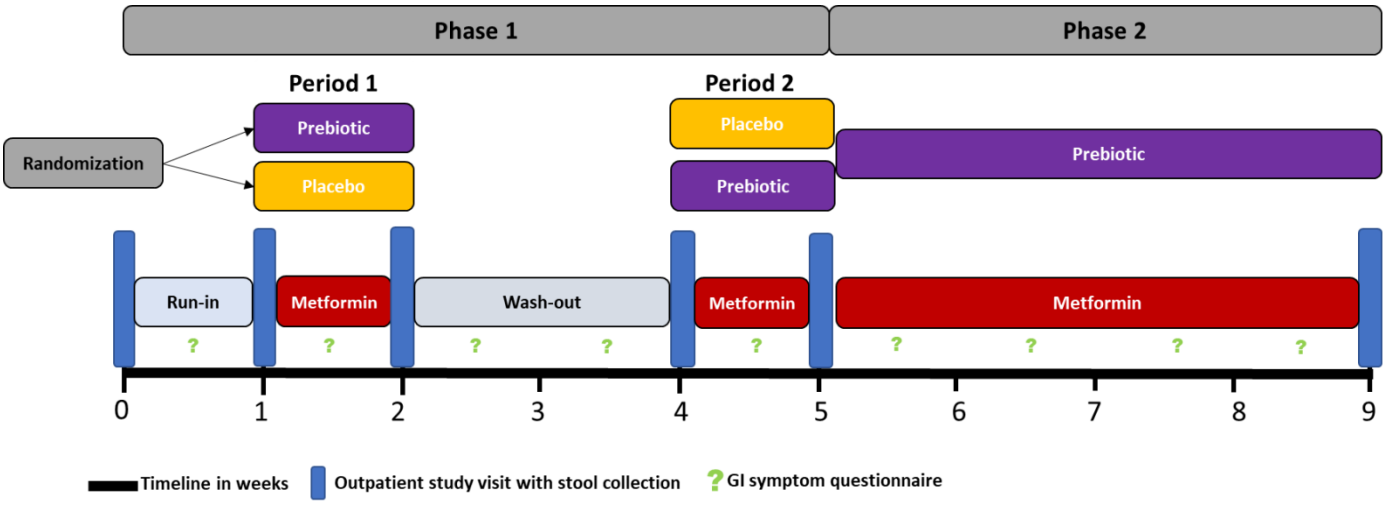

Figure 4. Study Design and Timeline

Phase 1: This is a randomized double-blind cross-over study of youth with T2D with two intervention periods (metformin + prebiotic MM BiomeBliss® ®, and metformin + placebo). Participants will have 5 study visits over the 5-week period. After screening history and physical examination are performed at visit 1, participants will enter a 1-week run-in period in which metformin therapy will be discontinued and blood glucose will be monitored twice daily via finger stick or with a continuous glucose monitor. The order of intervention will be randomized at Visit 2; when initiating metformin, participants will have 1-3 days of dose escalation, then arrive at a final dose of 850mg twice daily. After 7-9 days of treatment, participants will be evaluated as described in Table 1, then enter a 2-week washout period of prebiotic/placebo and metformin, and then cross over to the opposite intervention for 1 week.

Phase 2: This is an open-label 4-week extension of metformin + prebiotic MM. Participants will receive a 1-month supply of metformin and prebiotic at the end of Phase 1 (Visit 5) and be evaluated for measures of stool metabolite and glycemia at the end of the trial (Visit 6).

Every effort will be made to adhere to the proposed timelines, but some flexibility is required for scheduling of other studies, unanticipated equipment maintenance, etc. Scheduling variations will not be reported.

3.2 Study Protocol

Table 1 illustrates study procedures and timing over the 2 study phases. All visits will take place at the NIH Hatfield Clinical Center. Except for the screening visit, participants will be evaluated after an 8-10 hour overnight fast. Detailed descriptions of each procedure/ visit are given below.

Table 1 – Study Procedures and timeline

	Visit 1	Run in (7-10 days)	Visit 2	Period 1 (7-9 days)	Visit 3	Wash out (2-3 weeks)	Visit 4	Period 2 (7-9 days)	Visit 5	Open Label (4 weeks)	Visit 6
Histories and Questionnaires											
History/Physical	X		X		X		X		X		X
Quality of Life Questionnaire			X		X		X		X		X
Food Record Kept		X								X	
GI Symptom Questionnaire		Every 3 days		Daily		Every 3 days		Daily		Every 3 days	
Testing											
Mixed Meal Test and other blood tests					X				X		X
Urine Collection	X		X		X		X		X		X
Stool sample collected			X		X		X		X		X
DXA			X								
Daily CGM worn		X		X		X		X		X	
Accelerometer worn				X				X		X	
Study Agents											
Study Medications Given			X				X		X		
Pill/Bottle count					X				X		X
Meals given from metabolic kitchen			X				X				

Subject Identification: potential participants will be referred by local providers responsible for clinical care. This includes the Children's National Multidisciplinary Type-2 Diabetes clinic, as well as from other community providers to whom we will provide information about our study (see recruitment strategies, 8.4). Interested participants will be given our study information and will contact us via the study line or email. Participants will be phone screened to determine

baseline eligibility and additional study information. If participants are eligible, we will arrange a screening visit at the NIH.

Screening visit (Visit 1): After initial screening for eligibility and informed consent process completed, participants will undergo history and physical examination, routine bloodwork, and urine test to determine eligibility. Nutrition staff will also obtain body circumference measures, such as neck, waist and hip circumference. These measurements will be done over minimal clothing with a non-stretch measuring tape.

Participants will receive instructions on stool collection at home and stool collection kits - one to be mailed back to the study team for analysis prior to stopping their baseline metformin dose (unless they are able to provide a stool sample after consent while still at NIH), and one to collect a sample in the 24 hours prior to visit 2 if has a bowel movement. Patients will also be taught how to fill out daily surveys to assess GI symptoms (see details below). They will meet with a member of the nutrition team to select foods for their metabolic diet and to be educated on completing a 3-day food record. Participants will also be educated on foods we will ask them to avoid during the study, such as pre/pro-biotics (kombucha, kefir, yogurt, etc.) and foods containing non-nutritive sweeteners.

Period 1 (Drug-free run-in, Visit 2 and 3)

Drug-free run-in period: Participants will discontinue metformin for the 7-10 days preceding Visit 2, to assess their metabolic and gut microbiome response off anti-diabetic medications, as well as to wash out the immediate effect of metformin on the participants microbiota. The timing of this 1 week period was chosen because changes in gut microbiome have been observed in as little as 1 day and on average 1 week [33]. During this time, participants will complete daily GI symptom questionnaires and monitor their blood glucose and upload their readings daily while not taking metformin. Participants with persistently elevated fasting glucose readings and/or signs and symptoms of metabolic decompensation (e.g. vomiting, dehydration, lethargy, abdominal pain, ketonuria and acidosis) will be withdrawn from the study and restarted on his/her medication. During the drug-free run-in period, individuals who cannot either wear the continuous glucose monitor (CGM), or complete at least 2 finger stick blood glucose measurements daily will be withdrawn from the study.

Visit 2: This visit will take place 7-10 days after discontinuing metformin. Study personnel will review dosing and the completeness of GI questionnaires, as well as glucose trends with the participants. Participants will leave with a study metformin supply from the NIH pharmacy, as well as bottles of either placebo or prebiotic for a 1 week +15% supply and be instructed to take metformin and the powder mixed with a shake provided by the study team at the same time twice daily, with the first dose given with dinner that evening. Participants will take metformin 850mg with dinner on day 1-3and the dose will be increased to 850mg with breakfast and with dinner on day 3 and thereafter. Participants will fill out daily GI symptom questionnaires and will be instructed to contact the NIH study team if they are unable to tolerate the medication. Participants will be provided with 1 week of food to take home and consume on the week prior to Visit 3. The NIH Clinical Center Metabolic Kitchen will pack out meals for 7 days. Energy provided will be estimated using Mifflin St Jeor equation and an activity factor of 1.3 [34]. Participants will also leave with an accelerometer to be worn around the wrist 24 hours/day during the period between visits 2 and 3.

Visit 3: Participants will return to the NIH CC 7-9 days after visit 2. Stool and urine sample collection and bottle/ pill counts and assessment of uneaten food will be performed, and the accelerometer will be returned. Participants will undergo a MMT (see description below). Completeness of GI symptom questionnaires and glucose trends will be reviewed.

Period 2 (Washout, Visit 4 and 5)

Participants will be instructed to stop taking all metformin and MM/placebo powder during the washout period between visit 3 and 4; instructions for CGM and hyperglycemia precautions that were given at the screening visit will be reviewed with the family. The 2-3 week washout was chosen to minimize any carry-over effects and has been optimal for detecting gut microbiota after discontinuing therapy [9, 27].
Visit 4: Visit 4 will occur at the NIH CC 14-24 days after the beginning of the wash out period; The NIH Clinical Center Metabolic Kitchen will pack out frozen and non-perishable items to provide meals for 7 days. Participants will also be asked to wear the accelerometer on wrist until they return for visit 5.
Visit 5: Participants will bring a stool sample and study medications and have identical assessments as described in visit 3 above. They will also be reminded about keeping food records, which they will be asked to do prior to visit 6

Phase 2 (open-label study and Visit 6)
At the end of Visit 5, patients will leave with a 1-month supply of metformin and MM, to continue taking twice daily, as well as an accelerometer device daily for 4 weeks. **Final study Visit 6** – Participants will undergo assessments as outlined in Table 1 and receive counseling on resuming their medication regimen from prior to the study, as well as follow up nutrition counseling on healthy diet and lifestyle practices.

Rescue visit for GI intolerance
If participants are unable to tolerate 850mg twice daily during the study, they will be withdrawn from the study and have the option come in for a rescue visit, where they will provide a stool sample and obtain bloodwork. They will then be offered the opportunity to enter the open label arm using BiomeBliss® in combination with their highest tolerable metformin dose.

3.2.1 Study Procedures

- **GI symptom questionnaires**: A questionnaire derived from validated sets used for GI symptoms of irritable bowel syndrome [35] which previous investigators have used [27]. Assessments of stool consistency (not applicable, very hard, hard, formed, loose, watery), urgency to evacuate (no need to evacuate within 3 hours after dosing, need to evacuate within 3 hours, need to evacuate within 2 hours, need to evacuate within 1 hour), daily bowel movements (at least 1 movement every 3-4 days, at least 1 movement every 2 days, at least 1 movement per day, at least 2 movements per day), bloating sensation (not applicable, mild, moderate, severe), flatulence (less than normal, normal, moderately increased, greatly increased), and evacuation completeness (not applicable, incomplete, constipated), in addition to a King's stool chart, will be included (see Appendix A) [27]. This survey will be available in an online platform designed for mobile device but may also be filled out in paper form.
- **Blood glucose monitoring**: Blood glucose will be monitored with either fingerstick blood glucose twice daily (fasting and bedtime) or by using an FDA- approved continuous glucose monitoring device (CGM) (for example, the Freestyle Libre). The device will be used to monitor glucose in real-time, approximately every 5 minutes. The system consists of a small sensor, transmitter, and hand-held receiver. The small sensor, with a small needle attached, will be inserted subcutaneously. The transmitter, which is attached to the sensor, will send the measured glucose to the receiver. The sensor is changed at least once every two weeks. Participants will receive training on removing and replacing the sensor and education on the signs and symptoms of hyperglycemia (see Appendix B). Participants will be asked to upload their readings daily to be monitored by a member of the study team daily while participants are not taking metformin, and weekly while taking metformin. Participants will be asked to contact the study team immediately after confirming with a finger stick BG if their glucose is low (<50mg/dL), or if they have a fasting blood glucose reading of >200mg/dl, or a reading anytime of >300mg/dl. Participants with persistently elevated fasting glucose readings and/or signs and symptoms of metabolic decompensation (e.g. vomiting, dehydration, lethargy, abdominal pain, ketonuria and acidosis) will be withdrawn from the study and restarted on his/her medication.

- **Activity and Sleep Monitoring**: Free-living physical activity and sleep quality will be quantified using a small, non-invasive, portable watch accelerometer (GT3X+ by Actigraph Inc., Pensacola FL) worn on the participant's wrist. Physical activity levels, sleep duration and efficiency, amount of time spent in sedentary, moderate, vigorous intensity categories and estimated activity-associated energy expenditures will be extracted using established predictive equations. This is an FDA 510(k) cleared Class II medical device.
- **Urine sample collection**: urine will be collected at every visit for storage; for females, this same sample will be used for a pregnancy test
- **Stool sample collection:** Participants will bring stool samples from home collected in the previous 24 hours to be processed by the study team or stool samples will be collected during outpatient visits. The stool sample will be stored at 4C in a sterile plastic vial and processed same day (see Appendix C).
- **Mixed Meal Test (MMT):** One intravenous catheter will be placed in the arm for blood draws upon arrival on the morning of the MMT.A liquid meal to provide ~30% of the estimated daily calorie requirements for weight maintenance determined by the Mifflin St. Jeor equation with an activity factor of 1.3 [34]. Blood samples will be obtained at 0, 10, 20, 30, 40. 50, 60, 90, 120, 150 and 180 minutes to measure hormones and metabolites including glucose, lactate, free fatty acid, triglyceride, C-peptide, and insulin concentrations. Blood to measure markers of intestinal permeability will be obtained at time 0.
- **Quality of Life Questionnaire**: Participants will fill out the PedsQL quality of life questionnaire [32] (see Appendix D).
- **Food Records:** Participants will review their food records with a member of the nutrition team. If participants do not bring records to this appointment, the nutrition team will perform a 24-hour recall capturing foods consumed in the day prior to admission (see Appendix E).
- **DXA scan** – dual x-ray absorptiometry scan will be performed to evaluate body composition and determine fat mass and lean body mass.

3.3 Inclusion and Exclusion Criteria

3.3.1 Inclusion Criteria

1. Age 10-25 years
2. Pubertal or post-pubertal: Girls – Tanner stage IV-V breast; Boys – Testicular volume >10cc
3. Diagnosis of type 2 diabetes by ADA guidelines [36] or with established diagnosis previously treated with metformin.
4. Negative test for diabetes-related autoantibodies (glutamic acid decarboxylase 65 and tyrosine phosphatase-related islet antigen 2 (IA-2)) documented in NIH CRIS chart or via outside laboratory assessment within the last 10 years.
5. Hemoglobin A1C <8% at study initiation

3.3.2 Exclusion Criteria

1. Pregnancy or breastfeeding
2. Allergy to study medications
3. Allergy or self-reported intolerance to blueberry, pomegranate, or oats, soy, gluten or dairy products.
4. Chronic insulin therapy or insulin use within the last 3 months
5. Treatment with other medications which are known to affect the parameters under study, including antibiotics within the last month, immunosuppressants, proton-pump inhibitors, supraphysiologic systemic steroids, probiotic or prebiotic supplements
6. Heavy yogurt consumption (2 or more servings of ≥6 oz per day)
7. Chronic GI disease, gastric bypass surgery, cancer diagnosis or autoimmune disease

8. Metabolic derangement such as metabolic acidosis, severe hyperglycemia (fasting blood glucose ≥ 200mg/dL), and/or liver enzymes > three times the upper limit of normal.
9. Any other condition that, in the opinion of the investigators, will increase risk to the subject, or impede the accurate collection of study-related data.
10. Body weight ≥ 450lbs
11. Body weight ≤58kg
12. Hemoglobin concentration <10g/dL

3.3.3 Rationale for Inclusion Criteria

Age Range 10-25 years and pubertal status

The age range of 10-25 years was chosen to maximize recruitment and includes a wide range of youth afflicted with type 2 diabetes. All participants will be pubertal or post-pubertal youth (defined as Tanner stage IV-V for girls and testicular size 11-25cc for boys) to minimize any potential differences in gut microbiota associated with insulin resistance of puberty. We believe this approach is reasonable because we will have a well-defined cohort (by pubertal status). In addition, the broad content of the microbiome is of similar structure after 3 years old until later adulthood [29]
Pubertal status will be defined based on breast examination (Tanner stage IV-V) in girls and testicular examination (11-25cc) in boys. Pubic hair and bone age will not be used to characterize pubertal stage.

Diabetes diagnosis criteria and HbA1c <8%

Type 2 diabetes in youth will be diagnosed according to ADA criteria [36]and eligibility criteria were based on the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study design [37]. Youth with HbA1c ≥8% often have severe hyperglycemia and glucotoxicity which is associated with increased risk for dehydration and ketoacidosis and these conditions may be associated with. changes in the microbiome [38, 39].

3.3.4 Rationale for Exclusion Criteria

Pregnancy and Breastfeeding

Pregnancy and breastfeeding are associated with physiologic increases in insulin resistance, which would confound data interpretation. In addition, the DXA scan cannot safely be performed in pregnant women. Female participants of childbearing age who meet other eligibility requirements and wish to participate in the study will be informed of the potential risks to a pregnancy conceived while on any study medication. Participants who consent to participate will be asked to practice reliable birth control including systemic hormones and/or barrier methods. Patients who are pregnant and/or sexually active and not using adequate birth control will be excluded from enrollment in the trial. Pregnancy tests will be obtained from all female participants before inpatient visits and right before DXA scans. If a subject becomes pregnant while on study they will be removed from the study and will not be allowed to re-enroll on the study

Chronic insulin therapy

Participants who are currently taking insulin therapy or who are within 3 months of insulin treatment will be excluded from the study, as the run-in period would be riskier for these individuals.

Chronic GI diseases or medications/supplements affecting the GI tract

Individuals who have a disease, such as known autoimmunity, inflammatory bowel disease or celiac, may have altered gut microbiota due to that underlying condition. Certain medications are known or theorized to influence the gut milieu, and so to avoid confounding in this small study, we will exclude these patients as well. Additionally, yogurt or other pre/pro-biotic supplements will change the gut microbiome.

Any condition which may increase risk to the subject or impede accurate data collection

Examples include participants with a history of active thyroid disease, liver disease, pancreatitis, nephrotic syndrome or lupus.

Weight ≥ 450lbs
Individuals with weight ≥ 450lbs will be excluded because the maximum weight for the dual energy absorptiometry (DXA) scan is 450lbs.

3.4 Randomization

The NIH Clinical Center (CC) Pharmacy will perform randomization. The randomization schedules will be constructed by an independent statistician and shared with the NIH CC Pharmacy. The PI and study staff will not view the randomization schedule *a priori*. Subjects will be randomized as they enter the protocol as follows: The PI or study staff will notify the NIH CC pharmacy of the eligible enrolled participant and the pharmacy will assign the intervention arm according to the predetermined randomization schedule.

3.5 Study Agents/ Intervention

Three study agents will be used: metformin standard release 850mg oral tablet, BiomeBliss® powder, and placebo powder. Metformin will be used within the approved dosing regimens. BiomeBliss will be used according to its labeled instructions, see package label (Appendix C)

Blinding of the investigators and participants will be maintained throughout until the end of the study with the exception of our research nurse who can communicate with participants about any problems with the powder they may be taking. Investigators will be unblinded in the case of a participant needing to be withdrawn due to metformin intolerance; if the intolerance is noted during treatment with placebo, participants will be offered the option to immediately enter the second phase.

The following placebo was chosen to ensure appropriate blinding and similar sensory characteristics for the placebo and product: the placebo (Gatorade G2 (low sugar) powder, a mix of Glacier Freeze and Grape flavors The placebo will be formulated and packaged by NIH Clinical Center Pharmacy as outlined in the Table 2 below and based on the following justifications:

1. The non-fiber carbohydrate content and calories are comparable in the placebo and BiomeBliss. Although the total carbohydrate content is different, the calories provided, and the non-fiber carbohydrate content are similar between placebo and Biomebliss. It was important to ensure that there was no fiber in the proposed placebo because the fiber (beta glucan and inulin) is the active ingredient under study. We expect that the placebo should have no impact on stool consistency or frequency, so that we can best evaluate the effect that the study fiber supplement has on the stool.

2. Comparable non-nutritive sweetener (NNS) in placebo and BiomeBliss. The proposed placebo is sweetened with a non-nutritive sweetener rather than by sucrose alone to help keep the mass of each dose similar and isocaloric. We expect that stevia (a "natural" NNS) and the artificial NNSs, such as sucralose and acesulfame, will have comparable but negligible effects on glycemia in the short term [40]. However, we acknowledge that the effects of NNS on the microbiome is an emerging area of research. The data on the effects of non-nutritive sweeteners and their potential alterations of gut microbiota are controversial. Over the last decade, there is an increasing number of rodent and in vivo models indicating modest changes in gut phyla with NNS doses that far exceed the maximum acceptable daily intake [41-45]. The clinical impact of these changes is still unclear; human studies are scarce and conflicting. A handful of clinical trials, using acesulfame do not agree on whether this sweetener exerts a change in gut microbiota [46-48]. To our knowledge, there are no

human trials rigorously evaluating gut microbiome with stevia and sucralose [49]. Additionally, we expect that during the phase 2 when we expect to see changes in the microbiome, our participants will be consuming NNS in possibly higher quantities in their usual diet. We will be asking them to avoid diet sodas and other items known to be sweetened principally with NNS (see appendix G), but even foods that are not advertised as "diet" or low-sugar frequently contain NNS, so they will be impossible to avoid in a real-world study.

Table 2: Biome Bliss and G2 Placebo Nutrition Facts

	BiomeBliss	**Gatorade G2 Placebo Mixture** (4g Grape + 15g Glacier Freeze)
Calories per serving	60kcal (27g)	63 kcal (19g)
Ingredients	Inulin, Blueberry Extract, Beta-glucan (oats), Soy Protein Isolate, Pomegranate Flavor, Xanthan Gum, Citric Acid, Stevia Extract	**Glacier Freeze:** sugar, citric acid, salt, sodium citrate, natural flavor, monopotassium phosphate, modified food starch, calcium silicate, sucralose, acesulfame potassium, blue 1 **Grape:** sugar, citricacid, salt, sodium citrate, monopotassium phosphate, natural and artificial flavor, cornstarch, calcium silicate, sucralose, acesulfame potassium, blue 1, red 40
Dietary Fiber	9g	None
Total Carbohydrate	22g	17.5g
Non-fiber Carbohydrate	13g	17.5g

The NIH CC Pharmacy and the prebiotic fiber will be packaged and dispensed in a similar container. Each product will be mixed in a pre-specified shake, provided by the study team, immediately prior to consumption. Mixing the powders with the shake prior to consumption will assure that consistency is also similar.

3.5.1 Dose titration for gastrointestinal intolerance

At the start of each period, participants will take metformin 850mg daily, regardless of their dose prior to beginning the study. Participants will be titrated up to 850mg twice daily within 1-3days of Period 1 and 2. For participants who experience intolerable gastrointestinal side effects, metformin dose will be decreased by 850mg. Examples of such side effects include 3 or more diarrheal episodes or intense discomfort or cramping for more than 3 hours. If gastrointestinal symptoms resolve x 2 days, and at the discretion of the PI, the dose of metformin may be increased again to 850mg twice daily. Participants who are unable to tolerate 850mg daily will come for the rescue visit and be withdrawn from the study.

3.5.2 Dispensing and Storing Study Medications

The NIH CC Pharmacy will acquire and store metformin and BiomeBliss® powder for the study and will formulate and compound the placebo powder. The study medication will be coded and dispensed by the NIH CC Pharmacy. The

pharmacy will dispense the study medication according to the predetermined randomization table. The Randomization section contains additional details on the processes we will use to assign randomization. The storage of medications will be according to the package insert for each study drug. Medication will be supplied in 7 day +15% supply at Visit 2 and 4.

3.5.3 Compliance and Adherence

When potential participants are considering participation, the importance of compliance with the intervention assignment will be stressed. The ability to adhere to the protocol of the study, and take medication twice daily, as instructed, is one of the inclusion criteria of the study. Medication adherence will be determined by study personnel counting of pills and sachets that were dispensed at each medical visit and returned at the next medical visit (Visits 3 and 5). The percent of pills and sachets taken over each intervention period will be assessed. We will employ the following strategies:

1. Fifteen-percent extra of metformin tablets and powder sachets will be given to the participant to improve compliance, as the participants will not be told how many extra doses are sent (although they will be told that there are more than the exact number of pills so that we can be sure they are taking it). Participants will return the remaining doses and the number of unused tablets/sachets will be recorded.
2. Adherence to blood glucose monitoring will be assessed by reviewing CGM or glucometer downloads at every visit.
3. The participants will be contacted frequently by text message to encourage and assess compliance. Text and email-based reminders are an important adherence tool which can be tailored to the needs of the individual youth and family. Text messaging has been shown to improve outcomes in children with other chronic diseases, such as asthma [50] and sickle cell anemia [51] and in adults with type 2 diabetes [52].

3.6 Follow-up and Post-Study Treatment

The patient's clinically relevant data, available at the end of the study, will be shared with their diabetes provider with the consent of the participant and the parent. At the family's request, it will also be provided to his or her primary care provider.

Post-Study Obligations

There are no anticipated post-study obligations.

The study drugs (metformin and BiomeBliss®) will only be provided to participants while participating in this protocol. After completion or withdrawal from the study, if the patient would like to continue the medication, their clinically relevant data may be shared with their diabetes provider (with the consent of the participant and the parent) for further management.

4.0 Clinical and Laboratory Methods

4.1 Microbiome Analysis

Fecal samples will be collected in sterile cryovials and stored at 4C for less than 24 hours. The sample will be transferred to -80C and stored until further analysis for microbial diversity and phylla. This analysis will be performed in collaboration with Dr. Hariom Yadav at Wakeforest University. After thawing of fecal sample, fecal DNA will be isolated for microbial compositional analysis using PowerSoil® DNA Isolation Kit or comparable kit. Amplicon sequencing using next generation technology (bTEFAP®) will be used to for microbial sequencing using Illumina MiSeq and HiSeq platforms technologies. In brief, the 16S universal Eubacterial primers 27F AGAGTTTGATYMTGGCTCAG and 519R GTNTTACNGCGGCKGCTG will be utilized to evaluate the microbial ecology of each sample on the Illumina MiSeq with methods via the bTEFAP® DNA analysis service. A single-step 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) will be used under the following conditions: 94C for 3 minutes, followed by 28 cycles of

94C for 30 seconds; 53C for 40 seconds and 72C for 1 minute; after which a final elongation step at 72C for 5 minutes will be performed. Following PCR, all amplicon products from different samples will be mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples will be sequenced utilizing the Illumina MiSeq chemistry following manufacturer's protocols.

The Q25 sequence data derived from the sequencing process will be processed using a pipeline (MR DNA, Shallowater, TX). Sequences will be depleted of barcodes and primers then short sequences < 200bp are removed, sequences with ambiguous base calls removed, and sequences with homopolymer runs exceeding 6bp removed. Sequences are then denoised and chimeras removed. Operational taxonomic units (OTU) will be defined after removal of singleton sequences, clustering at 3% divergence (97% similarity)[53-56]. OTUs will be taxonomically classified using BLASTn against a curated GreenGenes/RDP/NCBI derived database[57] and will be compiled into each taxonomic level into both "counts" and "percentage" files. Count files contain the actual number of sequences while the percent files contain the relative (proportion) percentage of sequences within each sample that map to the designated taxonomic classification.

4.2 Short Chain Fatty Acid Analysis

Fecal samples will be grounded with a pellet pestle motor, followed by being resuspended in 1 mL PBS buffer (0.1 M, pH 7.4). After 4 h dissolution with 1 min vortex every 20 min, samples will be centrifuged (12, 000 g, 10 min) and passed through the 0.45 μm membrane filter. Cell-free samples were used for determining the concentrations of SCFA (lactate, acetate, propionate and butyrate) using a high-performance liquid chromatography (Waters-2695 Alliance HPLC system, Waters Corporation, Milford, MA, USA) with DAD detector at 210 nm, equipped with a Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA). Sample (10 μL) will be injected each time and H_2SO_4 (0.005 N) will be used to elute the column with a flow rate of 0.6 mL/min at 35ºC.

5.0 Statistical and Power Analyses

5.1 Sample Size Justification

Sample size estimates are based on the mean changes and standard deviations in GI tolerability score observed in the study of adult subjects taking the MM [27] including a mean tolerability score of 7 with standard deviation 4. Using alpha 0.05 with 80% power, we will need 10 participants to complete the study. Assuming a 50% attrition rate for this crossover study, we expect to recruit approximately 20 individuals. For the secondary outcome, our preliminary data reporting a mean change of 2.95±3.3 μmol/g of proprionate and 4.17±5.72 μmol/g of butyrate (Figure 3), 20 participants will achieve 90% power, assuming an α=0.05, and correlation coefficient of 0.5.

5.2 Analysis of Primary and Secondary Outcomes

The primary outcome, the participant's composite score of tolerability will be constructed using the principal component analysis (PCA) based on 4 GI side effect profile categories (stool consistency, urgency to evacuate, bloating sensation, and flatulence). PCA will be used to account for the expected high inter-patient variability. Comparison of mean tolerability score over 1 week will be with linear mixed models, adjusting for baseline score. Pre-specified covariates are: treatment period and sequence effects from the cross-over design.

The secondary outcome, change in mean stool SCFA over 1 and 4 weeks, will be analyzed using repeated measures of analysis variance (ANOVA) to consider correlation of observations within each participant. Participant and exploratory characteristics will be expressed as mean and standard deviation, median and interquartile range for continuous variables and frequency for categorical variables. Between group comparison of means will also be analyzed using repeated measures ANOVA. Spearman correlations will be used to compare relationships between continuous variables. A P-value of <0.05 will be used for statistical significance.

6.0 Collection and Storage of Human Specimens or Data

6.1 Research Use, Storage and Disposition of Human Subjects' Samples

For future reference and potential use, we will store all samples collected in this protocol in our locked freezers for an unlimited period of time. Samples will be labeled with coded identifiers linked to patient identity only via a secured database. Research records and data with personal identifiers will be stored in our locked offices, the medical record department, and the electronic study database. This material will additionally be protected by medical record and computer access procedures. Access to records and data associated with personal information will be restricted to the Principal Investigator, Co-Investigators, study support staff, study monitors and database support staff.

Subjects may request that unused samples be removed from our freezers and be destroyed. If no such request is made, we will keep samples until they are completely used or no longer of scientific value, at which time they will be destroyed. We do not plan to destroy personal medical information or stored data. The Principal Investigator will report loss or destruction of data or samples to the IRB.

6.2 Materials Transfer Information

Stored samples and/or data may be sent to outside collaborating laboratories, or shared with other NIH collaborating investigators, to study questions related to diabetes or its complications (including, for example: microbiome sequencing, stool metabolite analysis, diabetes, obesity, weight, and lipid metabolism). Samples may be sent to outside commercial laboratories for analysis. Samples and data sent to outside laboratories and collaborators for analysis and/or testing will contain only coded numbers, without personal identifiers. Materials Transfer Agreements will be completed before the exchange of samples and/or data with outside collaborators.

6.3 Genomic Data Sharing Plan

This protocol involves the collection of non-human genomic DNA. Fecal samples will be stored for analysis of microbial DNA. The participants will consent for their data to be shared through publicly accessible databases over the internet, NCBI or NIH's genebank. Data will be submitted at the time of publication and will include the relevant genomic data and corresponding phenotypic data. Users must agree to the conditions of use governing access to the public release data, including limitation of research to investigations consistent with the participants' consent, restrictions against attempting to identify study participants, destruction of the data after analyses are completed, reporting responsibilities, restrictions on redistribution of the data to third parties and proper acknowledgment of the data resource.

7.0 Multiple-site Studies

The primary site for study visits will be at the NIH Clinical Center. Primary enrollment and informed consent and assent will only occur at the NIH Clinical Center.

8.0 Human Subject Protection

8.1 Investigators and Roles

Please see Key Study Personnel document. Dr. Stephanie Chung (PI), Dr. Abby Meyers, Nurse Practitioner Michael Stagliano and Nurse Lilian Mabundo will obtain informed consent.

8.2 Informed Consent/Assent Procedures

Informed consent/ assent will be obtained as consistent with the requirements of SOP 12 and 14D. Written consent/assent will be obtained from each subject after detailed explanations of the planned procedures by the principal or a designated

associate investigator. Prospective subjects will be given the research information in layman's term and in language understandable to the subjects. The consent process will take place prior to any study procedures. Subjects have the right to withdraw participation from this protocol at any time.

8.2.1 Language for Minors

Written informed consent and assent will be obtained from the minor and his/her parent/guardian or (LAR) prior to any screening visits, study procedures or interventions. The Principal Investigator or other designated qualified protocol investigators will explain the study in language understandable to the parent/guardian or (LAR)). The investigator will also explain the study to the minor who is of a younger age and level of understanding. Enough time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. In accordance with 45 CFR 46.408, [the parent/guardian or (LAR)], will sign the current IRB approved informed consent. The minor ages 10-17yrs will provide written assent, as we expect that all of those enrolled will be capable of understanding and signing the assent document. The investigator will sign the assent as well. A copy of the consent and assent will be given to the minor and [his/her [parent/guardian or (LAR)] for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent CC medical record. The consent process will additionally be documented in the electronic medical record (CRIS).

For minors who reach the age of consent while enrolled in our protocol, the parent and the minor will be forewarned at the time of enrollment, that there will be a need to consent the minor on reaching the age of consent as consistent with the requirements of SOP-12 and 14D—Requirements for Informed Consent. This is because the prior parental permission and child assent are not equivalent to legally effective informed consent for the now-adult subject (consistent with NIH HRPP SOP 14D.8). All individuals will be given the opportunity to ask questions. In these situations, reconsenting will be performed at the NIH CC or using the telephone consent process.

8.2.2 Telephone consenting

When executing the consent process by telephone, the subject will receive a copy of the protocol consent in the mail prior to being consented. After they have had an opportunity to review the consent the investigator will contact the subject by telephone. The investigator will review the investigational nature of the protocol with the subject and answer questions. If the subject chooses to participate, the subject will sign and date the consent. The informed consent document will be mailed to the principal or associate investigator who led the discussion, who will sign and date and mail back a fully executed copy for the subject's records. The informed consent process will be documented on a progress note and a copy of the note and the original informed consent will be filed in the subject's record.

8.3 Rationale for Subject Selection

Type 2 diabetes in youth is a rapidly progressive and aggressive disease yet metformin therapy, the only oral- approved anti-diabetic therapy, is associated with GI sides effects in up to 80% of newly initiated patients and up to 30% of patients on chronic therapy. The burden of metformin-related GI side effects is magnified in youth because it is a potentially modifiable reason for medication non-adherence and subsequent poor glycemic control. Therefore, investigating a non-pharmacological supplement that may improve metformin tolerance in youth and young adults is of high clinical significance. Volunteers eligible for this study include mature adolescents and young adults with type 2 diabetes. Young adults will be included in the protocol to enhance recruitment and because the disease progression is similar to adolescents with type 2 diabetes. We will not recruit pre-pubertal or peri-pubertal children with diabetes because this increases the risk that they have type 1 diabetes (a condition not relevant to the aims of the current study).

8.3.1 Rationale for Exclusion of Vulnerable Populations

This study will involve recruitment of children age 10-17 years as described above. The study will not involve recruitment of other vulnerable populations including pregnant women, prisoners, adults who are or maybe unable to give consent because they do not meet the inclusion criteria for this study. We will protect participants as described in SOP 14A.

8.4 Recruitment Strategies

Tools used for the recruitment of subjects will include the posting of the study description on the NIH CC website, the NIH Record, the Clinical Center News, Children's National Medical Center (CNMC),newspaper advertisements and social media). Additionally, fliers will be placed on bulletin boards at different buildings throughout the NIH campus, the CNMC, George Washington University, the University of Maryland and other pediatrician or doctors' office sites in the Washington DC Metropolitan area. Recruitment material will be IRB approved prior to dissemination. Also, potential participants may be referred by local providers responsible for clinical care. This includes the Children's National Multidisciplinary Type-2 Diabetes clinic, as well as from other community providers to whom we will provide our study information and fliers.

8.4.1 Strategies to address the challenges of clinical trials in youth with type 2 diabetes

The research team is experienced in successfully recruiting youth with type 2 diabetes and familiar with the major challenges to pediatric research in type 2 diabetes in youth and young adults: (1) difficulty with recruiting eligible participants and (2) poor retention rates[58]. To maximize recruitment multiple techniques will be employed including: partnering with major organizations in the Washington DC metro area including CNMC, INOVA, Georgetown University, University of Maryland, Kaiser, and Johns Hopkins University, who we will meet with on a periodic basis and provide handouts for them to give to any of their patients who may be interested.

To improve retention in this study we will employ the following strategies including: periodic telephone, e-mail and/ or text message communication, as well as strategizing with individual families regarding their unique socio-economic barriers and offer alternative and supportive strategies to encourage study adherence (e.g. finding creative solutions to transportation issues).

8.5 Risks/Benefits Analysis including Considerations of Alternatives to Participation

This study involves r***esearch*** that is ***greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.***

8.5.1 Evaluation of Benefits

This research protocol is designed to elucidate mechanisms of the side effects caused by metformin and the ability of a supplemental product to improve those in patients who are already requiring metformin. Metformin is approved for the treatment of youth with type 2 diabetes 10 years or older and is standard of care. Limited data (Burton et al 2015) demonstrates that there may be both an improvement in GI symptoms as well as in glycemia for patients with type 2 diabetes, so there is a theoretical possibility that the MM will reduce GI side effects of metformin, and therefore quality of life, and improve blood sugars. Participating in the protocol procedures may also be beneficial to the individual because it may provide specific information about the individual's glycemic control and diabetes management, as well as more frequent interaction with the study team.

8.5.2 Evaluation of Risks

This research protocol will be associated with greater than minimal risk. The specific risks and our approach to minimize these risks are outlined below.

1. Blood Sampling: Peripheral blood draws (venipuncture) performed during this study for research will not exceed 10.5 mL/kg, or 550 mL (whichever is smaller) per 8-week period for adults. For pediatric patients, blood draws will not exceed 5 mL/kg in a single day, or 9.5 mL/kg or 550 mL (whichever is smaller) per 8-week period. The total blood volume for this study is 372 ml over the 9-week period. Patients may experience some discomfort at the site of the needle entry, and there is a risk of bruising at the site. There is a remote risk of fainting or local infection.

2. Intravenous Catheter Placement: The placement of intravenous catheters can be uncomfortable and pose the potential risk of bleeding, bruising and infection. All catheters will be placed under sterile conditions and universal precautions will be observed. Should any complications occur, they will be addressed immediately.

3. Risk associated with metformin
    i. Gastrointestinal upset: Nausea, vomiting, diarrhea, flatulence, abdominal discomfort and indigestion are common side effects associated with metformin use. To be eligible for this study, participants will have an indication to start metformin as first line therapy for type 2 diabetes, if they are not already taking it prior to the start of the study. The following actions will be taken to minimize the risk for gastrointestinal disturbance:
        a. Metformin dosing will be dose escalated over a 1-3-day time period
        b. Gastrointestinal symptoms will be recorded daily by participants and communicated with the study team with each submission of the online questionnaire.
        c. Participants who experience severe and/or recurrent gastrointestinal symptoms, defined as ≥ 3 diarrheal episodes or intense discomfort or cramping > 3 hours, will be withdrawn from the study.
    ii. Boxed Warning – Lactic acidosis (rare): The risk of lactic acidosis with metformin use is increased in elderly individuals with renal dysfunction or congenital heart failure. Participants with chronic renal or heart failure, or other chronic metabolic illnesses will not be recruited for this study.
    iii. Kidney problems: In individuals with kidney failure, diarrhea, nausea and vomiting may cause a loss of fluids and dehydration. Subjects with impaired renal function at baseline evaluation will not be recruited for this study.

4. Risk associated with drug-free periods
    i. Severe Hyperglycemia with metabolic decompensation: During the 7-10-day period between Visit 1 and Visit 2, and again in the 21-day period between Visit 3 and 4, youth may be at increased risk for severe hyperglycemia since the youth will be asked to stop their anti-diabetic medication. This risk is reduced because we will only enroll individuals who are in relatively good glycemic control (HbA1c <8%) who will be at lower risk for decompensation. Subjects will receive counseling on diet and lifestyle treatment to minimize this risk, as this is in keeping with current standard of care clinical guidelines as an important treatment modality for youth with type 2 diabetes [59].
    The following steps will be taken to minimize the risks of severe hyperglycemia:
        a. Participants at higher risk for severe hyperglycemia defined as those with HbA1c ≥8% will not be eligible to participate.
        b. All participants will receive up-to-date education on diet and lifestyle management by the PI and study staff.
        c. During the run-in drug free period all individuals will monitor glucose levels by CGM. Those who are unwilling to wear CGM will be required to check 2 fingerstick glucoses per day. Study staff will be in daily contact with participants to review trend uploads.

d. If CGM or glucometer readings show >200mg/dl fasting, or 300mg/dl bedtime is noted during the run-in period, they will notify the study PI immediately. Individuals with persistently elevated glucose levels and signs and symptoms of metabolic decompensation (e.g. vomiting, dehydration, lethargy, abdominal pain) will be withdrawn from the study and restarted on his/ her medication.
e. If vomiting, abdominal pain or dehydration is noted, participant will be evaluated by a medical professional or PI immediately for metabolic evaluation of acidosis and ketosis. If metabolic decompensation (acidosis or ketosis) is noted, participants will be promptly treated and withdrawn from the study.

5. Stool sampling: Stool sampling is not associated with any health risk but may be uncomfortable for some participants.

6. Radiation Exposure: Dual energy absorptiometry (DXA) scan. The DXA scan is a reliable and reproducible method to measure body composition, specifically body fat and lean body mass. The patient lies on a flat table with the x-ray source below the table and the detector above. Each scan takes about 5-7minutes. For the DXA scan the effective dose of radiation is 0.00006 rem, which is below the guideline of 5 rem (or 0.5 rem in children) per year allowed for research subjects by the NIH Radiation Safety Committee. The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. If participants want to learn more about radiation, they will be given the pamphlet: An Introduction to Radiation for NIH Research Subjects.

7. Activity and Sleep monitoring: There are no risk associated with these monitors, but participants may find them inconvenient to wear.

8. Continuous glucose monitoring system: According to the device manufacturer, there is minimal risk associated with the device. Possible side effects include but are not limited to local infection, inflammation, pain or discomfort, bleeding at the insertion site, bruising, itching. Because this protocol represents the first use of this device by our research team, we do not know the frequency of such complications of the device. A medical provider will be available should any of these problems occur.

9. Risk associated with prebiotic MM: The MM Biome Bliss is a fiber supplement that is available and marketed in the USA. In the dose prescribed for this study, the beta-glucan supplement will be equivalent to ~3cups of cooked oatmeal per day and provide ~ 40% of recommended daily fiber intake. This supplement can uncommonly be associated with minor gastrointestinal upset (stomach bloating, loose stools) and has not been associated with any serious adverse effects.

8.5.3 Alternatives to participation

Participation in clinical trials is completely voluntary. Refusal to participate will not affect a subject's ability to participate in other studies at NIH or elsewhere.

9.0 Adverse Event Reporting

9.1 Event Characterization and Reporting to the IRB

Unanticipated problems, non-compliance, and other reportable events will be reported to the NIH IRB as per NIH HRPP SOP "Reporting Research Events," Policy 801. All adverse events occurring during the study, including those observed

by or reported to the research team, will be recorded. All actual or suspected non-compliance, major deviations, unanticipated problems (UP), new information that might affect the willingness of a participant to enroll or remain in the study, or suspension or termination of research activities placed by the NIH or IC leadership will be reported to the NIH IRB as soon as possible but not more than 7 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director and IRB within 24 hours after the PI first learns of the event.

At the time of CR, a high-level summary (not a line item listing) of events will be submitted including: major and minor protocol deviations; noncompliance reported to the IRB that is not related to a protocol deviation; Adverse Events and Serious Adverse Events that do not meet the definition of an UP; and UPs reported to the IRB

9.2 Data and Safety Monitoring Plan

Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan. Audit and/or monitoring visit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

The collection, monitoring and analysis of adverse events will be the responsibility of the Principal Investigator and the investigative team.

As required by FDA 21 CFR 312.50, trial procedures will be subject to review to ensure compliance with the protocol and applicable regulatory requirements. Results will be reported to the Principal Investigator/Sponsor for further reporting to the FDA consistent with applicable regulations. The specific monitoring plan will be developed with the Principal Investigator and frequency of monitoring visits determined by such factors as study enrollment, data collection status and regulatory obligations. Monitoring visit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

9.3 Monitoring Subjects and Criteria for Withdrawal of Subjects from the study

9.3.1 Withdrawal Criteria

1. **Withdrawal of consent**. A subject wishes to withdraw from the study as stated in the informed consent (all subjects reserve the right to withdraw from the study without prejudice).
2. **Adverse event**. A subject experiences an adverse event that in the investigator's opinion necessitates withdrawal from the study. Specific examples of adverse events that would result in withdrawal are:
    a) Severe gastrointestinal intolerance to study drug (metformin therapy or prebiotic supplement) defined as ≥ 3 diarrheal episodes or intense discomfort or cramping > 3 hours.
    b) Diabetic ketoacidosis or severe hyperglycemia requiring insulin replacement.
    c) Severe hypoglycemia defined as: 1) severe symptomatic hypoglycemia with seizures or unconsciousness and blood glucose <50mg/dl or 2) fasting hypoglycemia <30mg/dl on two consecutive days with or without symptoms.
3. **Abnormal screening blood tests.** Subjects who have abnormal blood tests and who meet exclusion criteria will be withdrawn from the study by the investigator.
4. **Investigator decision**. An investigator feels it is in the subject's best interest to terminate participation. The detailed reasoning behind this decision will be documented.
5. **Protocol deviation**. Includes subject noncompliance with CGM during the run-in period, pregnancy, study entry criterion deviation, or start of a concomitant medication that would impede accurate study analysis. If a female participant's urine pregnancy test is positive, we will ask the girl's permission to inform her family so that she can get optimal medical care. During the drug-free run-in period, individuals who cannot complete at least 2

fingerstick blood glucose measures, wear CGM or who cannot be reached for >3 days during this period will be withdrawn from the study.

9.4 Protection of Participant's Privacy and Confidentiality

At the time of enrollment each subject is given a study-specific code name. The coding system is available only to study staff and kept in a secure, password protected database accessible only to study staff. The actual names of the subjects will not be provided or ever made available in any publication.

9.5 Investigational New Drug Determination

This dietary supplement will be purchased from the original manufacturer and dispensed by the research pharmacy. It is not being researched to market as a drug nor is it being investigated under the regulatory definition of a drug (cure, treat, diagnose, mitigate, or prevent a disease). The supplement is being used to decrease the side effects of metformin. The treatment of side effects is not considered a disease, and therefore per FDA part 312 does not meet criteria for requirement of an IND (see Appendix F).

10.0 Renumeration

All participants will receive financial compensation for their time per NIH Clinical Center guidelines for on-site visits (which occur at NIH), and additional compensation will be provided for specific procedures based on inconvenience units, as outlined in Table 3.

Table 3: Compensation per Visit/ Procedure

	Compensation	Estimated repeats	Total
Visit	$ 40	6	$ 240
MMT	$ 50	3	$ 150
DXA	$ 30	1	$ 30
Stool collection	$ 20	6	$ 120
GI Symptom Questionnaire	$ 1	30	$ 30
Completion Bonus	$ 250	1	$ 250
Total			**$ 820**

If a participant were to drop out, the participant will only receive payment for whatever visits and procedures they completed up to that point. If a participant does not complete daily GI symptoms questionnaires, he/she will be compensated for each that is completed. Compensation will be given in two installments: after Visit 5 and at the end of the study. **Total compensation for guardians accompanying minors for the entire study is $50 per visit x 6 visits = $300.** The guardians will only receive payments for whatever visits were completed and will be paid as a lump sum at the completion of the study.

11.0 Conflict of Interest

1. The National Institutes of Health reviews NIH employees at least yearly for conflicts of interest. The following link contains details on this process: http://ethics.od.nih.gov/forms/Protocol-Reveiw-Guide.pdf.
2. This protocol has investigators who are not NIH employees. They are expected to comply with their Institutions' conflict of interest policies.

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