FOOD STUDY PROTOCOL

Clinical Study Number: WB01-205

ClinicalTrials.gov Identifier: NCT04424888

Evaluating Use of Continuous Glucose Monitors in a Short-term 2x2-Crossover Study

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Study Site: Whole Biome, Inc., 933 20th Street, San Francisco, CA 94107

Type of Study: Food Study Research

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Version 1.0 / Original	28 February 2018
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I, the undersigned, have read and approve this protocol and agree on its content.

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James Bullard, Ph.D.

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Background:

Diabetes mellitus and its comorbidities are currently a global, public health crisis, with approximately 30 million people diagnosed with diabetes and an estimated 80 million additional people with prediabetes in the US alone. Although diabetes is a multifactorial disease, it is widely believed that the proliferation of diabetes worldwide can be largely attributed to the adoption of the Western diet. Recently, research has shown that the use of the Western diet has significant impacts on the gut microbiome, altering both the composition and activities of that microbiome (The microbiome refers to the bacteria, viruses, fungi, phage, and other microorganisms that reside in the human intestine).

An individual's gut microbiome contains 10 to 100-fold more genetic information than resides in the host's genome. There is increasing evidence that the gut microbiome offers an opportunity for the development of novel interventions for a variety of diseases by modulating the composition of the gut microbiome. Specific bacterial strains in the gut microbiome ferment indigestible dietary fibers in the colon to produce short chain fatty acids (SCFAs), such as acetate, propionate, and butyrate (Nohr-2013). Use of the Western diet leads to a reduction in both the number and density of butyrate-producing microbes. SCFAs, and butyrate in particular, bind to the G protein-coupled receptors, GPR119, GPR41 and GPR43, which trigger the downstream signaling pathway for GLP-1, a hormone known to play a key role in the development and progression of diabetes. These observations lead to a hypothesis that replenishing the content of butyrate microbes in the intestinal tracts of these individuals will have beneficial metabolic effects.

Whole Biome has developed a proprietary discovery platform to identify, characterize and manufacture beneficial bacterial strains. Whole Biome is developing a medical food containing a subset of commensal butyrate-producing strains and mucin regulating strains that can be grown under controlled conditions that meet the standards for Current Good Manufacturing Practice (cGMP), thus allowing them to be administered to humans.

Purpose:

The goal of this study is to characterize the performance of a Continuous Glucose Monitoring System in medical food studies. The *Freestyle Libre(™)* Glucose Sensors[1] will be used to follow subjects 10-day glucose trajectories over the course of a 5-week, double-blind, placebo-controlled, 2x2 crossover, medical food experiment. The medical food has been designed to increase butyrate production and promote the health of the colonic mucin layer.

[1] Abbott: https://www.freestylelibre.us/buying-guide

Study Product Safety:

All ingredients contained in the study product formulations used in this study have received Generally Recognized as Safe (GRAS) designation. They are commensal organisms that have been documented in multiple studies to inhabit the human GI tract of healthy individuals. The organisms were grown under controlled conditions consistent with Current Good Manufacturing Practices (cGMP) and employ no animal-derived products. The microbes and all ingredients utilized during manufacturing were food grade and qualified as Generally Recognized as Safe (GRAS). Each of the microbes have been characterized in detail (including whole genome sequencing). Each manufactured lot has also been tested with standard methods to ensure the absence of toxins, heavy metals, etc.

All strains have also been tested *in vivo* in a diet-induced obese mouse model and Sprague-Dawley rats. Each of these rodent models were dosed for 28 days at multiples ≥60 times (based on body weight) the amounts being administered in this study. In each study, safety was evaluated by clinical observation, routine chemistry, and hematology parameters, in addition to necropsy evaluation at study conclusion. In both studies, no clinical, laboratory, or necropsy observations were observed that suggest adverse effects.

In addition, a 5-person safety study was conducted with administration of 3 strains for 14 days. No adverse events or tolerability issues were reported.

Finally, both formulations being utilized in this study (WBF-0009 and WBF-0011) are currently being evaluated in an ongoing multi-site, double-blind, placebo-controlled, medical-food trial. This ongoing study is testing the product in approximately 60 subjects at six sites in the USA. It is currently still underway and to date there has been multiple subjects who have up to 12 weeks of exposure (in a 12-week trial). There have been no reports of significant related adverse events in this study to date.

Study Design:

A double-blinded, placebo-controlled 2x2-crossover experimental design is used to compare the following:

1. WBF-0009 (placebo: excipients only)
2. WBF-0011 (butyrate-producing strains + a mucin degrading strain)

	N	Duration per Period (days)	Period-1	Period-2
Arm-A	3	14	WBF-0009	WBF-0011
Arm-B	3	14	WBF-0011	WBF-0009

Table 1: Experimental setup; Two subjects will be randomized at a time to maintain balance across the arms.

Objective:

This study aims to characterize the performance of a Continuous Glucose Monitoring System in medical food studies. The *Freestyle Libre*(™) Glucose Sensors will be used to follow subject's 10-day glucose trajectories over the course of a 5-week, double-blind, placebo-controlled, 2x2 crossover, medical-food experiment. The medical food has been designed to increase butyrate production and promote the health of the colonic mucin layer.

Primary Endpoint:

To determine the operating characteristics, sources of nuisance variance and appropriate statistical tests when using Freestyle Libre(™) Glucose Sensors in an exploratory medical food studies.

Secondary Endpoints:

1. Change in body mass
2. Change in fecal qPCR measures of probiotic strain concentration
3. Change in fecal SCFA concentrations unadjusted and adjusted by glucose levels
4. Expected lifespan of CGM sensors
5. CGM-sensor scanning and photo logging compliance rates and usability feedback

A. Study Product Portion Selection:

Selection of study product portion size was guided by the exposure in Sprague-Dawley rats for 28 days and a human safety study as described above under Study Product Safety. In the human safety study, subjects consumed 7.0 x 10^9 CFU of strain WB-STR-0005, 4.0 x 10^9 CFU of strain WB-STR-0006, and 2.0 x 10^8 CFU of strain WB-STR-0009 daily for 7 days, followed by a 5-fold increase in consumption to 3.5 x 10^{10} CFU of strain WB-STR-0005, 2.0 x 10^{10} CFU of strain WB-STR-0006, and 1.0 x 10^9 CFU of strain WB-STR-0009 daily for an additional 7 days. The administered strains were detected in stool by the end of the first week of consumption, with an increase in quantity following the 5-fold increase in consumption.

B. Study Structure:

This is a double-blind, 2x2-crossover experiment consisting of two two-week treatment periods. Subjects are randomized to one of two arms; placebo-first or placebo-second. Upon completion of the first 14-day treatment period, all subjects undergo a 3-day washout period before beginning the second 14-day period. After the second treatment period, subjects undergo a 3-day washout period to complete the study. At completion of the study subjects will be interviewed using two structured questionnaires about the study design, procedures, date and changes in health (Appendix D) that they may have noticed during the course of the study.

C. Duration:

The total duration of a subject's participation will be approximately 5 weeks, including an *enrollment* visit occurring 2 days prior to the *baseline* visit.

Throughout the study, subjects will wear a 14-day continuous glucose sensor. This sensor will be applied on study day -2, 12, 26 at the same location on an individual's left arm.

On days -2, 12, 14, 26, 31 subjects will receive the Appendix A: Instructions for Collecting Stool Sample
On study days 1, 14, 18, 31 and 35, subjects will (1) deliver a fecal sample (self-collected over previous 36 hours), (2) take anthropometric measurements, e.g., weight, blood pressure, waist circumference, (3) as well as perform a Appendix C: Standardized Meal Test.
On study days 1 and 18 subjects receive study product.

D. Number of Subjects:

It is anticipated that approximately 10 subjects will be screened with ~6 subjects randomized to yield ~6 subjects completing the study (~3 subjects/arm).

E. Inclusion Criteria:

1. Age: 18 to 75 years of age
2. If female, must meet all the following criteria:
   2.1. Not pregnant or breastfeeding
   2.2. If of childbearing potential (including peri-menopausal women who have had a menstrual period within one year) must practice and be willing to continue to practice appropriate birth control (defined as a method which results in a low failure rate, i.e., less than 1% per year, when used consistently and correctly, such as double barrier methods [male condom with spermicide, with or without cervical cap or diaphragm], implants, injectable or oral contraceptives [must have been using for at least the last 3 months], some intrauterine contraceptive devices, tubal ligation, or in an established relationship with a vasectomized partner) during the entire duration of the study
3. Must be able to read, understand, and sign the informed consent forms (ICF) and, when applicable, an authorization to use and disclose protected health information form (consistent with Health Insurance Portability and Accountability Act of 1996 [HIPAA] legislation as modified in 2013)
   3.1. Must be able to communicate with the investigator, and understand and comply with protocol requirements
   3.2. Must be able to wear a CG patch and perform a scan no less than once every 8 hours for the duration of the sensor periods.

F. Exclusion Criteria:

1. Subjects who plan to use antibiotic, antifungal, antiparasitic, or antiviral treatment during the study
2. Subjects using a proton pump inhibitor must be on a stable dose that will be maintained throughout the study period
3. Subjects who plan to travel outside the United States during the projected study period

4. Subjects who have received an experimental drug within 30 days prior to study entry
5. Subjects with known wheat, soy, milk, peanut, or tree nut allergies
6. Subjects who have been diagnosed with a sexually transmitted disease including, but not limited to, HIV, syphilis, herpes, gonorrhea, hepatitis A, hepatitis B, and hepatitis C
7. History of any surgery on the gastrointestinal tract except appendectomy and cholecystectomy
8. Subjects with any condition that the investigator deems as a sound reason for disqualification from enrollment into the study

G. Study Schedule of Events

Day	Event Name	Subject Receives
-2 to 0	Enrollment Visit	Informed Consent Stool Collection Kit dispensed CGM Sensor & Reader placed
1	Visit 1 (Baseline 1)	Stool Collection Kit collected Meal Tolerance Test Randomization to Arm A or Arm B 14-days of study product (WBF-0011 or WBF-0009) Anthropometric Measurements
Day 1 to 14 On treatment first Arm		
12	Visit 2	CGM Sensor removed and new sensor (2) placed Stool Collection Kit dispensed
14	Visit 3	Period 1 Anthropometric Measurements Meal Tolerance Test Stool Collection Kit collected and dispensed Research Compensation 1/2
Day 15 to 17 Wash out period		
18	Visit 4 (Baseline 2)	Stool Collection Kit collected Anthropometric Measurements Meal Tolerance Test Dispensed alternate Arm study product 14-days of WBF-0011 or WBF-0009
Day 18 to 31 On treatment		
26	Visit 5	Stool Collection Kit dispensed CGM Sensor removed and new sensor (3) placed
31	Visit 6	End of treatment second Arm Period 2 Anthropometric Measurements Meal Tolerance Test Collect all study product Stool Collection Kit collected and dispensed
Day 32 to 34 Washout period		
35	Visit 7	Stool Collection Kit collected Research Compensation 2/2
Follow Up	Visit 8	Subject Interviews

H. Removal of Subjects from Study Participation:

1. Withdrawal of Consent - Subject wishes to withdraw from the study as outlined in the informed consent form. All subjects reserve the right to withdraw from the study without prejudice to themselves or their future medical care.
2. Adverse Event - Subject experiences an adverse event, which in the investigator's opinion necessitates withdrawal from the study.
3. Investigator Decision - Investigator assessment that it is in the subject's best interest to terminate participation.
4. Protocol Violation – Includes subject non-compliance, documented pregnancy, study entry criteria violation, and/or initiation of an unacceptable concomitant medication, especially antibiotics.
5. Lost to Follow-Up - Subject fails to return for study visits and cannot be reached with reasonable attempts.
6. Administrative Reason – Includes discontinuation of the study protocol by the sponsor, the Food and Drug Administration (FDA), or other regulatory authority, and/or discontinuation of the study site's participation in the study protocol.

Any withdrawal from the study will be fully documented. The documentation will include reasons for the withdrawal and details of any sequelae (followed through until the symptoms have resolved or returned to baseline levels) if the reason for withdrawal was an adverse event.

I. Subject Information and Samples:

Once screened and qualified for entry, subjects will be instructed as follows:

1. Take no new prescription or over-the-counter medications excluded by the protocol without prior notification of the investigator.
2. Remain on their usual diet and exercise regimen throughout the study unless instructed to change by the investigator.
3. Fast overnight for at least 8 hours (no food or beverage except water) prior to each visit. (Note: this includes tea and coffee.)

4. Do not donate blood for the duration of the study.

The sponsor should be contacted if the investigator is informed of any violations of these restrictions. The sponsor will decide whether a subject with restriction violations will be granted a deviation and allowed to continue study participation.

J. Study Food Product:

Study product will consist of coated capsules designed to pass through the stomach intact and disintegrate in the terminal portion of the small intestine. Subjects will be instructed to take 3 capsules of the food product 30 minutes before the morning and evening meals beginning with the evening meal on study-day one through the completion of 14 days dosing; and again on study day 18 through the completion of another 14 days dosing.

The capsules will be identical in general appearance, size, and color across all the treatment arms. Each formulation capsule will contain the designated commensal microbe mixture (Table 1); and inulin, sucrose, trehalose, glycerin, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, titanium dioxide, polyvinylpyrrolidone, skim milk, and colloidal silicon dioxide. Placebo (WBF-0009) capsules will contain colloidal silicon dioxide. All ingredients are GRAS certified.

1. Packaging and Storage

The study food product will be provided in sealed, labelled screw cap bottles. Each bottle will contain 45 capsules to support 7 days of administration (42 capsules), plus 3 excess capsules to allow for replacement of lost capsules, etc. Additionally, the capsules for all treatment arms, will have identical appearances to support the study blind.

The study food product should be refrigerated at 4 ^{o}C except when the subject removes the bottle to take out 3 capsules scheduled for ingestion 30 minutes before the morning and evening meals. Once 3 capsules have been removed, the bottle should be tightly capped and returned to the refrigerator immediately.

The total amount of food study product needed for the duration of the study is listed in the table below. This is assuming 3 subjects per arm per period.

	Period-1 Bottles Needed	Period-2 Bottles Needed	Period-1 Daily Doses Needed	Period-2 Daily Doses Needed
WBF-0009	6	6	84	84
WBF-0011	6	6	84	84

Table 2: Study Product given enrollment from Table 1.

2. Dispensing of Study Food Product

Study materials will be provided to subjects by the investigator, medically qualified sub-investigator, or other qualified study-site personnel. The study food product will be provided with a lunch bag and ice packs to keep the product cold until it can be refrigerated. Under no circumstance will the investigator or sub-investigator(s) allow the study product to be used other than as directed by the protocol or to be administered to any persons other than subjects who have signed an informed consent form and are participating in the study.

Study personnel will be responsible for instructing subjects to administer the correct amounts of study product, i.e. 3 capsules 30 minutes before each morning and evening meal.

3. Study Product Accountability

The investigator listed will be responsible for study accountability. **Under no circumstances will the investigator or sub-investigators allow the study product to be used other than as specified in the protocol.**

4. Amount Administration, Route and Schedule

Study food product will be self-administered by the subject 30 minutes prior to the morning and evening meals as described above.

Study product dispensing and returns will be recorded on a study product disposition form, to provide a complete accounting of the receipt, disposition, and return of study product. At the completion of the study, all remaining used and unused study product, empty containers, and copies of the completed study product disposition forms will be returned by the subject.

K. Study Methods:

Prior to study visits requiring a standardized meal test, subjects will be asked to fast overnight for at least 8 hours (i.e., no food or beverage except water). Subjects should report to the clinical study site in the morning of each scheduled visit (ideally between 0800 and 0900 hours). For all study visits which require the subjects to be fasting, the subjects should refrain from administering the morning portion of study product prior to the visit and bring the morning's portion of study product with them to the study-site to take in conjunction with their standardized meal.

Subjects will be utilizing an android app to read (scan) and record any meals consumed

during the study, including all food, drink and alcohol (not including tea, coffee and water). If subjects have an android smart-phone they can download the app onto their own smart-phone if they would like to. If they don't have an android smart-phone or if they prefer not to download the app for the study onto their own smart-phone they will be issued a loan smart-phone for the duration of the study.

Unique Study Procedure	Procedure Description
CGM sensor-instructions	• Subjects are instructed on using the patch and reader device. First, before distributing reader or applying patch, instruct the subject to refrain from using any data produced by the system in a 'treatment' context. Re-iterate that this is a research study and that this is not a medical device. Continuous glucose data will not be available to subjects until the end of the study. • Ensure subject is not likely to be swimming or submerged for more than 30 minutes at a time, subject will not be in extreme temperatures, will not remove the patch and will let us know if the patch has failed or fallen off during the study. Furthermore, if these events do occur subjects will be instructed to add a comment using the provided application. • Ensure that the subject can scan the sensor at least every 8 hours. Instruct subject that they may be notified via SMS (text message) if they have not scanned within the last 8 hours. Subjects should connect the reader devices to a wifi network at home or at work. If no wifi network is available to subject, ensure that subject brings reader device to study site at each visit. • All subjects should use the same location on the left arm to apply the patch, unless there are specific issues with the left arm, e.g., scarring, tattoos, etc. If subject is unable to use left arm, subject should consistently use right arm throughout the study. • After subjects have the sensor applied (see 'Apply-sensor'). Demonstrate scanning the sensor and confirm that device is correctly associated with subject. • The reader will stay with a subject for the entire study and will be stickered with a subject-specific barcode. • Demonstrate the distinct ways of scanning the patch (underarm/overarm). Instruct subject on location of sensor on reader device. • Demonstrate adding comments using reader device using both notes and photos. Instruct subject that photographs of the food to be consumed should be taken at every meal, before any food or beverage is consumed. This excludes water, tea and coffee, but includes alcoholic beverages. If the subject misses a consumption event, they should add a comment including the time of consumption, e.g.,'ate burrito -2 hours' or 'ate burrito 2 hours ago'

	• Instruct subject on procedure for a sensor being knocked partially free. Subject should lightly press and hold the sensor for a moment to reaffirm adhesive. Subject should add a comment that sensor was 'reseated'
Apply-sensor	• Sensor should be applied to the left arm unless subject had previously applied sensor to right arm or subject wishes to use right arm. Subject should not change arm or arm location during study. Follow instructions for patch application. Instructions can be found here: https://freestyleserver.com/Payloads/IFU/2017_oct/ART28734-018_rev-B-Web.pdf and here: https://www.freestylelibre.us/sites/all/themes/freestyle_libre/pdf/FreeStyle%20Libre%20-%20Sensor%20Adhesion%20Guide.pdf.
Register-reader	• Study staff to scan reader barcode and subject barcode for reader registration.
Distribute-SCK	• Give subject stool collection kit (SCK) and describe stool collection procedure – written instructions included. • Explain importance of attempting to collect the sample at the same time of day for each fecal collection event throughout the study. We know that the microbiome varies regularly with things like time of day, time since last meal, etc. For most people, the most consistent time to collect a sample is shortly after waking up. • Upon receiving the kit, subjects should be instructed to store the unused kit in the freezer, so that the ice-packs can completely freeze prior to stool collection and transporting the sample to Whole Biome. • 36-12 hours prior to subject's standardized-meal visit, subjects should remove the collection kit from the freezer and following the procedure outlined in: Appendix A. After collecting the sample, return the sealed bucket to the cooler and return the cooler to the freezer for at least 12 hours before transporting it to Whole Biome. • Instruct subject that collected SCK should not remain outside of freezer for more than 4 hours in transit to study site for drop off.
Standardized Meal	• At 4 time points throughout the study, subjects will perform a fasted meal-tolerance test (Arm 1 Baseline & Measurements, Arm 2 Baseline & Measurements). This test is administered following a 8-hour fast. • Following a 8-hour fast, subjects will perform a pre-meal scan and finger-stick measurement of their blood glucose using a provided sensor and strip. Subjects will then consume a standardized meal supplement drink (See Appendix C). Optional: At 30 minutes subjects will scan the sensor and take a final blood glucose measure at the study site. Subjects will be asked to continue to fast for an additional hour, scanning the sensor again at minute 60 and 90.
Anthropometric	• Height, weight, waist-circumference, blood pressure, heart rate, blood oxygen

Measurements	
Subject Interview	• Structured questionnaire used to interview each subject. This will be optional.

Table 5: Study Procedure Descriptions

L. Ethical Safety Considerations:

The overall risk to subjects participating in this study is low. The safety of the microbes in the study product formulations are all commensal organisms manufactured according to cGMP standards and employ no animal derived products. (See Section A above for detailed discussion.)

M. Safety Assessments:

Safety will be assessed throughout the study by examination of reported adverse events (AEs).

Reporting of Adverse Events

Adverse events will be reported in accordance with the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 4.03. The CTCAE grades the severity of the AE based upon Grades 1 through 5 and lists unique clinical descriptions of severity for each AE.

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated or limiting age-appropriate instrumental ADL.

Grade 3: Severe or medically significant but not immediately life-threatening hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

Grade 4: Life threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

In the event of the occurrence of a same category of three or more grade 3 CTCAE or two grade 4 CTCAE or one grade 5 CTCAE, the trial will be paused, evaluated for safety by the Safety Monitoring Committee and allowed to proceed only after the Committee determines the causality is not associated with the investigational food study product.

Adverse Events and Subject Withdrawals

Adverse Event

Any untoward medical occurrence associated with the use of a therapy in humans, whether considered treatment related or not treatment related.

Life-Threatening Any untoward medical occurrence that places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious Adverse Event (SAE) ANY SAE THAT OCCURS AFTER THE SIGNING OF THE ICF THROUGH 30 DAYS AFTER ADMINISTRATION OF THE LAST AMOUNT OF STUDY MEDICATION MUST BE REPORTED IMMEDIATELY (WITHIN 24 HOURS OF KNOWLEDGE) TO THE SPONSOR'S MEDICAL MONITOR (858-342-7057). FAX THE SAE REPORT FORM TO THE NUMBER LISTED ON THE FORM.

An adverse event is considered "Serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Any SAE, unexpected events, or death encountered during the study and for 30 days thereafter will be reported to the principal and/or co-investigators by the subject or their representative in the case of death. The investigator will immediately report the SAE/unexpected event or death within 24 hours of being notified to the sponsor in writing for review and assessment/causality confirmation, and to the IRB (per FDA requirements).

Unexpected Adverse Event

An adverse event is considered "unexpected" if it is not listed in the study product data information document or is not listed at the specificity or severity that has been previously observed.

FDA guidelines recognize that:

1. "Individual adverse event reports generally require an evaluation of their relevance and significance to the study, including an evaluation of other adverse events, before they can be considered an unanticipated problem,"

and

2. "All reports to the IRB of unanticipated problems should explain clearly why the event described represents a 'problem' for the study and why it is 'unanticipated.''

Investigators are required to report unexpected adverse events that fit the following criteria *within 10 working days* of the time the investigator has become informed of the event(s) to the IRB if applicable and sponsor.

The following guidelines provide further definition to unexpected adverse events:

- Event is **unexpected** (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB- approved research protocol and informed consent form, or the investigator brochure; and (b) the characteristics of the subject population being studied,
- **Related or possibly related** to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research **places subjects or others at a greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

If the adverse event is clearly not related to the study food product, device, procedures, or washout process, it would not represent a risk to other subjects in the research or a "problem" for the study and, therefore, does

not have to be reported to the IRB.

1. ***Intensity***

The intensity of each adverse event will be characterized as mild, moderate, or severe, as follows:

- MILD: Usually transient, requires no special treatment, and does not interfere with the subject's daily activities.
- MODERATE: Usually causes a low level of inconvenience or concern to the subject and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.
- SEVERE: Interrupts a subject's usual daily activities and generally requires systemic drug therapy or other treatment.

2. ***Causality***

The investigator will grade the association of the adverse event as UNRELATED or RELATED to study medication. The following criteria should be considered for determining relatedness:

- UNRELATED: The adverse event is judged to be produced by the subject's clinical state or by other therapies administered to the subject.
- RELATED: The adverse event is judged to be related to the administration of study medication.

N. Data Management:

All data collected will be entered onto standardized Case Report Forms (CRFs) and entered into a database that will allow summaries and the statistical analyses outlined in the statistical analysis plan to be conducted. Upon conclusion of the study, all materials will be archived.

O. Statistical Considerations:

Analysis Populations:

- **Intent-To-Treat (ITT):** The ITT Population will include all randomized subjects who receive at least one portion of randomized study product, i.e. Placebo (WBF-0009) or WBF-0011.
- **Evaluable:** The evaluable population will include all ITT subjects who have had adequate exposure to the randomized study product during the 5-week treatment period.

P. Safety Analyses:

All non-serious adverse events occurring from the time of randomization through the subject's final study visit will be recorded in the adverse event electronic CRF. Adverse events will be collected for subjects who terminate early from the study until the time of their withdrawal.

All serious adverse events occurring from the time of randomization through the subject's final study visit will be recorded on the Serious Adverse Event eform (eSAE). Serious adverse events will be collected for subjects who terminate early from the study until the time of their withdrawal.

All adverse events will be tabulated and ordered by decreasing frequency for all subjects by study arm. If appropriate, the incidence of adverse events will be compared by Fisher's exact test. Special attention will be given to subjects who discontinue treatment because of adverse events.

Additionally, treatment emergent adverse events (TEAEs) of special interest, i.e., specific gastrointestinal symptoms, will be summarized separately, if deemed necessary.

All vital signs, and any laboratory results will be listed by subject and visit, including scheduled and unscheduled/repeat measurements. Laboratory assessments that are outside of normal ranges and/or with potential clinical importance will be flagged. Baseline values, the values at each visit, and changes from baseline values will be summarized descriptively for each of the quantitative laboratory assessments.

Q. Sample Size and Power Calculation:

The primary purpose of this study is to enable subsequent power calculations; the sample size has been selected to minimize recruitment time and study costs while obtaining sufficient precision for subsequent experimental design.

R. Informed Consent:

Each participant will be provided with oral and written information describing the nature, purpose and duration of the study, procedures involved in the study, participation/termination conditions, and risks and benefits. Prior to initiation of any study-related procedures, subjects will sign and date the informed consent form (ICF) to participate in the study.

S. Final Report:

All data, including subject characteristics, methodology, and clinical findings, will be presented in a final clinical/statistical report to be prepared by the sponsor or designee.

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Appendix A: Instructions for Collecting Stool Sample

You will be given a stool collection kit to take home so that you can collect the sample at your convenience.

Stool Collection Kit Checklist:

1) A Stool Collection Device (SCD) consisting of 3 separate components (Figure 1)
   a) A Stability Frame for positioning the collection bucket on the toilet
   b) A barcoded Stool Collection Bucket
   c) A Stool Bucket Lid with a label for the date and time of day
2) Disposable Gloves - 2 pairs
3) Sanitary Wipes
4) Ballpoint Pen
5) Stool Collection Bucket Cooler & Ice Packs

The Stool Collection Bucket Cooler with Ice Packs, Bucket and Lid should be stored in your freezer upon arriving home.

Collecting a Sample:

Note: Always use disposable gloves when handling an open bucket.

1) Obtain the Stool Bucket and Lid from the Stool Collection Bucket Cooler in your freezer.
2) Completely empty bladder.
3) Remove all items from the biohazard bag.
4) Use a sanitary wipe to cleanse toilet seat and rim of toilet bowl.
5) Fill out the date (month/day/year) and check the box corresponding to the time of day on the bucket lid label with the provided ballpoint pen.
6) Remove the lid from the bucket and place the lid inside of the biohazard bag.
7) Assemble the SCD by inserting the Collection Bucket securely into the Stability Frame (Figure 1).
8) Lift the toilet seat and place the SCD horizontally on the edges of the toilet bowl. The narrow side of the frame should face towards the rear end of the toilet bowl (Figure 3A).
9) Lower the toilet seat to secure the SCD (Figure 4A/Figure 4B).
10) Sit on the toilet and arrange yourself so that the stool sample will fall directly into the bucket. **Note: Do not urinate or place toilet paper into the Collection Bucket.**

11) When finished, use toilet paper to clean yourself and discard used toilet paper outside of the bucket.
12) When complete, lift the toilet seat and remove the SCD from the toilet.
13) Flush the toilet.
14) Set the SCD on a flat surface.
15) Push down on the Stability Frame to detach it from the Collection Bucket. Discard frame.
16) Remove the lid from biohazard bag and push the lid onto the Collection Bucket. The lid is secure when a "snap" is heard.
17) Place the sealed Collection Bucket into the Stool Collection Bucket Cooler.
18) Return Stool Collection Bucket Cooler to your freezer.

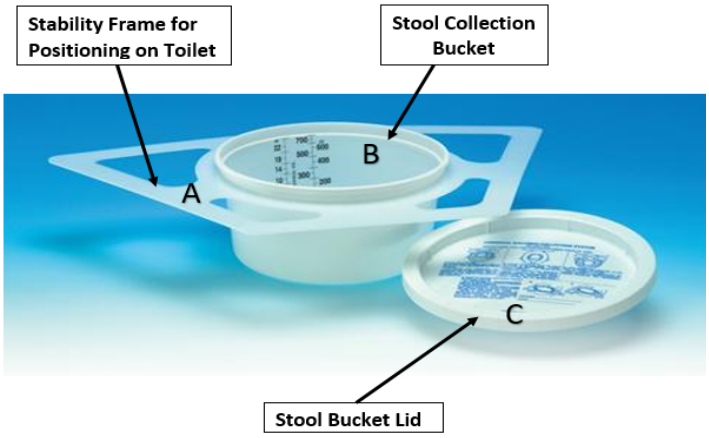

Figure C-1: Stool Collection Kit

Figure C-2: Stool Collection Kit Positioning

Appendix B: Pre-Screening Questionnaire

This pre-screening questionnaire will be utilized to pre-screen the subjects prior to scheduling an appointment for consent and screening to determine whether they are likely to qualify for the study. This will be delivered as an online form to complete.

Timestamp
Subject Email Address
Last HbA1c result
Date measured
Last Finger-stick glucose result (8-hour-fast)
Date measured
Current Weight (lb)
Current Height (in)
Are you willing to wear a continuous glucose monitor (CGM) for 6 weeks? This is a small device that is stuck to the upper arm. There will be 3 sensor applications during the study.
Are you willing to provide up to 5 stool samples throughout the study?
Are you willing to scan your CGM sensor at least once every 8 hours with an Android device application?
Are you willing to use an Android device application to maintain a food log by taking photographs of your meals before you eat them?
Are you willing to track your activity using an Android device application?
Are you willing to have your blood glucose tested using a finger-stick test up to 5 times throughout the study?
Are you available to come to Whole Biome on specific dates for the study visits ?

Thank you for your cooperation!

Appendix C: Standardized Meal Test:

At 4 times throughout the study, subjects will perform a fasted meal-tolerance test (Period-1 Baseline & Measurements, Period-2 Baseline & Measurements). This test is administered following a 6-hour fast.

Following a 6-hour fast, subjects will take a pre-meal scan and finger-stick measurement of their blood glucose using a provided sensor and strip. Subjects will then consume a standardized meal supplement drink (Boost).

Optional: At 30 minutes subjects will scan the sensor and take a final blood glucose measure at the study site. Subjects will be asked to continue to fast for an additional hour, scanning the sensor again at minute 60 and 90.

Appendix D: Structured Questionnaires for Interview

Key questions

- Overall
  - What did you like / notice in the overall study?
  - What did you NOT like / notice in the overall study?
- How did you feel in leg 1?
  - Any major changes?
  - What did you think you were on? Placebo or product? Why
- How did you feel in leg 2?
  - Any major changes?
  - What did you think you were on? Placebo or product? Why

- Product
  - Storing product
  - Taking product
  - Morning vs Night
  - Need to take product twice a day
  - Number of capsules required
  - Anything objectionable about the product?
  - Based on your experience, would be willing to continue using product on a constant basis?
- CGM
  - What worked / didn't work with Putting it on?
  - did it hurt?
    - When you put it on?
    - Wear it?
  - Any issues?
    - When putting / taking off a shirt?
  - What did you learn from the data?
  - Other learning?
  - Any Surprises?

- App
  - Taking readings
    - observations?
    - What worked?
    - What didn't?
  - Food diary

        - observations?
        - What worked?
        - What didn't?
- Behaviors
    - What behaviors changed for you?
    - Eating changes?
    - Mood changes?
    - Behavioural changes?
    - Social changes?
    - How did this work with others around?
    - How did this work in social settings?
    - How did this work when going out to eat?
  - Excitement
    - did you feel less excitement by the end of the study?
    - Did it affect the frequency of your scans, missed dose?
    - Ideas to keep excitement along the study?
    - Keeping in mind that a 6 weeks study is short compared to 202 for example

  - Future Studies
    - Would you mind tracking activity data
    - Would you have been more compliant if compensation was increased according to compliance
    - Would notifications/alerts during the study to remind you to scan, fast, etc. be helpful, annoying, ...?
    - How much would your compliance have been affected by having the application on your personal cell phone?
    - What information could be provided via the app to encourage usage, e.g., daily/hourly/weekly summaries? Recent meals?

  - Closing
    - Would you do another study similar to this one?
    - Would you recommend others to a study like this? On a scale of 1-10 with 1 being no and 10 being absolutely

Data Interviews

Interview Outline

Thank subjects for their ongoing involvement. Highlight the quality and value of the data that they have collected and the benefit their capacity to recall and verify a few details from the study.

Sensor

- Did you ever need to adjust or "re-affix" the sensor because of the sensor coming off due to a bump, a snag or some other reason? If yes, when did it occur and what were the circumstances?

- Do you feel that the individual sensors were positioned in the same location each time?

Boost Tests

Explain why boost tests are relevant for such a study.

date	event	subject
2018-03-19	PR1	7215852
2018-04-02	WS1	7215852
2018-04-06	PR2	7215852
2018-04-20	WS2	7215852
2018-03-19	PR1	7242473
2018-04-02	WS1	7242473
2018-04-06	PR2	7242473
2018-04-20	WS2	7242473
2018-03-19	PR1	9323892
2018-04-05	WS1	9323892
2018-04-10	PR2	9323892
2018-04-24	WS2	9323892
2018-03-20	PR1	95826107
2018-04-03	WS1	95826107
2018-04-06	PR2	95826107

2018-04-23	WS2	95826107
2018-03-20	PR1	98854106
2018-04-06	WS1	98854106
2018-04-12	PR2	98854106
2018-04-27	WS2	98854106
2018-03-20	PR1	911311275
2018-04-02	WS1	911311275
2018-04-06	PR2	911311275
2018-04-21	WS2	911311275

- Does the date and time of the boost test coincide with the date and time listed above? If the listed information is not correct, what changes should be made?

- Preceding each BTT, how long did you fast? For at least 5 hours? Coffee? Sugar? With milk/sugar?

- Preceding or during each BTT did you physically exert yourself, e.g., jogging, walking to work, etc. If yes, what was the exercise?

- Did you take a picture of the boost immediately before consuming the boost, if not, can your remember approximately when in relation to the picture you took the boost? Did you consume the boost in 1 minute, 10 minutes, 1 hour?

Sleep

- Is your sleep schedule regular?

- Did the character of your sleep change at any point is the study?

- Do you remember any instances during the study that you slept poorly, awoke during the night, etc?

- Is there a child in the house that necessitates you being up during the night?

- Do you remember any instances during the study that you ate during the night?

- How frequently do you eat the same meal for breakfast?
- Were there any studies days where you had a breakfast that differed significantly from your routine.
- During the study were there any occasions where had a meal that was much larger than usual? Is so, when did it occur.
- Are there any evenings during the study where you had more alcohol that usual?

Sport

- Do you remember any instances during the study that you physically exert yourself more than usual, e.g., long jogging, biking to work when late for a meeting, etc.

Observations

- Have you noticed change in mass/volume or characteristics of your stool during or after the study periods? During the study? During a specific period of the study? During or after the second wash-out period? If you did notice changes, were they reflected the previous stool samples collected?