Texas Christian University

Informed Consent to Participate in Research and Authorization to Collect, Use, and Share your Health Information

Title of Research: Supplementation with a Next-Generation Synbiotic in Individuals with Overweight or Obesity: A Triple-Blinded Randomized Controlled Clinical Trial
Principal Investigator: Elisa Marroquin Ph.D. and Ryan Porter Ph.D.
[Co-investigators:] Sarah McKinley-Barnard, Tim Ritter, Melissa Fernandez, Jessica Mrosla, Katelyn Harnen, Genevieve Aiwonegbe, Olivia Landis, Jade Nesbitt, Malia Shipsey, and Meggan Duncan

---

Overview: You are invited to participate in a research study. In order to participate, you must complete a screening questionnaire by following the QR code of the IRB-approved email, advertisement, or flier for this study. This questionnaire includes all the inclusion/exclusion criteria. Among the inclusion criteria are: be between 18-50 years of age and have a BMI between 25.0-40.0 (see the chart below).

You may not participate in this study if you:
1) are following a vegetarian, vegan, carnivore, or keto diet
2) currently taking metformin, GLP-1 agonists, insulin, or fiber
3) have taken antiacids, laxatives, probiotics, or medication that affects your immune system in the past month
4) pregnant, planning pregnancy during the study period, or lactating
5) have a history of inflammatory bowel disease, colon cancer, or chronic polyps
6) have been diagnosed with type 1 or type 2 diabetes
7) have active cancer
8) are currently participating in a weight loss intervention (dietetic or medication)
9) have used antibiotics, antifungals, or antivirals in the past 3 months
10) have had a history of recent (within 30 days) diarrhea illness
11) have a known allergy to any component of the study product
12) have had an acute inflammatory infection or inflammatory condition in the past 4 weeks
13) have had >10% weight variation in the past 6 months
14) have had any bariatric surgery

Height (inches)	Weight (pounds)
4'5"	100-160
4'6"	104-166
4'7"	108-172
4'8"	112-178
4'9"	116-185
4'10"	120-191
4'11"	124-198
5'0"	128-205

Height (inches)	Weight (pounds)
5'1"	133-211
5'2"	137-218
5'3"	141-226
5'4"	146-233
5'5"	150-240
5'6"	155-248
5'7"	160-255
5'8"	165-263

Height (inches)	Weight (pounds)
5'9"	169-271
5'10"	174-279
5'11"	179-287
6'0"	184-295
6'1"	190-303
6'2"	195-311
6'3"	200-320
6'4"	205-329

Height (inches)	Weight (pounds)
6'5"	211-337
6'6"	216-346
6'7"	220-355
6'8"	228-364
6'9"	233-373
6'10"	239-383
6'11"	245-392
7'0"	251-401

Study Details:
Our proposed triple-blinded, placebo controlled, randomized clinical trial is divided into two stages.

The first stage will consist of exploring the effect of a daily probiotic + prebiotic supplementation for 12 weeks on body composition, insulin sensitivity, depression, anxiety, and food cravings in 60 individuals with overweight or obesity who have no diagnosis of diabetes mellitus (DM). For this first stage, we hypothesize that this daily probiotic + prebiotic supplement will decrease adiposity, overall body weight, anxiety, depression, and food cravings and increase insulin sensitivity.

The second stage will only be conducted if enough external funds are ensured by using the results from the first stage as preliminary data. The second stage would consist on performing the genetic analysis of the microorganisms in your blood and your stools, as well as on analyzing the metabolites produced by these bacteria. The hypothesis of this second stage of the project is that the probiotic + prebiotic supplement will change blood and gut microbial composition as well as the presence and concentration of gut metabolites in blood. If a relationship exists between changes in body composition, insulin sensitivity, depression, anxiety, or cravings (first stage variables) with blood/gut microbial composition and derived-metabolites (second stage variables), we could show not only a cause-effect relationship but also the mechanism of action through which the supplement acts.

Participants: We are asking you to take part in this study because you meet our inclusion criteria and exclusion criteria. We want to evaluate if the probiotic + prebiotic supplement taken daily for 12 weeks affects your blood sugar, anxiety, depression, food cravings, and body composition. Participants are expected to consume 2 probiotic/placebo capsules per day, one in the morning and one in the evening with food. If you decide to be in this study, you will be one of 60 participants in this research study at TCU.

Voluntary Participation: Your participation is voluntary. You do not have to participate and may stop your participation at any time. Participants who complete the study receive a $50 Amazon card at the end of their 2nd visit and a $100 Amazon card at the end of their 3rd visit. In addition, participants will receive interpretation of their body composition scans, their glucose control results, and their psychological questionnaires at the end of the study.

Confidentiality: Even if we publish the findings from this study, we will keep your study information private and confidential. Only authorized, certified staff members will have the authority to look at your records which will be kept confidential in password secured computers and key secured offices.

What is the purpose of the research?
The purpose of this study is to find out the effects of a daily probiotic + prebiotic (a dietary supplement that contains both beneficial live bacteria and fiber that the bacteria eat) on body composition, blood sugar, depression, anxiety, and food cravings (first stage). Blood and stool samples will also be collected and if funds are available, it is possible that we will analyze gut microbial composition in both stool and blood samples, as well as metabolic analysis in blood (second stage). The individual ingredients and the probiotic + prebiotic as a whole have been used

in the past and have shown health benefits which will be kept confidential to prevent placebo effects. No adverse effects were observed in previous studies involving the probiotic + prebiotic.

What is my involvement for participating in this study?
Please see Table 1. Table of Procedures for a shorter description of this section (located at the end of this section)

In the first visit, you will come to the Kinesiology Department, located at Rickel building 259, where you will receive a complete explanation of the protocol and will process to sign the informed consent if agreeing to participate. Prior to signing, you will have your height and weight measured to ensure that you meet BMI inclusion criteria. Height will be measured using a standard stadiometer and weight will be measured using a standard calibrated digital scale. Your race/ethnicity will be asked to facilitate data interpretation. During this first visit, you will be provided with two stool sample collection kits, two 3-day diet logs, a 3-day supply of antibiotics (500 mg of generic Vancomycin every 8h, prescribed by Dr. Ritter), and a date for your second appointment. The 3-day antibiotic treatment prior to beginning the 12-week probiotic + prebiotic or placebo interventions is provided with the intention to help ensure enough space is available for the newly ingested bacteria. To prescribe you the antibiotic we will need to ask for your date of birth, presence of any allergies to medications, and preferred pharmacy. The cost of the generic antibiotic treatment will be reimbursed to the participants in their second visit. You will be instructed to:

1) write down everything you eat and drink for 3 days before the first stool collection
2) collect a stool sample before the start of the antibiotic regimen
3) consume the antibiotic for 3 days
4) write down everything you eat and drink in those 3 days of antibiotic consumption
5) collect a second stool sample within 12-48 hours after the last dose of the antibiotic
6) bring both stool samples and both diet logs to your second appointment
7) bring your generic antibiotic receipt (or empty antibiotic bottle) for reimbursement
8) fast 12 hours prior to the second appointment (refrain from the consumption of all food and liquids, except water). The first appointment is expected to take no greater than 45 minutes.

For the second visit (Week 1 begins the day of the 2nd visit), you will meet researchers at the Rickel building. This appointment is expected to take approximately 45 minutes and includes body weight measurement, two body composition analyses, three questionnaires for depression, anxiety, and cravings, and a blood draw. The blood draw will consist on the obtention of 4 tubes with a total of 26 mL. The time to complete each test is approximately as follows: 10 minutes for the blood draw, 10 minutes for the anxiety and depression survey, 25 minutes for the body composition tests. You will be asked to wear 1) fitted clothes, as loose clothes can affect the air pressure measurements of the BodPod (example of acceptable items include: compression clothes such as yoga pants, bike shorts, lycra/spandex clothing, swimsuits, sports bra, etc.), 2) a swim cap that we will provide to help compress any air pockets within the hair, 3) no shoes, and 4) no metal objects (piercings, rings, brassier with wire, etc.) due to high density misreading these can cause in DEXA. Participants can wear their typical clothes to get to the lab, bring their fitted clothes in a bag, and change clothing in the dressing room right before the test. You will be asked to void your bladder right before the test. Measurements will be conducted by a certified operators (Dr. Ryan Porter or Dr. Sarah McKinley-Barnard). Blood samples will be collected using sterile techniques and only by qualified investigators (Dr. Ryan Porter or Dr. Sarah McKinley-Barnard). Blood samples will be used to

measure fasting glucose, fasting insulin, and HbA1C (a marker of glucose control). Blood will be sampled using venipuncture. The blood samples will be collected using a traditional blood draw needle to extract blood from a vein in the frontal region of the arm. Two 10 mL vials of blood will be collected and centrifuged to separate the serum. One of those tubes will be stored in a -80°C freezer in the Exercise Physiology Laboratory (Rickel 259) whereas the other will be transported to BioReference for fasting glucose and insulin analysis. The 3 mL for EDTA analysis will also be transported to BioReference for HbA1C analysis. The Zymo collection tube (3 mL) will be stored at the Rickel 259 building. All blood collection vials and plasma/serum containers will be labeled using participant numbers (deidentified) for participant privacy. The leftover blood samples will be stored in the laboratory for 10 years from date of collection at which point they will be discarded as biohazard waste. You agree to allow your deidentified samples to be used for future research by signing the informed consent. These procedures have been approved by the TCU Institutional Biosafety Committee (IBC Protocol). At the end of the second appointment, you will be instructed to:

1) consume 2 probiotic/placebo capsules per day, one in the morning and one in the evening with food
2) write down everything you eat and drink for 72 hours before the last stool sample collection
3) collect a stool sample within 24 hours of finishing the last dose of the supplement intervention
4) bring the stool sample, diet record, and the remaining of your supplement or placebo to the third appointment
5) fast 12 hours prior to 3rd appointment (refrain from food & liquids, except water).

The third and final appointment (End of week 12th) is expected to also take approximately 45 minutes and will include a replication of all tests conducted in the second appointment (body weight measurement, two body composition tests, the anxiety, depression and cravings questionnaires, and a blood draw). You will turn in the remaining of your supplements or placebo for analysis of adherence and compliance. Once all samples have been collected researchers will give you the $100 Amazon gift card. Since this is a triple-blinded randomized clinical trial participant, researchers (including the PI), and statisticians will not know to which group you belong until after data analysis. A researcher, independent from this study, will save in different protected locations (Box, secured laptop, protected USB) the document containing the bottle IDs and the key group to which they belong (placebo or probiotic + prebiotic). Each person will receive 3 bottles (one per month) with the three of them having the same ID, this same ID will be used to identify all questionnaires and samples obtained from that person. This ID will also be different for each participant. The triple blinding nature of this study means that debriefing to you can only take part until after this point. Once all participants have been recruited and have finalized their interventions, and when all data has been analyzed, researchers can contact you back to verbally give you the debriefing statement. In this debriefing statement they will inform you which supplement/placebo you received. If enough external funds are ensured by using the results from the first stage as preliminary data, stage 2 will proceed.

We expect your participation to take three 45-minute appointments at TCU.

In addition to the procedures outlined above, we are asking you to allow us to obtain and store all

blood and stool samples at the beginning and at the end of the supplement intervention for potential future research. The samples will be stored in a -80° C at TCU for up to 10 years with the goal of obtaining NIH grant funding to analyze gut and blood microbiota analysis. Once stored, participants may not withdraw their samples for use in future research. With the intention to prevent lifestyle modifications that could affect blood and gut microbial composition, researchers will share and explain your body composition exams, blood work (glucose, insulin, and HbA1C), and psychological results (anxiety, depression, and cravings) until the end of the project. However, if staff members find that your blood or psychological values could jeopardize your well-being, they will contact you as soon as they become aware of such results. If we perform the second stage of the project (gut microbial analysis in blood and stool samples as well as metabolomic analysis in blood) those results will not be shared with you as gut microbiome interpretation at this point of time is not accurate.

We will de-identify your samples by using codes that don't include your initials or birthday. We will not release the code that links your ID to your personal identifying information for any reason. If stage two of this project is conducted, we will share your sample with researchers from other institutions for sample analysis.

Visit #	How do I prepare for this visit?	What will be done?	Time	Location
1st visit	1) 12h fasting (if you decide to enroll the study after having received an explanation of the procedures and after having had the opportunity to ask questions, fasting in this visit will save the need to schedule a 4th visit [prior to the 2nd visit in Week 1] just for fasting blood sample collection).	- Explanation of the study - Opportunity to ask questions - Signature of informed consent - Height and weight will be measured - Race will be asked - 1 blood draw of 4 tubes (26 mL total) - You will receive 2 stool collection kits, two 3-day food logs, a 3-day supply of antibiotics, and date for following appointment. - You will be instructed on how to collect each sample	45 minutes	Rickel building 256 and 259
2nd visit (Week 1 begins the day of the 2nd visit)	1) Fill out everything you eat/drink for 3 days before first stool sample collection 2) Collect a stool sample before the start of the antibiotic regimen 3) Consume antibiotics for 3 days and write down everything you eat and drink in these 3 days 4) Collect a stool sample within 12-48 hours after the last dose of the antibiotic 5) Schedule 2nd appointment and **bring evidence of having bought antibiotics** 6) **Bring both stool samples and both diet logs** with you to the 2nd appointment 7) **Fast 12 hours** prior to the 2nd appointment (refrain from the consumption of all food and liquids, except water). 8) Don't wear **clothes with metals** (women are recommended to use sports bra) or reflective materials. Wear or bring **fitted clothes** to change here (yoga pants, lycra/spandex clothing, bike shorts, etc.)	- Collection of the stool samples collected at home - Fill out Beck Anxiety and Depression Inventories, as well as the Cravings questionnaire - Body weight measurement - Body composition scans will be done - 1 blood draw of 4 tubes (26 mL total) - When the second visit has finalized you will receive a $50 Amazon card	45 minutes	Rickel building 256 and 259
3rd visit (end of week 12th)	1) **Consume the symbiotic** for 12 weeks (2 daily capsules, 1 in the morning and 1 at night with food). 2) Write everything you eat and drink for 72 h before the last stool collection 3) Collect a stool sample within 12-48 hours after the last dose of the synbiotic 4) Schedule your third appointment 5) **Bring the stool sample and diet record** with you to the third appointment 6) **Fast 12 hours** prior to the third appointment (refrain from the consumption of all food and liquids, except water). 7) Wear **clothes with no metals** (women are recommended to use sports bra) or reflective materials. Wear or bring **fitted clothes** to change here (yoga pants, lycra/spandex clothing, bike shorts, etc.). 8) **Bring the remaining supplement that was left over.**	- Fill out Beck Anxiety and Depression Inventories, as well as the Cravings questionnaire - Body weight measurement - Body composition scans will be done - 1 blood draw of 4 tubes (26 mL total) - Collection of the stool sample collected at home - When all samples have been collected you will receive a $100 Amazon card	45 minutes	Rickel building 256 and 259

Are there any alternatives and can I withdraw?
Participation for this study is completely voluntary. Participants may withdraw at any time; however, they will not receive compensation. Participants may contact the PI Elisa Marroquin (E.Marroquin@tcu.edu) or Jessica Mrosla (jessicamrosla@icloud.com) via email to request to withdraw from the study. If participants withdraw from their study the data collected up to that point will remain available for research purposes with the intention to use intent to treat analysis. Your decision to participate or not to participate will not affect your job at TCU or your student status, course grade, recommendations, or access to future courses or training opportunities.

What are the risks for participating in this study and how will they be minimized?
There are some risks you might experience from being in this study. The primary risks are radiation exposure from the DEXA scans, secondary infections due to the antibiotics, intestinal discomfort due to the symbiotic, breach of confidentiality, and risk of being asked questions that might be sensitive for you.

Participants should let the researchers know if they are in any other studies involving body composition scans to consider cumulative radiation exposure. There are a total of 2 DEXA scans in this study. This will expose you to low-dose radiation, the amount of radiation exposure is small and is about the same as one day of normal background radiation per scan. Exposure to radiation at low levels has the potential of causing cancer later in life. The risk of this occurring increases as the amount of exposure increases. The amount of radiation exposure in this study is very small and so the additional risk of causing radiation related effects does not change significantly by participating in this study. Prior to any body composition measurements, participants who are female and of childbearing age will be required to complete a pregnancy questionnaire. To minimize the radiation-related risks, females who become pregnant will be unenrolled from the study and will not undergo DEXA scanning.

Although the antibiotic intervention is short (3 days) and might not be enough to cause intestinal discomfort is possible that susceptible people may still experience gastric or intestinal symptoms such as bloating and diarrhea. Secondary infections to antibiotic treatment, such as *Clostridiodies difficile*, although unlikely are also possible. Short antibiotic interventions, however, cause minimal microbiota changes that are temporary and reversible (DOI: 10.1016/j.cell.2018.08.047). The antibiotic and dose chosen in this trial were the most highly used according to a systematic review our team published in 2022 in which we compared RCTs evaluating the effect of “antibiotic” versus “antibiotic + probiotics” (DOI: 10.1099/jmm.0.001625). Gastrointestinal discomfort at such short antibiotic interventions is unlikely, however, if present, symptoms tend to subside within a short period of time.

Daily probiotic + prebiotic supplementation might cause adverse gastrointestinal symptoms such as bloating. A previous study supplementing with this particular probiotic + prebiotic found no significant issues with tolerance and gastrointestinal disruption, therefore the risk for this study is deemed as low.

There is also a risk of being asked questions that might be sensitive for you and that pertain to your anxiety and depression levels. If you feel that answering the questionnaires caused psychological distress you have the following resources available:

- If you are a TCU student you can access to psychological health through several ways: psychiatric evaluations, 24/7 telephone counseling helpline, peer support communities, personalized interventions for student athletes, etc. (https://counseling.tcu.edu/student-services/#StudentAthletes)
- If you are not a TCU student, you can find free government support through Texas Health and Human Services by accessing the following link and filling out the form (https://resources.hhs.texas.gov/pages/find-services). This form will be used to provide you with the best phone number to contact based on your needs and based on your location. If you prefer to use a phone number you can call 855-937-2372 and they will help you to get in touch with a health professional.
- If you live in Tarrant County you can directly call MHMR of Tarrant County (My Health My Resources) at 800-866-2465.

Lastly, there is always a potential risk of data breach, however, only personnel that has been certified and approved by the IRB will have access to the participants and their related data. Samples, questionnaires, and other forms pertaining to the participants will be labeled with an ID that do not include any personal health information of the participant. The document containing the link between the names of the participants and their IDs will be saved in a password protected computer. Informed consents will be saved in the office of the PI which is locked at all times the PI is not present and which is located in a card locked department.

What are the benefits for participating in this study?
You may or may not benefit from this study. Although you will get body composition analyses, blood glucose control results, and psychological results at the end of the study there is no guarantee that your participation in this project will improve any of these parameters. This is because this is a randomized controlled trial and because this is a new population in which this supplement is being tested.

Will I be compensated for participating in this study?
You will receive a payment of $50.00 in the form of an Amazon gift card after your second visit and a $100 Amazon gift card at the end of the study. Payment will be provided once all samples and forms have been completed on each of the respective appointments. Participants who withdraw early will not receive compensation but will receive reimbursement for their generic antibiotics.

You will also receive a copy of your body composition scans which have a monetary market value of about $200.

Please tell the researchers if you have any adverse events or other problems related to your participation in the study. You should also contact your primary care physician for treatment. If your injury or sickness is an emergency, you should call 911 for an ambulance to take you to the emergency room. You or your insurance will be billed for whatever care you receive. Texas Christian University does not provide compensation or payment for any injury or physical harm that may occur as a result of being in this study. Also, Texas Christian University does not provide compensation for loss of employment, income, or emotional duress that may result from your injury or harm.

By signing this form, you do not give up your right to seek payment if you are harmed as a result of being in this study.

What are my costs to participate in the study?
Although you will be asked to pay the cost of the generic antibiotic treatment initially (which should have an estimated cost of $20-25), we will use your pharmacy ticket or empty antibiotic bottle to refund your money through an Amazon gift card of $50 processed at your second appointment (antibiotics will be limited to a cost no more than $50), so you will not be responsible for any costs to participate in this study. Your insurance will not be charged for any study-related testing; however, you are welcome to (but don't have to) use your insurance to buy the antibiotic.

How will my confidentiality be protected?
Every effort will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy. Your records may be reviewed by authorized University personnel or other individuals who will be bound by the same provisions of confidentiality.

With exemption of your informed consent, your identifiers will be removed from all forms and samples. Your information or samples could be used and/or distributed to another investigator for future research studies without additional consent from you or your Legally Authorized Representative.

We may publish what we learn from this study. If we do, we will not include your name. We will not publish anything that would let people know who you are.

Access to your health information is required to be part of this study. If you choose to take part in this study, you are giving us the authorization (i.e., your permission) to use the protected health information and information collected during the research that can identify you. The health information that we may collect and use for this research may include medical history that may be considered sensitive.

What will happen to the information collected about me after the study is over?
We will keep your research data to use for future research or other purpose. Your name and other information that can directly identify you will be kept secure and stored separately from the research data collected as part of the project.

We may share your research data with other investigators without asking for your consent again, but it will not contain information that could directly identify you.

Who should I contact if I have questions regarding the study or concerns regarding my rights as a study participant?
You can contact Jessica Mrosla at j.mrosla@tcu.edu or Dr. Elisa Marroquin at e.marroquin@tcu.edu or 817-257-1031 with any questions that you have about the study.

Dr. Brie Diamond, Chair, TCU Institutional Review Board, (817) 257-6152, b.diamond@tcu.edu; or Dr. Floyd Wormley, Associate Provost of Research, research@tcu.edu

HIPAA Authorization

This research uses or discloses Protected Health Information as defined by the Health Insurance Portability and Accountability Act (HIPAA). By signing this form, you are permitting Texas Christian University to use your health information for research purposes. The names of the TCU researchers who will gather this information from you are listed at top of this document, including the lead researcher. We will collect the following health information from you:

- Medical history of diabetes
- Weight
- Height
- Body composition
- Fasting Blood Glucose
- Fasting Insulin
- HbA1C
- Anxiety, Depression, and Craving Symptoms

By signing this document, you are agreeing to be in this study. Make sure you understand what the study is about before you sign. You will be given a copy of this document for your records. A copy also will be kept with the study records. If you have any questions about the study after you sign this document, you can contact the study team using the information provided above.

I understand what the study is about and my questions so far have been answered. I agree to take part in this study.

_____

Printed Participant Name

_____

Signature Date

_____

Printed Name of person obtaining consent

_____

Signature Date

Consent to be Contacted for Participation in Future Research

I give the researchers permission to keep my contact information and to contact me for future projects.

Yes _____ No _____

_____
Signature Date

Consent to Use Data/Biospecimens for Future Research

I agree that my information may be shared with other researchers for future research studies that may be similar to this study or may be completely different. The information shared with other researchers will not include any information that can directly identify me. Researchers will not contact me for additional permission to use this information.

Yes _____ No _____

_____
Signature Date