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COVER PAGE

Title: Understanding the Role of Gut Microbiota in Hyperphagia in Prader-Willi Syndrome

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INTERVENTIONAL RESEARCH PROTOCOL TEMPLATE

(HRP-503a)

STUDY INFORMATION

- **Title of Project:**
  Understanding the role of gut microbiota in hyperphagia in Prader-Willi syndrome

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- **Protocol Version and Date:**
  Version 6, October 7, 2022

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1.0 Research Design

1.1 Purpose/Specific Aims

To examine clinical relevance and test for a causative role of gut microbiota in the context of appetite regulation and food-related behavior in patients with hyperphagia syndrome

A. Objectives

To use high-fiber supplementation, an intervention known to create shifts in the gut microbiota towards a healthier structure, to explore the relationship between gut microbiota, appetite control and feeding behavior in patients with hyperphagia syndrome.

B. Hypotheses / Research Question(s)

We hypothesize that the gut microbiota in patients with hyperphagia is deficient in carbohydrate-utilizing short-chain fatty acid (SCFA) producers and dominated by bacteria that produce detrimental compounds. Such dysbiosis produces distinct profiles of bioactive compounds, including the proinflammatory lipopolysaccharide (LPS), that contribute to altered signaling in central appetite regulation.

1.2 Research Significance

Hyperphagia is a hallmark feature in PWS patients, with obsessive preoccupation with food and extreme overeating (energy intake of PWS patients may be 3-6 times that of controls (1, 2)) leading to obesity and the related co-morbidities that become one of the leading causes of death in this patient population (3). There is some evidence for hypothalamic dysfunction as an underlying mechanism for PWS-associated hyperphagia (4) but there is no effective treatment to normalize food-related behaviors. The emerging literature on the role of gut microbiota in the gut-brain axis may provide a novel perspective of feeding dysregulation associated with PWS and facilitate novel therapeutic approaches. In our previous study, high-fiber intervention induced significant shifts in the gut microbiota structure in PWS patients, an effect associated with improvements in metabolic parameters including body weight and glucose homeostasis and a modest improvement in the overall food-related behavior (5). Data from animal studies further support a plausible mechanism by which gut microbiota participates in the gut-brain axis to regulate food-related behavior (6): SCFAs generated from bacterial fermentation signal enteroendocrine cells for the production of appetite-regulating hormones including glucagon-like peptide-1 and peptide YY; LPS from Gram-negative bacteria disrupt the blood-brain barrier and thus enhances the effect of circulating cytokines on central feeding pathways. Other mechanisms that gut microbiota may regulate appetite include the production of neurotransmitters and neuromodulators (e.g., γ-amino butyric acid from *Bifidobacterium* and serotonin from *Enterococcus* spp.) that act on afferent axons locally or interact with the intestinal cells to modify neural signaling to the central nervous system.

1.3 Research Design and Methods

A. Research Procedures

RECRUITMENT. We are recruiting patients with hyperphagia aged 18-45 years who have not received growth hormone treatment in the previous 6 months. Study candidates will be recruited from Robert Wood Johnson Medical School (RWJMS) pediatric endocrine clinic as well as DEEP6, which applies artificial intelligence to identify patients meeting study criteria in the RWJMS outpatient electronic medical record.

ELIGIBILITY SCREENING.

For recruitment, individuals with patients with hyperphagia will be identified via Dr. Ian Marshalls RWJMS clinic and DEEP6. Patients identified on the DEEP6 list will be considered for the study if they are patients of Dr. Marshall. Once identified, the research staff in Rutgers will contact the patient and guardian via a phone call to provide an overview of the study, requirements for research participation, and review eligibility for the study **(Appendix A).** The study candidates and guardians will be informed whether they may be eligible for the study, and if so, a virtual informed consent

interview with the research team will be scheduled **(Appendix B).** Female candidates will be given two pregnancy test kits (Rapid Detection Pregnancy Test, Clearblue, Geneva, Switzerland) via mail and will perform the test on the day of the fMRI.

INFORMED CONSENT AND ELIGIBILITY SCREENING PART 2. The informed consent process will be conducted virtually through Rutgers Zoom, which is HIPPA protected. The study coordinator will explain the rationale, aim, expected outcomes significance of the research, the experimental procedures, what is required from the participants, and the benefits and risks associated with the study. Informed consent form and consent for health information release will be signed during the interview using DocuSign, a HIPPA compliant platform during the interview **(Appendix B).** The study candidates will receive copies of both signed documents. Upon completing these documents, the study coordinator will ask some additional questions regarding their medical history and other conditions to establish eligibility. Candidates will perform and present proof of a negative pregnancy test result the first study session.

TESTING AND INTERVENTION. Participants will attend a baseline visit at the Center for Advanced Human Brain Imaging Research (CAHBIR) at Rutgers University, during which functional magnetic resonance imaging (fMRI) coupled with a meal test will be performed to assess peripheral and central feeding pathways (7, 8). fMRI scans will be performed during resting state to assess functional connectivity of feeding related networks, with a specific focus on connectivity between the hypothalamus, insula, anterior cingulate, ventromedial/orbitofrontal prefrontal cortex and amygdala (9, 10). Activation to food (vs non-food) images will be assessed to index responsivity to appetitive feeding networks including the above regions and the ventral striatum using paradigms that have previously been found to be sensitive to trait and state in multiple studies (11-13). Thereafter participants will consume a liquid meal (525 kcal; Ensure Plus, Abbott). fMRI will be repeated immediately after the meal. Participants will attend a second visit at the IFNH, during which laboratory work coupled with a meal test will be performed to assess satiety hormones and inflammatory markers. Fasting blood draw will be taken, and the participants will consume a liquid meal (525 kcal; Ensure Plus, Abbot) followed by blood draws at 30, 60, 120, 180 and 240 min post-meal. Upon completion of baseline testing and providing a fecal sample, participants will consume NBT-NM108 (a mixture of inulin, Fibersol-2, and brans of oat, wheat, corn and sorghum; Notitia Biotechnologies) daily for 4 weeks. At the completion of the 4-week intervention, all sampling and testing will be repeated as per baseline. At baseline and end of intervention, participants will be interviewed with appetite and physical activity questionnaires **(Appendix C and G)** and requested to complete a 24-h food recall using MyFitnessPal, a mobile health app on their phone.

B. Data Points

- Gut microbiota composition: fecal samples will be collected at weeks 0 and 4.
- fMRI scans will be completed at baseline and end of intervention.
- Blood parameters (satiety hormones and inflammatory markers) at minutes 0, 30, 60, 120, 180, and 240 post-meal at week 0 and week 4.
- Appetite and physical activity questionnaires at weeks 0 and 4.
- Calorie count calculated via 24-h food recall on MyFitnessPal at weeks 0 and 4.

C. Study Duration

It will take each participant 28 days to complete the study. The overall duration of the study is one year.

D. Endpoints

1. Primary Outcome
   - Gut microbiota composition at Weeks 0 and 4
2. Secondary outcomes:

- Weeks 0, 1, 2, 3, and 4
    a. Weight measured in pounds
- Weeks 0 and 4
    a. Hormones related to satiety and hunger include ghrelin, peptide YY (PYY), leptin, glp-1/GIP, insulin, glucose
    b. Inflammatory markers include c-reactive protein (CRP), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-), adiponectin
    c. Outcome of fMRI showing responsivity of appetite feeding networks in the brain
    d. Caloric intake recorded in MyFitnessPal application
    e. Appetite behavior measured by hyperphagia questionnaire and visual analog scale (VAS)
    f. Physical activity measured by physical activity questionnaire

1.4 Preliminary Data

Dietary fibers modulated gut microbiota and conferred clinical benefits in PWS patients. Children aged 3-16 years old (N=17 with PWS and N=12 with simple obesity) were prescribed a diet enriched with wholegrains, traditional Chinese medicinal foods and prebiotics (WTP diet) for 30-90 days (5). This diet was specifically formulated to include a large amount of dietary fibers with diverse physicochemical structures to maximize carbohydrate fermentation by the gut microbiota. The diet induced significant and yet similar shifts in the overall gut microbiota structure in both groups, an effect associated with improvements in metabolic parameters including body weight and glucose homeostasis (Figure 1). Importantly, based on a hyperphagia questionnaire, we observed a modest but non-significant improvement in the overall food-related behavior in PWS patients (P=0.074).

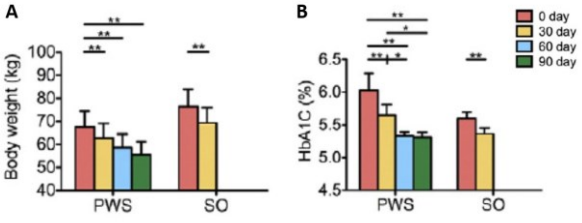

Figure 1. Body weight (A) and HbA1c (B) changes during 30-90 days of high-fiber intervention. PWS: Prader-Willi syndrome (N=17); SO: simple obesity (N=12).

Our network analysis identified guild-level structure in the gut microbiota (Figure 2). When supplemented with dietary fiber, gut bacteria exhibited co-abundance patterns and formed genome interaction groups (GIGs) or "guilds" (an ecological group in which members exploit an environmental resource in a similar way and show co-abundant behavior). Three guilds, which included strains of beneficial bacteria such as *Bifidobacterium* were negatively correlated with body weight and HbA1c, while 9 guilds which contained many opportunistic pathogens such as LPS producers were positively associated with disease parameters. Taken together, we showed that high-fiber diet was a feasible and effective way to modulate gut microbiota in PWS patients, which conferred clinical improvements and may also alleviate hyperphagia.

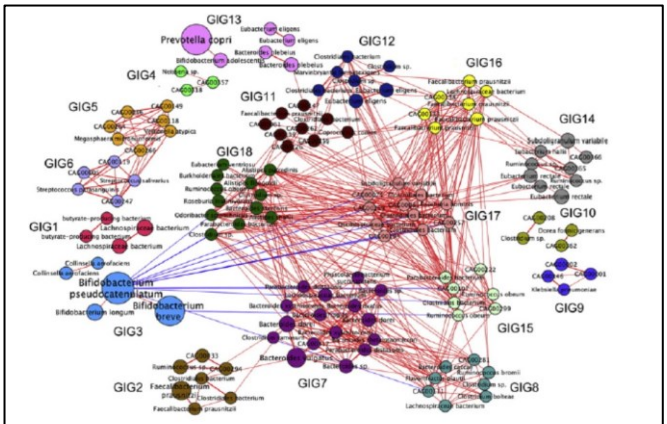

Figure 2. Network analysis of the bacterial genomes. Genomes of the same color were grouped in the same guild. Size of the nodes indicated the abundance of genomes. Red and blue lines denoted positive and negative correlations respectively.

Interestingly, transplantation of the pre- and post-intervention gut microbiota from the same patient to germ-free mice induced opposite responses in the recipient mice. While these mice had no genetic defects and were maintained on a standard chow diet, receiving the pre-intervention PWS gut microbiota alone was sufficient to induce inflammation, excessive fat accumulation and liver steatosis whereas the post-

intervention gut microbiota was without effect. This piece of causality evidence showed that at least some of the major metabolic phenotypes associated with PWS can be transferred to germ-free mice along with the transplanted gut microbiota (5, 15).

Dietary fibers selectively promoted acetate and butyrate producers and structured a healthier gut ecosystem. Patients with type 2 diabetes were randomized to receive either the high-fiber WTP diet (W group; N=27) or usual care (U group; N=16) for 84 days (16). We identified 141 bacterial genomes that had the genetic capacity for fermenting dietary fibers. Intriguingly, only 15 of them were able to take advantage of the fibers and increased their abundance, while 79 remained unchanged and 47 were suppressed during the high-fiber intervention. All 15 positive responders possessed the pathways to produce acetate, and some could also produce butyrate. When the gut microbiota was dominated by these bacteria, there was a concomitant reduction in bacteria that produced metabolically detrimental compounds (e.g. LPS, hydrogen sulfide and indole) and collectively these microbial changes led to improved metabolic outcomes (Figure 3). Acetate and butyrate can stimulate the production of GLP-1 and PYY; LPS can induce systemic inflammation; indole and hydrogen sulfide can inhibit the production of GLP-1 and PYY (17, 18). Shifting of the abundance of producers of these bioactive compounds may drive the improvement of metabolic phenotypes. It would be interesting to assess if appetite regulation and feeding behavior are also modulated by these high fermentable fiber-induced changes in gut microbiota.

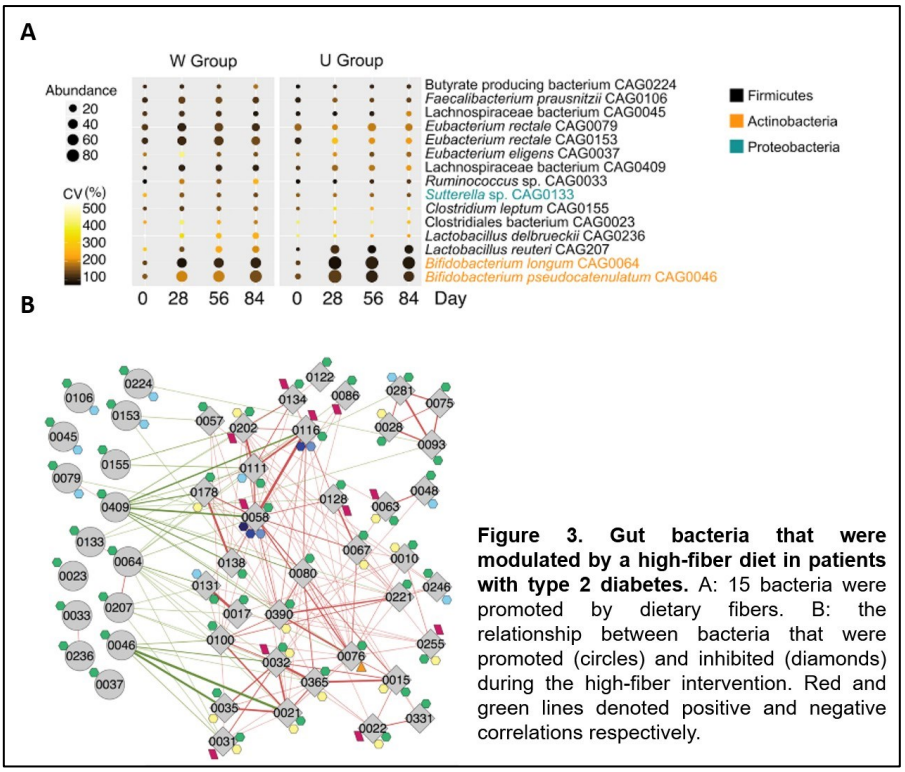

Figure 3. Gut bacteria that were modulated by a high-fiber diet in patients with type 2 diabetes. A: 15 bacteria were promoted by dietary fibers. B: the relationship between bacteria that were promoted (circles) and inhibited (diamonds) during the high-fiber intervention. Red and green lines denoted positive and negative correlations respectively.

1.5 Sample Size Justification

No power calculation is performed for this pilot study. We aim to recruit 10 patients with hyperphagia(5 male and 5 female) to capture a reasonable degree of variations in gut microbiota composition.

1.6 Study Variables

A. Independent Variables, Interventions, or Predictor Variables

NBT-NM108

B. Dependent Variables or Outcome Measures

Gut microbiota composition, weight, fMRI, total caloric intake, hyperphagia behavior, physical activity behavior and hormones of satiety

1.7 Drugs/Devices/Biologics

A. Schedule and Administration

Participants will receive NBT-NM108 prepared as muffin (each contains 30 g of the product) for 4 weeks. The dosage will be 2 muffins a day. This dosage of NBT-NM108 will provide 24 g/day of dietary fibers of diverse physicochemical structures as sources of fermentable carbohydrates for the gut microbiota. Participants will be given a supplement diary to keep track of consumption of muffins along with potential side effects (**Appendix H).**

B. Drug/Device Accountability and Storage Methods

NBT-NM108 will be manufactured by Safe Sterilization USA (138 Sylvania Place, South Plainfield, NJ), which is an FDA FSMA compliant GMP/NSF certified third party toll manufacturing facility and supplied by Notitia Biotechnologies. NBT-NM108 will be prepared into muffins by a ready to eat manufacturer and provided to participants.

1.8 Specimen Collection

A. Primary Specimen Collection

- **Types of Specimens**:
  1) Stool. Each participant will be provided with kits to collect fecal samples at home in Week 0 and Week 4. The fecal sample collection kit has been cleared by FDA for at-home use. Instructions and supplies will be provided to collect these samples at home **(Appendix D).**
  2) Blood. Venous blood draws (10 ml each) will be taken during the baseline and Week 4 post-intervention meal tests (T=0, 30, 60, 120, 180 and 240 min of each test) at IFNH by qualified professionals including phlebotomists, registered nurses and physicians.

- **Annotation**: Data to be annotated or associated with each specimen will include participant code, age, sex, ethnicity, medical history, and medications.

- **Transport**:
  1) Stool. Participants will return the samples by mailing them to the research team in prepaid shipping boxes. They will either schedule a FedEx pickup or drop off the package at a local FedEx office. The shipping packages are DOT (Department of Transportation) and IATA (International Air Transport Association) compliant.
  2) Blood. The samples will be processed and analyzed by Dr. Malin's lab located at the IFNH.

- **Processing**:
  1) Stool. Samples will be processed by members of PI Dr. Zhao's lab listed in this protocol.
  2) Blood. Samples will be processed by Dr.Malin's laboratory.

- **Storage**: All samples will be stored in locked -80°C freezers in laboratories in Lipman Hall (76 Lipman Dr, New Brunswick, NJ 08901) and New Jersey Institute for Food, Nutrition and Health (61 Dudley Rd, New Brunswick, NJ 08901) of Rutgers University until analysis. Samples will only be accessible by authorized research personnel named in this protocol. Specimens will be stored until they are no longer in a fit state for scientific analyses.

- **Disposition**: When specimens are no longer in a fit state for scientific analyses, they will be destroyed by incineration by authorized research personnel named in this protocol.

B. Secondary Specimen Collection

Not applicable.

1.9 Data Collection

A. Primary Data Collection

- **Location**:
  - Participants will be interviewed with Appetite and physical activity questionnaires and diet 24-hour recall at the clinical room at IFNH building.
  - fMRI will be conducted at the CAHBIR (116 RWJMS Staged Research Building).
  - Lab draws will be performed at IFNH or CAHBIR
- **Process of Data Collection**:
  - Study coordinators will interview participants with questions in the appetite and physical activity questionnaires and for 24-hour recall.
  - fMRI Session: Participants will complete an online MRI safety screening form prior to the study day **(Appendix E)**. The form must be reviewed by the certified MRI Technologist prior to entry into the scanner room. The individual will be positioned in the magnet and will receive an anatomical scan followed by resting state and food picture fMRI scans. They will then be taken out of the scanner, consume a meal and then have a brief anatomical scan for purposes of alignment with their high-resolution MRI and a repeat of their fMRI scans.
- **Timing and Frequency**: The interviews and scanning will be conducted at Week 0 and Week 4.
- **Procedures for Audio/Visual Recording**: N/A

Study Instruments:

- See **Appendix C** for the appetite questionnaire
- See **Appendix G for the physical activity questionnaire**
- MyFitnessPal application will be used to calculate caloric intake from 24-h diet recall

Ethnographic Studies, Interviews, Or Observation: N/A

Subject Identifiers: participants' names and birthday will be linked with the data collected.

B. Secondary Data Collection

NA

- **Type of Records**: NA
- **Location**: NA
- **Inclusion/Exclusion**: NA
- **Data Abstraction Form(s):** NA

1.10 Timetable/Schedule of Events

We expect to complete this trial within one year upon institutional approval. Detailed timeline is as follows:

	Preparations	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Institutional approval, staff training and material development													
Recruitment													
Intervention, sample measurement and clinical data collection													
Bioinformatics and data analysis													
Results dissemination													

2.0 Project Management

2.1 Research Staff and Qualifications

PI Dr. Keerthana Kesavarapu (DO) is the Director of Medical Obesity and Associate Professor of Gastroenterology and Hepatology at Rutgers-Robert Wood Johnson Medical School. She is a board-certified specialist in adult gastroenterology and obesity. Dr. Kesavarapu will actively recruit patients, participate in helping the study team design the assessment visit protocol, problem-solve with the study team on any issues that may arise and assist in analysis and interpretation of data.

Co-principal investigator Dr. Liping Zhao (BSc, MSc, PhD) is the Eveleigh-Fenton Chair of Applied Microbiology in the School of Environmental and Biological Sciences and the Director of Center for Nutrition, Microbiome & Health at New Jersey Institute for Food, Nutrition and Health (IFNH). Dr. Zhao's research applies molecular and genomic tools in understanding systems biology and predictive manipulation of the gut microbial communities, with his recent research primarily focuses on using dietary interventions to restore and maintain a health gut microbiota as a key strategy to improve metabolic health. With extensive experience in using high-fiber diets in clinical trials to modulate the gut microbiota, Dr. Zhao leads experimental design and will contribute to data analysis and interpretation.

Co-Investigator Dr. Steven Malin (PhD) is an Associate Professor in the Department of Kinesiology & Health with a dual appointment in the Department of Medicine. He is also the Director of the Applied Metabolism & Physiology Laboratory. He has published several studies that examined the effects of exercise, diet, pharmacology and weight loss surgery on insulin sensitivity, appetite regulation and clinical outcomes.

Co-Investigator Dr. David Zald (PhD) is the Director of CAHBIR at Rutgers Brain Health Institute. His research focuses on understanding the neural and neuropharmacological substrates of emotion and cognition, and the manner in which individual differences in the functioning of these systems impacts temperament, personality and psychopathology. Dr. Zald will provide supervision of stimulation paradigms and analysis and lead the interpretation of neuroimaging results.

Co-Investigator Dr. Ian Marshall (MD) is the Chief of the Division of Pediatric Endocrinology Division and Associate Professor of Pediatrics at Rutgers-Robert Wood Johnson Medical School. He is a board-certified specialist in Pediatric Endocrinology and have spent over 15 years in the care of pediatric and adult patients with PWS. Dr. Marshall will actively recruit patients, participate in helping the study team design the assessment visit protocol, problem-solve with the study team on any issues that may arise regarding any aspects of the study, and assist in the analysis and interpretation of data

Dr. Guojun Wu (BSc, PhD) is a Postdoctoral Research Associate of the Zhao Lab at the Department of Biochemistry and Microbiology. Dr. Wu was awarded his PhD in Microbiology from Shanghai Jiao Tong University (China) in March 2018. With initial training in bioinformatics, his research primarily focuses on mining high-throughput sequencing datasets to dissect the interactions between gut microbial community, diet and human health. Dr. Wu will lead bioinformatics and statistical analysis.

Dr. Shreya Ghosh (BSc, MSc, PhD) is a Postdoctoral Research Associate in the Zhao Lab at the Department of Biochemistry and Microbiology. Dr. Ghosh was awarded her PhD in Microbiology from Shanghai Jiao Tong University (China) in March 2022. She has experience in designing and conducting animal experiments to investigate the role of dietary interventions in metabolic health by interacting with the gut microbiota.

Graduate Students Tristan Ragland, Ying Wang, Rishika Navlani, Matt Pelton and Yongjia Gong contribute to experimental design and research material development. They will assist with screening, informed consent, data monitoring, follow-up phone calls, sample processing and data analysis.

2.2 Research Staff Training

All investigators have successfully completed the Collaborative Institutional Training Initiative (CITI) training.

2.3 Other Resources

Gut microbiome analysis will be performed at the Microbiome Core of the New Jersey Institute for Food, Nutrition and Health of Rutgers University. The Core owns a next-generation sequencing platform (Ion GeneStudio S5, ThermoFisher Scientific) to perform 16S rRNA gene sequencing and a 2X Intel Xeon Skylake 6130 Node (22M Cache, 2.666 MHz, 192 GB RAM, 5 TB storage) of the high-performance computing cluster Amarel at Rutgers University for statistical analyses and bioinformatics. fMRI will be performed at CAHBIR of the Robert Wood Johnson Medical Center. The proposed study will have access to a state-of-the art Siemens 3T MRI scanner which allows for high resolution multi-band collection optimized for imaging regions in ventral brain regions involved in feeding. The image processing facilities at CAHBIR is connected to Rutgers' high-performance computing facilities to allow rapid parallel processing of imaging data.

2.4 Research Sites

- Brain Health Institute (683 Hoes Lane West, Piscataway, NJ 08854)
- New Jersey Institute for Food, Nutrition and Health (61 Dudley Rd, New Brunswick, NJ 08901)
- Zhao Lab (76 Lipman Dr, New Brunswick, NJ 08901)

3.0 Multi-Center Research

Not applicable

4.0 Subject Considerations

4.1 Subject Selection and Enrollment Considerations

A. Recruitment Details

Patients with the diagnosis of PWS and/or hyperphagia will be recruited from Dr. Ian Marshall's pediatric endocrinology RWJMS clinic and DEEP6 software.

B. Subject Screening

- **Inclusion Criteria**
  - Aged between 18-45 (inclusive)
  - Confirmed PWS with genetic testing or documented ICD9/10 coding for hyperphagia
  - No growth hormone treatment in the previous 6 months
  - Body weight < 300 lbs.
- **Exclusion Criteria**
  - History of other gastrointestinal disorders such as small intestinal bacterial overgrowth, celiac disease, inflammatory bowel disease, or irritable bowel syndrome.
  - Pregnancy or breastfeeding
  - Prior gastrointestinal or bariatric surgery
  - Immunocompromised e.g., cancer treatment, bone marrow/organ transplant, immune deficiency, poorly controlled HIV/AIDS, prolonged use of steroids or other immunosuppressant medications
  - Antibiotic administration in the previous 30 days
  - Administration of pre/probiotic supplements or antibiotics.
  - Growth hormone administration in the previous 6 months
  - Have access to a smartphone, tablet, computer, or other qualifying internet-enabled device and be able to follow instructions.

- Individuals who are not proficient in English
- Contraindications for MRI scanning, including Ferrous material implanted in or on the body, including flakes or filings, surgical clips, bullets, or electrical devices such as a pacemaker, or nonremovable ferrous jewelry (fillings in teeth and permanent retainers are permitted). Individuals with surgical pins or plates above the neck are excluded. Surgical pins or plates below the neck are exclusions, except when the material is fixed to bone, and considered acceptable by the *Reference Manual for Magnetic Resonance Safety. Implants and Devices, 2020 Edition*. Almost all recent orthopedic implants are made of materials that are not ferromagnetic and therefore are safe for scanning, and even though some screws are still made of ferromagnetic materials these are firmly screwed into bone. In cases where the material is unknown or deemed unsafe for scanning by the *Reference Manual for Magnetic Resonance Safety. Implants and Devices,* the participant will be excluded. History of eye injury involving metallic materials, shavings in eyes, or welding without a face mask. Lead/iron tattoos and tattoos performed by a nonprofessional artist if the pigment material is unknown. Claustrophobia (history of significant anxiety in closed places). Back problem that would prevent the subject from laying still comfortably for up to 60 minutes.

C. Privacy Protections

Should any participants desire not to interact with or provide personal information to specific members of the research team, they may report such a need to the PI and the arrangement of the research personnel will be adjusted accordingly. Participants will not be required to provide a reason if they desire not to do so. If changing the research personnel is not possible, the PI will discuss with the subjects about alternative solutions.

Each participant will be assigned a unique identification code. A master code identifier which links the identification code and personal information will be maintained separately from the study data. Subject information is only accessible to named investigators in this protocol and authorized research personnel. All data and materials collected during this study are for research purposes only, and the data will be kept in strict confidence. De-identified information and biospecimens collected for this research may be used by or distributed to investigators for other research without obtaining additional informed consent from you.

Individuals must include their name on the MRI screening form, which includes private health information. This form is only visible to the study staff and the MRI technologist.

4.2 Obtaining Identifiable Information About Non-Subjects

Not applicable

4.3 Number of Subjects

A. Total Number of Subjects

10 patients; 5 male and 5 female

B. Total Number of Subjects If Multicenter Study

N/A

C. Feasibility

DEEP6 identified 26 patients via EPIC system who had the diagnosis of PWS with only 5 patients meeting our inclusion criteria. For this reason we are expanding our diagnosis coding to include hyperphagia syndrome. Given hyperphagia is a hallmark feature of PWS and the particular behavior we are aiming to study, this expansion should improve our subject pool and allow us to enroll 10 patients.

4.4 Consent Procedures

A. Consent Process

- **Location of Consent Process**
  The electronic consent process will take place during virtual interviews on Rutgers Zoom, a HIPPA compliant platform. Individuals who are willing to participate in this study will provide their electronic informed consent along with their guardians on DocuSign, a HIPPA compliant platform during the interview.

- **Ongoing Consent**
  Not applicable

- **Individual Roles for Researchers Involved in Consent**
  Study coordinators will conduct a consent interview to explain all aspects of the study and answer any questions the prospective participants may have.

- **Consent Discussion Duration**
  The consent discussion will take approximately 30 min.

- **Coercion or Undue Influence**
  In order to minimize the possibility of coercion or undue influence, the consent process will not be conducted by research personnel who have any known relationship or conflict of interest with the prospective participants.

- **Subject Understanding**
  Throughout the consent process the prospective participants will be encouraged to ask any questions should they feel the need to do so. The consent process will be divided into a series of small sections. Members of the research team will summarize each section, use probe questions to check for sufficient understanding before proceeding to the next section.

- **Protecting Privacy**
  Should any participants desire not to interact with or provide personal information to specific members of the research team, they may report such a need to the PI and the arrangement of the research personnel will be adjusted accordingly. Participants will not be required to provide a reason if they desire not to do so. Should changing of the research personnel is not possible, the PI will discuss with the subjects about alternative solutions.

  Each participant will be assigned a unique identification code. A master code identifier which links the identification code and personal information will be maintained separately from the study data. Subject information and de-identified study data are only accessible to named investigators in this protocol and authorized research personnel. All data and materials collected during this study are for research purpose only, and the data will be kept in strict confidence. No information will be given to anyone without permission from the subjects. Should an incidental finding appear on the subject's MRI, the MRI will be shared with a neuroradiologist from University Radiology Group. No name will appear on the MRI to protect the participant's privacy.

B. Waiver or Alteration of Consent Process

- **Waiver or Alteration Details**
  We are requesting an alteration of the consent process. Prospective participants will be provided with a copy of the consent form prior to a virtual consent interview. During this interview, the study coordinator will go through the contents of the consent form with the all

applicable parties (subject, surrogate, witness, and research team member) will provide signatures on the consent form if the prospective participants are willing to provide information for eligibility screening. This is being done to minimize in-person contact during the pandemic. The alteration refers to the utilization of virtual consent.

- **Destruction of Identifiers**
  The master code identifier, which contains personal information of subjects and the participant code assigned to them, will be deleted from the server 6 years after study completion.

- **Use of Deception/Concealment**
  Not applicable

C. Documentation of Consent

- **Documenting Consent**
  Prospective participants and the study coordinator who conducts the consent process will view and provide electronic signatures on the consent form in real-time through DocuSign, and both parties will keep a copy of the form.

- **Waiver of Documentation of Consent (i.e., will not obtain subject's signature)**
  Not applicable

4.5 Special Consent Populations

A. Enrolling Minors-Subjects Who Are Not Yet Adults
N/A

B. Enrolling Wards of the State
N/A

C. Enrolling Non-English-Speaking Subjects
N/A

D. Enrolling Adults Lacking Decision-Making Capacity (Surrogate Consent)

Given the nature of PWS, all patients will lack decision-making capacity and require surrogate consent.

Subjects will be assessed by Dr. Kunal Shah, not affiliated with the study team. He will assess if they are able to understand the details of the research and if she/he can make a decision about taking part in the study. He will document this determination in the electronic medical record. If the answer is yes, consent will be obtained by the individual.

If the subject does not have capacity to make informed decisions, Dr. Marshall will be notified and will obtain their Advance Directive from the patient or surrogate who has accompanied them to their clinic visit. If the advanced directive exists and indicates that the potential subject would not want to take part in proposed medical research, the subject will not be considered for the study. If the advanced directive does not exist or does not indicate that the subject would object to taking part of proposed medical research, The determination of consent will be provided to the patient and a surrogate.

Surrogate will be chosen from the highest category of surrogate decision-making priority. The surrogate will be contacted by phone. If one or two or more available persons in the same order of priority expresses opposition to the participation of the subject in the study, the investigator must exclude the subject from the study. If two or more available persons are in different orders of priority, refusal to consent by a potential surrogate who is of higher priority controls and will not be superseded by the consent of a person who is of a lower priority.

Once identified, the research staff in Rutgers will contact the surrogate via a phone call to provide an overview of the study, requirements for research participation, and review eligibility for the study **(Appendix A).** If the surrogate provides assent to participation and candidates are eligible for the study, a virtual informed consent interview with the research team will be scheduled **(Appendix B).** Subjects who are able to understand the concept of participating in a particular study will be asked to assent to the study; those that are unable to understand the concept of participating in a particular study will not be asked to assent.
For studies at RWJUH, the Chaplain is the designated witness for studies utilizing surrogate consent.
If subject express dissent to participation or if a surrogate withdraws consent, subject will be withdrawn from the study. No further testing will take place and high-fiber supplement will be stopped.

E. Special Consent Considerations
N/A

4.6 Economic Burden and/or Compensation for Subjects

A. Expenses
We do not expect any expenses the participants will incur by taking part in the research. The compensation should cover travel costs.

B. Compensation/Incentives
Compensation will be awarded via ClinCards. Participants will receive compensation for the following milestones:

- $25.00 after the completion of sample collection and shipment at Day 0
- $25.00 after the completion of sample collection and shipment at Day 28
- $30.00 after the completion of questionnaire at Day 0
- $30.00 after the completion of questionnaire at Day 28
- $50.00 after the completion of fMRI examination at Day 0
- $50.00 after the completion of fMRI examination at Day 28
- $50.00 after the completion of blood draw at Day 0
- $50.00 after the completion of blood draw at Day 28

C Compensation Documentation
Compensation will be awarded via ClinCards. Compensation will be tracked utilizing an excel sheet, which will also be stored in an encrypted, password-protected file.

4.7 Risks of Harm/Potential for Benefits to Subjects

A. Description of Risks of Harm to Subjects

- **Reasonably Foreseeable Risks of Harm**
  Measuring fasting blood glucose during the study both involves a finger prick that pierces the skin. There may be a bruise, bleeding, or infection, at the place where the skin is pierced. However, infection is rare. Collecting fecal samples at home may impose inconvenience for the participants and their families. Participants taking NBT-BN108 may experience gastrointestinal symptoms (e.g. abdominal pain/discomfort, constipation, or bloating). These discomforts are

minor and should not last longer than a few hours. This product is low in digestible and simple carbohydrates and may reduce food intake.

There are no known health risks associated with the magnetic field produced by this 3.0T scanner in healthy adults. The FDA has indicated that they consider MR imaging on machines up to 4.0T to pose no risk (https://www.fda.gov/medical-devices/cdrh-research-programs/magnetic-resonance-imaging-mri-safety-and-effectiveness). Sammet (2016) provides discussion of MRI safety issues, each of which can be avoided with proper management. These include:1) attractive forces and torque caused by the magnet, 2) thermal effects caused by the radiofrequency fields, 3) peripheral nerve stimulation due to switching gradients, 4) hearing damage caused by the acoustic noise of the gradient system, 5) questions of risk for a fetus, 6) claustrophobia and other discomforts. These are briefly detailed below.

Attractive Forces: Ferrous material can be pulled toward the magnet, causing injury to a subject. These forces can cause metallic implants to be attracted to the magnet. The magnet can erase credit cards and other types of swipe cards.

Thermal Effects: The radiofrequency field can cause heating of ferrous material that are in the field, this is a concern for metal implants, and tattoos on the skin that specifically contain metal (iron).

Peripheral Nerve Stimulation (PNS): Although none of the planned scans involve parameters that should cause any peripheral nerve stimulation (tingling sensation caused by stimulation of a sensory nerve or motor twitch caused by stimulation of a motor nerve), it is hypothetically possible for this to occur in a scan in which magnetic gradients are being rapidly switched because the gradient cause a neuron near the surface of the skin or muscle to “fire”. While the gradient performance of the magnet is not expected to produce PNS within the operational limits defined by Siemens, they remain a possibility for a small minority of sequences. Note that PNS is not considered an adverse event because when it occurs, it is an issue only of patient comfort rather than a safety concern.

Hearing damage: The gradient system noise often reaches 100dB. Sustained exposure to this volume of noise without noise reduction devices can cause hearing damage.
Claustrophobia, and

Additional Discomforts: Some participants experience anxiety due to the enclosed nature of the space in the scanner and head coil. Some potential minor discomforts associated with the procedures include back discomfort from lying in one position.

Incidental Findings: An incidental finding could arise during scanning. Learning of an incidental finding could cause psychological distress.

- **Risk of Harm from an Intervention on a Subject with an Existing Condition**
  Although 3.0 T MRI is safe for healthy individuals several existing conditions, or situations can increase risk, or can increase the risk of discomfort. Persons at primary risk from exposure to the magnet include persons with metal surgical implants, and anyone with implanted electric/magnetic devices (e.g. a pacemaker, aneurism clips, IUDs). Such individuals are therefore excluded from the study.

- **Other Foreseeable Risks of Harm**
  N/A

- **Observation and Sensitive Information**
  N/A

B. Procedures which Risk Harm to Embryo, Fetus, and/or Pregnant Subjects
N/A

C. Risks of Harm to Non-Subjects
Fecal sample collection at home may impose inconvenience for individuals in the same household.

D. Assessment of Social Behavior Considerations
N/A

E. Minimizing Risks of Harm
Participants will be provided with oral and written instructions and supplies to collect fecal samples at home without direct physical contact with the specimens throughout the process **(Appendix D).** The specimens will be stored in a sealed container, a leak-proof secondary package (a sealable plastic bag lined with absorbent pads) and an outer package (e.g. a shipping box). Participants are not required to store the samples at home; they will mail the specimens to the research team using the shipping supplies provided.

To minimize adverse effects due to NBT-NM108, they will also be advised to drink enough fluids throughout the study (approximately eight 8-ounce glasses of fluid a day including the amount of water taken together with NBT-NM108) to avoid constipation. Participants will be asked to monitor potential adverse events daily. If participants report an adverse event, a member of the study team will contact them to ask follow-up questions about the degree of severity (mild, moderate, or severe) and the frequency of the adverse events. The study physician (PI Dr. Kesavarapu) will assess the adverse events and, if necessary, she will instruct the participants to discontinue treatment.

Measuring fasting blood glucose during the study both involves a finger prick that pierces the skin. There may be a bruise, bleeding, or infection, at the place where the skin is pierced. However, infection is rare. To minimize adverse effects, venipuncture will be done by a certified venupuncturist.

Minimizing risks from the fMRI
Exclusion of individuals with contra-indications for scanning: Individuals at primary risk for the exposure to high magnetic fields due to the presence of potentially dangerous ferrous materials in the body are excluded. This eliminates the risk of attractive forces or thermal heating. Participants are told of these exclusions during recruitment and must complete a comprehensive screen of any potential metal on their body. This screening form is reviewed by a member of the team with level 2 training, and it is confirmed by the scanner operator before the participant is allowed in the scanner room. For participants who possess orthopedic screws or pins that are firmly in bone, participants can only be scanned if the type of screw or pin is considered safe according to the *Reference Manual for Magnetic Resonance Safety. Implants and Devices, 2020 Edition*
Participants are withdrawn from the study if it is determined there is any contra-indication for scanning.

Avoidance of other risks from the high magnetic field: The MRI suite is coded in terms of zones. The entire scanner suite has controlled access, and only individuals with appropriate training (see staff training) are allowed to permit people into the scanner control room or MRI and only after they have been screened. Subjects are reminded to remove any jewelry before entering the scanner room and are screened with a handheld metal detector before entering the scanner room. Subjects are given a box/locker to place their wallet, keys and other personal belongings before entering the scanner room. All study personnel have undergone safety training and must adhere to safety rules to limit potential risks in the scanner suite.

Even if a participant passes screening for the presence of ferrous implants or tattoos, participants with orthopedic hardware are told to squeeze a ball so that they can be removed from the scanner in the unlikely event that they feel sensations such as warming at the site of an orthopedic screw or tattoo.

Avoidance of claustrophobia: Participants are excluded if they report a history of claustrophobia, and they are warned in advance that the space is cramped and that they will have a head coil placed around their head. If an individual does become claustrophobic during the scan, they can inform the study staff and discontinue the study at any time. Subjects will be given a ball to squeeze so that they can stop the scan at any time.

Avoidance of physical discomfort from lying in the scanner: Individuals with a history of chronic or recent acute back pain are excluded from the study. Subjects are warned that they will need to lie on their back in a still position for up to 90 minutes. Pillows and cushions are used to keep the subject comfortable and the scan operator always checks that the person is comfortable before closing the scan room door. If the subject does need to move during the scan, they can be repositioned as long as it is done between scans.

Avoidance of discomfort from volume of the scanner: Subjects are given earplugs and noise reducing headphones to limit the volume of the scanner.
Incidental Findings: If an incidental finding is observed during a scan, it will not be initially shown to the subject. After scanning, the scan data will be electronically submitted to the University Radiology Group imaging server, where it will be read by a neuroradiologist who will provide a brief written report. If the neuroradiologist determines the scan has clinical significance that warrants attention, the PI will inform the participant and after a release of information form is completed, will provide the imaging data and report to a physician of the participant's choice.

Hospitalization during study
If patients are hospitalized during the study, they will be removed from the study and discontinue all data and sample collection. Subjects will need to contact the study PI, Keerthana Kesavarapu, D.O. at (732)235-7784 if hospitalized during the study.

- **Certificate of Confidentiality**
  N/A

- **Provisions to Protect the Privacy Interests of Subjects**
  Should any participants desire not to interact with or provide personal information to specific members of the research team, they may report such a need to the PI and the arrangement of the research personnel will be adjusted accordingly. Participants will not be required to provide a reason if they desire not to do so. If changing of research personnel is not possible, the PI will discuss with the subjects about alternative solutions.

  Each participant will be assigned a unique identification code. A master code identifier which links the identification code and personal information will be maintained separately from the study data. Subject information and de-identified study data are only accessible to named investigators in this protocol and authorized research personnel. All data and materials collected during this study are for research purposes only, and the data will be kept in strict confidence. No information will be given to anyone without permission from the subjects.

F. Potential Direct Benefits to Subjects

There are no known direct benefits to subjects.

5.0 Special Considerations

5.1 Health Insurance Portability and Accountability Act (HIPAA)

We will ask participants for their consent so that designated members of the research team can review his/her medical record to retrieve medical history, medications, clinical and laboratory data related to PWS or hyperphagia. All received health information will be stored in an encrypted password-protected file which is stored on the university's secure server and will only be accessible to authorized research personnel.

5.2 Family Educational Rights and Privacy Act (FERPA)

N/A

5.3 Code of Federal Regulations Title 45 Part 46 (Vulnerable Populations)

A. Special Populations

N/A

5.4 General Data Protection Regulation (GDPR)

N/A

5.5 NJ Access to Medical Research Act (Surrogate Consent)

N/A

6.0 Data Management Plan

6.1 Data Analysis

Microbiome sequencing data will be analyzed based on the amplicon sequence variants (ASVs) [28] to determine the gut microbiota composition using QIIME 2 [29]. Shannon index and ASV richness will be used to assess alpha diversity. Principal coordinate analysis will be used to compare and visualize dissimilarity in gut microbiota structure between samples based on beta diversity (Weighted and Unweighted UniFrac distance) and statistical differences will be tested using permutational multivariate analysis of variance (PERMANOVA). PICRUSt 2 will be used to predict the functions of individual ASVs and the collective functions of the gut microbiota at the gene content and pathway levels [30]. Global functional profiles of the gut microbiota will be compared and visualized by principal component analysis. Repeat measures correlation will be used to assess the relationships between ASVs [31], followed by ASV clustering based on co-abundance patterns. Multivariate methods such as MaAsLin2, that allow adjustment for confounding variables and model the covariates as random effects, will be applied to interpret the relationship between ASVs / ASV co-abundance groups / functions and the clinical metadata.

Difference in continuous variables of metadata, such as measurements of hormones, blood inflammatory markers and total caloric intake, between week 0 and week 4 will be tested by Paired t-test (two-tailed) if meeting normal distribution or Paired Wilcoxon Signed-Rank test (two-tailed) if not meeting normal distribution. Difference in the continuous variables of metadata between male and female will be tested by Student's t-test (two-tailed) if meeting normal distribution or Mann-Whitney test if not meeting normal distribution. Fisher's exact test will be used to compare proportions of categorical metadata, such as data in the appetite and physical activity questionnaires, between male and female, and between Week 0 and Week 4. The false discovery rate will be applied for multiple testing, where appropriate.

Functional MRI data will be preprocessed with fMRI/PREP and analyzed with Statistical Parametric Mapping. Functional connectivity in the resting state will be extracted in the feeding related networks using the Conn Toolbox with a specific focus on connectivity between anatomically defined ROIs in the hypothalamus, insula, anterior cingulate, ventromedial/orbitofrontal prefrontal cortex, ventral striatum and amygdala. Activation to food (vs non-food) images will be assessed for responsivity in appetitive feeding networks by first modelling food stimulus presentations vs. non food presentations on a voxel wise basis and then extracting activations in anatomically defined regions of interest. The extracted data will then be analyzed as dependent variables following procedures like those described above.

6.2 Data Security

The study data will include two key components: 1) a master code identifier that contains the personal information of the participants and the participant code assigned to them; and 2) study data files that contain all the de-identified data generated in this trial. All received health information will be stored in an encrypted password-protected file which is stored on the university's secure server and will only be accessible to authorized research personnel. The data will only be accessible to authorized research personnel.

The master code identifier and the data files will be kept in two separate locations. The master code identifier is only accessible to the Principal Investigator and research personnel who schedule appointments and shipments. Data in hard copies and the master code identifier will be kept for 6 years after study completion, then any hard copies will be shredded, and the master code identifier will be permanently deleted from the server. As study data will then exist in de-identified form, the electronic data files will be kept indefinitely for utilization in future research. Data will not be distributed to other investigators for future research.

6.3 Data and Safety Monitoring

A. Data/Safety Monitoring Plan

A Data and Safety Monitoring Board (DSMB) will approve the protocol before the study is initiated, and after 30% of the participants complete 14 days of the intervention to review study progress including recruitment, retention, adherence, adverse events and study outcomes, as well as to ensure that participant safety is addressed adequately. Reports of adverse events will be used to assess the safety of the study.

B. Data/Safety Monitoring Board Details

The DSMB will include Rutgers University faculty members with expertise in medicine, epidemiology and/or statistics who are not directly involved with the study. The Board will meet before the study is initiated, and after 30% of the participants complete 14 days of the intervention to review study progress and provide recommendations related to continuing, modifying or terminating the study. Any serious adverse event that is life-threatening or results in death, that is possibly, probably, or related to the research protocol, will trigger an immediate suspension of the research. All details about the DSMB are found in **Appendix F.**

6.4 Reporting Results

A. Individual Subjects' Results

No individual study results will be released to participants.

B. Aggregate Results

No aggregate study results will be released to participants.

C. Professional Reporting

Preliminary results will be presented in local and international academic conferences. The final study findings will be published in peer-reviewed academic journals.

D. Clinical Trials Registration, Results Reporting and Consent Posting
This trial needs to be registered on ClinicalTrials.gov

6.5 Secondary Use of the Data

Fecal samples, blood samples, and de-identified data collected from participants may be stored in Lipman Hall, Rutgers University for future research by the principal investigator,

7.0 Research Repositories – Specimens and/or Data

N/A

8.0 Approvals/Authorizations

Approval from Institutional Biosafety Committee of Rutgers University for fecal sample processing and microbiota profiling has been obtained under Co-PI Dr. Zhao's program of work titled "Personalized gut microbiota-targeted dietary intervention for patients with type 2 diabetes" at Rutgers University (#17-051).

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