

The effects of diet and lifestyle on quality of life and methylation-related biomarkers *in vivo*.

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Protocol

Study Title: Methylation Diet and Lifestyle (MDL) Study

PI: Ryan Bradley, ND, MPH and Kara Fitzgerald, ND

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PROTOCOL

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A. ABSTRACT

The maintenance of health and the progression of disease are associated with an individual's genetic make-up and environmental factors, including lifestyle choices (such as diet, exercise, behaviors, stressors, sleep, tobacco and alcohol use), environmental exposures and socioeconomic determinants. Environmental factors have been shown to influence, sometimes rapidly, epigenetic processes thereby influencing genetic expression.¹ Regulation of the human genome by the epigenome is now regarded as a cornerstone, heritable, physiologic process, playing a key role in phenotypic expression of health and disease.

DNA methylation is a well-researched, primary epigenetic process.² Aberrant DNA methylation resulting in hyper- or hypomethylated regions of the genome, generally results in inhibition or

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expression of certain genes and has been associated with the pathogenesis of numerous conditions, ranging from inflammation and accelerated aging, to cancer, autoimmunity, diabetes, heart disease, dementia, allergic disease, posttraumatic stress disease and others.³⁻⁶ Likewise, certain healthy diet and lifestyle habits have been demonstrated to favorably influence DNA methylation patterns.^{7,8}

Understanding that environmental factors can potently and sometimes rapidly, favorably or negatively influence epigenetic expression, a short-term diet and lifestyle intervention may significantly augment DNA methylation expression.

The purpose of this study is to evaluate a 9-week diet and lifestyle intervention on patient-reported quality of life, symptoms, and DNA and biochemical methylation-related biomarkers in healthy males ages 50-72.

B. SPECIFIC AIMS

B.1 Primary Aim:

To evaluate a 9-week diet and lifestyle intervention on patient-reported quality of life.

Hypothesis: Quality of life will improve as a result a 9-week diet and lifestyle intervention, relative to a wait-list control condition.

Outcome measure: Changes in patient-reported quality of life, measured by the PROMIS-29 (Patient-Reported Outcomes Measurement Information System-29) questionnaire.

B.2 Secondary Aim:

To evaluate changes in patient-reported symptoms after a 9-week diet and lifestyle intervention.

Hypothesis: Severity of patient-reported symptoms will decrease following a 9-week diet and lifestyle intervention.

Outcome measures: Changes in patient-reported symptoms, measured by the MYMOP (Measure Yourself Medical Outcome Profile) questionnaire, NUNM Multi-system side effects/symptoms questionnaire, and the Medical Symptom Questionnaire (MSQ).

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B.3 Tertiary Aim:

To assess changes in methylation at CpG sites of DNA after a 9-week diet and lifestyle intervention.

Hypothesis: Methylation at CpG sites associated with chronic inflammation will change after the 9-week diet and lifestyle intervention.

Outcome measure: Number of CpG methylation sites, measured by the Infinium MethylationEPIC Kit (Illumina, Inc.)

B.4 Exploratory Aims:

To evaluate changes in biomarkers associated with methylation.

Outcome measures: Changes in the methylation related biomarkers as measured by Doctor's Data Methylation Index Panel, Methylation Profile, and Folate Vitamer Panel.

C. BACKGROUND AND SIGNIFICANCE

C.1. Methylation background and significance

Methylation, happening in every cell, virtually continuously, signifies the enzymatic addition of a single carbon compound on a substrate and regulates gene expression, protein function, and RNA processing.⁹ Folate vitamer, 5-methyltetrahydrofolate (5-MTHF) acts as key substrate within these pathways by transferring a methyl group to cobalamin by the enzyme methylenetetrahydrofolate reductase (MTHFR). This new compound, methylcobalamin, is a coenzyme used by methionine synthase and transfers a methyl group to homocysteine, creating methionine. Methionine undergoes a structural transformation under the presence adenosine triphosphate (ATP) to become S-adenosyl methionine (SAME). SAME, the universal methyl donor, is essential for over a 100 methylation reactions by transferring a methyl group to DNA, proteins, phospholipids, amino acids and neurotransmitters.

Research into the epigenome demonstrates that DNA methylation is a cornerstone regulator of gene expression. Genetic methylation is reliant on SAME availability. Myriad environmental factors, as outlined above, also influence genetic methylation either directly or through altered SAME availability.

Imbalances in methylation pathways due to single nucleotide polymorphisms (SNPs) is a rapidly emerging area of investigation implicated in numerous disease processes.¹⁰ While genetic polymorphisms alter enzyme function, it may increase risk of certain diseases.¹⁰⁻¹² Investigation into a single nucleotide polymorphism of MTHFR has gained popularity. Individuals with a point

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mutation on MTHFR gene, result in higher plasma levels of homocysteine, a risk factor associated with atherosclerosis and coronary artery diseases.^{13,14} Hyperhomocysteinemia is significantly inversely correlated with DNA hypomethylation and exacerbated by folate deficiency.¹⁵

C.2. Diet and lifestyle intervention as a means to support proper methylation

Inadequate diet and physical inactivity are indicated as two of the top 5 modifiable risk factors in several chronic diseases as well as associated with all-cause mortality.¹⁶ A diet lacking adequate intake of essential nutrients and antioxidants, as well as lack of exercise increase biomarkers associated with oxidative stress, endothelial dysfunction, and DNA hypomethylation.^{17,18} Oxidative overload places stress on cellular metabolic function, and is correlated with an increased risk of chronic diseases, such as cancer, cardiovascular and neurodegenerative diseases.¹⁹⁻²¹

With regard to the epigenome, poor diet has been associated with accelerated aging as evidenced by Horvath's Epigenetic Clock looking at DNA methylation patterns.⁸ Oxidative stress has also been shown to limit methyl donor availability, thereby impacting DNA methylation, by promoting transsulfuration and glutathione synthesis while inhibiting the methylation cycle. Oxidative damage to the epigenome further contributes to aberrant DNA methylation patterns.²²

A nutrient-rich diet and regular, moderate, aerobic exercise reduces oxidative stress, and risk of disease, by increasing antioxidants and activity of antioxidant enzymes, while also altering gene specific DNA methylation levels.^{7,23,24} Phytochemicals in various plant foods have demonstrated the ability to modulate DNA methylation. These include quercetin, found in red onion and apple skins, green tea, and red grapes; epigallocatechin-3-O-gallate (EGCG), found highest in green and white tea; genistein, found in soybeans; apigenin, found in parsley, artichoke, and celery; and curcumin, found in turmeric.²⁵ In addition, regular physical activity has shown to induce epigenetic changes in DNA methylation and lower homocysteine levels.^{26,27}

To date, this would be the first known clinical trial investigating diet and lifestyle intervention on patient-reported quality of life, symptoms, and DNA and biochemical methylation-related biomarkers in healthy males ages 50-72.

D. RESEARCH DESIGN AND METHODS STUDY FLOW

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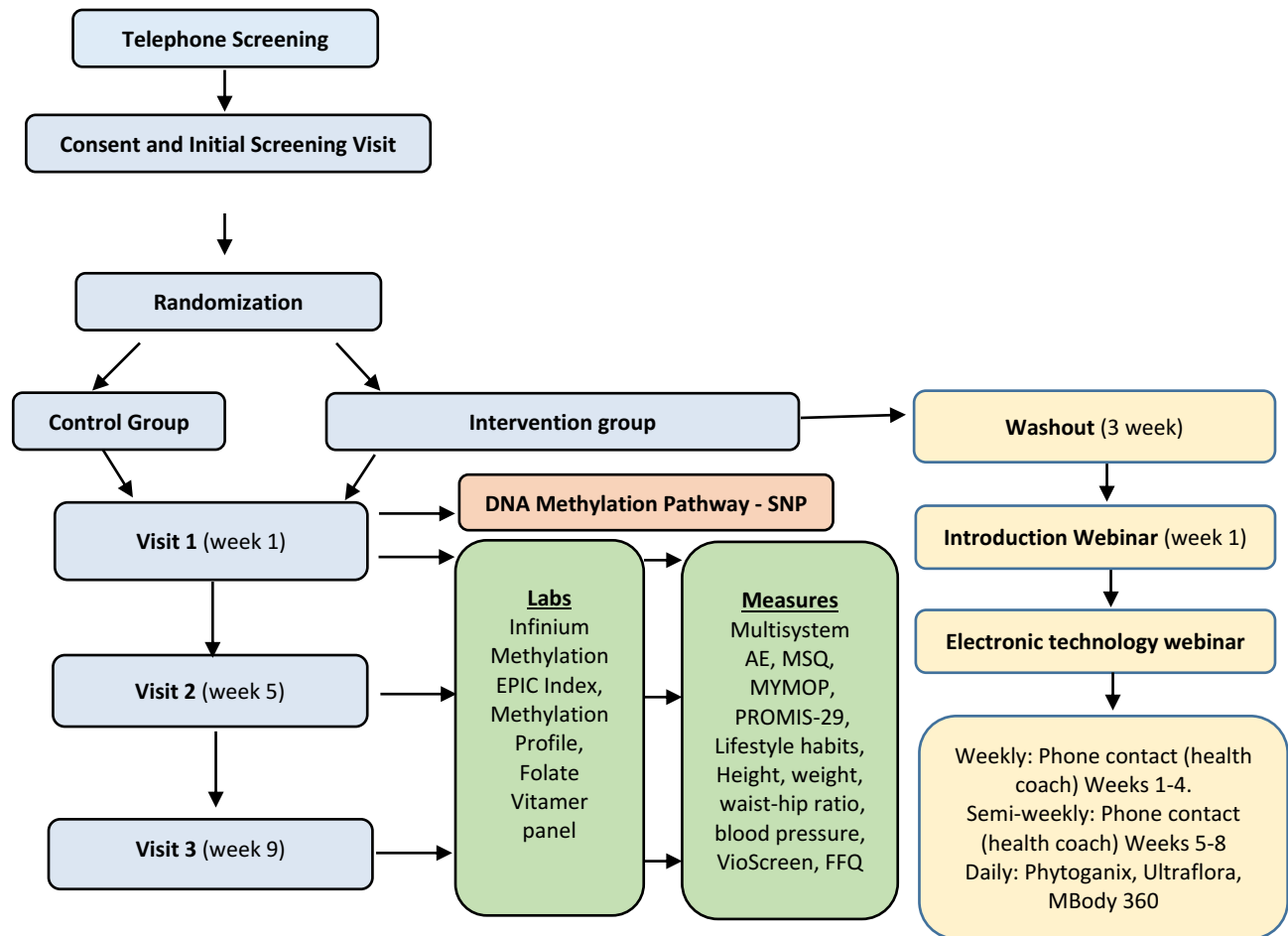
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D.1. Overview and Design

This study is a preliminary randomized control trial evaluating a 9-week diet and lifestyle intervention on quality of life, patient-reported symptoms, and biomarkers associated with methylation. We will recruit forty-eight participants with 24 randomized to each study group. Refer to Figure 1 for an overview of the study flow.

Figure 1. Study Flow Overview



D.2 Study Intervention

Study participants will be randomized into an intervention or control group. The intervention group will undergo a 3-week washout period following the discontinuation of any non-medically required nutrition or herbal supplements. The intervention group will follow detailed diet and lifestyle recommendations over a 9-week period. Two webinars at the start of the

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intervention phase will provide information to participants on proper food and exercise choices, as well as how to access and use the required electronic resources (apps).

The control group will undergo the same testing measures as the intervention group, but will not have access to the education information or be instructed to change diet or lifestyle factors. They will have access to the information after the study is complete.

D.2.1 Diet Recommendations

Specific dietary guidelines will be outlined for participants and should include the following:

Per week	Per day
<p>3 servings of liver</p> <ul style="list-style-type: none"> • (1 serving = 3 oz) • Choose preferably organic liver <p>5-10 eggs</p> <ul style="list-style-type: none"> • Ideally free-range, organic, omega-3 enriched 	<p>2 cups of dark leafy greens</p> <ul style="list-style-type: none"> • Measured raw, chopped, and packed • Including kale, Swiss chard, collards, spinach, dandelion, mustard greens • Does not include salad greens such as romaine, iceberg, Spring mix <p>2 cups cruciferous vegetables</p> <ul style="list-style-type: none"> • Measured raw, chopped, and packed • Including broccoli, cabbage, cauliflower, Brussels sprouts, bok choy, arugula, kale, watercress, rutabaga, kohlrabi, radish, turnip
General	
<p>Choose organic at least half of the time.</p> <p>Stay hydrated</p> <p>Don't eat between 7pm and 7am, and at least 2 hours before bed</p> <p>Include healthy oils</p> <ul style="list-style-type: none"> • Balance types of fat • E.g. coconut, olive, flaxseed and pumpkin seed oil <p>Avoid dairy, grains, legumes/beans</p> <p>Minimize plastic food containers</p>	<p>3 cups colorful, low glycemic vegetables of your choosing</p> <p>1-2 medium beet</p> <p>4 tbsp (1/4 cup) pumpkin seeds (or pumpkin seed butter)</p> <p>4 tbsp (1/4 cup) sunflower seeds (or sunflower seed butter)</p> <p>1+ serving methylation adaptogens:</p> <ul style="list-style-type: none"> • 1/2 cup berries (wild preferred) • 1/2 tsp rosemary • 1/2 tsp turmeric • 2 medium cloves garlic • 2 cups green tea (brewed 10 minutes) • 3 cups oolong tea (brewed 10 minutes) <p>6 oz animal protein</p> <ul style="list-style-type: none"> • grass-fed, pastured, organic and hormone/antibiotic-free <p>2 servings of low glycemic fruit</p>

These recommendations serve as a guidance, while individual intake may vary based on preference. Throughout the study, participants will be provided 2-week menu plans with recipes for all meals, snacks, smoothies.

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Refer to Appendix A for detailed nutrition recommendations.

D.2.2 Exercise Recommendations

Participants will be encouraged to exercise a minimum of 30 minutes at least 5 days per week at an intensity of 60-80% of maximum perceived exertion. Possible types of physical activity include, but not limited to, brisk walking, light jogging, hiking, dancing, yardwork, bicycling, and swimming.

D.2.3 Stress Management Recommendations

Participants will utilize the Cleveland Clinic's Stress Free Now application to engage in a variety of guided stress reduction techniques, including meditation and mindful breathing. Recommended frequency is twice daily, preferably morning and evening.

D.2.4. Lifestyle Recommendations

Participants are encouraged to average a minimum of 7 hours of sleep per night.

D.2.5. Study Supplements

Participants will be provided with two supplements. Details on the products are outlined in section D.12 of the protocol. Participants will take 2 servings of PhytoGanix and 2 capsules of UltraFlora Intensive Care probiotic daily, in divided doses.

D.2.6. Control group

The control group will be waitlisted during the study intervention phase. They will be provided with a copy of the intervention webinars and educational handouts after the study is concluded.

D.2.7. Tools to measure study variables and assist with adherence:

D.2.7.1. Mbody360 app

MBODY360 is a HIPAA-Compliant Web-based complete health & wellness coaching platform that connects practitioners to their patients and clients in real-time and tracks patient's engagement & compliance. It includes secure video, text chatting, and group coaching tools. Participants will track their sleep, supplement usage, exercise, stress management. Participant progress will be monitored daily by health coaches using the Mbody 360 app.

Participants will be prompted to answer the following questions each day:

- Did you eat according to program guidance?
- What did you eat that was not according to the program?

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- Did you complete at least 30 minutes of moderate intensity physical activity today?
- Did you complete the stress management program today? For how long?
- How many hours of sleep did you get last night?
- Did you take any new medications, prescription or over-the-counter, today?
 - *If yes, which new medication did you take?
- Did you take your study probiotic supplement today?
- Did you take your Phytoganix supplement today?

D.2.7.2. VioScreen

VioScreen is a validated food frequency questionnaire software program, which tracks food intake over the past 90 days. It will take approximately 20 minutes for participants to input their dietary information. Developed by the Nutrition Coordinating Center at the University of Minnesota, it utilizes a standardized food and nutrient database. All participants will complete this at Visits 1, 2 and 3.

D.2.7.3. Stress Free Now

Stress Free Now by the Cleveland Clinic is an application that seven different relaxation techniques, including mindfulness and meditation. Each technique lasts approximately 10 minutes.

D.2.7.4. Zoom

A web-based communication program, Zoom will be utilized for group webinars and Q&A sessions throughout the study.

D.2.7.5. Study Specific Food Frequency Questionnaire

Not all foods recommended and consumed during the study intervention may be captured by VioScreen. An additional food frequency questionnaire (FFQ), with approximately 250 foods and beverages, was created to assess consumption. It has been designed to be consistent with the validated block Brief FFQ by NutritionQuest. This will be administered electronically via RedCap online database to all study participants at Visits 1, 2, and 3.

D.2.7.5. Adherence phone calls

Participants will be contacted via phone once per week during the first 4 weeks of the study and every other week for the remainder of the study. Designated health coaches will assess for adherence to the study intervention including dietary, stress management, and exercise recommendations. Participants will be able to ask questions pertaining to the study intervention.

Refer to Appendix B for detailed script of these phone calls.

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D.3 Description of Participant Population

D.3.1. Participant Characteristics

Sample size: n=48

We will enroll and randomize up to 48 people which will allow for 20% attrition such that we have an evaluated sample size of 40 to allow for withdraws or dropouts.

Age: 50-72

Gender: Males only

Minorities: all racial and ethnic backgrounds will be recruited from the general population without preference or discrimination.

Vulnerable Populations (e.g. children, pregnant women, cognitively impaired): No vulnerable populations will be enrolled.

D.3.2. Inclusion and Exclusion Criteria

Individuals must meet the following participation criteria:

Inclusion criteria:

- Males, ages 50-72
- Willing to adhere to 9 weeks of a dietary and lifestyle program including specific nutrition and exercise guidelines
- Willing to avoid any over-the-counter medications, supplements or herbal products for the length of the study, except short-term use (<1 week) use at least 1 week before scheduled study visits
- Willing to have blood drawn three times and abstain from food or beverage intake for 10-12 hours before blood draws
- Willing to provide saliva samples
- Willing to track food intake, sleep, stress management techniques, and exercise daily
- Willing to drink a nutrient-enriched beverage and take a encapsulated probiotic daily
- Willing and able to use electronic devices and connect to the internet
- Able to speak, read and understand English

Exclusion criteria:

- Currently taking any of the following prescription medications
 - Proton pump inhibitors: omeprazole (Prilosec, Prilosec OTC); aspirin and omeprazole (Yosprala); lansoprazole (Prevacid, Prevacid IV, Prevacid 24-Hour); dexlansoprazole (Dexilent, Dexilent Solutab); rabeprazole (Aciphex, Aciphex Sprinkle); pantoprazole (Protonix)

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- H2-blockers: nizatidine (Axid, Axid AR, Axid Pulvules); famotidine (Pepcid, Pepcid AC); cimetidine (Tagamet, Tagamet HB); ranitidine (Zantac)
- These classes of medications are excluded due to direct (due to nutrient requirements for metabolism) and indirect (through impaired digestion and assimilation of nutrients).
- Use of nutrition supplements or herbal products not prescribed by a licensed healthcare provider for a medical condition
- Currently following a prescribed dietary/lifestyle program or initiate within the 30 days prior to baseline
- Initiation of or changes to an exercise regimen within 30 days prior to baseline
- Use of nicotine, marijuana or cannabinoids (including CBD products) or recreational drugs/substances (such as but not limited to cocaine, phencyclidine [PCP], and methamphetamine) current/within the last 30 days or use during the study
- Have a diagnosis of cardiovascular disease, kidney disease, liver disease, diabetes, autoimmune disease, high blood pressure, or cancer (does not include *basal cell carcinoma, squamous cell carcinoma, and/or carcinoma in situ of the cervix*).
- Have a diagnosis of an immunodeficiency condition, such as Human Immunodeficiency Virus (HIV) infection or Acquired Immune Deficiency Syndrome (AIDS)
- Have a diagnosis of neurodegenerative conditions such as, amyotrophic lateral sclerosis (ALS), Parkinson’s disease, Multiple Sclerosis, or Alzheimer’s disease.
- Excessive alcohol consumption (more than 4 drinks per day or 14 per week on average)
- Known sensitivity, intolerance or allergy to ingredients in the study supplements or in the recommended dietary therapy
- Currently receiving intravenous nutrient therapy
- Currently participating in another interventional research study or participated in another interventional study within the last 3 weeks prior to baseline

D.4 Outcome Measures

Table 1 below provides a summary of study outcomes, associated measures, and a brief description. More detailed descriptions of each measure are provided below Table 1.

Table 1.		
OUTCOME	MEASURE	DESCRIPTION
Primary Outcome		
Health-related Quality of Life	Patient Reported Outcomes Measurement Information (PROMIS-29)	PROMIS-29 is a validated, 29 question survey divided into seven sub-domains of function including physical functioning, social function, pain interference, pain intensity, sleep, depression, and anxiety.

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Secondary Outcome		
Change(s) in patient-reported symptoms	Measure Yourself Medical Outcome Profile (MYMOP)	MYMOP is a patient-centered outcome measure that allows for the participant to self-select their top health concern(s) or symptom(s) they are dealing with in their own words. The participant can also choose an activity that is limited by that health concern.
	NUNM Multi-system AE questionnaire	The NUNM Multi-system Symptom/Adverse Event Questionnaire is a standardized ninety-one point monitoring form that requires asking questions pertaining to the following organ systems: eyes/ears/nose/throat, gastrointestinal, neurological/musculoskeletal, psychological/general, cardiopulmonary, skin, genitourinary and whole body systems.
Tertiary Outcome		
Methylation status of CpG sites	Infinium Methylation EPIC Index by Illumina	Epigenetic profile that assesses methylation on over 850,000 CpG DNA sites
Exploratory Outcomes		
Changes in the methylation related biomarkers	Methylation Profile by Doctor's Data	Functional assessment of the phenotypic expression of common SNPs and provides a "methylation index," a ratio of SAM to SAH.
	Folate Vitamer Panel by Doctor's Data	Measures folate congeners affecting enzyme function in methylation pathways including unmodified Folic Acid (UMFA), 5-Methyltetrahydrofolate (5-MTHF), tetrahydrofolate (THF), folinic Acid (5-CHO-THF)
Change(s) in patient-reported symptoms	Medical Symptoms Questionnaire (MSQ)	The MSQ is a 71-question measure that measures various health areas including: Digestive tract, Eyes, Energy/Activity, Emotions, Head, Heart, Joint/Muscles,

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		Lungs, Mind, Mouth/Throat, Nose, Skin, Weight, and Other.
Measurement of Potential Confounders	DNA Methylation Pathway Profile	Assesses changes to SNPs - DNA genotype which may affect enzymatic function which include, methionine metabolism, detoxification, hormones, and vitamin D.

D.4.1. Primary Outcome: Patient-reported Quality of Life

Patient Reported Outcomes Measurement Information - PROMIS – 29 Profile

The Patient Reported Outcomes Measurement Information System (PROMIS) is a collection of patient-reported health status inventories funded by the NIH. PROMIS provides a standardized, reliable, and valid measure of health status that assesses physical, mental, and social well-being from the patient perspective. The PROMIS-29 Profile is a collection of self-report short forms containing items from 7 PROMIS domains (Physical Function, Anxiety, Depression, Fatigue, Sleep Disturbance, Ability to Participate in Social Roles and Activities, Pain Interference, and Pain Intensity). The PROMIS-29 Profile consists of 4 questions per domain rated on a 5-point rating scale along with a 1 question Pain Intensity section rated on an 11-point scale. The domains are assessed over the past 7 days except for the Physical Function domain which has no specified time frame.

PROMIS-29

Score	Severity
29-52	None-minimal
51-75	Mild
76-100	Moderate
101-125	Moderately severe
126-150	Severe

PROMIS-29 provides a continuous symptom severity score (7 categories). Total raw scores range from 29 to 150. A raw score is created from each subscale (except Pain Intensity) that makes up the Profile except the Pain Intensity item. Scores are translated into T-scores which are referenced to mean score levels in the general U.S. population. Lower PROMIS scores have been shown to predict reduced health related quality of life for multiple conditions.

D.4.2. Secondary Outcome: Patient-reported Symptom Change

D.4.2.1. Primary Measure: Measure Yourself Medical Outcome Profile (MYMOP)

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Measure Yourself Medical Outcome Profile (MYMOP) is a patient centered outcome measure that allows for the participant to self-select their top health concern(s) or symptom(s) they are dealing with in their own words. The participant can also choose an activity that is limited by that health concern. The participant is then asked about their own general wellbeing. Each of these questions is scored on a 0-6 scale. The medications that the participant is taking related to the specific symptoms and their desire to cut down the medication is also recorded.

Scoring of the MYMOP is an average of all of the profile questions answered. Participants only have to choose one symptom and answer the well-being profile question. If a second related symptom and activity level are identified all of the scores are included in the average final score.

MYMOP has been shown to be an effective outcome measurement for health concerns, especially in a diverse population and has more sensitivity to change compared to the SF-36.^{29,30}

D.4.2.2. Secondary Measure: NUNM Multi-system Symptom/Adverse Events Questionnaire

The NUNM Multi-system Symptom/Adverse Event Questionnaire is a standardized ninety-one point monitoring form that requires asking questions pertaining to the following organ systems: eyes/ears/nose/throat, gastrointestinal, neurological/ musculoskeletal, psychological/general, cardiopulmonary, skin, genitourinary and whole body systems.

Table 3. Grading of Items on the NUNM Adverse Event Monitoring Form	
Grade	Definition
0=Not Present	No AE, Function within normal limits
1=Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; no intervention	No intervention; asymptomatic lab or radiographic findings; marginal clinical significance
2=Moderate; minimal, local or noninvasive intervention indicated	AE limited activities of daily living (ADLS)
3=Severe or medically significant but not immediately life-threatening	AE significantly limited basic self-care, AE required initial hospitalization or prolongation of hospitalization
4=Life-threatening	Life-threatening consequences AE
5=Fatal	Fatal AE

Changes in a current symptom(s) by a grade of 2 or higher, or a new symptom(s) graded 2 or higher will be included in analysis to compare symptom changes between groups.

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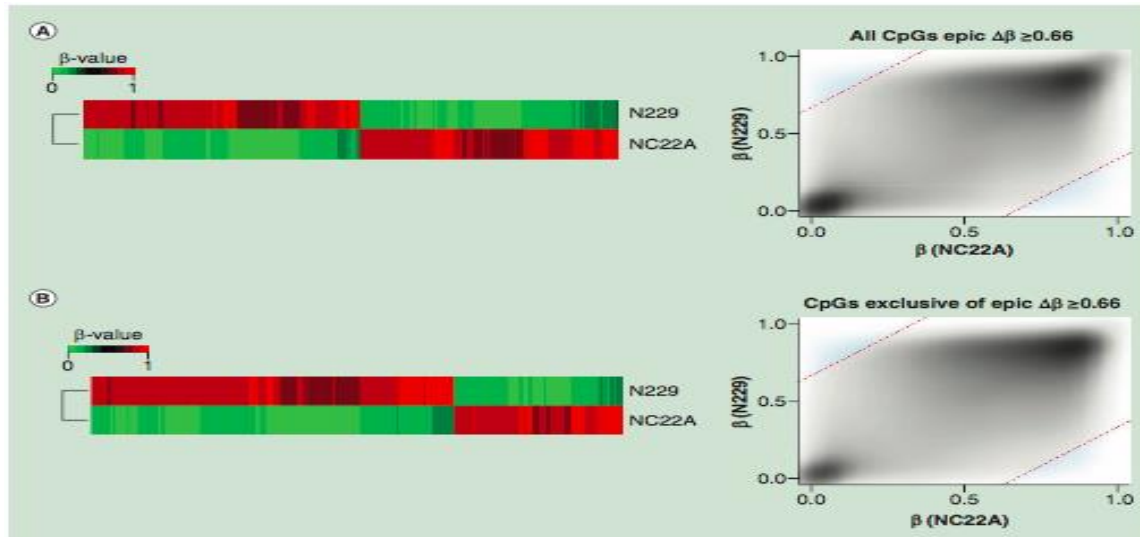
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D.4.3. Tertiary Outcome: Changes in methylation at CpG sites of DNA

The tertiary outcome will be the assessment of methylation changes at “CpG” sites on DNA using a commercial available assay kit. CpG sites are sites on DNA in which a cytosine base is separated from a guanine base by only one phosphate; CpG sites are known sites for regulation by methylation. The Infinium MethylationEPIC Kit by Illumina uses the latest technology to assess methylation status of over 850,000 DNA sites across the genome. Using DNA provided from a saliva sample, this assay measured changes in methylation at “CpG” sites of DNA, i.e., whether or not sites for methylation are, or are not, methylated. For a given time point, the degree of methylation at each site (i.e., β) is presented as a color ranging from “green” (i.e., $\beta = 0$, low methylation) to “red” (i.e., $\beta = 1$, high methylation). We will compare the methylation state of participants at baseline to their status at the end of the study. “Differential” methylation is considered a change in CpG site methylation of $\Delta\beta \geq 0.66$. See Figure 2 below for a typical representation of the results of the assay.²⁸

Figure 2: Typical Demonstration of Results Applying the Infinium MethylationEPIC Kit



A saliva sample will collect approximately 2 mL (~1 teaspoon) into a cryovial. Saliva samples will be labeled only with a study ID, date of collection, and visit number and frozen to -70 °C.

At the conclusion of the study, all samples will be batched shipped to: Yale University Center for Genome Analysis Attn: Guilin Wang, PhD, Associate Director of Microarray; 300 Heffernan Dr. B-36, West Haven, CT 06516.

D.4.4. Exploratory Outcomes: Changes in biomarkers associated with methylation

D.4.4.1. Methylation Profile – Doctor’s Data

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Methionine is an important amino acid involved in the methylation of DNA, proteins, and neurotransmitters. Impaired methionine metabolism may be caused by genetic changes, nutritional deficiencies, aging and environmental toxins and associated with various health conditions such as cardiovascular disease. This test measures various intermediates in methionine metabolism, including cystathionine, cysteine, homocysteine, methionine, S-Adenosylhomocysteine (SAH), S-Adenosylmethionine (SAM), SAM/SAH ratio, and adenosine. Abnormal levels may indicate single nucleotide polymorphisms to specific enzymes.

A venous blood draw will collect 6 mL of blood (~1 tablespoon) into an EDTA tube (purple top).

D.4.4.2. Folate Vitamer Panel – Doctor’s Data

Folate exists in several different chemical forms which are most commonly involved in one carbon metabolism. Abnormal levels of folate vitamers may indicate single nucleotide polymorphisms affecting enzyme function in methylation pathways. This test measures folate congeners including unmodified Folic Acid (UMFA), 5-Methyltetrahydrofolate (5-MTHF), tetrahydrofolate (THF), folinic Acid (5-CHO-THF).

A venous blood draw will collect 6 mL of blood (~1 tablespoon) into an EDTA tube (purple top).

D.4.4.3. Tertiary Measure: Medical Symptoms Questionnaire (MSQ)

The MSQ is a 71 question survey that measures various health areas including: Digestive tract, Eyes, Energy/Activity, Emotions, Head, Heart, Joint/Muscles, Lungs, Mind, Mouth/Throat, Nose, Skin, Weight, and Other. Each question is measured on a 5 point rating scale ranging from Never or almost never have symptom to frequently have it and effect is severe. Scoring: Optimal scoring is less than 10, Mild Toxicity: 10-50, Moderate Toxicity: 50-100, Severe Toxicity: over 100.

This instrument is a non-validated tool utilized in the functional medicine community to assess health. The current study may have the potential to validate the MSQ by comparing results to the PROMIS-29 and NUNM Multi-system Symptom/Adverse Event Questionnaire.

D.4.5. Measurement of Potential Confounders

DNA Methylation Pathway Profile – Doctor’s Data

This test assesses changes to DNA genotype, called single nucleotide polymorphisms (SNPs), which may affect enzymatic function. Potential biochemical pathways impacted include, methionine metabolism, detoxification, hormones, and vitamin D. This is a blood spot test and requires collection of 5 drops of blood after a finger stick.

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PI: Ryan Bradley, ND, MPH and Kara Fitzgerald, ND

IRB #: RB100217

Approval Date: 2.9.18

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D.5 Participant Schedule

D.5.1 Overview

Individuals will be screened over the phone and at one in-person visit. Eligible participants who choose to enroll in the study will attend a total of 4 in-person visits. Participants will be randomized into either the intervention or control group after the screening visit. All study visits will be conducted at the Helfgott Research Institute in Portland, OR. Refer to Table 4 for an outline of the study visits.

Table 4: Study Procedures

	Phone Screening	Screening Visit	Study Visit 1 (Week 1)	Study Visit 2 (Week 5)	Study Visit 3 (Week 9)
Eligibility screening	X				
Consent for study participation		X			
Eligibility verification		X			
Demographic form		X			
W-9		X			
Anthropometrics/Vitals: height, weight, waist-hip ratio, heart rate, blood pressure, temperature, respiratory rate		X	X	X	X
Questionnaires: <ul style="list-style-type: none"> • NUNM Multi-system Symptom/Adverse Event • MSQ • MYMOP • PROMIS-29 • Lifestyle Habits • VioScreen • Study specific FFQ 			X	X	X
Blood Draw, blood spot card and saliva sample			X	X	X

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DNA Methylation Pathway Profile - SNP Panel			X		
Infinium Methylation EPIC Index			X		X
Methylation Profile			X	X	X
Folate Vitamer Panel			X	X	X
Estimated Time to Complete	15 minutes	45 minutes	90 minutes	90 minutes	90 minutes

D.5.2. Recruitment

Recruitment will be conducted in the general community surrounding Portland, OR using flyers, online, newspaper, and electronic newsletters.

D.5.3. Telephone Screening

Potential participants will be screened over the phone prior to coming in for the first visit. A standardized telephone script will be used. The telephone screening call will take approximately 10-15 minutes. Participants who meet eligibility criteria over the phone will be scheduled for a screening visit. The consent form will be provided to participants prior to the screening visit via email; they will be instructed to review the form ahead of time but not to sign it until the screening visit where they can ask questions about the study. If an individual is eligible for the study, enrollment will happen on a rolling basis.

D.5.4. Screening Visit

All study visits will take place at the Helfgott Research Institute in Portland, OR. The objective of the Screening Visit is to explain the study procedures, secure informed consent, and verify eligibility for the trial. Eligibility will be verified by reviewing responses to the eligibility questionnaire. The screening visit is estimated to take 45 minutes.

The following will be performed/obtained at screening:

- Signed and dated informed consent
- Eligibility Questionnaire
- Verification that the participant meets the inclusion and exclusion criteria;
- W-9 – once determined eligible
- Demographics (including age, gender, ethnicity, and race);
- Anthropometrics and vitals (including height, weight, pulse rate, blood pressure);

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Sample half size servings of the study supplement drink will be provided while participants are completing the eligibility questionnaire. Upon sampling the shakes, participants will be asked if they are willing to take the product twice per day for the study period.

D.5.5. Informed Consent

Consent will be obtained from participants at the beginning of the screening visit. The consent form will include the purpose of the study, a description of the procedures, a list of risks and benefits, an assurance of confidentiality, an assurance of withdrawal without prejudice, a statement about payment policies for research related injuries, study incentives, and a telephone number for answering questions about the research, all explained at an 8th grade reading level. Participants will also be given information on their right to privacy for personal health information based on HIPAA regulations. Procedures for data sharing using de-identified data will be explained later in this protocol.

D.5.6. Randomization

Once a participant is deemed eligible and provided signed consent, the study staff will determine randomization order using a random number generator (randomization.com). After enrollment, the study coordinator will select the next sequential envelope from the locked filing cabinet within the study site office. The study staff will write the current date and participant initials on the bottom right corner of the envelope and open the envelope and retrieve its contents. The envelope contents will show which cohort the participant is assigned to, with intervention or control. This will be assigned to the participant ID number and entered into REDCap.

D.5.7. Washout Period

Study participants assigned to the intervention group will be asked to refrain from taking any over-the-counter supplements, medications or herbal products, except short-term use (<1 week) and at least 1 week before scheduled study visits, alcohol, nicotine, marijuana or cannabinoids (including CBD products) or recreational drugs/substances for 3 weeks before the baseline visit. Written instructions will be provided.

D.5.8. Study Visits

There will be 3 in-person study visits for all participants during the 9-week study.

Baseline/Study Visit 1 (Week 1)

The following procedures, evaluation and/or assessments will be obtained at Study Visit 1:

- Height and weight
- Vital signs including heart rate, blood pressure, respiration rate and temperature

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- Questionnaires including Lifestyle Habits, PROMIS-29, MYMOP, MSQ, and NUNM Adverse Events, VioScreen
- Blood draw to collect 2 tubes of blood
- Finger stick to collect 5 drops of blood
- Saliva sample
- If in intervention group, will receive study supplements for first 4 weeks and instructions on how and when to take
- Case report form completed by study staff
- Waist-to-hip ratio will be calculated using the following methods:
 - Participant with stand up straight and breathe out. Use a tape measure to check the distance around the smallest part of the waist, just above the umbilicus. This is the waist circumference.
 - Measure the distance around the largest part of the hips — the widest part of the buttocks. This is the hip circumference.
 - Calculate the waist-to-hip ratio by dividing the waist circumference by the hip circumference.

Study Visit 2 (Week 5)

The following procedures, evaluation and/or assessments will be obtained at Study Visit 2:

- Weight
- Vital signs including heart rate, blood pressure, respiration rate and temperature
- Questionnaires including Lifestyle Habits, PROMIS-29, MYMOP, MSQ, and NUNM Adverse Events, VioScreen
- Blood draw to collect 2 tubes of blood
- Saliva sample
- If in intervention group, will receive study supplements for remaining 4 weeks of study
- Case report form completed by study staff

Study Visit 3 (Week 9)

The following procedures, evaluation and/or assessments will be obtained at Study Visit 3:

- Weight
- Waist-to-hip ratio will be calculated as previous defined in Study Visit 1
- Vital signs including heart rate, blood pressure, respiration rate and temperature.
- Questionnaires including Lifestyle Habits, PROMIS-29, MYMOP, MSQ, and NUNM Adverse Events, VioScreen
- Blood draw to collect 2 tubes of blood
- Saliva sample
- If in intervention group, return unused supplements
- Case report form completed by study staff

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D.5.9. Educational Webinars

Weekly, live webinars will provide information on the diet, exercise, stress management, and lifestyle recommendations. Prior to initiating any of these changes, an introductory webinar will provide an overview of the study intervention. An additional webinar will provide detailed instruction on how to access and utilize two electronic applications – MBody360 and Stress Free Now. All webinars will be delivered via Zoom, a free online communication application.

Refer to Appendix C for detailed information on webinar content

D.6. Study Withdrawal and/or Early Termination

Participants will be informed that they have the right to withdraw from the study at any time, without affecting their relationship with NUNM. Participants may also be discontinued from the study if the investigators determine it is in the best interest of the participant.

Information about study withdrawal or premature discontinuation from the study will be captured throughout the study. Study withdrawal or premature discontinuation will be categorized as follows:

- Participant began taking an excluded medication or supplement
- The Principal/Clinical Investigator decided that withdrawal/discontinuation was in the best interest of the participant due to an adverse event, non-compliance, or another reason
- The participant requests withdrawal from the study for reasons other than above
- The participant failed to return for study visits

If a participant withdraws before the second visit, they will be replaced, however participants who withdraw between the second and third visits will not be replaced. Every effort will be made to collect a final salivary sample and all final labs from all participants, even if they withdraw from the study early.

D.7. Study Products

D.7.1. PhytoGanix (tropical fruit flavor)

Organic Superfruits & Greens Powder Drink Mix is a food-based phytonutrient blend designed to support intestinal health and immune function.

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Serving Size: 1 Scoop (10.4 grams)

Ingredient	Amount per serving
Calories	40
Total Carbohydrate	9 g
Dietary Fiber	1 g
Sugars	1 g
Protein	1 g
Vitamin C (as ascorbic acid)	100 mg
Calcium	20 mg
Iron	1.4 mg
Sodium	30 mg
Organic Seed and Prebiotic Blend:	5.53 g
Isomalto-Oligosaccharide, Flax Seed Powder, Chia Seed Powder, Quinoa (<i>Chenopodium quinoa</i> Willd.) Sprout Powder	
Organic Veggie Blend:	2.35 g
Spirulina Whole Algae Powder, Carrot Root Powder, Broccoli Head Powder, Cauliflower Head Powder, Spinach Leaf Powder, Parsley Leaf Powder, Bamboo Shoot Extract, Beet Root Powder	
Organic Fruit Blend:	1.2 g
Apple Fruit Powder, Pineapple Fruit Powder, Strawberry Fruit Powder, Raspberry Fruit Powder, Blackberry Fruit Powder, Blueberry Fruit Powder	
Herbal Support Blend:	300 mg
Milk Thistle Seed Extract, Turmeric Rhizome Extract, Green Tea Leaf Extract, Reishi Mushroom Whole Plant Powder, Maitake Mushroom Whole Plant Powder, Shiitake Mushroom Whole Plant Powder, Japanese Knotweed Root Extract, Quercetin Dihydrate, Organic Maca Root Powder, Organic Cassia Bark Powder, Dong Quai Root Powder, Ginger Root Powder, Garlic Bulb Powder	
Enzyme Blend (including protease, amylase, lipase, and cellulase)	200 mg
<i>Lactobacillus acidophilus</i> VK3 Strain	200 mg

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D.7.2. UltraFlora Intensive Care

Probiotic designed to help maintain healthy flora and bowel function during dietary changes, promote the integrity of the GI intestinal barrier, and support a healthy immune response.

Ingredient	Amount per serving
<u>Lactobacillus plantarum 299v Strain</u>	20 billion live Strain organisms ^{††}

Ingredients: *Lactobacillus plantarum* 299v Strain, Potato starch, capsule (hydroxypropylmethylcellulose), *Lactobacillus plantarum* 299v (soy), and magnesium stearate (vegetable).

D.7.2. Product Descriptions

PhytoGanix contains organic fruit, vegetables, chia, quinoa sprouts, herbs, plant enzymes, prebiotics and probiotics. PhytoGanix Intensive will be provided and packed by Metagenics, Inc. in individual serving packets. It should be stored at room temperature in a cool, dry place.

UltraFlora Intensive Care contains the *L. plantarum* 299v strain of probiotics to help relieve intestinal irritation and bowel discomfort, promote the integrity of the GI intestinal barrier, and support a healthy immune response. UltraFlora Intensive Care will be provided and packed by Metagenics, Inc. in glass jars containing 30 capsules. It should be stored at room temperature in a cool, dry place or keep refrigerated.

The study products will be labeled with the following information: the product name, the study title, the IRB #, dosing information, the phone # of the Study Coordinator and Clinical Investigator.

D.7.3. Product Dispensing

Participants assigned to the intervention group will be given ~60 packets of PhytoGanix (adequate to provide 2 servings per day until Visit 2) and 2 bottles of UltraFlora Intensive Care at Study Visit 1. They will be instructed to wait to take the supplements until after the Introduction Webinar during the first week of the study. This will cover the approximately 56 dosages needed until Visit 2, plus extra dosages to allow for any loss of product. Participants will be provided with written instructions on taking the study product. At Visit 2, participants will return any unused study product in exchange for 2 additional bottles of each study product to cover the approximately 56 doses needed from Visit 2 until Visit 3. At Visit 3, participants will return any unused study product.

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D.7.4. Product Administration

Participants will be instructed to blend, shake, or briskly stir 1 level scoops of PhytoGanix into 8 ounces of water or juice two times daily. Participants will be instructed to take 2 capsules of UltraFlora intensive Care with 8 ounces of water once a day.

D.7.5. Product Adherence

Participants will be contacted by phone weekly Weeks 1-4 and semi-weekly Weeks 5-8 by the health coach team to check in on their progress. If participants report issues with product adherence product, the health coach team will discuss strategies with the participant to increase adherence. (For example, they may suggest posting note on the refrigerator or keeping the study product jar in a location such as a kitchen counter or dining table in order to incorporate taking the study product into daily routines.)

Adherence will be quantified by the study investigators who will count unused capsules and weigh unused study product after it is returned at the 5 week and 9 week study visits.

D.8. Blood and Saliva Collection and Analysis

Fasting blood samples and saliva samples will be obtained at the baseline, 5 week and 9 week visits. All study participants will be assigned a unique study ID number, and all blood samples, saliva samples, and laboratory paperwork will be identified with that number.

Blood collection will consist of a venous blood draw into of approximately 12 mL of blood (~2 tablespoons) into two EDTA (purple top) tubes and one finger stick collecting 5 drops of blood for a blood spot test.

These samples will be picked up by courier on the same day they are collected and transported to Doctor's Data, Inc for analysis.

Saliva sample collection will include approximately 2 mL collected from each participant. Participants will have abstained from food or beverages, except water, overnight prior to the collection. Participants will simply spit into a screw topped cryovial labeled with their study ID, date of collection, study time point, and approximate volume. If necessary, participants will be allowed up to 8 oz., of water in order to provide the sample, waiting at least 15 minutes between water intake and the sample collection. If necessary, cotton buccal cushions/swabs will be provided for participants to chew on to induce salivation.

E. HUMAN SUBJECTS RESEARCH

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E.1. Potential Risks

E.1.1. Blood Draws

There will be three blood draws during this study. The main risks associated with drawing blood are infection, bruising, redness, pain, discomfort, or bleeding at the needle puncture site. Occasionally, individuals may feel lightheaded or faint during or immediately after a blood sample is collected. There is also a rare risk of swelling around the vein.

E.1.2. Study Product Ingestion

Most of the ingredients are essential macronutrients and micronutrients. Risks associated with any dietary supplement include gastrointestinal symptoms such as gas, bloating, digestive upset and change in bowel movement frequency or consistency. Individuals with known sensitivities, intolerances, and allergies to any of the product ingredients will be excluded from the study.

The product ingredients are approved for human use by the FDA under GRAS (Generally Recognized as Safe) status and are frequently consumed in commonly eaten foods, spices, flavorings, and beverages. The study products are described in sections D.7.1. and D.7.2.

Lactobacillus species are usually well-tolerated when taken orally. *Lactobacillus actobacillus acidophilus* and the *Lactobacillus plantarum 299v strain* have been used safely in studies lasting from one week to six months.

E.1.3. Confidentiality

There is a small risk that information about a study participant could be inadvertently disclosed to non-study personnel.

E.2. Protection Against Risks

E.2.1. Blood Draws

Study protocol and procedures to minimize risks associated with blood draws are in place. Blood samples will be obtained by certified phlebotomists, or licensed physicians in medical facilities, which includes procedures and staff to handle medical emergencies.

E.2.2. Study Product Ingestion

Prospective participants will be screened for eligibility to minimize potential contraindications to the study product as well as determine general health status. Adverse event monitoring will occur throughout the study.

E.2.3. Confidentiality

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Throughout the study, measures to ensure the privacy of information on study participants will be maintained. Details of Confidentiality and Data security are outlined in section G.1.

Participants will be told about any new information that may affect their willingness to participate in the study.

E.3. Potential Benefit

Participants may not receive a direct benefit from their participation in this research. Participants may experience improved quality of life through adherence to the lifestyle recommendations included in the proposed intervention, however their composite effects are unknown. Participants may experience improved cardiovascular health through adherence to the physical activity and stress management recommendations in the proposed intervention.

F. Importance of Knowledge to be Gained

The proposed research will benefit society by producing proof-of-concept data on the efficacy of diet and lifestyle factors to alter epigenetic signaling after a short-term intervention. Demonstrating alterations in methylation sites in pathways with known associations with disease risk may lead to improvements in disease prevention initiatives and interventions toward the improved health of the public.

G. DATA COLLECTION, SOURCES AND ANALYSIS

G.1. Confidentiality and Data Security

Throughout the study, measures to ensure the privacy of information on study participants will be maintained. All study investigators and staff have been certified in human subjects research and have received training in HIPAA regulations. Participants and staff will be informed of the confidentiality of information and assured that data will be used only for statistical purposes and group analyses in which the individual cannot be identified. No data beyond what is stated in the Informed Consent will be sought without authorization from the participant. Information on illnesses and hospitalizations will not be sought from hospitals or doctors without a signed medical release from the subject. Conversely, no information on any individual will be released to anyone other than study personnel without a signed medical release from the participant, or where appropriate, the next of kin or a physician in case of a life threatening emergency to the subject. All study personnel will be instructed not to discuss any participants with persons other than study personnel.

Each participant will be assigned a unique alpha-numeric ID upon screening. All blood tubes, and associated paperwork will be marked using the unique ID to protect patient confidentiality. All data resulting from study visits will be collected on standardized case report forms (CRFs). Data will be transferred from CRFs to a secure REDCap database for data management. REDCap

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is a secure, web-based application that supports electronic data capture for research studies. REDCap features include: 1) intuitive data entry features; 2) audit trails for tracking data manipulation and export; 3) user-based privileges that support HIPAA compliance; 4) seamless data export to common statistical packages; and 5) procedures for importing data from external sources. The REDCap database will be accessible only by the study team. Data will be destroyed 3 years after completion of the study. Computer files will be deleted from the server and paper files will be destroyed using a professional document shredding service.

All completed forms will be kept in locked files to which only project personnel have access. These files will also be in locked rooms. Data from paper forms will be manually entered into REDCap and kept as electronic files. Electronic files will be password protected with access given to study personnel only. All analyses will be conducted by study staff at the Helfgott Research Institute. The study biostatistician will only access de-identified data exported from REDCap to Excel or SPSS. Only de-identified data will be presented in any reports, presentations or manuscripts.

G.2. Informed Consent

Consent will be obtained from the participants for the study protocol. The Consent Form will include the purpose of the study, a description of the procedures, a list of risks and benefits, an assurance of confidentiality, an assurance of withdrawal without prejudice, study incentives, and telephone numbers for answering questions about the research. Participants will also be given information on their right to privacy for personal health information based on HIPAA regulations. Procedures for data sharing using de-identified data will be explained.

G.3. Adverse Event Monitoring

If an Adverse event (AE) is reported at Study Visit 1 or Study Visit 2, the Study Coordinator will contact the CI and/or the PI as soon as possible, i.e., during the appointment, for AEs graded as “moderate” or more severe (Grade 3 or higher). If the Study Coordinator is unable to reach the CI/PI within the appointment, the participant will be contacted by the PI as soon as possible within the same day. The Study Coordinator and CI will complete the “Standardized Adverse Events Questionnaire: Spontaneous Reporting” form (Appendix A) in order to capture details regarding the adverse event and any action taken.

If a participant calls the Study Coordinator or the Principal/Clinical Investigator to report AEs, a similar procedure will be followed, which will include completion of the “Standardized Adverse Events Questionnaire: Spontaneous Reporting” form.

G.3.1. Risk Classification: Low

All procedures are considered “low risk” as per the NUNM Helfgott Research Institute Procedure/Intervention Risk Classification list. These low-risk procedures include administering

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questionnaires, and venipuncture/blood draws on non-pregnant adults. Participants will be told about any new information that may affect their willingness to participate in the study.

G.3.2. Reporting to the NUNM Institutional Review Board

All adverse events will be recorded in the Adverse Event Log. Anticipated adverse events will be reported to IRB during continuing review. Unanticipated problems will be reported to IRB within 10 working days of the investigators being notified of the event. Serious adverse events will be reported to IRB within 24 hours of the investigators being notified of the event.

G.3.3. Adverse Event Definitions

Adverse Event (AE): Any untoward medical occurrence in a clinical investigation subject. An AE can therefore be an unfavorable and unintended sign (including abnormal laboratory findings), symptoms (nausea and loose stools), or disease temporally associated with the study, whether or not related to the study procedure, or a worsening of a pre-existing condition or illness.

Serious Adverse Event (SAE): Any untoward medical occurrence that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or results in a congenital anomaly or birth defect. Other important medical events requiring medical or surgical intervention to prevent a serious outcome would also be considered SAEs.

Unanticipated Problems (UP): Any incident, experience, or outcome that meets all of the following criteria: 1) 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 document; and (b) the characteristics of the subject population being studied; 2) related or possibly related to participation in the research and 3) 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.

G.4. Statistical Analysis

PROMIS 29 T-scores will be presented as mean (SD), for each group at each of the three data collection time points. For the primary aim, we will compare changes in PROMIS-29 T-scores between groups, using a linear mixed model with random intercept to test for a Group*Visit interaction. If there is a significant main effect, we will also present uncorrected contrasts comparing changes from baseline to Week 5, and from baseline to Week 9, between groups.

Although PROMIS T-scores are not particularly susceptible to extreme outliers, data will be examined for any outlying PROMIS scores or changes in PROMIS scores. If these scores are deemed to be in error, then they will be treated as missing in all analyses. Otherwise, scores

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will be retained in the main analysis; but we will conduct separate sensitivity analyses to test for effects of outlying data on results.

Mixed models incorporate all available data, without excluding any participants from analysis. Nevertheless, any participants who have baseline data only will be excluded from analysis. As an exploratory follow-up analysis, we will re-analyze changes from Week 1 to Week 9, using data from completers only.

For the secondary aim, the total score of the MSQ will be analyzed similarly to PROMIS-29. Since MYMOP asks for two symptoms without distinction, we will classify each problem as either the *Primary* problem (the problem with the highest symptom rating at baseline; or, in the case of a tie, the first problem listed) or the *Secondary* problem. We will also calculate the *average* of the two problem scores, at each collection time point. If only one problem is listed, then this will be used as both the Primary and Average, for that individual, with Secondary score missing. Changes in MYMOP severity scores will be compared between groups using a non-parametric Mann-Whitney test. Separate comparisons will be made between changes over the first 4 weeks of the study and changes over the entire 9-week intervention period.

G.5 SPONSOR RESPONSIBILITIES FOR INTERVENTIONAL PRODUCT(S)

The sponsor, Metagenics, is responsible for supplying the interventional product(s) in accordance with Good Manufacturing Practices (GMP). These products will be fully characterized, properly coded and suitably packaged in a way to provide protection against deterioration with appropriate labelling. Sufficient samples of each batch and a record of analyses and characteristics will be kept by the sponsor for reference so that, if necessary, an independent laboratory is able to re-check the interventional product(s), e.g. for quality control or bioequivalence. Records of the quantities of interventional products supplied will be maintained with batch or serial numbers by sponsor. The sponsor will ensure that the investigator is able to establish a system within their institution for adequate and safe handling, storage, use during the course of the study and, if appropriate, return or destruction of the interventional product(s) upon completion of the study.

G.6 SPONSOR MONITORING

The sponsor, Metagenics, will appoint suitable and appropriately trained monitor(s) and clinical research support personnel to conduct regular site visits starting with recruitment and continuing on a schedule that is mutually agreed upon by the study coordinator and sponsor. Follow up information from these visits will be provided to site, sponsor and to Principal Investigators in a timely manner for their reference purposes.

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Sponsor will ensure to provide ongoing training to their study personnel so that they are suitably qualified and to keep them up to date with new developments in clinical research.

H. Incentives

Participants will earn \$50.00 for each week they participate in the study, up to \$450 if they participate for the full 9 weeks. Checks will be mailed after visit 2 (week 5) and visit 3 (week 9), compensated for each week they participated.

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