Official Title: Western Versus Prudent Diet Feeding Study in Adult Women With Asthma

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JHM IRB - eForm A – Protocol

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1. ABSTRACT

Background: In the U.S., increased intake of fast food and highly processed foods (a "Western-style" diet) has been coincident with the dramatic rise in asthma. Observational studies demonstrate a link between a dietary pattern and asthma morbidity at the level of the individual, and limited trials of counselling for a healthier diet have shown promise in improving asthma health.

Objectives: The objective of the study is to demonstrate the feasibility of a randomized, controlled trial of complete dietary replacement (feeding study) in a population of adults with asthma, and to provide measures of variability in the proposed outcomes (symptoms, lung function, markers of inflammation and oxidative stress) to inform sample size calculation for a larger, definitive trial.

Methods: The study is a feasibility trial of a prudent (high in fruit and vegetables, whole grains, low-fat dairy) versus Western-style (high in processed foods, fast food, sweets) diet intervention in adults with asthma. Eight to twelve adult women (ages 18-55) who meet eligibility criteria will be enrolled. At baseline, demographic information, asthma symptom scores, usual dietary intake, anthropometric measures and DEXA, spirometry, FENO, blood, urine, nasal, and sputum samples will be collected. Participants will then be randomized to either the Western-style diet or a prudent diet. Prior to initiating the diet, they will have assessment of select biologic outcomes (FENO, spirometry, symptom assessment, biomarkers of oxidative stress and inflammation in urine and serum, and nasal swab). After 6 days of the randomized diet, they will repeat these assessments and undergo induced sputum for local (pulmonary) biomarkers of inflammation and oxidative stress. The participants will then have a 4-8 week washout period, and repeat the same order of assessments prior to and after receiving the alternate diet assignment. During each of the 6-day interventions, participants will record asthma-related symptoms via a standardized questionnaire. At the final session, participants will provide feedback on the acceptability and palatability of the dietary interventions and assessments. As this is a feasibility study, we will use results to assess the process and procedures necessary to scale the intervention to a larger study population.

Implications: Study findings will provide insight into feasibility of a larger-scale clinical trial investigating the effect of these diet patterns on adult asthma. We expect to gain insights into the

acceptability of intervention duration, tolerability of study visits and participant flow, and sample size necessary for a larger, definitive clinical trial.

2. OBJECTIVES

The primary objective for this study is to determine the feasibility of a randomized, crossover trial of a Western-style compared to a prudent dietary intervention among Baltimore City adults with asthma. Our secondary objective is to gather preliminary data on markers of systemic and airway inflammation and oxidative stress, lung function, and changes in the microbiome induced by diet to inform sample size for a definitive trial.

3. BACKGROUND

The rising burden of asthma has trended in parallel with dramatic shifts of dietary habits towards a "Western" diet and away from a "prudent" diet (high in fruits, vegetables, whole grains, n3-PUFAs (found in fish, seafood, some plant-based oils), low fat dairy products, and overall antioxidant levels). These trends are particularly relevant to low-income, African American populations who, in addition to their particular vulnerability to asthma, tend to have poorer quality diets, lower in fiber, fruits, and vegetables, and higher in fat and sugar. These correlations have prompted further evaluation, though dietary investigations in asthma have primarily included a focus on individual micronutrients. These include "anti-inflammatory" and antioxidant vitamins (A, C, D, E), minerals (zinc, selenium), flavonoids, and unsaturated fatty acids. While each factor has demonstrated some positive association with improved asthma or wheeze, nearly all have conflicting evidence in the peer-reviewed literature and, disappointingly, randomized trials have shown inconsistent or negative results with single-micronutrient supplementation. The complexity of diet as an exposure variable may be difficult to accomplish by isolating nutrients**.** For example, while one medium grapefruit contains 200% daily value (DV) of vitamin C, it also contains 70% DV vitamin A, 8% DV calcium, and 16% DV fiber, and has a filling effect that may defer intake of other foods. Unanticipated correlations between nutrients may falsely diminish associations in controlled, statistical analyses, and fail to identify potentially synergistic effects. Because of these issues, there has been increased interest in evaluating whole foods and dietary patterns. To date, nutritional research studies examining dietary patterns have focused heavily on the relationship to other chronic diseases. Whole food intake and dietary patterns have been predictive of multiple disease states, including obesity, cardiovascular disease, and cancer, and there is evidence of an association between healthier, recommended dietary patterns and wellness in these examples. Indeed, evidence exists as to the success of this approach in asthma. A diet rich in antioxidants and low in saturated fats (the Mediterranean diet, similar to the prudent diet but typified by foods endemic to Mediterranean countries, i.e. olive oil, fish, wine) has been proposed as a "respiratory healthy" diet, possibly by attenuating the inflammatory response through high levels of antioxidant intake. Cross-sectional studies suggest better symptom control with adherence to this diet and others similar in composition. Observational work outside of the U.S. demonstrates the positive effects of a "healthy" diet pattern and the negative effects of an "unhealthy" diet pattern on asthma morbidity and respiratory health.

Several small trials of whole food and diet pattern intervention have shown promise. In Australia LG Wood, et al conducted a trial with 137 asthmatic adults who were asked to consume either <1 serving fruit and 2 servings vegetables daily (low antioxidant, LAO) versus 2 servings fruit and 5

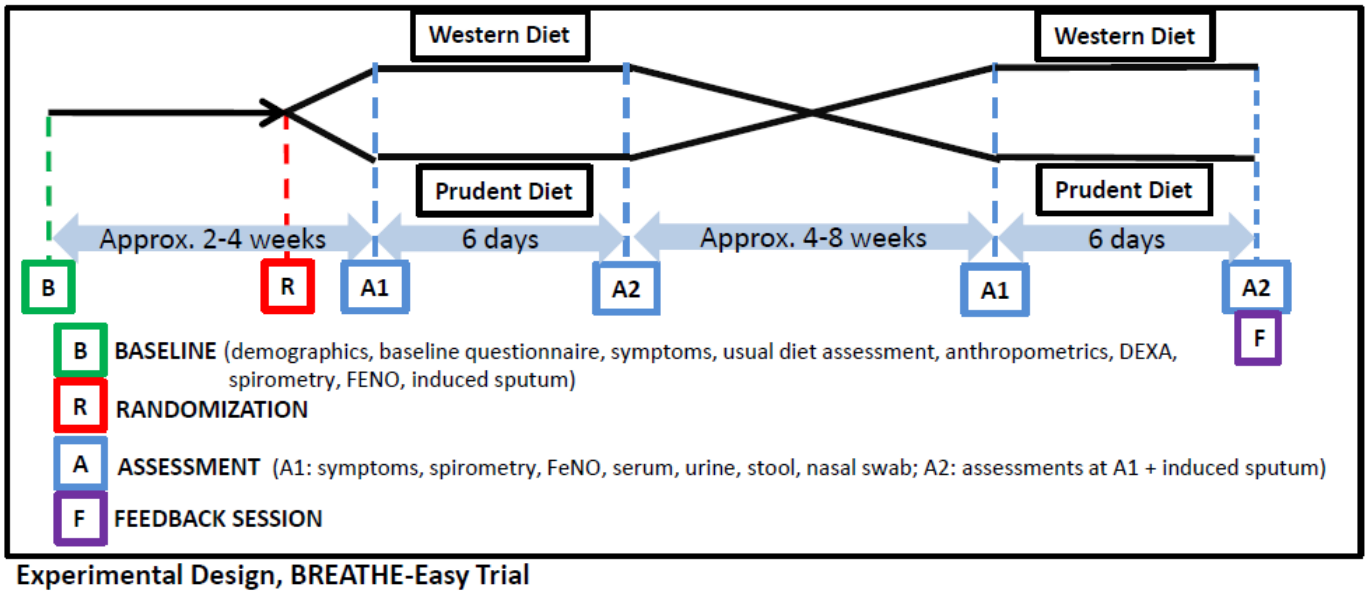
servings vegetables daily (high antioxidant, HAO). Percent predicted FEV_1 and time to exacerbation were significantly reduced in the LAO compared to the HAO group. The same investigators found that a single high-fat/high-energy meal increased neutrophilic airway inflammation and reduced airway bronchodilator recovery in adult asthmatics. Two small, pilot trials of healthy diets in U.S. populations demonstrate encouraging improvements in asthma outcomes including symptoms, lung function, and serum inflammatory markers. Studies thus far have been limited by either scale-ability (due to duration and therefore cost of studied intervention) or underrepresentation of low-income, African-Americans who have the greatest burden of asthma prevalence and morbidity. Furthermore, the prevalence of adult asthma is 70% higher in females than males, females carry a higher rate of asthma attacks than males, and obesity is a greater risk factor for asthma among adult women compared to adult men. There is a need to define interventions in populations affected the most by asthma, in order to have the greatest impact.

The current study is designed to assess the feasibility of a larger-scale intervention trial comparing a Western-style diet to a prudent diet intervention in an urban population disproportionately affected by asthma. Similarly, because females with obesity represent a high-risk group for asthma incidence and prevalence, and may represent a unique phenotype as compared to males and non-obese individuals, we propose to limit this small pilot to females with obesity.

4. STUDY PROCEDURES

a. Study design summary

Figure 1. Study Design



Experimental Design, BREATHE-Easy Trial

We will conduct a single-blinded (study staff only), randomized crossover pilot trial to evaluate the feasibility of a Western and prudent diet feeding study in Baltimore City adults with asthma (Figure 1). The results from this study will provide data to inform a larger trial evaluating the differential effects of a Western-style (high in processed foods, fast food, sweets) diet and a prudent (high in

fruit and vegetables, whole grains, low-fat dairy) diet on symptoms, lung function, systemic inflammation, and local airway inflammation among adult asthmatics.

Potential study subjects will undergo phone screening prior to invitation for initial clinic visit. Participants will be consented at initial clinic visit, after which they will complete additional eligibility testing (urine pregnancy testing, urinary cotinine measurement, induced sputum collection, and if eligible undergo baseline assessments (demographic information, baseline questionnaire, usual dietary intake evaluation, anthropometric measures, DEXA, spirometry, FeNO, blood, urine, and nasal sample collection). Participants will then be randomized to initiate a Western-style diet or prudent diet and scheduled a date to return for their first assessment (A1) prior to initiating the assigned intervention. FeNO, spirometry, symptoms/questionnaires, and nasal swab, urine, stool/fecal strip, and serum collection will occur at first assessment. Participants will then receive 3 days of meals to take home. Participants will return at day 3 to retrieve the last 3 days of meals (or alternatively, the last 3 days of meals will be delivered to their place of residence), and will return again at day 6 to repeat the assessments at A1 + sputum induction. During each of the Western and prudent intervention diet periods, participants will complete daily questionnaires/diaries on food consumed, symptoms, and other potential exposures that may affect asthma control. Participants will then have an approximately 4-8 week washout period during which they will be instructed to resume their regular diet before continuing with the alternate dietary intervention with mirrored assessments. At the second A2 visit, participants will be asked to answer questions regarding acceptability and tolerability of study schedule and participant flow, and to report comments on study acceptability ad libitum.

i. Recruitment

We will recruit 8-12 obese adult females with asthma, meeting the inclusion and exclusion criteria below. Participants will be recruited using a database of previous study participants in the Center for Childhood Asthma in the Urban Environment (which conducts research in child and adult population) and other Johns Hopkins asthma cohorts currently approved by the Johns Hopkins Institutional Review Board. All potential subjects in this database have consented to having their contact information maintained in a database and to be contacted about participating in research studies.

We will also recruit patients from the Principal Investigator's and Co-Investigator's Pulmonary Clinics who have agreed to be contacted about research studies. In addition, we will recruit potential subjects who are referred from other physicians and have given permission to be contacted. These potential participants will not be approached by research staff or investigators unless they have given permission to their doctors and this permission has been documented in the medical record. All such potential subjects will be given information about the study by their doctor along with study team contact information. They may initiate contact with the study team unless they have given permission to be contacted by a study team member and it is documented in their medical record.

We will also recruit patients at community events and with IRB-approved fliers.

We will also send letters regarding the study to the families of children who have enrolled in our pediatric asthma studies within the Center for Childhood Asthma in the Urban Environment. These families have demonstrated an active interest in research and a high proportion of family members of these children also have asthma. The letter will provide an "opt-out" option where female adult family members may choose not to be contacted by phone. If no "opt-out" letter is returned, we will contact the families to assess interest.

ii. Participant eligibility, baseline visit, and randomization

Participant eligibility is determined by an initial telephone screening call and in an in-person screening visit.

Phone Screening: Potential participants will be asked brief questions on major eligibility criteria (see Section 5), including age, sex, medical history, medications, smoking history and status, food storage and preparation equipment available to the potential participant, and willingness to consume foods planned for the study.

Screening/Baseline exam*:* All potential participants who pass the phone screen will be invited to consent and complete the in-person screening, which includes an assessment of asthma symptoms via the **Asthma Control Test** (ACT), measurement of **body mass index** (BMI), a **urine cotinine** test and, for women of childbearing potential, a **urine pregnancy test**. Individuals with a negative urine cotinine who are not pregnant will complete initial spirometry and sputum induction.
Spirometry and Induced Sputum Details:

1) **Spirometry** will be performed according to American Thoracic Society guidelines using a KOKO (Pulmonary Data Services, Inc., Louisville, CO) pneumotachometer to obtain FEV_1, FVC, FEF_{25-75}, and PEF. After spirometry is obtained, participants will be administered 2 puffs of albuterol via a spacer. After 15-45 minutes, spirometry will be repeated. With each spirometry session, 3 sets of FEV_1 values will be obtained. The highest FEV_1 measurement post albuterol administration will be used to determine eligibility for induced sputum (must be >60% of predicted value based on standard reference equations adjusted for age, race, gender, and height).
2) **Sputum induction**: Participants will be instructed through an induced sputum procedure via hypertonic saline nebulization. Sputum inductions and processing will be performed following published protocols. Briefly, after baseline spirometry, an ultrasonic nebulizer will be used to deliver hypertonic saline for at most a total of 20 minutes. The subject will be asked to expectorate sputum into a specimen cup placed on ice. Lung function will be monitored to ensure participant safety. If there is a fall in FEV_1 of $\geq$ 20% compared baseline FEV_1 prior to sputum induction at any point or if the patient is unwilling or unable to continue, sputum induction will be terminated and albuterol administered as needed.

Those who are able to successfully produce an adequate sputum sample will be enrolled in the full trial and asked to complete the baseline evaluation on the same day.

The baseline evaluation will include the following:

1) **Collection of demographic information**, including name, address, date of birth, and medical history
2) **Baseline questionnaire**, including questions on common exposures known or hypothesized to exacerbate asthma (i.e. cleaning products, home environment, etc).
3) **Symptom report** via questionnaires. These include the Asthma Quality of Life Questionnaire (AQLQ), Asthma Symptom Utility Index (ASUI), and Asthma Control Test (ACT). The AQLQ has been validated for individuals 15 and older and is designed to measure daily impact of asthma in patients' lives. The ASUI is a validated 2-week recall questionnaire that addresses issues of asthma control weighted by impact on functional status. The ACT is a validated 4-week recall of symptoms and functioning.
4) **Other questionnaires** aimed at assessing self-efficacy in disease management, including the validated PROMIS-29 and EQ-5D-5L.
5) **Instruction for evaluation of usual diet** via a 3-day food diary, to be completed at home over the ensuing 3 days.
6) **Assessment of daily caloric** needs by a registered dietician, on which to base calories provided during 6-day feeding intervention.
7) **Dual Energy X-ray Absorptiometry (DEXA) Scan** at the Johns Hopkins Hospital Broadway Adult Outpatient CRU.
8) **Anthropometric measures** of height, weight, waist circumference, chest circumference, and neck circumference
9) **Fractional exhaled nitric oxide (FeNO) measurement:** Exhaled nitric oxide is a known marker of pulmonary inflammation and will provide a means of assessing pulmonary oxidative stress and inflammation. Measurement of exhaled nitric oxide will be obtained according to ATS guidelines and prior to lung function whenever possible. Nitric oxide concentrations will be measured using a chemiluminescent analyzer (NIOX Mino, Aerocrine, Sweden). This equipment is FDA-approved for clinical use in asthma management.
10) **Serum collection:** Serum will be collected via phlebotomy, a routine clinical procedure performed by drawing blood from a vein in the forearm, in order to investigate serum biomarkers indicating inflammation and oxidative stress, such as IL-5, IL-13, RANTES, superoxide anion, and lipid peroxides. Any excess serum will be stored for future analyses.
11) **Urine collection:** Prior to enrollment, all participants will submit a urine sample for pregnancy testing, and urine will also be collected for analysis at each assessment visit. Urine biomarkers, including LTE_4 and F-isoprostane, will be measured to assess oxidative stress and inflammation. We will also measure other markers of inflammation/chemical exposure that may affect asthma control. Any excess urine will be stored for future analyses.
12) **Nasal swab collection:** Nasal swab (and optionally, oropharyngeal and skin swab) collection will occur at each assessment visit, before and after each dietary intervention, to obtain a sample for cellular examination. Analysis of biomarkers in nasal specimens will include (1) cell count and differential, (2) cytokines, (3) chemokines, and other markers of inflammation, (4) microbial assessment.

***Randomization**:* Randomization will occur within approximately one to two weeks of enrollment. Participants will be randomized using a computerized random assignment table with permuted blocks. Participants will be aware of their assignment as the study cannot be blinded.

iii. Intervention

Participants will be provided with all meals for 6 days through the Nutrition Core at the ICTR. Meals will be calorically balanced such that the "prudent" versus "Western-style" intervention will contain similar caloric counts.

Participants will be instructed not to consume meals or snacks outside of those provided and will be asked to visit the research clinic at most twice to pick up their meals: once at initial assessment, and mid-way through the intervention period, they may either return to the clinic to pick up the remainder of their meals or their meals will be delivered to their home. For each day of this controlled feeding, participants will also complete a **daily diary**. The daily diary asks about study foods not eaten, non-study foods eaten, and beverages consumed over the past day. Participants will also report **daily symptom assessment** with validated symptom scales and rescue inhaler use daily during the 6-day dietary intervention, as well as exposures that may affect asthma control.

A washout period of approximately 4-8 weeks separates each intervention period; the washout period allows ad libitum food intake.

Description of Diets:
Energy values and macronutrient distribution (as percent of kilocalories) will be similar for the prudent and Western diet with a goal of 35-37% Fat, 45-47% Carbohydrate, and 16-18% Protein. Differences between the diets are defined in the table below.

Nutrient or Food Pattern	Western Diet	Prudent Diet
Saturated fat (% of kcals)	≥10	<8
Omega-6:Omega-3 ratio	≥13:1	≤5:1
Whole grains (% of grain servings)	0	100
Fruit (servings/day)	≤1	≥3
Vegetable, not potato (servings/day)	≤1	≥3
Nuts (1 oz. serving/day)	0	≥1
Fish (3 ounce serving 4/6 days)	0	2
Processed meat (ounces/day)	≥3	0
Sweets (30-125 gram serving/day)	≥1	0
Sugar sweetened beverages (fluid ounces/day)	≥8	0

Food Production and Distribution:
Meals will be prepared and distributed in the research kitchen of the Nutrition Core at the ICTR. The research kitchen is inspected annually by the Baltimore City Health Department. Managers and staff of the ICTR research kitchen are certified through ServeSafe. Participants will be instructed in food safety.

Promotion of Adherence: Efforts to promote adherence begin at the earliest stages of the study. During the phone and in-person screenings, participants are repeatedly provided with information about key features of the study. During the phone screening, participants will complete the Diet Screening Form, which includes a detailed list of foods provided. Individuals must be willing to eat

each of these foods; otherwise, they are excluded. Key contacts with nutrition staff include an in-person evaluation by a dietitian. The intent of these efforts is to identify and exclude, prior to randomization, participants who are unwilling or unable to comply with the feeding protocol.

Efforts to promote adherence center on making the foods palatable and convenient to the participant lifestyles; maintaining easy access to staff; and providing available, daily, supportive contacts. Acceptance of the controlled feeding protocol is increased by allowing participants to consume pre-approved selected beverages, as well as an unlimited amount of water and artificially sweetened soft drinks.

Assessment of Adherence

Adherence assessment includes both self-reported and objective measures. The subjective measures are used to determine suitability for randomization and subsequently to counsel participants and promote adherence during the trial. Self-reported measures are obtained from information provided on a daily diary and from subjective judgment by clinic personnel. Each day, an overall compliance score (0=compliant; 1, 2, or 3 for various degrees of non-compliance) is calculated based on staff observation and information from the daily diary. Adherence will also be assessed at each post-intervention assessment via objective measures, including serum biomarkers (lipids, carotenoids, and free fatty acids), and on-site meal consumption of the first meals within each intervention. Comparison pre- and post-intervention will provide objective measures of adherence to provided intervention.

iv. Assessments

Assessments will include the following (refer to Figure 1):

Assessment 1 (before the dietary intervention period)

1) **Spirometry:** Spirometry will be performed according to ATS guidelines to obtain FEV_1, FVC, FEF_{25-75}, and PEF. After spirometry is obtained, participants will be administered 2 puffs of albuterol via a spacer. After 15-45 minutes, spirometry will be repeated. At each examination, 3 sets of FEV_1 values will be obtained, and the highest FEV_1 measurement will be used as the baseline measurement for FEV_1 recovery.
2) **Fractional exhaled nitric oxide (FeNO) measurement:** Exhaled nitric oxide is a known marker of pulmonary inflammation and will provide a means of assessing pulmonary oxidative stress and inflammation. Measurement of exhaled nitric oxide will be obtained according to ATS guidelines and prior to lung function whenever possible. Nitric oxide concentrations will be measured using a chemiluminescent analyzer (NIOX Mino, Aerocrine, Sweden). This equipment is FDA-approved for clinical use in asthma management.
3) **Serum collection:** Serum will be collected via phlebotomy, a routine clinical procedure performed by drawing blood from a vein in the forearm, in order to investigate serum biomarkers indicating inflammation and oxidative stress. Any excess serum will be stored for future analyses.
4) **Urine collection:** Prior to enrollment, all participants will submit a urine sample for pregnancy testing, and urine will also be collected for analysis at each assessment visit. Urine biomarkers, including LTE_4 and F-isoprostane, will be measured to assess oxidative stress and inflammation.

We will also measure other markers of inflammation/chemical exposure that may affect asthma control. Any excess urine will be stored for future analyses.

5) **Stool/Fecal strip sample:** One sample will be collected to analyze gut microbiome at baseline and during each follow-up visit. Sample will be stored at -80°C until further analysis of gut microbiome.
6) **Symptom report** via questionnaires including the Asthma Quality of Life Questionnaire (AQLQ), Asthma Symptom Utility Index (ASUI), and the Asthma Control Test (ACT). The AQLQ has been validated for individuals 15 and older and is designed to measure daily impact of asthma in patients' lives. The ASUI is a validated 2-week recall questionnaire that addresses issues of asthma control weighted by impact on functional status. Participants will also complete daily symptom diaries during the 6 days of intervention. The ACT is a validated 4-week recall of symptoms and functioning.

7) **Nasal swab collection:** Nasal swab (and optionally, oropharyngeal and skin swab) collection will occur at each assessment visit, before and after each dietary intervention, to obtain a sample for cellular examination. Analysis of biomarkers in nasal specimens will include (1) cell count and differential, (2) cytokines, (3) chemokines, and other markers of inflammation, (4) microbial assessment.

Daily Diaries:
During the course of each feeding period, participants will complete daily diaries (questions and format supplied) to document data such as adherence to the diet, use of cleaning products, symptoms and albuterol use. Participants will be contacted daily with reminders to complete the diaries and to allow for any participant questions or concerns. Telephone calls or text messages will be used for this purpose.

Assessment 2 (after the dietary intervention period) will include all of the above assessments, plus:

8) **Sputum induction**: Participants will be instructed through an induced sputum procedure via hypertonic saline nebulization. Sputum inductions and processing will be performed following published protocols. Briefly, after baseline spirometry, an ultrasonic nebulizer will be used to deliver hypertonic saline for at most a total of 20 minutes. The subject will be asked to expectorate sputum into a specimen cup placed on ice. Lung function will be monitored to ensure participant safety. If there is a fall in FEV_1 of $\geq$ 20% compared baseline FEV_1 prior to sputum induction at any point or if the patient is unwilling or unable to continue, sputum induction will be terminated and albuterol administered as needed.
9) **Feedback session:** We will solicit feedback on acceptability of intervention and satisfaction with study structure, including study instruments administered to optimize participant experience and retention for an anticipated, larger-scale trial.

b. **Study duration and number of study visits required of research participants**

Each study participant will be enrolled for a maximum of approximately 16 weeks, and with rolling enrollment we anticipate completion of all study visits within 1 year. Each research participant will be required to attend a baseline clinic visit and four assessment clinic visits. In addition, during each of the Western and prudent intervention periods, the participants will be required to pick up their meals at most twice: once at initial assessment, and mid-way through the intervention period, they

may either return to the clinic to pick up the remainder of their meals or their meals will be delivered to their home. In total, each research participant will be required to make five to seven study-related visits.

c. **Blinding, including justification for blinding or not blinding the trial, if applicable.**

It is not possible to blind participants to this protocol, as they will be aware which diet they have been assigned. Participants will be randomized using a computerized random assignment table with permuted blocks to receive either the "Western" or "prudent" diet first, and they will receive the alternate diet after the washout period. We will blind investigators and all study staff outside of the nutrition CRU staff to diet assignment.

d. **Justification of why participants will not receive routine care or will have current therapy stopped.**

All patients enrolled in this study will continue to have their usual care for asthma as determined by their primary care provider or their usual asthma caregiver. The study protocol will not interfere with participants receiving their previous routine medical care. A copy of all complete blood count results along with a copy of all pulmonary function test results obtained during their visits will be given to the participants at the end of the study accompanied by a letter for their PCP.

e. **Justification for inclusion of a placebo or non-treatment group.**

Each participant will receive both dietary interventions, the "Western" diet and the "prudent" diet.

f. **Definition of treatment failure or participant removal criteria.**

We do not anticipate that participants will experience any significant side effects from ingestion of the study diets.

At any time, the subject's participation will stop if he/she requests not to proceed with the study or at the determination of the Principal Investigator.

g. **Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.**

If a participant's participation in the study ends prematurely, no additional dietary measures will be taken. Participants will resume their typical diet once the study concludes or when the participant exits the study.

5. INCLUSION/EXCLUSION CRITERIA

Inclusion criteria:

1) Age 18-55
2) Female
3) Obese (BMI$\geq$30kg/m^2)
4) Not breastfeeding
5) Diagnosis of asthma, defined as

- Physician diagnosis of asthma, and
- Current treatment for asthma by a healthcare provider within the preceding twelve months. (Current asthma treatment defined as regular use of asthma medications. Asthma medications include short and long acting adrenergic bronchodilators, bronchodilator combinations, inhaled anticholinergics, inhaled corticosteroids, cromolyn sodium and nedocromil, leukotriene modifiers and methylxanthines.)

6) Stable asthma, defined by no asthma exacerbation (ED visit, course of increased systemic steroids, or urgent health care visit for asthma) during the prior four weeks.
7) Symptomatic asthma (Asthma Control Test <20 at baseline screening visit)
8) Non-smoker, defined by no cigarettes in the past year and a negative urine cotinine
9) No other major pulmonary disease such as cystic fibrosis or COPD
10) Willing to eat study diet and nothing else for each of the 6 days of controlled feeding

Exclusion criteria:

1) Chronic oral steroid therapy (daily)
2) Oral corticosteroid use within the past 4 weeks
3) Respiratory tract infection within the past 4 weeks
4) Significant medical issues such as heart disease or poorly controlled hypertension, type 1 diabetes, poorly controlled type 2 diabetes, or hypothyroidism that would interfere with collection of outcome measures or present safety issues in the opinion of the Principal Investigator
5) Pregnancy (self-report), planning a pregnancy, or nursing/breastfeeding mothers
6) Food allergy that interferes with ability to complete the study
7) Food preferences, intolerances, or dietary requirements that would interfere with diet adherence
8) Taking vitamin supplements
9) Planned dietary changes during the study period
10) Use of coumadin
11) Consumption of more than 14 alcoholic drinks per week, or consumption of 6 or more drinks on an occasion, one or more occasions per week
12) FEV_1<1.5L or <60% predicted post bronchodilator administration
13) Inability to perform acceptable spirometry
14) Any condition or compliance issue which in the opinion of the investigator might interfere with participation in the study
15) Lack of appropriate food refrigeration and preparation equipment (oven or microwave)
16) Inability to produce adequate sputum following induction with hypertonic saline neb

6. DRUGS/SUBSTANCES/DEVICES

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Albuterol is a commercially available, FDA approved drug. We will administer albuterol according to ATS guidelines and as described elsewhere on this form.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Not applicable.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

Not applicable.

7. STUDY STATISTICS

a. Primary outcome variable

Primary Outcome: Feasibility of intervention and study procedures. We will measure this via qualitative interviews at the feedback sessions.

b. Secondary outcome variables

Secondary Outcomes: We will also assess compliance with the dietary intervention via change in dietary markers; examples include serum lipids, serum fatty acids, and serum carotenoids. We will assess success of the study procedures based on success at obtaining serial induced sputum at follow-up visits. Based on previous findings that have demonstrated increases in inflammation (both Th1 and Th2) and oxidative stress among individuals with asthma or in response to diet exposure, we have selected a panel of biomarkers to be measured in study participants utilizing the resources and staff of the CCAUE. We will measure markers of Th1 inflammation (including sputum neutrophil %, neutrophil elastase, IL-8, and IL-6; serum TNF-alpha and IFN-gamma), markers of Th2 inflammation (including sputum eosinophil %, IL-5, and IL-13; FeNO; serum eosinophil %), and markers of oxidative stress (including sputum 8-isoprostane, serum RANTES, and urine 8-isoprostane). We will also evaluate stool samples, nasal swab samples, and sputum samples for microbiome and changes with each dietary intervention. We will also look at change in reported symptoms, self-efficacy in managing disease, and lung function between the Western and prudent diets. Lastly, we will also evaluate temporal variability in exposures (such as cleaning agents) that could be linked to respiratory effects and/or to other related health outcomes, including oxidative stress.

c. Statistical plan including sample size justification and interim data analysis

Analysis Plan: Participant feedback will be analyzed via a deductive approach for content analysis. We will examine for themes within the interview data which either support the current approach or suggest that procedure modification could improve feasibility and participant experience in a future trial. The distributions of all quantitative variables will be examined for outliers and missing data points. Next, the distributions of serum lipids, carotenoids, fatty acids, FEV_1, FeNO, and sputum neutrophil % will be summarized and displayed for each time point. Post-challenge levels of these outcome variables will be compared between prudent and Western diet interventions using paired t-tests, displaying the calculated differences as percentages and absolute differences. Group 1 will be defined as the group randomized first to the Western diet, and Group 2 will be defined as the group randomized first to the prudent diet. We will also evaluate for evidence of carryover effect (measurable effect from order of intervention), although the washout period has been carefully selected to prevent this. We will examine differences in the outcome variables by order of intervention occurrence and apply the t-test. If the outcomes do not appear normally distributed, we

will transform them so that they meet assumptions of normality. In addition, we will calculate the intraclass correlation coefficient (ICC) using mixed effects models to assess the temporal variability of target biomarkers. The ICC is a measure of reproducibility and commonly used to assess the suitability of biomarkers to properly characterize exposures.

Power Calculations: Power calculations cannot be completed for the primary, qualitative outcome of feasibility.

Sample Size: We propose a small study as a feasibility trial to generate preliminary data for a future, larger intervention that will be powered to detect clinical endpoints.

Study Duration: The duration of intervention, 6 days, is shorter than most other diet intervention studies to date. Our prior intervention was of one-month duration, with outcomes similar at two weeks and one month. Other trials of diet intervention in asthma have also been at least two weeks in duration, though this duration of meal replacement is costly and difficult to scale. Importantly, just four hours after a high-fat diet challenge, meaningful changes in lung function and inflammatory markers have been detected, suggesting the presence of short-term effects on asthma health. We intend to further evaluate these short-term effects, both a novel and important aspect of the proposal that will inform duration and timing of outcome assessment in the planned, future trial. Based on variability of the secondary outcomes assessed in this trial and degree and directionality of change seen, we anticipate that this pilot will inform both feasibility as well as necessary duration of a future, definitive trial.

As this is a pilot study, we do not intend to complete interim analyses.

d. Early stopping rules

As this is a pilot study, there will be no formal criteria for stopping the study early, however participants may choose to leave the study early or may leave the study early at the determination of the Principal Investigator.

8. RISKS

a. Medical risks, listing all procedures, their major and minor risks and expected frequency, and
b. Steps taken to minimize the risks.

Urine collection: Urine collection is considered a very low risk procedure. Urine will be collected the morning of the in-person screening visit and the morning of each of the four assessment visits.

Questionnaires: The only risk involved in completing the questionnaires is the potential for breach of confidentiality. Unique identifiers will be assigned to participants and these will be used to label all paperwork, including questionnaires. Some participants may also feel uncomfortable answering personal questions, but all participants will be offered the opportunity to refrain from answering any questions they do not want to answer. Participants will answer questionnaires/logs at the baseline visit, and during each of the intervention periods.

Dual Energy X-ray Absorptiometry (DEXA) scan: The DEXA scan is considered a safe test and is widely used in clinical practice for estimation of bone mineral density in screening for osteoporosis, and it is also widely used in research studies including the National Health and Nutrition Examination Survey as conducted by the Centers for Disease Control and Prevention. The risks of this scan are directly related to the radiation dose received by the participant. We estimate that the dose of radiation received during a DEXA total body scan is around 0.001 rem per DEXA scan, a miniscule dose compared to the dose of 0.04 rem for an abdominal x-ray (Huda, 1996). Women capable of bearing children will receive a urine pregnancy test prior to the DEXA scan to exclude pregnant women from this procedure. The DEXA scan will only be performed once per participant, at the baseline visit.

Spirometry: Spirometry is a routine clinical procedure that entails little risk. One minor risk is the slight discomfort of forceful exhalation, but this will be minimized by effective coaching and administration by trained study personnel. Spirometry will be performed at the baseline visit and during each of the four assessment visits.

The drug albuterol will be administered during spirometry and can lead to tremor, nervousness, tachycardia, palpitations, and headache. These reactions are transient and rare (< 5%) with the proposed doses used for this study, and if they occur, we will monitor the patient until they return to baseline. High dose albuterol may cause arrhythmias and hypokalemia. However, these reactions are very unlikely with the doses used for this study.

Induced sputum: Hypertonic saline nebulization can cause bronchospasm; to prevent this, albuterol will be administered prior to the procedure, hypertonic saline will be administered in escalating doses, and lung function will be measured throughout the protocol to prevent excessive responses. If there is a fall in post-bronchodilator FEV_1 of $\geq$ 20% compared to FEV_1 prior to sputum induction at any point or if the patient is unwilling or unable to continue, sputum induction will be terminated and albuterol administered as needed. Sputum induction will not be performed or will be terminated in subjects with post-bronchodilator FEV_1 < 60% predicted. Risks are minimized by conducting the test in a medically supervised environment with trained personnel, and a physician will be available in the clinic during the procedure. Oxygen and albuterol via metered dose inhaler will be available and additional therapy will be administered as deemed appropriate depending on the circumstance. In the event of an emergency, the JHH Adult Code team will be called to respond. Induced sputum will be conducted at the baseline visit and during each of the two assessment visits following each dietary intervention.

Stool/fecal strip collection: Stool/fecal strip collection is considered a very low risk procedure. Samples will be collected during each of the four assessment visits.

FeNO measurement: FeNO measurement is considered a very low risk procedure. Measurement of exhaled nitric oxide will be obtained according to ATS guidelines at each of the four assessment visits.

Serum collection via venipuncture: Phlebotomy is a routine clinical procedure. There is a risk of minor discomfort or bruising at the site of the blood draw. In addition, some people experience lightheadedness. Very rarely, an infection can develop at the venipuncture site. In order to reduce

risks, a trained staff member who is experienced in phlebotomy will perform the venipuncture. Phlebotomy will be performed at each of the four assessment visits.

Nasal swab collection: The main risk of cell sampling is minor discomfort while the sample is being collected, though the discomfort should subside immediately after the sample is taken. There is a slight risk of some nasal bleeding during or just after the sample is collected, and rarely, an infection may occur at the sampling site. In order to minimize these risks, a staff member who is experienced and trained in nasal sample collection will perform the procedure. Nasal swab collection will be performed at each of the four assessment visits.

Dietary intervention: The two periods of dietary interventions pose minimal risk. Participants may experience some bloating and other minor gastrointestinal (GI) discomforts related to the high fruit, dairy, and fiber content of the intervention diets. Our experience suggests that GI discomfort is generally minor and subsides quickly, and GI problems often resolve soon after changes in diet. Participants are monitored for reactions to the diets and, if necessary, the diet can be modified or terminated.

c. Plan for reporting unanticipated problems or study deviations

Any serious or unexpected adverse event will be reported to the IRB according to institutional reporting requirements; other adverse events will be reported to the IRB at the time of continuing review. Protocol deviations will be reported to the IRB according to institutional reporting requirements: minor or administrative deviations to the protocol will be reported with the continuing review; major, non-emergent deviations will be submitted for review; and emergent deviations will be reported as soon as possible and at least within 5 days to the IRB.

d. Legal risks such as the risks that would be associated with breach of confidentiality

The risk of breach of confidentiality will be minimized by using unique identifiers for participants and keeping the record that links the identifier to the participant in a locked file cabinet or office and/or password protected database accessible only to the investigators and study staff.

e. Financial risks to the participants.

In the rare event that a participant has a reaction to diet intervention and he/she seeks medical care, he/she will be responsible for payment of those services. The subject will not be charged for any of the proposed procedures and will be compensated for time spent in the study.

9. BENEFITS

a. Description of the probable benefits for the participant and for society.

The potential benefits of this study for the participant include the introduction of healthy meals into their diet and a broader understanding of the relationship between their diet and asthma symptoms. Benefits for society include the identification of important relationships between dietary patterns

and asthma morbidity, and the potential future implementation of scalable dietary interventions for asthma.

10. PAYMENT AND RENUMERATION

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants can earn up to $380 total:

- $50 after completing the in-person screening *and all* subsequent baseline measurements and questionnaires ($15 for completing urine pregnancy test and urine cotinine only, $20 for completing urine pregnancy test, urine cotinine, and spirometry only, $25 for completing urine pregnancy test, urine cotinine, spirometry, and induced sputum only)
- $70 after completing the first period of dietary intervention (+ $10 bonus for completing all surveys)
- $70 after completing the second period of dietary intervention (+ $10 bonus for completing all surveys)
- $25 for every stool sample you're able to provide at the 4 Assessment visits (+ $50 bonus for providing a stool sample at all 4 visits)
- $20 bonus after completing the entire study including the feedback session

Participants will receive Compensation to cover any travel-related expenses; this may include cab fare, bus fare, or cost of parking garage ticket.
Participants will receive all of their meals during the dietary intervention periods. We will provide a meal of the participants choosing from the hospital cafeteria following the baseline visit and each of the assessment 2 visits.
There are no penalties for not completing the protocol.

11. COSTS

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

All costs that are associated with the study procedures are covered by the investigators.