

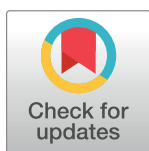
RESEARCH ARTICLE

A pilot feeding study for adults with asthma: The healthy eating better breathing trial

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Abstract

Rationale

Evidence from observational studies and to a lesser extent clinical trials suggest that a healthy diet may improve symptoms and lung function in patients with asthma. We conducted a pilot study to determine the feasibility of conducting a larger scale dietary trial and to provide preliminary evidence on the impact of a healthy diet on asthma outcomes.

Methods

In a randomized, two period cross-over trial, participants with asthma received a 4-week dietary intervention followed by a usual diet (or vice versa), separated by a 4-week washout. The dietary intervention was a healthy diet rich in unsaturated fat. During the dietary intervention, participants ate three meals per week on site at the Johns Hopkins ProHealth Research Center. All remaining meals and snacks were provided for participants to consume off-site. During the control diet, participants were instructed to continue their usual dietary intake. Relevant biomarkers and asthma clinical outcomes were assessed at 0, 2, and 4 weeks after starting each arm of the study.

Results

Eleven participants were randomized, and seven completed the full study protocol. Among these seven participants, average age was 42 years, six were female, and six were African American. Participant self-report of dietary intake revealed significant increases in fruit, vegetable, and omega-3 fatty acid intake with the dietary intervention compared to usual diet. Serum carotenoids (eg. lutein and beta-cryptoxanthin) increased in the intervention versus control. Total cholesterol decreased in the intervention versus control diet. There was no consistent effect on asthma outcomes.

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Conclusions

The findings suggest that a feeding trial in participants with asthma is feasible. Larger trials are needed to definitively assess the potential benefits of dietary interventions on pulmonary symptoms and function in patients with asthma.

Introduction

Observational studies have demonstrated harmful effects of the “Western” diet pattern in asthma and conversely a protective effect of healthier diets (such as a “Prudent” or Mediterranean diet pattern) [1–3]. “Western” diet patterns are characterized by high intake of processed meats and fast foods, whereas healthier diets such as the Mediterranean diet are characterized by higher intake of fruits, vegetables, whole grains, lean meats, fish, nuts, and omega-3 fatty acids.

Clinical trials are needed to confirm the effects, if any, of diet on lung symptoms and function in patients with asthma. A limited number of trials explore the effects of dietary counseling on asthma morbidity, with promising results [4–6]. However, no trial to date has investigated the effects of diet on lung symptoms and function using a feeding study design, i.e. providing all meals and snacks to study participants. This approach provides the strongest efficacy data to assess the effects of dietary changes on outcomes. As such, we applied this rigorous study design, as has been done previously to investigate the effect of diet on cardiovascular disease risk factors [7–9].

One of the most widely-recognized feeding studies is the Dietary Approaches to Stop Hypertension (DASH) Trial. A subsequent feeding study, termed the Optimal Macronutrient Intake Trial to Prevent Heart Disease (OmniHeart Trial), tested a DASH-style diet intervention which is rich in unsaturated fat and similar to a Mediterranean diet [7,10]. This diet features high fruit, vegetable, and low-fat dairy intake, with replacement of carbohydrates with olive oil, canola oil, and other monounsaturated fats [11]. The feasibility of testing this diet in adults with asthma is important and cannot be inferred from feeding studies in the general population, given a higher prevalence of food sensitization and food allergy among asthmatics [12,13]. Using a randomized, two period crossover trial of a four week “respiratory healthy” diet intervention versus usual diet as control, we investigated the feasibility of conducting a feeding study in adults with asthma (the Healthy Eating Better Breathing Trial).

Methods

Subjects

Adults age 18–50 years with stable asthma were recruited via mailings, flyers in the community, and contacting participants in previous Johns Hopkins asthma studies. Eligibility was determined at a screening visit where informed consent was obtained and criteria included stable, active asthma. Asthma was defined as self-reported physician diagnosis and treatment for asthma within the 12 months prior to enrollment, and stability was defined as no asthma exacerbation (emergency department visit, systemic steroid treatment, or urgent care visit) or respiratory infection in the 4 weeks prior to enrollment. Participants were excluded if they were active smokers, pregnant or breastfeeding, used systemic corticosteroids or warfarin, had another major pulmonary diagnosis or significant systemic illness, reported high alcohol consumption (over 14 drinks per week or six or more drinks on one or more occasions per week),

reported food allergy, weighed over 350 pounds or had changed weight by over ten pounds in the two months prior to screening, or reported so few symptoms at screening (Asthma Control Test score of 20 or more) that a change in symptoms was unlikely to be detected. To be eligible, individuals also had to agree to eat at least one meal per day at the study site, three to four days per week for four weeks during the intervention diet.

Study design

This pilot study used a randomized two period, two treatment crossover design. A four week washout separated the two diets, each of which lasted 4 weeks. A convenience sample of 12 participants was proposed for this feasibility trial. Participants were enrolled by a dedicated study coordinator between September and November of 2012. Participants were randomized to begin with either the control or intervention diet. The random allocation sequence was generated by members of the data core and provided to research staff at the ProHealth facility. During the intervention diet, participants were instructed to consume only foods provided by the study, which included three meals and one snack daily. Food during the intervention diet was prepared and provided by the nutritional staff in the research kitchen at the ProHealth facility. Nutrient targets were based on the “diet rich in unsaturated fat” arm of the OmniHeart Trial, described in detail previously [7,10]. Sample menus can be found in the Online Supplement (Table A in [S1 File](#)). Supplied calories were adjusted to achieve stable weight, with anticipated daily caloric needs based on weight and estimated energy expenditure. During the intervention diet, participants ate lunch at the ProHealth facility in Baltimore three to four times per week and received enough for the meals between the in-person visits. Additional meals and snacks were provided to cover unanticipated events (e.g. weather delays) that may have resulted in schedule changes in order to ensure that participants always had access to study food. During the control diet and washout, participants were instructed to consume their typical diet; no meals were provided, and they were not required to visit the ProHealth facility for meals.

Procedures

Prior to randomization, asthma status was reassessed, and a usual diet assessment was completed via three 24-hour diet recalls. At 0, 2, and 4 weeks of each four-week diet, participants completed the following assessments: Asthma Control Test (ACT) questionnaire [14,15], Asthma-Specific Quality of Life Questionnaire (AQLQ-S) [16–18], Asthma Symptoms Utility Index (ASUI) [19,20], and spirometry and exhaled nitric oxide (eNO) measurements according to ATS guidelines [21,22]. Fasting blood samples were collected at these same timepoints for evaluation of serum carotenoids by high performance liquid chromatography (GENOX Labs) and lipids by spectrophotometry (Quest Diagnostics). To provide further insight into dietary change, participants completed three 24-hour diet recalls during each of the intervention and control diets.

Outcome measures

Adherence to the dietary intervention was assessed via daily self-reported adherence to the diet, serum carotenoid levels, serum lipids, and 24-hour recalls of dietary intake. Outcomes relevant to asthma morbidity included the ACT, AQLQ, and ASUI questionnaires, spirometry [23], and eNO.

Statistical analysis

Given sample size and non-normality of sample population data, all summary statistics are presented as medians and interquartile ranges. Biomarkers and asthma clinical outcomes are presented as change at week four (primary analysis) or week two (online supplement) from value obtained at week zero of each respective intervention or control diet. As three 24 hour dietary recalls were performed during each of the screening, intervention diet, and control diet windows, the approach slightly differed; the three recalls were averaged for each week, and changes were presented as differences between the intervention or control diet and the screening week. Individual estimates are graphically represented as change from screening to control and screening to intervention diet. The Wilcoxon signed rank test is used to provide pairwise comparisons of the change in data points across each of the control and intervention diets. A p-value of <0.05 is considered statistically-significant. Statistical analyses were performed using STATA version 13 (College Station, TX) and R 3.0.3 (Vienna, Austria) statistical software.

This trial is registered at clinicaltrials.gov, identifier NCT02904655. The full trial protocol can be accessed through the Johns Hopkins Institutional Review Board or by contacting the corresponding author and is available in the Online Supplement (Appendix A in [S1 File](#)). The protocol was approved by the Johns Hopkins Medicine Institutional Review Board (NA_00071879). All participants provided written, informed consent. The data upon which these analyses are based is provided in the Online Supplement (Tables B,C,D,E in [S1 File](#)). For participant privacy, given small sample size, data with the potential for participant identification are withheld. For access to an anonymized data set, please contact the corresponding author (MCM). Funding was provided by the National Institute of Environmental Health Sciences and the Environmental Protection Agency.

Results

Nineteen adults were screened and 11 participants were enrolled and randomized. Reasons for screen-failure included active smoking, ACT score of 20 or greater at the time of screening, and inability to commit to the time required for study visits. Four participants discontinued the study after randomization; one prior to initiating the intervention for reasons undisclosed by the participant, and three during the intervention feeding diet. Two participants did not like the meals, and one reported a possible food allergy that was not recalled initially during screening. Seven participants completed the full protocol and their data was used for all analyses ([Fig 1](#)).

Participants were predominantly female (6 of 7) and African-American (6 of 7) ([Table 1](#)). All participants were obese, with a median body mass index of 38 kg/m^2 , and five reported a low-income.

Self-report of dietary intake and measurement of biomarkers suggested participant adherence to the dietary intervention. Four out of seven participants denied snacking or meals outside of the study diet. Of the three participants who reported consuming food items outside of the meals provided, this were reported at most on 3 out of the 28 days of the intervention. Participants reported a higher intake of fruits and vegetables during the intervention diet as compared to the control diet, with a median increase of over 2.5 servings of fruit and over 3 servings of vegetables per day ([Table 2](#) and [Fig 2A](#)). This increase was corroborated biologically via increases in the several serum carotenoids. For example, lutein and beta-cryptoxanthin increased during the intervention diet as compared to during the control diet at 4 weeks (median lutein -0.001 versus $0.05 \text{ } \mu\text{g/ml}$; median beta-cryptoxanthin 0.001 versus

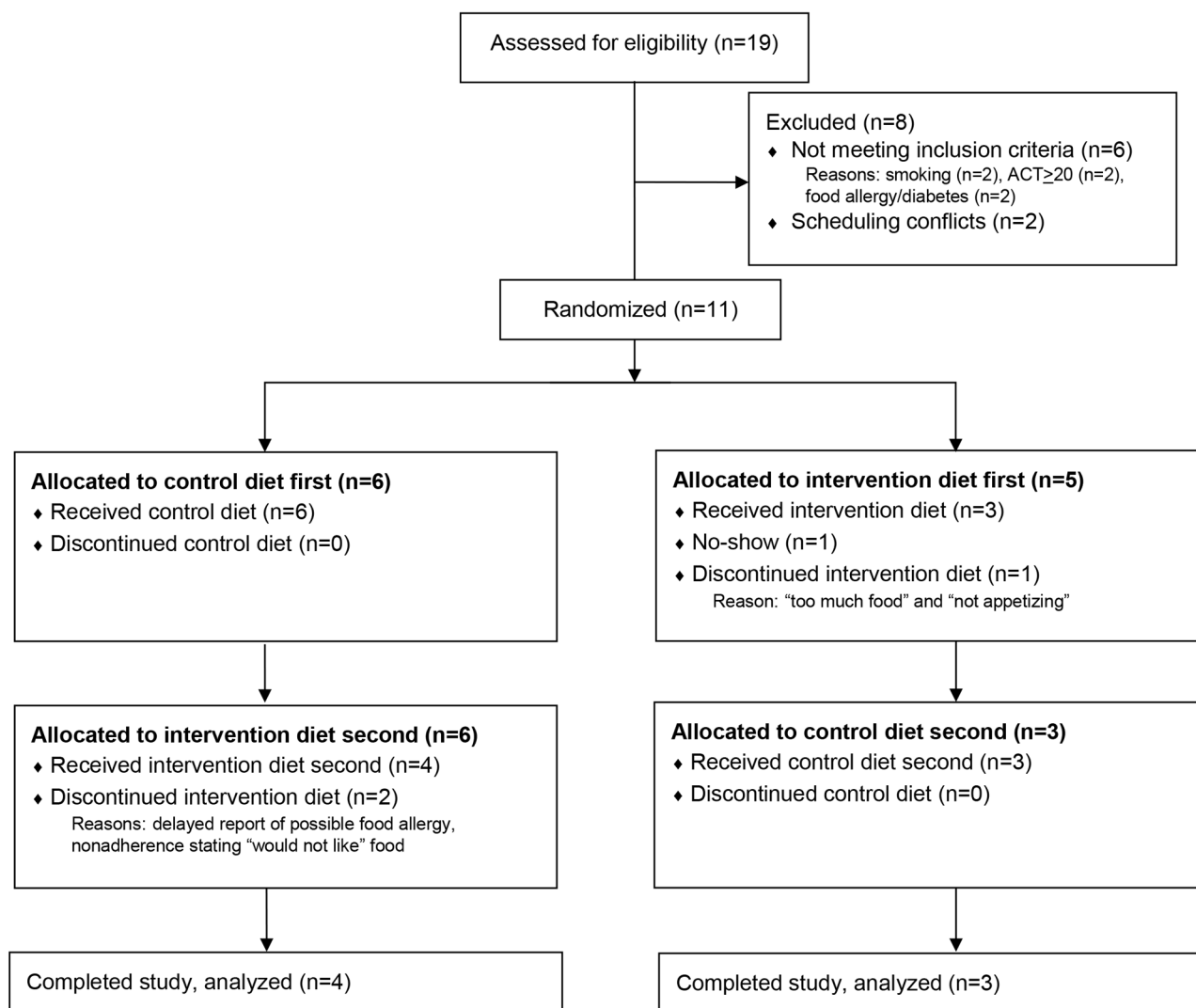


Fig 1. Consort diagram. Participants screened and enrolled.

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0.02 μ g/ml respectively, Table 3 and Fig 2C). Results were similar at the two and four-week marks (Table F in S1 File).

Notable nutrient changes with the intervention included an increase in percentage of intake from monounsaturated fat and decrease in the intake of polyunsaturated fat during the intervention diet as compared to during the control diet (Table 2). Dietary intake, reported by participants in 24-hour recalls during the intervention diet, approximated the composition of the "diet rich in unsaturated fat" arm of the OmniHeart Trial (Table 4). Additionally, participants reported an increase in omega-3-fatty acid intake during the intervention diet as compared to during the control diet (median increase 0.48g versus decrease 0.52g, Table 2 and Fig 2B), consistent with transition to a more Mediterranean-style diet during the intervention.

Adherence to the dietary intervention was also supported by changes in measurements in serum cholesterol. A drop in total cholesterol was observed in the study group after 4 weeks of intervention compared to 4 weeks of control (median decrease 13mg/dl versus increase of 2mg/dl, Fig 2D), along with a trend towards decrease in low density lipoprotein levels

Table 1. Participant characteristics.

Characteristic	n = 7
Age in years	42 (37–50)
Sex, number female	6
Race	
Caucasian	1
Black/African-American	6
Education	
< High school	3
High School	2
Some College	2
Income	
Under \$10,000	3
\$10,000–49,999	2
>\$50,000	2
BMI	38 (33–39)
Ever smoker	2

Absolute numbers and median (25%-75%tile) provided for continuous data; Both ever-smokers reported less than 10 pack-years

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(Table 4). Median triglyceride levels were not affected. Summary statistics of the change in outcomes of the course of the control and intervention diets were similar at two weeks (online Supplement, S6 Table).

Asthma outcomes assessed using standardized questionnaires, lung function, and FeNO demonstrated variability in response to intervention with modest sample size. Of the outcomes assessed, ACT scores and lung function appeared most responsive to the intervention in the hypothesized direction (Table 5, Fig 3A and 3B). Summary statistics of the change in outcomes of the course of the control and intervention diets were similar at two weeks (Table G in [S1 File](#)).

Discussion

To our knowledge, we report findings from the first feeding study (complete provision of meals) in participants with asthma. The study results suggest that the rigorous deployment of a dietary intervention providing complete meals is feasible in an urban population with asthma, as demonstrated by self-report of dietary change, increases in serum carotenoids and reduction in total cholesterol (biomarkers of adherence) with the intervention. Challenges encountered during the course of the study, including participant enrollment, will be useful to inform a future trial.

While previous studies demonstrate an ability to enact dietary change and suggest a positive effect on asthma outcomes, the Healthy Eating Better Breathing study adds a novel perspective. First, our method of effecting dietary change is unique to the asthma literature. Complete dietary replacement is a highly precise method to alter dietary intake, and though it is labor and cost intensive, it may provide investigators with the highest assurance of participant compliance with the dietary intervention. Second, the majority of our sample was African-American, 5 of 7 were from a low socioeconomic background, and overall subjects demonstrated a median BMI of 38 kg/m². Our findings demonstrate the ability to achieve dietary change in the context of a clinical trial in an urban population that carries a disproportionate burden of

Table 2. Participant-Reported dietary intake from 24 Hour dietary recalls.

	Screening	Control Diet	Intervention Diet	Δ between Diets	P-value
Dietary Intake (servings/day)					
Fruit	0.01	0.79	3.28	2.56	0.02
	(0–0.92)	(0.34–1.25)	(2.25–3.81)	(2.25–2.81)	
Vegetables	1.66	2.16	5.60	3.15	0.02
	(1.16–2.07)	(0.98–3.01)	(3.69–6.53)	(2.68–4.28)	
Whole Grains	0.16	0.88	0.81	0.02	1.00
	(0–1.15)	(0.03–1.27)	(0.53–1.11)	(-1.27–0.53)	
Refined Grains	3.79	3.04	2.28	-0.30	0.40
	(1.56–4.17)	(1.23–4.07)	(1.65–2.74)	(-2.07–1.05)	
Mixed Grains	0	0	0.41	0.33	0.27
	(0–1.01)	(0–0.68)	(0.16–0.52)	(-0.78–0.42)	
Dairy	0.92	0.52	0.95	0.31	0.13
	(0.62–2.72)	(0.43–1.22)	(0.58–2.00)	(0.07–0.78)	
Total Meat	3.07	2.50	2.07	0.54	0.87
	(2.50–4.04)	(0.69–3.47)	(1.76–3.38)	(-1.40–1.18)	
Lean Meat	1.67	0.47	1.86	0.76	0.13
	(0.78–2.36)	(0.02–1.16)	(1.00–3.05)	(-0.33–3.03)	
Processed Meats	0.63	0.08	0	-0.08	0.10
	(0.24–1.57)	(0–1.18)	(0–0)	(-1.18–0)	
Fish/Seafood	1.31	1.00	1.52	0.18	0.27
	(0–3.91)	(0.61–1.61)	(0.61–2.63)	(-0.35–1.47)	
Oil	1.17	0.64	0.67	-0.38	0.34
	(0.78–1.92)	(0.30–2.17)	(0.24–1.64)	(-2.02–0.99)	
Nuts	0	0.64	3.77	1.13	0.18
	(0–0)	(0–3.53)	(1.13–5.68)	(-0.96–5.36)	
Sweets	2.79	1.13	0.93	-0.05	0.40
	(0.51–4.55)	(0.69–3.75)	(0.58–1.08)	(-3.30–0.49)	
Nutrients					
Total fat (%)	31.45	34.39	40.44	3.07	0.06
	(28.30–42.83)	(27.10–40.59)	(35.58–44.70)	(1.80–11.32)	
Saturated (%)	9.73	9.26	7.84	-1.27	0.09
	(8.01–14.57)	(8.63–12.51)	(7.20–11.10)	(-3.05–0.08)	
Monounsaturated (%)	13.56	15.26	18.95	4.28	0.04
	(13.27–19.44)	(11.41–18.00)	(18.23–22.31)	(0.89–8.61)	
Polyunsaturated (%)	6.73	10.75	9.12	1.63	0.06
	(6.01–9.61)	(10.20–13.05)	(6.43–10.79)	(-0.17–6.56)	
Carbohydrate (%)	45.47	49.24	40.00	-3.43	0.24
	(38.47–49.49)	(39.66–50.31)	(38.72–45.49)	(-13.88–0.38)	
Protein (%)	18.66	16.23	17.26	0.14	0.61
	(14.59–23.62)	(15.78–17.31)	(16.39–20.45)	(-0.91–2.68)	
Cholesterol (mg)	338.83	220.90	144.29	-87.68	0.13
	(128.81–366.06)	(126.65–245.40)	(92.48–156.37)	(-131.16–18.36)	
Omega-3 Fatty Acids (g)	1.46	1.36	2.03	0.55	0.02
	(1.03–2.08)	(0.84–1.57)	(1.39–2.72)	(0.46–1.45)	

Data are presented as median (25%–75%tile). Average daily intake computed from mean of 3x 24-hour recalls during each diet. P-value for change between diets.

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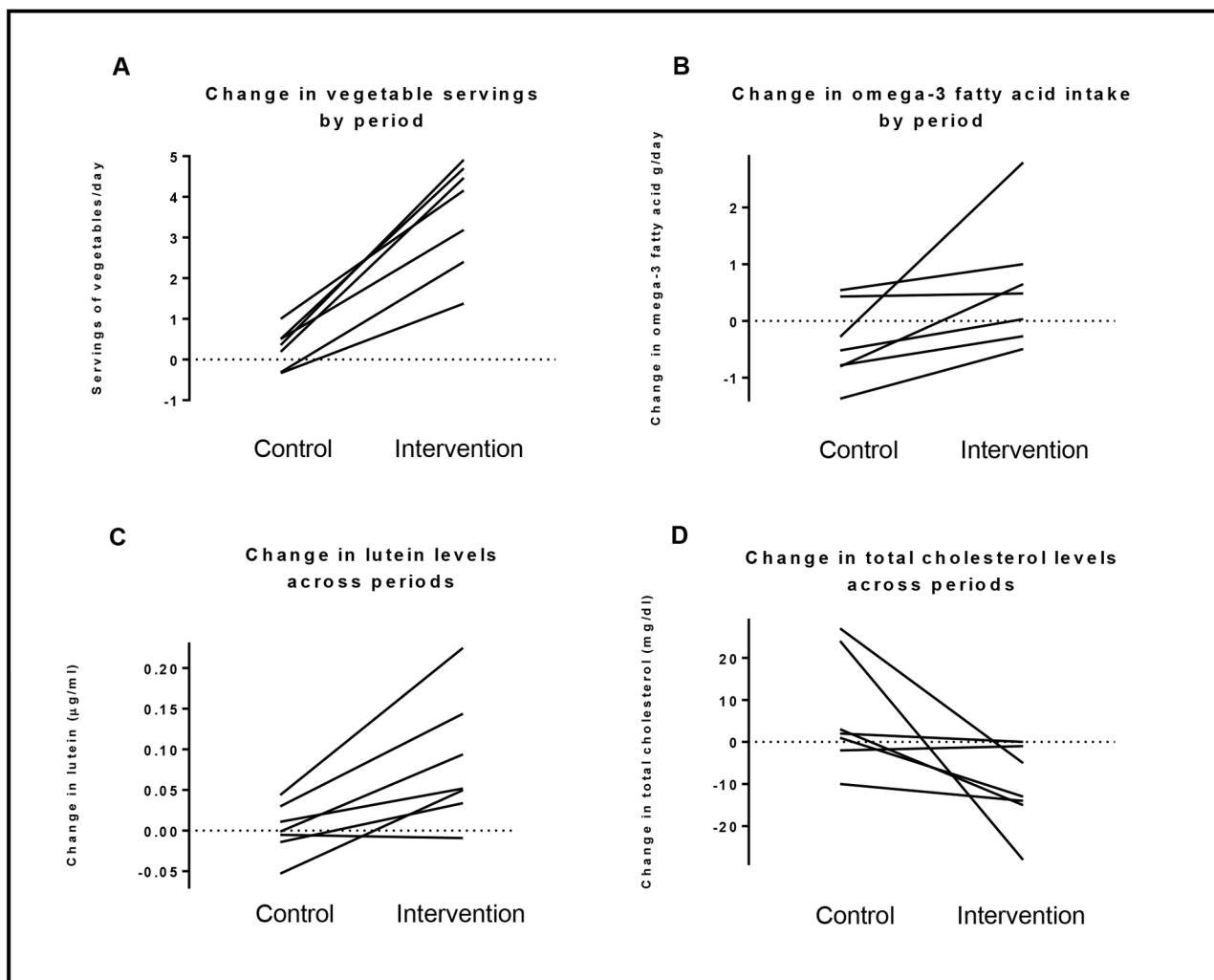


Fig 2. Select adherence outcomes. Data in each graph represent the change in value from week 0 to week 4 of the control diet and the change in value from week 0 to week 4 of the intervention diet. Points are linked by lines connecting responses within individual participants, with the following outcomes represented: (A) vegetable servings; (B) omega-3 fatty acid intake; (C) lutein levels; (D) total cholesterol levels.

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asthma prevalence and morbidity [24], who may therefore stand to benefit the most from asthma interventions and are underrepresented in trials to date. Third, the outcomes were assessed at both two and four weeks. Biomarker and metabolic changes were seen as early as two weeks and remained consistent at four weeks. These findings suggest that making measurements as early as two weeks may be a good use of resources in a future study and also demonstrate the ability to sustain the intervention diet for as long as one month using the feeding trial design.

The successes of meal provision as a method of dietary change may be attributable to multiple factors. Participants were financially compensated for their significant time commitment to the trial, including consumption of several meals per week at the facility. All meals were pre-prepared and provided by the research kitchen, simultaneously reducing the burden of food preparation for the participant and eliminating the need for participants to apply knowledge of intervention diet composition to succeed in dietary change. Several participants reported that they had never tasted some of the foods in the intervention diet prior to study

Table 3. Change in serum markers of adherence.

	Baseline	Δ during Control Diet	Δ during Intervention Diet	
Serum Carotenoids (μg/ml)				
Lutein	0.10	-0.001	0.05	0.03
	(0.09–0.13)	(-0.01–0.02)	(0.04–0.12)	
Zeaxanthin	0.04	-0.001	-0.002	0.30
	(0.03–0.05)	(-0.002–0.01)	(-0.004–0.01)	
Lycopene	0.21	-0.02	-0.04	0.38
	(0.11–0.25)	(-0.02–0.02)	(-0.05–0)	
Retinyl palmitate	0.01	0	0.02	0.42
	(0–0.02)	(0–0.004)	(0–0.02)	
Alpha-carotene	0.01	-0.001	0.03	0.24
	(0–0.02)	(-0.006–0.005)	(0.002–0.04)	
Beta-carotene	0.08	0.01	0.06	0.11
	(0.07–0.09)	(-0.01–0.01)	(0.01–0.10)	
Beta-cryptoxanthin	0.03	0.001	0.02	0.03
	(0.03–0.05)	(-0.003–0.006)	(0.01–0.05)	
Retinol	0.46	0.03	-0.03	0.58
	(0.36–0.55)	(-0.01–0.05)	(-0.09–0.02)	
Lipids (mg/dl)				
LDL	79	1	1	0.06
	(64–88)	(0–16)	(-4.5–3)	
HDL	58	-3	-7	0.08
	(53–68)	(-3.5–0)	(-19.5– -5.5)	
Triglycerides	76	2	2	0.94
	(56–92)	(0–16)	(-10.5–11)	
Total cholesterol	153.5	2	-13	0.03
	(142–166)	(-0.5–13.5)	(-14.5– -3)	

Data are presented as median (25%–75%tile). Change from 0 to 4 weeks.

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enrollment. Had participants been instructed to prepare and consume these foods independently, incomplete understanding of or hesitancy to try preparation techniques could have provided an obstacle to adherence and reduced the potential to achieve nutrient targets. Lastly, participants remarked that they enjoyed eating meals at the clinical research unit with other study subjects, and reported a sense of community with fellow participants in the study.

Table 4. Comparison of BREATHE 24-hour recall data to OmniHeart diet composition.

	BREATHE Intervention Diet, Participant-Reported Median Intake	OmniHeart Unsaturated Fat Diet, Goal Intake*
Total fat (%)	40	37
Saturated (%)	8	6
Monounsaturated (%)	19	21
Polyunsaturated (%)	11	10
Carbohydrate (%)	40	48
Protein (%)	17	15
Cholesterol (mg)	144	150

*Reference: (32)

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Table 5. Change in asthma morbidity outcomes.

	Baseline	Δ during Control Diet	Δ during Intervention Diet	P-value
ACT	20	0	2	0.24
	(18.25–21)	(-1-1.5)	(0.5–6.5)	
ASUI*	0.75	-0.125	-0.5	0.79
	(0.5–1)	(-0.25–0)	(-0.78–0)	
AQLQ [†]	8.5	-5	-0.5	0.37
	(4.5–13.5)	(-8.5- -1.5)	(-1.25–0.75)	
FEV ₁ (% predicted)	86.5	-8	-2	0.15
	(76.75–97)	(-10.5- -3)	(-3.5–0.5)	
FEV ₁ /FVC ratio	0.87	0.005	0.01	0.81
	(0.82–0.89)	(-0.002–0.016)	(0.003–0.03)	
eNO (ppb)	45	-2	2	0.61
	(23–44)	(-4.5–5)	(-12.5–4)	

Data are presented as median (25%-75%tile). ACT: Asthma Control Test (range 5–25, higher score denoting better asthma control); ASUI: Asthma Symptom Utility Index (range 0–1, higher score denoting fewer symptoms); AQLQ: Asthma Quality of Life Questionnaire (range 0–60, higher score denoting worse quality of life); eNO: exhaled nitric oxide, ppb: parts per billion

*n = 6;

[†]n = 4.

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The modest sample size of subjects completing the study limits the ability to draw conclusions regarding the impact of the healthy diet intervention on asthma health. Limited evidence from other small diet intervention trials, primarily involving dietary counseling with or without vouchers for food purchase, suggested trends in asthma improvement. A study in Australia randomized 79 adults with stable asthma to a counseling intervention of either high or low fruit and vegetable intake. Compliance with the dietary requirements of the intervention, assessed by 24-hour recall, was unmet by 20% of the participants. High intake of fruits and

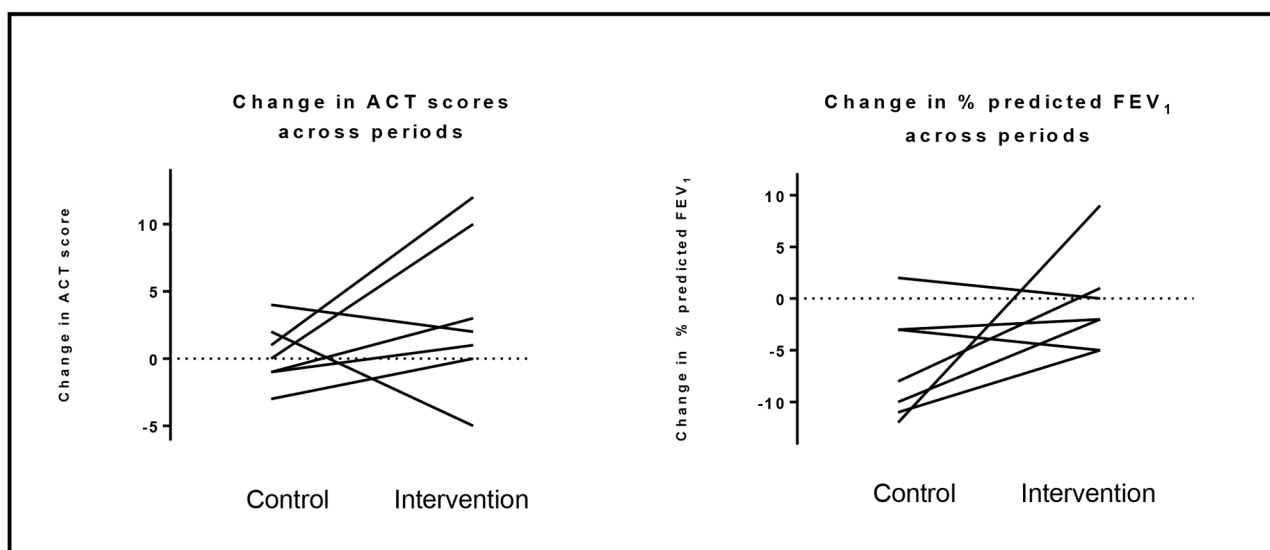


Fig 3. Select asthma morbidity outcomes. Data in each graph represent the change in value from week 0 to week 4 of the control diet and the change in value from week 0 to week 4 of the intervention diet. Points are linked by lines connecting responses within individual participants.

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vegetables was associated with a significant increase in serum carotenoids, whereas low intake was associated with a significant drop in serum carotenoids and in FEV₁ [5]. A second study in New Zealand randomized 35 adults with symptomatic asthma to one of three Mediterranean Diet interventions; over 40 hours of dietary consultation sessions plus provision of olive oil and food vouchers, two hours of dietary consultation plus provision of olive oil and food vouchers, or one dietary consultation, recipes, and free food at the end of the trial [6]. Markers of adherence (Mediterranean Diet Score, serum ascorbic acid, total cholesterol and LDL cholesterol) were influenced favorably with the highest intensity intervention. The study was underpowered to detect clinical endpoints in asthma, though clinically important improvements were seen in AQLQ categories [6]. Lastly, a recent US trial randomized 90 adult participants with uncontrolled asthma to usual care or intense dietary counselling to modify diet to the DASH (Dietary Approaches to Stop Hypertension) Diet [4,8,25]. Odds of reaching goal fruit and vegetable intake in the intervention group, though improved by 1.9 servings per day at 6 months, was not statistically different than the control group. Modest, clinically significant improvements were noted in the Asthma Control Questionnaire [26] and mini-AQLQ [27].

Though a feeding study is unique in the asthma-diet literature and overcomes several potential barriers to diet adherence, this type of study presents several challenges. First, four participants decided to drop-out of the trial after randomization; at least three of these were directly related to food preferences and expected tolerability of the intervention diet. This pilot study, in contrast to the DASH and OmniHeart trials, did not include a pre-randomization, run-in phase, which provides potential participants with firsthand knowledge of the diets and feeding procedures. Either a run-in phase or an intensive discussion of the intervention diet during the consent and enrollment process, including specific menus, ingredients, and quantities of food provided, is therefore suggested to prevent enrollment of individuals at greater risk of drop-out and therefore improve resource allocation in the study. Second, complete meal replacement is expensive and labor intensive. Due to the cost of preparing and delivering the intervention, sample size was limited. However, the use of crossover design increased statistical power as each participant served as an internal control. Participant compensation, an additional cost, is typical for clinical trials but may have positively skewed initial enrollment in the study. Third, though we attempted to enroll participants with stable but symptomatic asthma at baseline, at subsequent baseline exams participants enrolled in the trial, on average, demonstrated well-controlled asthma (ACT median 20). Therefore clinical effects of a dietary intervention may have been too subtle to detect. Fourth, while we provided all meals during the intervention diet with instructions to consume all food and only the food provided, three participants reported several days of nonadherence to provided meals. Still, measurable changes in serum biomarkers, lipids, and non-significant trends in asthma health outcomes were observed.

Notably, the total timeline of this trial was shorter than others published, and may have affected magnitude of effect on clinical outcomes. Two of the previous dietary intervention trials in asthma assess outcomes at the earliest timepoint of three months [6,25], however consistent with our results at two and four weeks, in the 14-week Australian trial of dietary counselling to manipulate fruit and vegetable intake, differences in serum carotenoid values as well as in FEV₁ and FVC were noted as early as two weeks [5]. Furthermore, trials of dietary change in cardiovascular health have demonstrated a reduction in lipid levels at four weeks comparable to that at six weeks (The OmniHeart Trial, [10]), suggesting that serum metabolic markers reach a new steady state at sometime between zero and four weeks. Additionally, dietary challenge with a high fat meal has produced an effect on lung function as early as 4 hours post intervention, notably with an exaggerated negative effect on lung function among obese asthmatics such as those enrolled in our study [28]. We propose that feeding trials have the

potential to provide the highest level of evidence for the effects of a predefined diet on asthma outcomes, and provide a significant opportunity to advance understanding of the effects of diet manipulation on asthma health. There is an express need to build an evidence base for the effect of nutrition on asthma through efficacy studies, both to substantiate a link and refine the diet most effective in improving asthma health. Strong evidence of efficacy provides a foundation for effectiveness and implementation studies that are likewise crucial to this work and move successful interventions to reach a broader population, particularly in areas with limited resources to enact such change. Increasing availability of food delivery and services that focus on and market healthy meals, in addition to targeting facilities where meals are served to attendees, may provide unique opportunities to implement meal replacement on a broader scale.

Feeding trials offer a means to investigate dietary patterns in a highly standardized and rigorous approach, and have been used successfully to study effects of dietary change in other chronic diseases. The findings presented here suggest that a feeding trial in participants with asthma is highly feasible. Given significant questions that remain regarding the effect of dietary pattern change on asthma health, larger trials are needed to evaluate clinical endpoints.

Supporting information

S1 File. **Table A.** Sample Weekly Menu; **Table B.** Data: Serum Markers of Adherence; **Table C.** Data: Asthma Morbidity Outcomes; **Table D.** Data: Reported Dietary Intake in Foods; **Table E.** Data: Reported Dietary Intake in Nutrients; **Table F.** Change in Serum Markers of Adherence at 2 Weeks; **Table G.** Change in Asthma Morbidity Outcomes at 2 Weeks; **Appendix A.** Study Protocol.
(DOCX)

S1 CONSORT Checklist.
(DOC)

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1 Online Supplement

2 Table A. Sample Weekly Menu

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Breakfast	Breakfast	Breakfast	Breakfast	Breakfast	Breakfast	Breakfast
Broccoli and Potato Frittata; Watermelon; Bread; Orange Juice; Olive Oil; Margarine	Instant Oatmeal; Yogurt- Light; Pecans; Raisins; Milk -Skim	Cheerios; Milk – Skim; Almonds; Banana	Blueberry Waffles; Fresh Strawberries; Almonds; Orange Juice	Lemon Chickpea Muffin; Pecans; Fresh Orange; Olive Oil; Margarine; Milk - Skim	Creamy Quinoa Cereal; Almonds; Milk – Skim	Banana Raspberry Muffin; Yogurt- Light; Pecans; Vegetable Juice; Olive Oil; Margarine
Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch
Chicken Ginger Stir Fry; Unsalted Peanuts; Brown Rice; Fresh Pineapple	Chicken Salad/Whole Wheat Bread; Cherry Tomatoes; Spinach; Mandarin Orange; Salad/Canola Oil Rice Vinegar; Almonds	Tuna Salad; Bread; Asian Slaw with Olive Oil; Ambrosia Salad	White Chicken; Corn Chili; Greek Vegetable Salad/Herb Vinaigrette; Broccoli; 2% Cottage Cheese; Peaches	Greek Tuna Pockets; Feta Cheese; Broccoli Salad with Olive Oil; Watermelon	Provolone Grilled Cheese Sandwich- with Olive Oil; Tomato Basil Soup; Cole Slaw; Banana	Herbed Beef Pasta; Mozzarella Cheese-reduced fat; Celery/Carrot Sticks; Fresh Orange
Dinner	Dinner	Dinner	Dinner	Dinner	Dinner	Dinner
Pork; Yams; Collards with Olive Oil; Olive Oil; Margarine	Cozy Spiced Beef; Broccoli; Ginger Peach Melba	Mesquite Lime Chicken; Stewed Tomatoes; Black Olives; Turnip Greens With Olive Oil; Pears	Meatloaf; Mashed Potatoes; Kale with Olive Oil; Olive Oil; Margarine; Cantaloupe	Rosemary Baked Chicken; Mushroom Barley Pilaf; Cooked Spinach; Apricot Halves	Creamy Horseradish Salmon; Black Bean, Mango/Citrus Salsa; Ginger Orange Carrot	Baked Cod – Panko Crumbs and Olive Oil; Brown Rice; Spiced Roasted Brussels Sprouts; Dried Apricots
Snack	Snack	Snack	Snack	Snack	Snack	Snack
Unsweetened Applesauce; Wheat Crackers; Peanut Butter; Milk - Skim	Cheddar Cheese – reduced fat; Carrots; Celery; Orange Juice	Raisins; Unsalted Peanuts; Orange Juice	Pears; Carrot- Spice Muffins; Olive Oil; Margarine; Milk- Skim	Strawberry Salsa; Cinnamon Tortilla Chips; Unsalted Peanuts	Yogurt-Light; Cantaloupe	Prunes; Unsalted Peanuts; Milk - Skim

3

4

Table B. Data: Serum Markers of Adherence

Participant	Week	C/I	Serum Carotenoids (µg/ml)								Serum Lipids (mg/ml)			
			lutein	zeaxanthin	lycopene	retinyl palmitate	α-carotene	β-carotene	β-cryptoxanthin	retinol	LDL	HDL	triglycerides	TC
1	0	I	0.06	0.026	0.253	1.00E-04	0.0017	0.086	0.028	0.329	82	45	77	142
	2	I	0.073	0.031	0.162	1.00E-04	1.00E-04	0.099	0.032	0.333	81	44	74	140
	4	I	0.051	0.022	0.084	0.022	1.00E-04	0.076	0.028	0.173	85	26	90	129
	0	C	0.087	0.043	0.212	1.00E-04	0.002	0.085	0.03	0.328	77	53	56	141
	2	C	0.118	0.059	0.34	1.00E-04	0.011	0.108	0.049	0.55	80	55	70	149
	4	C	0.082	0.042	0.188	1.00E-04	0.001	0.093	0.037	0.388	78	52	60	142
2	0	C	0.048	0.019	0.027	0.022	1.00E-04	0.071	0.008	0.411	47	53	68	114
	2	C	0.077	0.017	0.025	0.034	1.00E-04	0.089	0.013	0.476	47	54	76	116
	4	C	0.059	0.016	0.038	0.022	0.0096	0.075	0.0096	0.406	48	50	70	112
	0	I	0.052	0.016	0.092	1.00E-04	0.002	0.078	0.014	0.419	56	48	52	114
	2	I	0.135	0.024	0.068	1.00E-04	0.004	0.095	0.017	0.44	58	44	48	112
	4	I	0.104	0.02	0.056	1.00E-04	0.007	0.102	0.027	0.425	57	44	61	113
3	0	I	0.09	0.037	0.212	1.00E-04	0.002	0.065	0.026	0.349	58	71	75	144
	2	I	0.237	0.047	0.151	0.021	0.017	0.15	0.086	0.396	50	70	86	137
	4	I	0.234	0.06	0.232	1.00E-04	0.05	0.203	0.099	0.322	61	71	62	144
	0	C	0.147	0.07	0.25	0.021	0.009	0.075	0.047	0.473	61	70	81	147
	2	C	0.216	0.101	0.461	0.019	0.022	0.115	0.054	0.557	70	53	66	136
	4	C	0.177	0.074	0.358	0.021	0.01	0.112	0.047	0.617	71	62	80	149
4	0	C	0.1	0.042	0.3	0.02	0.009	0.07	0.096	0.641	81	60	96	160
	2	C	0.119	0.046	0.231	0.017	0.005	0.065	0.082	0.566	86	56	91	160
	4	C	0.144	0.061	0.323	0.028	0.004	0.077	0.086	0.623	80	57	128	163
	0	I	0.13	0.052	0.203	0.025	0.066	0.123	0.086	0.556	94	61	79	171
	2	I	0.337	0.072	0.319	0.06	0.066	0.157	0.088	0.666	75	53	125	153
	4	I	0.355	0.092	0.184	1.00E-04	0.034	0.103	0.084	0.764	88	54	71	156
5	0	C	0.105	0.038	0.318	0.021	0.021	0.18	0.049	0.293	99	77	37	183
	2	C	0.101	0.046	0.285	0.021	0.016	0.19	0.136	0.335	123	81	39	212
	4	C	0.091	0.036	0.212	0.028	0.015	0.148	0.071	0.325	121	78	38	207
	0	I	0.119	0.047	0.335	1.00E-04	0.03	0.232	0.065	0.445	127	88	44	224
	2	I	0.188	0.051	0.287	1.00E-04	0.058	0.374	0.101	0.358	128	68	55	207
	4	I	0.153	0.039	0.275	0.022	0.063	0.332	0.115	0.489	124	63	46	196
6	0	I	0.089	0.03	0.147	1.00E-04	0.024	0.082	0.013	0.551	89	58	96	166
	2	I	0.218	0.037	0.135	0.03	0.054	0.193	0.063	0.595	101	45	114	169
	4	I	0.139	0.027	0.109	0.02	0.062	0.178	0.034	0.465	96	51	71	161
	0	C	0.145	0.049	0.09	0.023	0.016	0.095	0.027	0.669	74	58	57	143
	2	C	0.124	0.038	0.071	1.00E-04	0.029	0.14	0.021	0.629	91	60	59	163
	4	C	0.092	0.03	0.07	1.00E-04	0.027	0.104	0.02	0.626	97	62	54	170
7	0	C	0.12	0.034	0.124	1.00E-04	0.026	0.077	0.03	0.511	84	53	144	166
	2	C	0.119	0.04	0.15	1.00E-04	0.021	0.063	0.034	0.519	82	56	165	171
	4	C	0.119	0.042	0.107	1.00E-04	0.02	0.056	0.035	0.542	73	49	172	156
	0	I	0.145	0.044	0.106	0.022	0.017	0.072	0.045	0.608	75	53	160	160

	2	I	0.315	0.059	0.107	0.037	0.029	0.126	0.087	0.715	56	40	184	133
	4	I	0.239	0.042	0.148	0.04	0.044	0.136	0.096	0.511	47	33	331	146

Serum carotenoids and lipids by participant, week of measurement, and during control (C) or intervention (I) diets. Order of C or I is randomization order.

Table C. Data: Asthma Morbidity Outcomes

Participant	Week	C/I	Asthma Morbidity Outcomes					
			ACT	ASUI	AQLQ	FEV ₁ (% predicted)	FEV ₁ /FVC ratio	eNO (ppb)
1	0	I	14	0	0	101	0.7769231	8
	2	I	25	0.25	0	96	0.7741935	10
	4	I	24	0	0	101	0.7829457	15
	0	C	23	0	0	93	0.775	8
	2	C	24	0	0	102	0.7846154	9
	4	C	23	0	0	95	0.7723577	6
2	0	C	16	.	.	73	0.8588235	28
	2	C	19	0.5	12	78	0.975	19
	4	C	20	0.5	10	62	0.984127	23
	0	I	18	1.5	9	80	0.9876543	40
	2	I	18	1.25	4	93	1.0108696	25
	4	I	20	0.25	4	75	0.9868421	17
3	0	I	19	1.25	15	85	0.8762887	88
	2	I	13	0.5	33	103	0.8728814	90
	4	I	14	0.75	18	83	0.7904762	74
	0	C	19	0.25	13	97	0.8738739	28
	2	C	21	0.25	12	88	0.8073395	36
	4	C	21	0	5	87	0.8365385	34
4	0	C	21	0.75	9	99	0.8918919	131
	2	C	21	0.75	9	98	0.8828829	87
	4	C	20	0.75	7	96	0.8971963	95
	0	I	21	0.75	8	102	0.9189189	109
	2	I	23	0.25	7	101	0.9181818	71
	4	I	24	0	6	97	0.9326923	114
5	0	C	20	1	.	61	0.7625	33
	2	C	16	1	24	54	0.7605634	17
	4	C	17	0.5	19	58	0.7733333	37
	0	I	20	0.5	24	76	0.8085106	34
	2	I	21	1	16	76	0.8351648	27
	4	I	20	1	10	74	0.8314607	23
6	0	I	12	2	22	73	0.8690476	21
	2	I	21	0.5	14	79	1.0821918	28
	4	I	24	1	.	82	0.9647059	23
	0	C	23	0.5	0	79	1.025974	20
	2	C	24	0.25	1	76	1.0555556	13
	4	C	24	0.5	2	67	1.046875	16
7	0	C	23	0.5	16	97	0.8660714	45
	2	C	22	0.5	7	91	0.8584906	58
	4	C	22	0.25	6	89	0.8640777	57
	0	I	21	0.75	6	88	0.8543689	36
	2	I	25	0.5	2	90	0.9	59
	4	I	22	0.75	5	89	0.89	39

Asthma morbidity outcomes by participant, week of measurement, and during control (C) or intervention (I) diets. Order of C or I is randomization order.

Table D. Data: Reported Dietary Intake in Foods

		Dietary Intake (servings/day)												
Participant	Time	fruit	vegetables	whole grains	refined grains	mixed grains	dairy	total meat	lean meat	processed meats	fish/ seafood	oil	nuts	sweets
1	S	1.263333	1.160667	1.727667	.417	0	.4243333	0	0	0	3.905333	0	0	0
	I	2.226333	2.538	1.073667	.614	1	.5103333	1.756	1	0	1.258667	.6666667	.6666667	.8623333
	C	1.590333	.8273333	1.207333	.5283334	.6666667	.4423333	.5786666	.2423333	.0783333	1.613	0	0	.032
2	S	0	1.291333	0	9.319667	0	2.720333	4.013	1.668333	1.566333	0	.976	0	11.534
	I	2.253667	3.694333	.529	2.000667	.2186667	.581	1.693333	.4536667	0	1.517333	3	1.128667	.448
	C	0	.977	0	4.068	1	1.222333	2.500667	1.160333	.6816667	0	.2986667	0	3.75
3	S	.0063333	2.002333	0	1.888333	0	.9916667	2.496333	2.257667	.2386667	2.591	.78	0	2.794667
	I	3.142667	6.16	1.105333	1.654	0	3.129	4.014667	3.245333	0	.6113333	.6666667	5.998667	.5786666
	C	.3353333	3.006	.8836667	4.364333	0	.1246667	11.16833	0	3.298333	.6113333	1.836333	.635	6.536667
4	S	.9166667	.8966666	.908	1.562667	1.007667	.9216667	4.044667	.9103333	2.545667	0	1.167667	0	.514
	I	3.807667	5.599667	0	2.282333	.1646667	1.996333	3.376667	3.05	.0463333	.5173333	.2423333	5.073	1.078
	C	1.25	1.402667	1.268333	1.232	0	1.219	2.834667	.0246667	0	1	.6236667	3.527333	1
5	S	0	2.067667	0	3.789	.8926667	.8613333	2.945333	2.357333	.335	4.702	1.414	0	.851
	I	4.379333	6.535333	.5516667	3.318667	.411	.9533334	2.074667	1.858	0	2.034667	1.635333	3.770333	1.177333
	C	.643	2.258	.5333334	1.368667	0	.516	3.47	2.192333	1.175667	.7173333	.6446667	5.15	.686
6	S	0	2.790333	1.149333	4.166	0	.6226667	10.90967	4.379	.9173334	0	3.781	1.763667	4.208333
	I	3.664	7.700334	.806	2.500333	.4213333	.7403333	2.058	1.011333	0	3.273	0	2.568667	1.079
	C	.883	3.149333	2.333333	3.7	0	.4343333	.682	.682	0	3.091667	2.166667	3.527333	1.128667
7	S	.3333333	1.655333	.159	3.844	2.141	2.911	3.074	.7766667	.6276667	1.313	1.922	0	4.552667
	I	3.288333	4.842333	1.552	2.739	.5186667	1.462	2.557667	1.884	0	2.633667	.6666667	5.677	.9313333
	C	.7853333	2.160667	.0286667	3.039667	0	1.188333	1.458	.4703333	0	1.159667	2.683667	0	1.808667

Reported dietary intake by participant during screening (S), control (C), or intervention (I) diets. Each data point represents mean of 3x24 hour recalls during that time diet.

Table E. Data: Reported Dietary Intake in Nutrients

Participant		Nutrients							
		Total fat (%)	Saturated fat (%)	Monounsaturated fat (%)	Polyunsaturated fat (%)	Carbohydrate (%)	Protein (%)	Cholesterol (mg/day)	Omega-3 fatty acids (g/day)
1	S	21.806	7.338667	9.083667	5.383667	45.46533	32.68333	82.96934	1.461
	I	28.739	7.846333	12.19933	8.693334	50.697	20.447	92.47567	1.192333
	C	24.81533	8.199667	11.312	5.303667	50.31267	24.86533	56.61667	0.680333
2	S	28.47067	8.993667	13.467	6.01	56.949	14.528	380.609	1.361333
	I	44.697	9.338	22.30733	13.05167	39.99833	15.28567	89.75367	1.393
	C	27.102	9.260333	11.41333	6.428333	57.103	15.78233	220.8993	0.839667
3	S	28.30333	8.009667	13.56433	6.729333	49.48567	22.17433	128.8057	1.027333
	I	43.97767	11.235	22.28067	10.462	38.71833	17.26233	157.539	2.026333
	C	41.805	14.285	18.00433	9.515667	42.14967	14.67133	436.1203	1.567667
4	S	42.82667	14.57	19.805	8.451667	38.46567	18.662	366.061	1.011333
	I	45.71567	11.09867	23.867	10.75	35.36133	18.91233	156.37	1.496
	C	34.39267	10.012	15.26133	9.119333	49.23667	16.22967	162.188	1.439667
5	S	45.372	15.26633	19.43767	10.668	31.02733	23.622	285.666	2.075667
	I	38.08767	7.363667	18.22633	12.49767	45.48733	16.43067	115.0423	2.723
	C	40.59	8.631667	19.292	12.66633	30.454	16.28667	245.398	1.274
6	S	35.39633	9.734334	16.053	9.609	45.81033	18.64067	356.56	2.725333
	I	35.581	6.967333	18.417	10.19667	42.85367	21.61167	145.0087	2.230667
	C	33.778	8.847	14.13633	10.79467	50.2	15.99533	126.6497	1.355
7	S	31.453	11.537	13.27	6.646	43.69067	14.59033	338.829	1.914333
	I	40.44667	7.204334	18.95	14.29233	39.038	16.39467	144.292	4.703667
	C	37.381	12.51233	17.13767	7.731	39.65567	17.30567	231.9733	1.633667

Reported dietary intake by participant during screening (S), control (C), or intervention (I) diets. Each data point represents mean of 3x24 hour recalls during that time diet.

Table F. Change in Serum Markers of Adherence at 2 Weeks

	Baseline	Δ during Control Diet from Baseline	Δ during Intervention Diet from Baseline	
Serum Carotenoids (μg/ml)				
Lutein	0.10 (0.09-0.13)	0.02 (-0.003-0.03)	0.13 (0.08-0.16)	0.03
Zeaxanthin	0.04 (0.03-0.05)	0.01 (0.001-0.01)	0.008 (0.006-0.01)	0.81
Lycopene	0.21 (0.11-0.25)	-0.002 (-0.03-0.08)	-0.02 (-0.05- -0.006)	0.21
Retinyl palmitate	0.01 (0-0.02)	0 (-0.003-0)	0.02 (0-0.03)	0.11
Alpha-carotene	0.01 (0-0.02)	0 (-0.005-0.01)	0.01 (0.001-0.02)	0.11
Beta-carotene	0.08 (0.07-0.09)	0.02 (0.003-0.03)	0.05 (0.03-0.10)	0.08
Beta-cryptoxanthin	0.03 (0.03-0.05)	0.005 (-0.001-0.01)	0.04 (0.004-0.05)	0.38
Retinol	0.46 (0.36-0.55)	0.04 (-0.02-0.07)	0.04 (0.01-0.08)	0.94
Lipids (mg/dl)				
LDL	79 (64-88)	5 (1.5-13)	-1 (-13.5-1.5)	0.03
HDL	58 (53-68)	2 (-1.5-2.5)	-8 (-13- -2.5)	0.18
Triglycerides	76 (56-92)	2 (-1.5-11)	11 (4 - 21)	0.38
Total cholesterol	153.5 (142-166)	5 (1-14)	-7 (-17.5 - -2)	0.04

Data are presented as median (25%-75%tile).

Table G. Change in Asthma Morbidity Outcomes at 2 Weeks

	Baseline	Δ during Control Diet from Baseline	Δ during Intervention Diet from Baseline	P-value
ACT	20 (18.25-21)	1 (-1-2)	2 (0.5-6.5)	0.31
ASUI [*]	0.63 (0.44-0.81)	0 (0-0)	-0.38 (-0.69-0.13)	0.29
AQLQ [§]	8.5 (1.5-14.5)	0 (-1-0)	-1 (-4-0)	0.86
FEV ₁ (% predicted)	86.5 (76.75-97)	-3 (-6.5-2)	2 (-0.5-9.5)	0.29
FEV ₁ /FVC ratio	0.87 (0.82-0.89)	-0.002 (-0.008-0.02)	0.02 (-0.002-0.04)	0.38
eNO (ppb)	45 (23-44)	-7 (-12.5-4.5)	2 (-11-4.5)	0.20

Data are presented as median (25%-75%tile). ACT: Asthma Control Test (range 5-25, higher score denoting better asthma control); ASUI: Asthma Symptom Utility Index (range 0-1, higher score denoting fewer symptoms); AQLQ: Asthma Quality of Life Questionnaire (range 0-60, higher score denoting worse quality of life); eNO: exhaled nitric oxide, ppb: parts per billion ^{*}n=6; [§]n=5.

Appendix A. Study Protocol

Dietary Interventions in Asthma Treatment: Healthy Eating Better Breathing Asthma Diet Pilot Study

1. Abstract

Background: A Mediterranean-like diet (MED DIET) has been shown to improve the risk profile of an inflammatory disease, cardiovascular disease, and so may have similar effects on another inflammatory disease, asthma.

Objectives: The objective of the study is to demonstrate feasibility of a randomized, controlled clinical trial of a dietary intervention in a population of adults with asthma in preparation for a large definitive trial aimed at improving asthma clinical outcomes and improving markers of inflammation and oxidative stress.

Methods: The study is a pilot study of a randomized crossover trial to assess the feasibility of doing a randomized crossover and to understand variances of clinical and inflammatory outcomes to inform the design of a larger trial. Ten to twelve adults (ages 18-50) who meet eligibility criteria will be randomized to either the MED DIET or a control diet, the participant's usual diet (control). At baseline, FENO, FEV₁, nasal epithelial gene expression, symptoms, and biomarkers of oxidative stress and inflammation in urine, nasal lavage fluid, and serum will be assessed. Participants will receive either the MED DIET or will continue with their regular diet for 4 weeks. They will be randomized into 1 of 2 sequences, and will then have assessment of outcomes (FENO, FEV₁, symptoms, nasal epithelial gene expression, and biomarkers of oxidative stress and inflammation in urine, nasal lavage fluid, and serum) again at 2 weeks and at 4 weeks after starting the diet. The participants will then have a 2-8 week washout period and then have repeat assessment of FENO, FEV₁, symptoms, nasal epithelial gene expression, and biomarkers of oxidative stress and inflammation in urine, nasal lavage fluid, and serum. They will then begin the alternate dietary intervention (MED DIET or control), depending on which they received first, and again have assessment of outcome measures at 2 and 4 weeks. As this is a feasibility study, we will assess the process and procedures necessary to perform a dietary intervention in a study population of adults with asthma.

Implications: Findings from this study will provide insight as to the feasibility of conducting a clinical trial of a dietary intervention in a population of adults with asthma. The results may provide preliminary evidence of the effect of a Mediterranean-like dietary intervention on lung function, inflammation, oxidative stress, and symptoms in asthma and provide insight into the duration of the intervention and the sample size needed to conduct a definitive trial.

2. Objectives

The primary objective of this study is to determine the feasibility of a randomized, crossover trial of a Mediterranean-like dietary intervention compared to a usual diet on lung function, oxidative stress, inflammation and symptoms in asthma. The completion of this study will inform the design for a larger trial.

3. Background

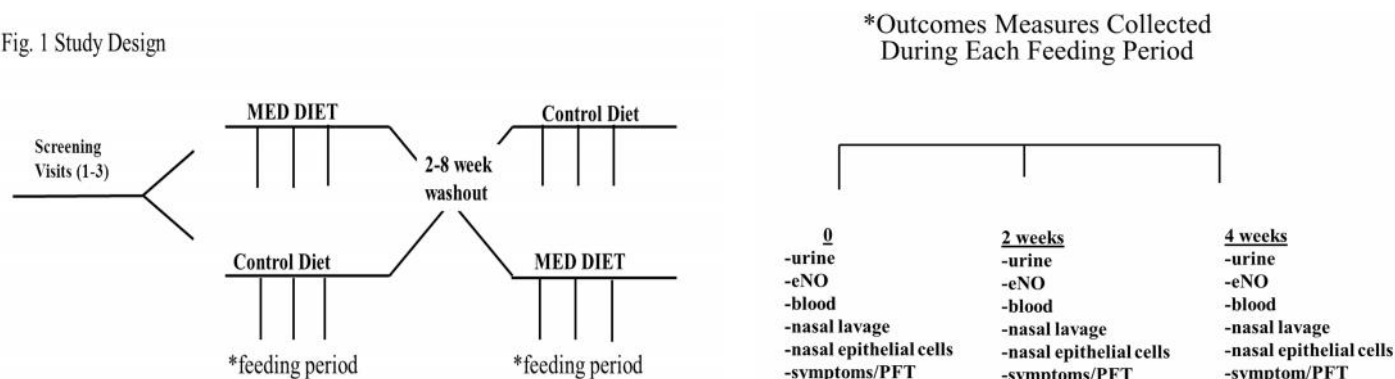
A Mediterranean-like diet has been proposed as a “respiratory healthy” diet. The traditional Mediterranean diet is characterized by an increased intake of plant foods such as fruits and vegetables, bread and cereals (primarily whole grain), legumes and nuts, with olive oil serving as the principal fat source. It is rich in both antioxidants and cis-monounsaturated fatty acids and provides an important source of dietary antioxidants. Adherence to the Mediterranean diet has been shown to be protective for clinically significant asthma and allergic disease in multiple cross-sectional observational studies. In a cross-sectional survey of 1,784 pre-school aged children, adherence with the Mediterranean diet was a protective factor for current wheezing.¹ Similarly, in a cross-sectional observational study of 690 children aged 7-18 years, adherence to a traditional Mediterranean diet was associated with reduced incidence of allergic rhinitis and a trend towards decreased incidence of wheezing.² In a study of 174 asthmatics with a mean age of 40 years, high adherence to the Mediterranean diet had a 78% reduction in the risk of having uncontrolled asthma (defined as FEV₁ >80% predicted, exhaled nitric oxide >35 ppb or asthma control questionnaire score > 1.0) after controlling for gender, age, education, inhaled corticosteroid use and energy intake (OR = 0.22; 95% CI = 0.05-0.85; p=0.028).³ Recent studies that have measured adherence to a traditional Mediterranean diet have shown better control of asthma in adults and improvement in asthma symptoms and rhinitis in children.⁴ As research focuses on favorable dietary patterns, a countering question is whether there exists an unfavorable diet that worsens asthma, specifically in susceptible minority populations. An observational study conducted in ~56,000 females in France showed that a dietary pattern loaded heavily with pizza/salty pies, dessert, cured meats and pasta, representing a diet high in fat, simple sugars and carbohydrates was associated with asthma severity.⁵ Specifically, this “western diet”, a diet similar to that common in inner city Baltimore African-Americans, was associated with increased risk of reporting frequent asthma attacks, whereas the “nuts and wine” diet, which was loaded heavily with nuts and seeds, olives, wines and fortified wine, was associated with a decreased risk of reporting frequent asthma attacks. To date, associations between asthma health and dietary intake have largely been investigated using observational studies.

The current study is designed to assess the feasibility of a large-scale dietary intervention trial comparing a Mediterranean-like diet to a Western-style diet. The dietary intervention includes a Mediterranean-like diet that approximates that from one of the three arms (the diet is rich in unsaturated fats) studied in the Optimal Macro-Nutrient Intake Health Trial to Prevent Heart Disease (OMNI-Heart) Trial⁶. We will compare this to a control diet that represents the participant’s usual diet.

4. Study Procedures

a. Study Design Summary

Fig. 1 Study Design



Randomized, crossover trial

Potential study subjects will undergo screening for eligibility at 2-3 clinic screening visits. At screening visit 1, they will have urine cotinine and questionnaires. At screening visit 2, they will complete questionnaires. At screening visit 3, they will be randomized to the MED DIET or control diet, defined as their regular diet (Figure 1). FENO, lung function, nasal epithelial gene expression, urinary oxidative stress (OS) biomarkers, serum inflammatory and OS biomarkers, and symptoms will be assessed at clinic visits before, 2 weeks after, and 4 weeks after starting each dietary intervention. Participants will then have a 2-8 week washout period during which they will resume their regular diet before continuing with the alternate dietary intervention. If the participant began on the controlled diet the washout may be shortened to 2 weeks as needed for logistical purposes.

b. Recruitment

The primary recruiting tool will be mass mailing of flyers and letters with follow-up telephone calls. This approach has been used in our current and completed asthma studies. Mailing will include letters in which participants will be given the choice to opt out from being further contacted and participants who do not opt out may receive a follow-up telephone call. Potential participants will be identified through research databases [NA_00026977: Effect of Fenzian treatment on symptoms, pulmonary function and Albuterol/Salbutamol use in patients with mild to moderate persistent asthma. A multicenter SHAM controlled clinical trial; screen failures from NA_00035701: Spiromics that expressed an interest in being contacted for future studies; NA_00033424: Recruitment Database of Volunteers for Research Studies in the Division of Allergy and Clinical Immunology; NA_000006137: Future Subject Database].

The IRB approved flyer will be passed out at community events (ie: health fairs, markets and fairs) and throughout the neighborhood community (mall kiosks, bus stops).

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 also 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.

Patients from Johns Hopkins and Johns Hopkins Community Physicians Clinics will also be a source of recruitment and will receive letters with follow-up telephone calls in which patients will be given the choice to opt out from being contacted and patients who do not opt out may receive a follow-up telephone call. We will recruit at community events. This approach has been successfully used in previous asthma studies.

c. Eligibility criteria

The following inclusion/exclusion criteria that must be met at screening visits to proceed with further study activities are:

Inclusion Criteria:

- 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]
- 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.
- Non-smoker, defined by no cigarettes in the past 1 month and a negative urine cotinine
- Not breastfeeding
- Age 18-50 years
- No other major pulmonary disease such as cystic fibrosis or COPD
- Willing to eat at least one on-site meal/day, 3-4 days a week, and willing to eat study diets and nothing else for the 4 weeks of controlled feeding

Exclusion Criteria:

- Chronic oral steroid therapy (daily)
- Oral corticosteroid use within past 4 weeks
- Respiratory tract infection within past 4 weeks

- 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
- Pregnancy (self-report), planning a pregnancy, or nursing/breastfeeding mothers
- Food allergy
- 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
- Food preferences, intolerances, or dietary requirements that would interfere with diet adherence
- Weight over 350 pounds
- Weight loss or gain of 10 pounds or more during prior 2 months
- Any condition or compliance issue which in the opinion of the investigator might interfere with participation in the study
- Use of warfarin

d.Participant eligibility visits

Participant eligibility is determined by an initial telephone or in-person pre-screening process and in a series of 2-3 formal screening visits, each of which includes questionnaires and clinical measurements. Data collected in the screening visits also provide baseline levels used to describe participants.

Pre-Screen Contact: This contact is conducted over the phone or in-person. The pre-screening form includes brief questions on major eligibility criteria. Eligible and interested individuals from the pre-screen contact are scheduled for the first formal screening visit.

Screening Visit 1 (SV1): The study protocol and eligibility criteria will be reviewed with the participant. If the participant appears to be potentially eligible and willing to participate, the informed consent process will take place. SV1 includes:

- A rapid urine cotinine test will be performed for all participants to verify non-smoking status.
- Measurement of height and weight
- A screening questionnaire will be administered by study staff and will capture socio-demographic information, pulmonary and allergic history, smoking history, medications, use of birth control for women, and review of dietary habits, food allergies, and preferences (used to assess a participant's ability and willingness to consume all diets and to adhere to the feeding protocol).
- Asthma Control Test (ACT)

Screening Visit 2* (SV2): A second screening visit will be conducted to increase the likelihood of adherence to the study protocol and will include collection of baseline data. Participants will receive a tour of the feeding site and an orientation to the study protocol. SV2 includes:

- Asthma health questionnaires

- Tour of facility and orientation session
- Caloric questionnaire

Screening Visit 3*(SV3): A third screening visit will be conducted to increase the likelihood of adherence to the study protocol and will include collection of baseline data. Participants will receive study protocol of procedures brochure. SV3 includes:

- Pre and post-bronchodilator spirometry
- Collection of blood, urine, nasal lavage, exhaled nitric oxide testing and nasal epithelial cells
- Randomization

*In some cases SV2 and SV3 maybe combined for logistical considerations.

Randomization: Participants will be randomized at SV3. Participants will be aware of their assignment as the study cannot be blinded.

e.Intervention

Description of Diets

The nutrient targets of the MED DIET intervention in current study will be based on those from the unsaturated fat diet based on an intervention arm used in Omni heart. After randomization, participants are either continued on their usual diet or fed the intervention diet. The MED DIET is low in saturated fat and cholesterol, emphasizing unsaturated fat (21% of kcal), predominantly monounsaturated fat. This diet uses nuts, seeds, and oils such as olive, canola, and safflower oil to meet its target fat distributions. Specially formulated fat products are not used. For each diet, five calorie levels are prepared (1600, 2000, 2500, 3000 and 3500 kcal). The macronutrient profile is identical at each calorie level. The micronutrient levels are based on a Linear Index Model that indexes micronutrient levels to energy levels⁷. This model is based on actual population consumption data and thus provides a realistic range of micronutrient intakes at lower and higher calorie levels, rather than a fixed ratio applied to all calorie levels (e.g. 4,700 mg of K per 2,100 kcal, or 2,238 mg of K per 1,000 kcal). Participants will be assigned a calorie level based on questionnaire administered at SV2. The calorie level will be adjusted as needed during the study protocol to maintain the baseline weight.

Food Production and Distribution

Food production and distribution will occur at the ProHealth Clinical Research Unit using procedures that have been successfully employed in previous studies⁶⁻⁸ Once menus are formulated, all foods are identified and selected to promote consistency. Specific national brands are selected, as well as purchasing specifications for meats and produce. Food production is also conducted according to respective state or county public health guidelines and JCAHO regulations. Quality control procedures developed in the DASH trials are used to monitor food procurement, preparation, and distribution. Research kitchens are monitored for safety, sanitation and equipment accuracy.

The process of preparing foods is a labor intensive process. In this process, food service workers weigh, portion, and package individual food items for the three diets, according to the calorie levels of the participants being fed. The cooks prepare all cooked items (e.g. casseroles, meats); in the process, they measure raw product, cook the items following standard hygienic procedures, and store the individualized portions. Some portion-controlled packaged food items are used for production efficiency. A diet technician assembles the trays for on-site meals and the bags and coolers for distribution of off-site meals, by diet and calorie level. A second person independently rechecks the assembled foods to confirm the accuracy of food delivery.

The feeding protocol is very similar to that used in the DASH and DASH-Sodium trials. All study food is provided to participants who are instructed to eat all their food and to consume no additional food other than approved selected beverages. Participants are required to eat one meal (the main meal of the day) at the feeding center for 4 days a week for the first 2 weeks of the 4 week MED DIET intervention. The remaining food for the day and weekend food are provided for consumption off-site. During the third, and fourth weeks of the MED DIET, participants will eat 1 main meal on-site for three days a week and will be provided with the remainder of their food for consumption off-site. On any given feeding visit, participants are expected to complete a daily food diary and be weighed. The daily diary asks about study foods not eaten, non-study foods eaten, and beverages consumed over the past day.

A dietitian case manager meets participants at the feeding center, receives and reviews their progress, elicits general feedback, tracks weight, and adjusts the calorie level, if needed. The participant then proceeds to the dining area to eat the on-site meal. A meal monitor checks the completed tray and provides the take-home meals. On each day of feeding, participants complete a daily diary, which elicits information about food and beverage consumption over the preceding day, including non-alcohol beverages consumed, alcohol beverages consumed, unit foods eaten, study foods not eaten, non-study foods eaten, and vitamin/supplement consumed.

Promotion of Adherence

Efforts to promote adherence begin at the earliest stages of the study. During screening and orientation, participants are repeatedly provided with information about key features of the study. At the screening visit, they are provided a detailed list of foods provided in the diets. Individuals must be willing to eat each of these foods; otherwise, they are excluded. Key contacts with dietary 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 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 of 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. The objective measures, available at the end of the study, are used to document the overall success of our procedures. Objective measures include serum carotenoids, meal attendance and on-site meal consumption; and body weight.

Intervention Periods

Each of two feeding periods lasts 4 weeks. During the MED DIET intervention period, participants are provided all of their food, snacks and most beverages. For each day of this controlled feeding, participants also complete a daily diary. During the last week of the last feeding period, participants complete an anonymous adherence questionnaire. During the control diet period, participants will consume their regular diet. A washout period of at least 2-8 weeks separates each period; the washout period allows ad libitum food intake. Participants are fed sufficient calories to maintain their weight. Participants have serum, urine, ENO, nasal lavage, nasal cells collected and questionnaires completed before and at approximately 2 and 4 weeks after each intervention. Table 2 displays major measurements and data collection activities by visit. The following sections describe our approach to measuring all outcomes variables and other selected measurements.

f.Study Procedure Methods

Intervention

During the MED DIET intervention, participants are provided all of their food, snacks and most beverages. On weekdays, participants will eat some of their major meals on-site, either lunch or dinner, and receive the remainder of their meals to be eaten off-site. For weekend meals, they are provided all of their food on the preceding Thursday or Friday. For each day of controlled feeding, participants complete a daily diary which asks about study food not eaten, non-study food eaten, the number of caffeinated beverages consumed, and the number of alcohol beverages consumed.

Table 1: Study Data Collection Table

	Pre-screening call	Screening visit 1	Screening visit 2	Screening visit 3	Intervention 1			Washout	Intervention 2		
Visit		SV1	SV2	SV3	IV1.1	IV1.2	IV2.3		IV2.1	IV2.2	IV2.3
Time (weeks)* these times may vary	1	1-2			3-6			7-16	17-20		
Consent/eligibility		X									
Randomization			X*	X*							
Tour/Information Session		X									
Asthma Questionnaires		X	X*	X*		X	X		X	X	X
Urine cotinine		X									
Urine			X*	X*		X	X		X	X	X
FENO			X*	X*		X	X		X	X	X
Blood Draw			X*	X*		X	X		X	X	X
Nasal Lavage			X*	X*		X	X		X	X	X
Nasal Epithelial Cell Collection			X*	X*		X	X		X	X	X
PFTs			X*	X*		X	X		X	X	X
Environment Assessment					X				X		
Telephone- 24 hour diet		XXX			XXX				XXX		
Asthma Daily Diary+					X+				X+		

* These data may be collected at either SV2 or SV3. If more than 2 weeks have elapsed between the collection of baseline clinical and inflammatory data that occurs at SV2 or SV3 and IV1, these procedures will be repeated at IV1.

+This diary will be collected daily.

g. Questionnaires:

The following questionnaires will be administered:

Asthma Questionnaires

- The Asthma Control Test (ACT) is a validated questionnaire⁹. It integrates common indicators of asthma control including cough, nocturnal awakenings, frequency of medication use, and frequency of symptoms. It is a sensitive measure of asthma control, a difference of 3 reflects a clinically meaningful difference in asthma control. The instrument is well validated and is a short questionnaire with easy to administer questions that are easily understood.
- The Asthma Symptom Utility Index (ASUI)¹⁰ is a validated 2-week recall questionnaire that addresses issues of asthma control weighted by impact on functional status.
- Asthma specific quality of life is measured with the Marks Atcham Quality of Life Questionnaire (Marks AQLQ) which has been validated for individuals 15 and older. The questionnaire is designed to measure daily impact of asthma in patients' lives.¹¹
- Juniper Asthma Control Questionnaire (ACQ) will be administered at all visits. ACQ is a validated instrument that integrates common indicators of asthma control including use of bronchodilators, nocturnal symptoms, cough, activity level, and pulmonary function.¹² The questionnaire takes less than 5 minutes to complete.

Asthma Daily Diary

Participants will complete an asthma diary during the study period.¹³ Participants will complete an asthma diary during the study period that quantifies daytime and nocturnal asthma symptoms and has been validated for use as an outcome measure in clinical trials.

Environmental Assessment

Participants will complete an environmental assessment questionnaire that has been successfully used in our previous environmental epidemiologic studies in Baltimore at IV1.2 and IV2.2.

24 Hour Dietary Recall

24 hour diet recall surveys will be administered by telephone by research staff from the Penn State Diet Assessment Center. Participants will have a goal of three 24 hour dietary recall surveys administered at baseline and during each intervention period, for a goal of nine 24 hour dietary recalls. Each set of three recalls will include one weekend day. The participant will be contacted on 3 separate occasions via phone by interview staff at the Penn State Diet Assessment Center. The phone calls will be made on both weekdays and weekend days and will last about 20 minutes. During this call the interviewer will ask standard sets of questions on food and drinks that the participant consumed in the previous 24 hours. To help estimate the food amounts, participants will be provided with a Food Amount Booklet to reference during the phone assessments.

Weight is measured by trained, certified staff using a calibrated balance beam scale. Weight will be recorded during screening at the V1 visit (to determine eligibility and to estimate calorie requirements) and 3-4 times a week while on the MED DIET intervention (to adjust calorie intake in order to maintain weight).

Height (collected once at the V1 visit) is measured by trained staff using a stadiometer.

Lung Function

Spirometry will be performed at the baseline screening visit and at 5 follow up visits (see Table 1). Spirometry will be performed according to ATS guidelines to obtain FEV₁, FVC, FEF₂₅₋₇₅, and PEF.¹⁶⁻¹⁷ After spirometry is obtained, participants will be administered 2 puffs of albuterol via a spacer. After 15-45 minutes, spirometry will be repeated.

Urine

Urine will be collected at the baseline screening visit and as a part of the 5 follow up visits (see Table 1). At the baseline screening visit, it will be collected for rapid urine cotinine measurement on all participants. Rapid urine cotinine testing will be performed with NicAlert test strips and a NicAlert of 4 or greater will exclude a participant from proceeding. Urinary biomarkers will also be measured to assess oxidative stress and inflammation at baseline and at the additional 5 follow up visits. LTE₄ and F-isoprostane have emerged as robust markers of oxidative stress and inflammation¹⁰ and will be quantified by ELISA in Dr. Biswal's or Dr. Wills-Karp's laboratories or by an outside commercial laboratory, along with other markers related to asthma and allergy.

Blood

At baseline, blood will be analyzed for allergen specific IgE levels to cockroach, mouse, cat, dog, dust mites, orchard grass, and ragweed using the ImmunoCAP system (Pharmacia Diagnostics, Uppsala, Sweden).

Blood will be collected at the screening visit 2 and on each of the 5 follow up visits for the following:

- (1) Serum inflammatory markers and oxidative stress markers, such as IL-5, IL-13, RANTES, superoxide anion, lipid peroxides
- (2) Basophil activation
- (3) White blood cell superoxide anion production
- (4) Complete blood count with differential
- (5) Comprehensive metabolic panel with lipids and iron
- (6) Serum carotenoids

Cytokines/chemokines and markers of oxidative stress will be measured in the Biswal Laboratory or the Wills-Karp Laboratory at the Johns Hopkins School of Public Health. Basophil activation will be measured in the Shreffler Laboratory at Mass General Hospital.

h.Biospecimens and Biomarkers

Biomarkers will be measured in the following biospecimens: nasal lavage, nasal epithelial cells, serum, blood, urine, and FENO. These will include various markers of inflammation and oxidative stress (see below).

FENO: Exhaled nitric oxide is a known marker of pulmonary inflammation and will provide a means of assessing pulmonary oxidative stress and inflammation. FENO will be measured during each of the 6 clinic visits. Measurement of exhaled nitric oxide will be obtained according to the American Thoracic Society Guidelines.¹⁷ 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.

Nasal Epithelial Cell Collection: Nasal epithelial cells will be obtained during each clinic visit. The sampling will be done with the Rhinoprobe, a commercially available, disposable curette designed for such sampling, and the cell sample will be used to measure Phase II enzyme gene expression. The nasal cavity will be held open using an autoclaved nasal speculum. The curette will be inserted into the nose under direct visual inspection. Gentle pressure will be applied to the curette as it is drawn gently along the inferior surface of the inferior nasal turbinate 10 times. The curette will then be placed in an eppendorf tube containing lysis buffer. RNA will be extracted for analysis of gene expression using RT-qPCR, such as the phase II enzymes, GCLM, GCLC, HO-1, as well as expression of other genes related to asthma or allergy.

Nasal Lavage Procedure: Nasal lavage will be performed at each clinic visit. To undergo nasal lavage, a participant will be instructed to inspire deeply and hold his or her breath so that the soft palate seals off the nasopharynx. The participant will then extend his or her neck backwards, at a 45 degree angle, while the lavage solution is instilled in the two nostrils with the use of a pipette. The lavage solution dwells in the nasal passages for 10 seconds, while the study participant holds his or her breath. Next, the participant will be instructed to lean forward and expel the lavage fluid into an emesis basin. The return fluids will be shaken and kept on ice until they are centrifuged. Following centrifugation, pellets from the lavages will be used for cytology. The supernatants of lavages will be used for analysis of mediators and cytokines.

Analysis of Biomarkers in Nasal Specimens: The following biomarkers/correlative assays will be run on nasal lavage specimens in either our laboratory, the Biswal laboratory in the JHSPH: (1) cell count and differential, (2) cytokines and chemokines. The percentage of eosinophils, neutrophils, and mononuclear cells in nasal lavage fluid will be quantified. The supernatant will be stored frozen until assayed for cytokines, chemokines, and other markers of inflammation. Cytokines/chemokines will be measured by ELISA using multi-plex antibody bead kits from Lincoplex (St. Charles, Missouri) according to the manufacturer's instructions or other comparable immunoassay. Tryptase levels will be quantified by fluoroenzyme immunoassay in the Matsui laboratory (ImmunoCAP Tryptase, Pharmacia Diagnostics, Uppsala, Sweden).

i. Blinding, including justification

It is not possible to blind participants to this protocol as they will consume the intervention diet at the feeding center and the control diet will consist of their usual diet. Participants will be randomized using a computerized randomization scheme to receive either the MED DIET or the control diet first and they will receive the alternate diet after the washout period. The nutrition core at ProHealth will dispense the dietary interventions to the study participants. The study staffs who will have access to the randomization scheme are Roger Peng, PhD (co-investigator and biostatistician) and the database programmer from the Data Core and the staff in the Nutrition Core at ProHealth.

j. Justification why participants will not receive routine care or will have current therapy stopped

The study protocol will not interfere with participants receiving their previous routine medical care, and asthma care. A copy of all lab (blood) 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.

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

Each participant will receive the intervention and the control diet in this crossover study. The participant's regular diet will serve as the control to provide a comparison which represents the diet typically consumed by the individual.

l. Definition of treatment failure or participant removal criteria

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

At any time, the subject's participation will stop if he/she requests not to proceed with the study.

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

Participants will resume their typical diet once the study concludes or when the participant stops the study.

5. Inclusion/Exclusion Criteria

Inclusion Criteria:

- 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]

- 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.
- Non-smoker, defined by no cigarettes in the past 1 month and a negative urine cotinine
- Not breastfeeding
- Age 18-50 years
- No other major pulmonary disease such as cystic fibrosis or COPD
- Willing to eat at least one on-site meal a day, 3-4 days a week, and willing to eat study diets and nothing else for the 4 weeks of controlled feeding

Exclusion Criteria:

- Chronic oral steroid therapy (daily)
- Oral corticosteroid use within past 4 weeks
- Respiratory tract infection within past 4 weeks
- 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
- Pregnancy(self-report), planning a pregnancy, or nursing/breastfeeding mothers
- Food allergy
- 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
- Food preferences, intolerances, or dietary requirements that would interfere with diet adherence
- Weight over 350 pounds
- Weight loss or gain of 10 pounds or more during prior 2 months
- Any condition or compliance issue which in the opinion of the investigator might interfere with participation in the study
- Use of warfarin

6. Drugs/Substances/Devices

7. Study Statistics

a.Primary outcome variable:

As this is a pilot study, our outcomes of interest are related to process and procedures necessary to successfully deliver a dietary intervention to a population of adults with asthma. We have chosen a convenience sample of approximately 12 participants for this feasibility study. To assess feasibility, we will assess numbers of participants screened for each enrolled, reasons for screen fails, rate of enrollment, and numbers of drop-outs. We will assess adherence to the Mediterranean dietary intervention using both self-reported and objective measures. Self-reported measures are obtained from information provided on a daily diary and from subjective judgment of 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. The objective measures include serum carotenoid values, meal attendance, on-site meal consumption; and body weight. We will measure clinical and biological endpoints to inform the design of a larger, more definitive trial, including asthma symptoms scores and lung function.

b.Early stopping rules

As this is a pilot study there will be no formal criteria for stopping the study early, and there is no plan for interim analysis for individuals. However participants may choose leave the study early or at the determination of the Principal Investigator.

8. Risks

a.Medical risks, listing all procedures, their major and minor risks and expected frequency

Questionnaires: The principal risk associated with questionnaires is loss of confidentiality.

Spirometry: The minor risk is the slight discomfort of forceful exhalation.

The drug albuterol 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.

Venipuncture: 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.

Nasal cell collection: A risk of cell sampling is mild discomfort while the sample is being collected; this feeling should go away immediately after the sample is taken. There is a slight risk of some nasal bleeding during or just after the sample is collected. Rarely an infection may occur at the sampling site

Nasal Lavage: Minimal discomfort and a small risk of aspiration of the lavage fluid.

Dietary intervention (MED DIET) The diet interventions pose minimal risk. Participants may experience some bloating and other minor gastrointestinal discomforts related to the high fruit, dairy and fiber content of the intervention diets. It has been our experience that these problems resolve soon after changes in diet. For those persons with lactose intolerance, we provide lactaid. Our experience suggests that GI discomfort is generally minor and subsides quickly. Participants are monitored for reactions to the diets and, if necessary, the diet can be modified or terminated.

b.Steps taken to minimize the risks

Questionnaires: Unique identifiers will be assigned to participants and these will be used to label all paperwork, including questionnaires.

Spirometry: Will be performed by trained study personnel.

Venipuncture: A staff member who is experienced in phlebotomy will perform venipuncture.

Nasal cell collection: A staff member experienced and trained in nasal epithelial cell collection will perform the procedure.

Nasal Lavage: A staff member experienced and trained in nasal lavage will perform the procedure. To reduce the risk of aspiration a small volume of lactated ringers 10 ml (5 ml per nostril) will be instilled into the nasal cavity and subjects will be instructed to hold their breath to seal off the nasopharynx.

Dietary intervention (MED DIET): GI discomfort is generally minor and subsides quickly. 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. All costs associated with diet intervention, blood drawing, questionnaires and spirometry are covered by the investigators.

9. Benefits

The results of the study will lend important insight into the role of diet in asthma clinical symptoms, lung function, and inflammation and oxidative stress. The study may also provide insight as to the potential for treating asthma with dietary interventions.

10. Payment and Remuneration

A study participant will receive compensation according to the table below. In addition, participants will be reimbursed for any transportation fees that are needed in extenuating circumstances for travel to and from the ProHealth Site for study purposes. They will also receive all of their meals during the MED DIET intervention periods. Participants will receive an insulated bag to transport study food to and from ProHealth and will be able to keep bag at the end of the study.

Study Activity/Procedure	Compensation
Completion of dietary intervention and associated study procedures	\$150/intervention period x 2 intervention periods = \$300
Telephone completion of 24 hour diet recall (9)	\$180
Bonus for completing entire study	\$100
Total Possible Compensation	\$580

11. Costs

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

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3
	2b	Specific objectives or hypotheses	3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4-5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	4
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	4
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	4
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	NA

		assessing outcomes) and how	
Statistical methods	11b	If relevant, description of the similarity of interventions	4-5
	12a	Statistical methods used to compare groups for primary and secondary outcomes	6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	28
	13b	For each group, losses and exclusions after randomisation, together with reasons	28
Recruitment	14a	Dates defining the periods of recruitment and follow-up	4
	14b	Why the trial ended or was stopped	4
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	15
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	15
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	16-19
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	16-19
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	16-19
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	11
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	11
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11
Other information			
Registration	23	Registration number and name of trial registry	6
Protocol	24	Where the full trial protocol can be accessed, if available	6
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	1

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming; for those and for up to date references relevant to this checklist, see www.consort-statement.org.