SOLUBLE FIBRE FOR RESPIRATORY HEALTH

RESEARCH PROTOCOL

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Sponsor:
Australian Health and Nutrition Association Limited trading as Sanitarium Health & Wellbeing Company

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Glossary of Terms

ACQ	Asthma Control Questionnaire
AE	Adverse Event
AHR	Airway Hyper-Responsiveness
ANZCTR	Australian and New Zealand Clinical Trials Registry
AQLQ	Asthma-related Quality of Life Questionnaire
CRP	C-reactive protein
CTN	Clinical Trial Notification
DQES	Dietary Questionnaire for Epidemiological Studies
FeNO	Fraction of exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 second
FFAR	Free Fatty Acid Receptor
FFQ	Food Frequency Questionnaire
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GINA	Global Initiative for Asthma
GPR	G-Protein coupled Receptor
GS-MS	Gas Chromatography-Mass Spectrometry
GSRS	Gastrointestinal Symptom Rating Scale
HAT	Histone Acetyltransferase
HDAC	Histone Deacetylase
HMRI	Hunter Medical Research Institute
HNEH	Hunter New England Health
IL	Interleukin
LMM	Linear Mixed Model
MCID	Minimal Clinically Important Difference
NFκB	Nuclear factor kappa B
NK	Natural killer
OCS	Oral Corticosteroids
PBMC	Peripheral Blood Mononuclear Cell
PEF	Peak Expiratory Flow
SAE	Serious Adverse Event
SCFA	Short-chain Fatty Acid
SF36	36-Item Short Form (General) Health Survey
TGA	Therapeutic Goods Administration
TNF	Tumour necrosis factor

SYNOPSIS

Title: Soluble Fibre for Respiratory Health (OLI-2 study)

Sponsor: Australia Health and Nutrition Association Limited trading as Sanitarium Health & Wellbeing Company

Primary Outcome: Asthma control (Asthma Control Questionnaire, ACQ7)

Secondary Outcomes:
Airway inflammation (airway cell counts, exhaled nitric oxide)
Lung function (spirometry)
Airway hyper-responsiveness (hypertonic saline challenge)
Plasma short-chain fatty acid (total SCFA, acetate, butyrate, propionate by GC-MS)
Gastrointestinal Symptoms Rating Scale scores (modified GSRS)
Dietary intake at baseline (FFQ)
Faecal microbiome

Number of Participants: n=32

Study Duration: September 2018 – November 2019

Study Population:
Males and females aged >18 years
Doctor diagnosis of asthma and confirmed airflow variability
Poorly controlled asthma, defined as ACQ7 >0.75 units
No recent (past month) respiratory tract infection
No respiratory conditions other than asthma
Dietary modifications unsuitable
No diagnosis of active gastrointestinal disease
No fibre, prebiotic or probiotic supplement use within the previous 2 weeks. If appropriate to do so, supplements that are not being taken for a health condition may be washed out for 24 weeks before commencing the study.
Never or ex-smokers (ceased smoking >6 months prior)
Not pregnant or breastfeeding
No chronic or excessive alcohol consumption
No unexplained weight loss (>5% body weight) in the past 6 months
Not currently using systemic corticosteroid, immunosuppressive or antibiotic drugs
No terminal illness
No diagnosis of galactosemia or allergy to ragweed

Study Design: This study is a double-blind, randomised, placebo-controlled, 4-way crossover trial in 32 asthmatic adults. Subjects will complete 4 x 2 week interventions, with a 2 week run in period and 2 week washout between interventions (intervention order randomly allocated). The study is testing a soluble fibre supplement at high and low doses compared to placebo.

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2. Purpose of Study

Soluble fibre is a source of plasma short chain fatty acids (SCFA), which are known to have anti-inflammatory properties. This study will investigate the effects of soluble fibre supplementation on asthma control and airway inflammation in clinically stable asthmatic adults, who are poorly controlled at baseline.

3. Aims

In asthmatics who are poorly controlled at baseline, we aim to examine the effect of soluble fibre supplementation on:

1. Asthma control
2. Airway inflammation, lung function, airway hyper responsiveness, plasma short chain fatty acids (SCFA), gastrointestinal symptoms and faecal microbiota.

4. Background and Preliminary Studies

Asthma affects approximately 300 million people worldwide(1) and in Australia, asthma prevalence is approximately 10% (2). Asthma was the eleventh-leading contributor to the overall burden of disease in 2003, resulting in the loss of 63,100 years of healthy life (3). Inhaled glucocorticoids are the mainstay of asthma management; however, as the side effects, cost (>$300m per year) and non-compliance pose a significant burden, non-pharmacological approaches to managing asthma are urgently needed.

Asthma prevalence has increased in westernised countries in recent decades, suggesting that environmental influences such as dietary change may contribute to asthma development and progression. Epidemiological studies report that asthma risk is increased with consumption of western style diets (4-6), which are characterised by a high intake of processed foods, resulting in fibre consumption that is well below recommended levels (7, 8).

Soluble fibre – a source of short chain fatty acids (SCFA):

Dietary fibre is a complex carbohydrate found in plant-based foods, which exists in both soluble and insoluble forms. Soluble fibre is partially, or completely, fermented by commensal bacteria in the colon to produce the biologically active SCFA. The key SCFAs are butyrate, the major energy source for colonocytes; propionate, which is mostly absorbed by the liver; and acetate, the primary SCFA that enters the circulation. SCFA production is dependent on host gut microbiome composition, with *Bifidobacterium* and *Lactobacillus* amongst the most potent producers of SCFA (9). SCFA production is also dependent on the type of fibre consumed. β2-1 fructans, which include inulin and fructo-oligosaccharides, are amongst the most highly fermentable soluble fibres and are potent sources of SCFA (10, 11). In addition to providing substrates for fermentation, some soluble fibres, including inulin, act as prebiotics and enhance SCFA production by preferentially stimulating the growth of SCFA-producing bacteria, in as little as one week (9). SCFA are attracting much attention due to their anti-inflammatory properties.

Anti-inflammatory mechanisms of SCFA:

i. Free fatty acid receptor activation: Soluble fibre-derived SCFAs can bind to two major receptors, the free fatty acid receptor (FFAR)2 also known as G-protein-coupled receptor (GPR)43 and FFAR3, also known as GPR41. Animal studies have shown that SCFAs can induce the development of regulatory T cells via GPR43 activation (12). Furthermore, in mouse models of allergic airway inflammation, fibre-rich diets increase plasma SCFA levels and reduce airway inflammation after an allergen challenge, dependent on GPR41 (13). In adults with asthma, we have demonstrated for the first time that a single dose of soluble fibre stimulates GPR41/43 expression and modulates immune responses in human airways (14).

ii. Epigenetic regulation: Histone acetylation, which promotes gene transcription, can also be modified by SCFA. Histone acetylation is catalysed by histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs). SCFAs are well known HDAC inhibitors and reduce inflammation by suppressing NFκB. We have collaborated on a comprehensive study using a mouse model of asthma, which showed that SCFA suppress HDAC activity and that inhibition of HDAC9 has anti-inflammatory effects in the airways (15).

Clinical Supplementation trials to boost SCFA and reduce inflammation

Several human trials have used soluble fibre supplements – specifically inulin or oligosaccharides - to modulate the microbiome and/or immune responses (e.g. T cell differentiation and inflammatory cytokine expression) (16). Inulin and/or oligosaccharide supplementation (5.5-10 g/day for 8-12 weeks) has been shown to reduce serum CRP, TNF-α and IL-6 in overweight subjects and Type 2 diabetics (17-19). In a double-blind, placebo controlled trial in 44 elderly subjects, using 5.5g oligosaccharides/day for 10wks (20), there was an increase in *Bifidobacteria* numbers, phagocytosis and NK cell activity and a decrease in *ex-vivo* production of IL-6, IL-1β and TNF-α in whole cell cultures stimulated by LPS (20).

We have completed a 7-day trial examining the effects of soluble fibre (12g/day inulin) in asthma. Asthmatic adults consumed soluble fibre (inulin, 12g/day), soluble fibre + probiotic, or placebo for 7 days. Inulin supplementation led to an increase in beneficial bacteria, *Bifidobacterium and Anaerostipes*, asthma control (ACQ) improved, and airway eosinophils decreased. Importantly, following inulin supplementation, in subjects with poorly controlled asthma at baseline (n=12), 100% of subjects had an improvement in asthma control, with 50% reaching the minimum clinically important difference (MCID, 0.5) (21).

Next Steps

Our pilot study has identified a potential anti-inflammatory role of soluble fibre (inulin) in the airways. In subsequent work we have identified an alternative form of soluble fibre (blend of inulin, fructooligosaccharides and galactooligosaccharides), with superior ability to increase plasma SCFA (the key bioactive metabolites of soluble fibre). Here we aim to test the efficacy of this alternative formulation, at low and high doses, in asthmatics who are poorly controlled at baseline, and are thus most likely to gain benefit from the intervention.

5. Research Plan

5.1. Study Design

A double-blind, randomised, placebo-controlled, 4-way crossover trial including clinically stable asthmatic adults, will be used to assess the efficacy of an oligosaccharide blend in improving asthma control as measured by the Asthma Control Questionnaire (ACQ7). The effect of the oligosaccharide blend on airway inflammation, lung function, airway hyper responsiveness, plasma short chain fatty acid (SCFA) production, gastrointestinal symptoms and faecal microbiota will also be examined. The trial will be conducted in accordance with Good Clinical Practice (GCP) standards and approved by the Hunter New England Health Human Research Ethics Committee, and registered with the University of Newcastle Human Research Ethics Committee and the Australian New Zealand Clinical Trials Registry (ANZCTR).

Inclusion Criteria

Males and females aged >18 years
Doctor diagnosis of asthma and confirmed airflow variability
Poorly controlled asthma, defined as ACQ7 >0.75 units

Exclusion Criteria

Recent (past month) respiratory tract infection; respiratory conditions other than asthma; dietary modifications unsuitable; non-adherence to prescribed asthma medications; diagnosis of diabetes or active gastrointestinal disease; fibre, prebiotic or probiotic supplement use - If appropriate to do so, supplements that are not being taken for a health condition may be washed out for 2-4 weeks before commencing the study. No current smokers (last 6 months); pregnancy or breastfeeding; chronic or excessive alcohol consumption; unexplained weight loss (>5% body weight) in the past 6 months; use of systemic corticosteroids, immunosuppressive or antibiotic drugs within the previous 4 weeks; terminal illness; galactosemia or ragweed allergy.

5.2. Methods and Procedures

5.2.1. Participant Recruitment

Participants will be recruited via the Hunter Medical Research Institute (HMRI) research register and HMRI newsletter. Other recruitment strategies will include media release, social media and radio advertising and placing recruitment flyers in the HMRI building, John Hunter Hospital, University of Newcastle notice boards, local pharmacy's, shopping centres and doctors surgeries. Initially, interested potential participants will be contacted by telephone and provided with the participant information and consent form, either by post or email, and given time to consider whether or not they would like to participate and discuss the project with others such as their treating doctor. After 1-2 weeks the study coordinator will phone the potential participant to determine their interest and answer any questions. If they are interested, a telephone screen would then take place and, if appropriate, a mutually

convenient time arranged for a face to face screening visit. All participants will be consented in writing prior to attending the screening visit.

5.2.2. Intervention

Subjects will be instructed to mix powder sachets with water (250mL, room temperature) and drink immediately each morning and evening. Powder sachets will contain:

1. High dose 1: 12 g/d of total oligosaccharides via (1x6g oligosaccharides and 1x6g placebo) dose in morning and (1x6g oligosaccharides) dose in evening.
2. High dose 2: 12g total oligosaccharides via 1x12g oligosaccharides dose in morning and 1x6g placebo dose in evening.
3. Low dose: 1x6g total oligosaccharides via (1x6g oligosaccharides and 1x6g placebo) in morning and 1x6g dose placebo dose in evening.
4. Placebo control: 18 g/d or equivalent weight to investigational product of maltodextrin powder via 1x12g dose in morning and 1x6g dose in evening.

5.2.3. Study Visits

Participants will be screened for study eligibility by telephone prior to study commencement and will then attend a screening visit and eligible subjects will be entered into the study. They will commence the study background diet for 2 weeks, then attend the HMRI clinic on 8 occasions, before and after each 2-week intervention (high dose 1, high dose 2, low dose and placebo control), which will be separated by 2 weeks (***Fig 3***). During each intervention phase, participants will be telephoned for motivational purposes, to record compliance with the intervention, the study diet and their prescribed asthma medications, and to establish whether any adverse effects have been experienced.

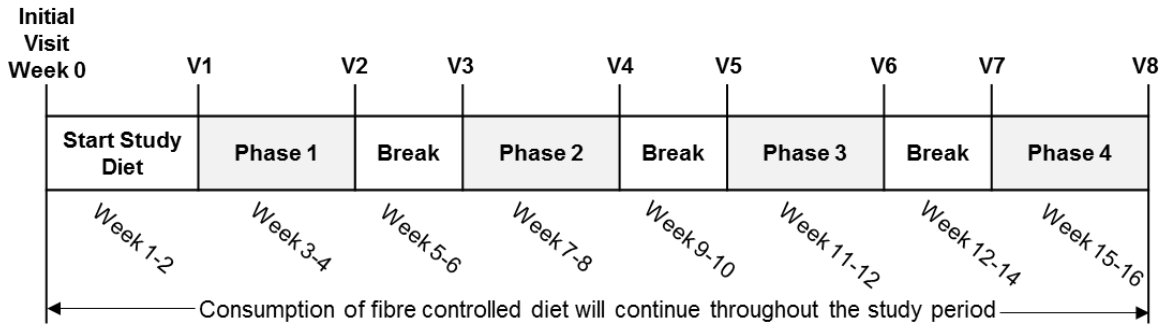

Figure 3. Study visit schedule.

Initial Visit (V0): Participants will attend the Clinical Trials Facility on Level 4 at the Hunter Medical Research Institute (HMRI). At this screening/baseline visit, participants will have the opportunity to ask and have their questions satisfactorily answered by the study coordinator before signing the Consent Form. Participants will then be screened for study eligibility. Medical history, respiratory and non-respiratory medication use, smoking history, exacerbation history and asthma control will be recorded. An allergy skin prick test including a ragweed allergen will be performed. Lung function will be measured by spirometry. If there is no evidence of airflow variability, hypertonic saline challenge will be performed. Subjects will be required to withhold their asthma medications for a defined time prior to the visit. Details of withholding times are listed in Table 1. Alternatively, participants may be provided with a peak flow meter to record their peak expiratory flow (PEF) twice-daily over a period of at least one week. Participants will receive instructions on limiting their intake of soluble fibre in the two weeks prior to commencing the study and for the entire duration of the study, should they be eligible for enrolment (see description of study diet below). We will also confirm adherence to prescribed asthma medication during the 2-weeks run-in period. If asthma medication adherence is less than 80%, participants will be withdrawn from the study prior to randomisation. Participants who exacerbate and use a course of antibiotics and/or oral steroids (e.g., Prednisone) during the study will be asked to stop taking the study supplement until they have fully recovered from their flare-up. Participants will be asked to recommence taking the study supplement 4 weeks after completing their course of antibiotic and/or prednisone. Study visits will also be rescheduled accordingly. Subjects who require a change to their maintenance inhaled corticosteroid dose during the study will be withdrawn.

All study visits (Visits 1 to 8): Eligible participants will be randomised and booked to attend the clinic for Visits 1-8 at mutually convenient times. Participants will be phoned prior to their appointment to confirm that they are able to attend and to remind them to withhold their asthma medications for a defined time prior to the test (Table 1). Participants will be provided with a standardized non-fermentable evening meal (supermarket supplied frozen pasta meal), which they will be asked to consume the night before each of their study visits (V1-V8) (on the evening prior to visits 2, 4 and 6 participants will also need to consume their study supplements). Participants will then need to fast for the next 12-hours. During this fasting period, participants can drink water as desired. On the following morning, participants will attend the clinic at HMRI (prior to visits 2, 4, and 6 subjects will consume the study supplement 2 hours before their clinic appointment) for approximately 2.5 hours for their scheduled appointments.

On the morning of the study visit, participants will arrive at the HMRI clinic after a 12-hour overnight fast. They will have a fasting venous blood sample collected, exhaled nitric oxide measured, spirometry and combined hypertonic saline challenge/sputum induction will be performed. Participants will also be assessed for asthma control (Asthma Control Questionnaire (ACQ7)), asthma related quality of life (AQLQ), general health (SF36), gastrointestinal symptoms (Gastrointestinal Symptoms Rating Scale (GSRS)) and 24 hour food recall. At visit 1 only, participants will also be asked to complete a food frequency questionnaire to assess usual dietary intake. At the completion of visits 1, 3, 5 and 7 participants will receive their study supplements to be taken twice each day for 2 weeks. Participants will be shown how to mix powder sachets with water (room temperature) and drink immediately. For the duration of the study, participants will complete a study diary to record adherence with the study supplements and asthma medications and adverse effects.

Participants will be reimbursed $100 upon completion of the study for travel costs associated with participation in this study.

5.2.4. Study Diet

For 2 weeks prior to visit 1 and for the duration of the study, participants will be instructed by a study dietitian to consume a low soluble fibre background diet. This will be achieved by avoidance of soluble fibre-rich foods including oats, oat bran, beans, seeds and limiting fruit and vegetable intake to no more than 2 serves/day and consuming ½ cup of bran based cereal per day (~ 13g fibre, ~90% insoluble fibre) to avoid constipation. Participants will also be instructed to avoid sources of probiotics such as yoghurt and fermented milk drinks. Participants will also be instructed to avoid fibre supplements such as psyllium, Metamucil, Benefibre or Fybogel and foods such as asparagus, jerusalem artichokes and bananas as they naturally contain prebiotics (inulin). Education will be provided by a dietitian to identify foods that have added soluble fibre, such as bread, and will be asked to avoid these foods. We will explain to subjects that they can consume all other foods including 2 serves of fruit or vegetables as they would normally.

Table 1. Withholding times for asthma medications prior to all visits.

6 Hours	24 Hours	5 Days	
Airomir	Foradile	Aller G	Telfast
Asmol	Neulin SR	Andrumin	Vallergan
Atrovent	Oxis	Avil	Zadine
Atrovent Forte	Serevent	Avil Retard	Zyrtec
Bricanyl	Singulair	Benadryl	
Combivent	Slo-Bid	Claratyne	
Epaq	Spiriva	Demazin	
Intal	Symbicort	Disolyn	
Intal Forte	Seretide	Panquil	
Respolin	Flutiform	Periactin	
Tilade	Austyn	Phenergan	
Ventolin	Neulin	Polaramine	

5.2.5. Randomisation

The order of the interventions will be determined by randomisation. Eligible participants will be assigned a unique study number according to a computer generated randomisation schedule. The randomisation service will be managed by an independent statistician at HMRI. Randomisation coding will use a 4x4 Latin square method. During the treatment phase, both the participants and the investigators will be blinded to the allocation.

5.2.6. Adherence

Adherence to the study intervention will be monitored at each visit by sachet countback and 24-hr food recall (to check background diet). Participants will also record supplements consumed and asthma medications used in the study diary which will be checked at each visit. Subjects who consume <80% of the study supplements will be excluded from the per protocol analysis.

5.2.7. Outcome Measures

Primary: Asthma control (Asthma Control Questionnaire, ACQ7)

Secondary: Airway inflammation (airway cell counts, exhaled nitric oxide), lung function (spirometry), airway hyper-responsiveness (hypertonic saline challenge), plasma short-chain fatty acid (total SCFA, acetate, butyrate, propionate by GC-MS), Gastrointestinal Symptoms Rating Scale scores (GSRS), Asthma Quality of Life (AQLQ), General health Status (SF36), faecal microbiota and dietary intake at baseline (FFQ).

5.2.8. Clinical Assessment

Asthma control

The validated Juniper Asthma Control Questionnaire (ACQ7) (22) will be used to assess asthma control. The ACQ measures asthma control over the previous week and is comprised of seven items that are equally weighted. Participants will be asked to respond to the first six items (five of which record symptoms and one of which assesses rescue short-acting β2-agonist use) using a seven-point scale (0 = no impairment, 6 = maximum impairment). The final item provides a score for lung function (FEV1) based on spirometry results. The overall ACQ score, between 0 (totally controlled asthma) and 7 (severely uncontrolled asthma), is calculated as the mean of the seven item scores. A score of 0-0.75 indicates well-controlled asthma, >0.75-1.5 indicates partially controlled asthma and a score of >1.5 indicates poorly controlled asthma. An ACQ change of ≥0.5 is considered clinically significant. The ACQ-6 score will be calculated as the mean of the first 6 items, to allow assessment of asthma control without consideration of lung function.

Blood pressure

An appropriately sized blood pressure cuff will be placed firmly around the upper arm of the participant, centred over the brachial artery. After resting quietly in a seated position for 10 min, four consecutive blood pressure and heart rate readings will be taken at one min intervals by a single observer using an electronic vital signs monitor (Welch Allyn, 6000 Series). The first reading will be discarded, and an average of the remaining measurements recorded for analysis.

Spirometry, Saline Challenge and Sputum Induction

Asthma medications are to be withheld (short acting β2-agonists and anti-cholinergics for 6 hours, long-acting β2-agonists, combination inhalers and long acting theophylline for 24 hours, and short acting theophylline for 12 hours) prior to measuring dynamic lung function using a MedGraphics spirometer (CPFS/D and BreezeSuite software, MedGraphics, Minnesota, USA). Predicted values for forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) will be calculated using National Health and Nutrition Examination Survey (NHANES) III data 39, which accounts for age, gender, race and height. Prior to conducting the assessment, the room temperature, barometric pressure and humidity is to be recorded, and the spirometer calibrated.

Participants will perform the manoeuvre seated in a chair and wearing a disposable nose clip. A new disposable mouthpiece will be attached to the spirometer, and after completing 1-2 tidal breaths, the participant is instructed to inhale to total lung capacity, then forcefully exhale to

residual volume. This step is repeated until three reproducible (within 150ml) and technically acceptable results are obtained, with a maximum of eight attempts performed.
Sputum induction coupled with bronchial provocation challenge (to assess airway hyper responsiveness (AHR)) will also be performed at each visit over a standardised 15.5 minutes nebulisation protocol (30s, 1min, 2mins, 4mins, 4mins, 4mins). To determine AHR, the participant is exposed to a mist of hypertonic (4.5%) saline created by a nebulizer (ULTRA-NEB™ ultrasonic nebulizer, DeVilbiss, Model 2000) 40. This test will commence immediately after spirometry is performed. The participant is instructed to inhale the saline aerosol through the mouthpiece for incremental time periods in doubling doses, for no longer than 15.5 mins. FEV1 is measured after each period of saline inhalation and compared to the baseline value. If the participant's FEV1 falls ≥15% below their initial baseline value, the challenge is complete, as this is indicative of AHR. Participants with AHR are administered 400mcg salbutamol via a spacer, and their lung function assessed after 15mins.
During the challenge participants will be encouraged to cough and clear their throat after each period of saline inhalation. This will help to dislodge sputum from their chest wall. The participant is instructed to empty the contents of their mouth into a specimen jar. Sputum induction will continue until either the completion of 15.5 minutes of inhalation, If the participants FEV1 falls ≥15% again during the test, or if the participant declines to continue.

Sputum Cell Counts
Sputum will be obtained by induction during hypertonic saline challenge. Lower respiratory sputum portions will be selected and dispersed using dithiothreitol. Total cell counts and cell viability (trypan blue) will be performed, followed by cytospins for differential cell counts. Supernatant will be stored at -70^{o}C for potential future analysis of additional inflammatory markers.

Exhaled Nitric Oxide (eNO) Measurement
eNO correlates with eosinophilic airway inflammation. eNO will be measured using a portable handheld electrochemical analyser NIOX VERO® (Aerocrine, Sweden) and reported in parts per billion (ppb). Participants will be instructed to empty their breath away from the mouthpiece, take a normal size breath in through the mouthpiece, and exhale in one single breath at a constant and steady rate. eNO is always to be measured prior to spirometry as forced breathing manoeuvres may alter eNO results.

Blood SCFA and Inflammation Markers
A total of 27 mL blood will be collected by venepuncture at each visit in the HMRI building by an experienced phlebotomist. The volume of blood to be collected is <0.5% of total blood volume, and thus represents no hemodynamic risk to participants. A 9mL aliquot of whole blood will be centrifuged at 4^{o}C, 3000rpm for 10 minutes and plasma removed and stored at -80^{o}C. Plasma samples will be analysed by gas chromatography with flame ionisation detector for plasma short chain fatty acids, including acetate, butyrate and propionate. Plasma will also be stored for potential future analysis of additional inflammatory markers. The remaining 18 ml of blood will be used for assessment of immune responses in isolated peripheral blood mononuclear cells (PBMCs).

Gastrointestinal Symptoms

Gastrointestinal symptoms will be assessed using the modified Gastrointestinal Symptoms Rating Scale(GSRS) (23). The GSRS is a 15-item questionnaire which is used to verify the presence and intensity of gastrointestinal symptoms which are categorised into 5 symptom clusters; indigestion, reflux, diarrhoea, abdominal pain and constipation.

Asthma-related Quality of Life (AQLQ)

The perceived impact of asthma on participants' quality of life will be measured and monitored using the 32-tem questionnaire (24).

General Health Status (SF36)

Participants will complete the SF36 at each visit (1-8) to measure and monitor changes in general health, including physical and mental well-being and functioning (25).

Faecal Microbiota

Faeces samples will be collected by participants at home using the faeces collection kit provided and stored in the home freezer at -20^{o}C until the next clinic visit. Samples will be brought into the clinic in the esky provided and samples will be stored at -20^{o}C until dispatched for off-site analysis.

Dietary Intake

Participants will complete a food frequency questionnaire (FFQ) to assess usual dietary intake at baseline (DQES, Victorian Cancer Council), and at each visit (1-8) a 24 hour food recall will be collected by a research assistant trained in collecting this information.

Allergy Skin Prick Test

Participants will have an allergy skin prick test done at the screening visit. In this test, we will place a drop of each allergen on the skin of the participant's forearm. Then the skin is gently scratched so the allergen goes under the skin's surface. We will then closely watch the skin for 15 minutes for signs of a reaction, usually swelling and redness of the site. Common aeroallergens will be used to determine atopy status (dust mite, cockroach, cat, household moulds and grass mix). A ragweed allergen will also be used to assess allergy to ragweed. If any participant experiences a significant reaction (≥3mm) to the ragweed allergen they will be excluded from the study.

5.2.9. Laboratory methods

Blood Samples

(a) Processing

Blood will be collected into EDTA tubes, centrifuged at 20˚C, 3000 rpm for 10 minutes then plasma will be collected and stored at -80˚C. Remaining blood cells will be used for isolation of PBMCs using the Lymphoprep™ (STEMCELL Technologies, Australia) density gradient method, as per the manufacturer's recommendations. Cell counts and viability tests with trypan blue staining were performed. Isolated PBMCs will be used for cell culture experiments, with 2×10^{6} PBMC stored in buffer RLT for RNA extraction and gene expression analysis.

(b) PBMC Cell Culture

Isolated PBMCs will be seeded in 24-well tissue culture plates (Sigma, Australia) at a final concentration of 1×10^6 cells/well. Cells will be stimulated in duplicate with 5ng/mL lipopolysaccharide (LPS, *E. coli*), and 20 MOI rhinovirus A1 (RVA1/Newcastle/2018, 2.36×10^8 $TCID_{50}$/mL). Plates will be incubated at 35°C 5% CO_2 for a total of 48 h. Tumour-necrosis factor-α (TNF-α), interleukin-10 (IL-10), and interferon-γ (IFN-γ) concentrations in cell free supernatant will be measured using DuoSet ELISA assays (R&D Systems, Australia).

(c) Plasma SCFA

SCFAs will be measured in plasma by gas chromatography (GC). Plasma samples (200uL), containing heptanoic acid (internal standard, 100 nmol) will be extracted with ether (3mL). The ether layer will be transferred to a clean tube containing 0.2 M sodium hydroxide (50uL). The ether is then removed and the aqueous solution washed again with ether (3mL). The aqueous solution is then transferred to a GC vial and acidified by addition of 1 M phosphoric acid (30uL). The mixture is injected onto a GC column (Zebron FFAP 30 m x 530 um x 1.0 um) and SCFAs (acetate, propionate and butyrate) are quantified against calibration mixtures extracted in the same way.

Induced Sputum Samples

(a) Processing

Sputum collected by induction during hypertonic saline challenge will be processed within 30 minutes of collection. Mucus plugs will be dispersed using 0.1% dithiothreitol, then total cell counts (TCC) and cell viability (using trypan blue exclusion) will be performed. Cytospins will be prepared and stained (May-Grunwald Giemsa) for differential cell counts, on 400 non-squamous cells as previously described 41. Using standard morphological criteria, cells will be classified as neutrophils, eosinophil, columnar epithelial, squamous epithelial, macrophages and lymphocytes.

(b) Phenotyping

Sputum cell counts of neutrophils and eosinophils will be used to classify subjects by asthma inflammatory phenotype 42. This is done by converting the cell counts to a percentage of total cells in the sample. The following classifications are used: Eosinophilic asthma: Sputum eosinophils ≥3.0%, Neutrophilic asthma: Sputum neutrophils >61%, Paucigranulocytic asthma: Sputum eosinophils <3.0% and sputum neutrophils <61%, Mixed granulocytic asthma: Sputum eosinophils ≥3.0% and sputum neutrophils ≥61%.

(c) Molecular Testing

100µL of sputum plugs will be selected and homogenised in buffer RLT (Qiagen, Hilden, Germany), then stored at -80˚C for RNA extraction and gene expression analysis.

Faecal samples

(a) Collection and Processing

Frozen stool samples will be obtained from participants at each clinic visit and stored at -20°C until shipped on dry-ice for offsite analysis.

Gene expression analysis

RNA will be extracted from sputum cells and PBMCs using RNeasy Mini Kit (Qiagen, Hilden, Germany) and quantitated using the Quant-iT RiboGreen RNA Assay Kit (Molecular Probes Inc, Invitrogen, Eugene, OR, USA) as per manufacturer's instructions. RNA will then be converted to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) via standard Taqman methods. Taqman qPCR primer and probes for genes of interest (PBMC: Class I and IIa HDAC subtypes (*HDAC1-5, 7-9*), *FFAR2*, *FFAR3*; Sputum: *HDAC9*, *IL4*, *IL5*, *IL13)* will be combined with cDNA and Taqman gene expression master mix as per manufacturer's instructions in duplicate real-time PCR reactions (7500 Real Time PCR System: Applied Biosystems). Analysis will be performed on the change in cycle threshold (∆Ct) between the target gene compared with the housekeeping gene 18S rRNA, calculated using 2-ΔΔCt relative to 18S and the mean of the baseline value (26).

5.2.10. Statistical Analysis

Data will be analysed with STATA 15 (StataCorp, College Station, Texas, USA) and reported as mean ± standard deviation or median [interquartile range]. Following the intervention period, changes within each treatment compared to baseline will be examined using paired Student's t test (normally distributed data) or Wilcoxon signed-rank test (non-normally distributed data). Per protocol analysis will be performed by linear mixed effects modelling (LMM's) fit by restricted maximum likelihood, to assess the difference in the change in outcomes from baseline, between treatments and placebo. Treatment group (high dose 1, high dose 2, low dose or placebo) and time (treated as categorical with levels at pre and post-treatment) will be specified as fixed effects with an interaction term. Subject ID will be included in the model to designate repeated measures. LMMs use all data available at each time point; therefore missing data imputation will not b be performed. We will also perform the above analysis in the subgroup of participants who have eosinophilic asthma at baseline. Associations between clinical outcomes, inflammatory biomarkers and plasma SCFA will be explored using Pearson or Spearman's rank correlation coefficient. Significance will be accepted if $p<0.05$.

5.2.11. Sample Size Estimate

Sample size has been informed by data from our previous 7-day inulin trial, using data from the sub-group of n=12 participants with baseline ACQ7≥0.75. With a sample size of n=26, and using $\alpha=0.0125$ to account for comparison of 4 groups, we will have 100% power to detect a change in our primary outcome (ACQ7; $\Delta=0.37$, SD=0.22). Allowing for 20% dropouts, we need to recruit 32 subjects.

Table 2. Schedule of Visits and Study Procedures.

	Visit Number	Visit 0	2-weeks run in period	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Design	Time point	**Screen**		**Day 0**	**Day 14**	**Day 28**	**Day 42**	**Day 56**	**Day 70**	**Day 84**	**Day 98**
	Visit Duration	1.5 hrs		2.5 hrs	2.5 hrs	2.5 hrs	2.5 hrs	2.5 hrs	2.5 hrs	2.5 hrs	2.5 hrs
	Randomisation			✓							
Questions	Informed consent	✓									
	Medical history	✓									
	Medication use	✓									
	Smoking history	✓									
	Exacerbation history	✓									
	Asthma control questionnaire (ACQ7)	✓		✓	✓	✓	✓	✓	✓	✓	✓
	Asthma related quality of life (AQLQ)			✓	✓	✓	✓	✓	✓	✓	✓
	Quality of Life (SF36)			✓	✓	✓	✓	✓	✓	✓	✓
	Instructions for fibre controlled diet	✓									
	Adherence with prescribed asthma medications	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Adherence with background diet		✓	✓	✓	✓	✓	✓	✓	✓	✓
	Adherence with study supplement				✓	✓	✓	✓	✓	✓	✓
	Food Frequency Questionnaire			✓							
	Gastrointestinal symptoms rating scale scores	✓		✓	✓	✓	✓	✓	✓	✓	✓
Procedures	Height	✓									
	Weight	✓		✓	✓	✓	✓	✓	✓	✓	✓
	Blood pressure	✓		✓	✓	✓	✓	✓	✓	✓	✓
	Exhaled nitric oxide measurement			✓	✓	✓	✓	✓	✓	✓	✓
	Spirometry	✓		✓	✓	✓	✓	✓	✓	✓	✓
	Combined saline challenge/induction	✓*		✓	✓	✓	✓	✓	✓	✓	✓
	Peak flow variability	✓*									
	Issue faeces collection kit	✓		✓	✓	✓	✓	✓	✓	✓	
	Issue meals for evening before clinic visit	✓		✓	✓	✓	✓	✓	✓	✓	
	Issue study supplement			✓		✓		✓		✓	
	Blood collection and storage			✓	✓	✓	✓	✓	✓	✓	✓
	Skin prick test	✓									

**Only if there is no evidence of airflow variability*

6. Safety and Ethical Considerations

6.1. Safety

The conduct of the study will be in accordance with the Code of Good Clinical Practice (GCP) and the NHMRC National Statement of Ethical Conduct in Research. The Investigators and study personnel will meet regularly to monitor recruitment, progress, adverse events and data entry throughout the study.

Participants will be closely monitored while undergoing testing. Only qualified staff who are fully trained in the operation of equipment, clinical procedures and response to emergencies will complete evaluations. Participant safety will be ensured at each visit and those who experience a significant worsening of their asthma symptoms (change in ACQ >1.0) will be referred to their treating physician.

The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. The sputum test can cause coughing, some minor chest discomfort and wheezing. This is brief and promptly responds to reliever medication, which will be provided for participants. The study supplementation contains ingredients that are approved foods and considered safe and suitable for use in food in Australia. Some subjects may experience mild side effects of flatulence, bloating, cramps or loose stools, for 12-24 hours after consuming the study supplements.

With the participant's permission, abnormal findings from routine clinical tests will be forwarded to their GP for follow up.

6.2. Reporting of Adverse Events

An Adverse Event (AE) is defined as any untoward medical occurrence in a participant during the assessments conducted at each clinic visit and during the study intervention that may or may not be related to the study protocol and/or investigational product. A Serious Adverse Event (SAE) is defined as any untoward serious medical occurrence at any dose that results in death, or is life-threatening or requires inpatient hospitalisation, or results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect, or is a medically important event or reaction.

The study coordinator will inform the principal investigator of all AEs and SAEs, who will then follow procedures for unblinding as necessary and notify the relevant bodies. Depending on the nature of AE or SAE, it may be necessary for treatment to cease and/or for the participant to be withdrawn from the study.

All SAE's that may be related or unrelated to the investigational product will be documented in the SAE Form, Case Report Form and will be reported within 72 hours to the Human Research Ethics Committee, the sponsor and the TGA. Any unforeseen AE, or complaints from participants in the research, or about the research, will be documented in the AE Form and Case Report Form and will be reported to the Human Research Ethics Committee.

6.3. Quality Control and Quality Assurance

Investigators and study coordinators will be qualified and appropriately trained in the assessments of the study. Data collection will be monitored by principal investigators. A weekly update on the study's progress will be scheduled with the principal investigator and study coordinators for monitoring purposes.

6.4. Discontinuation

Participants will be discontinued from the study:

- Where indicated by the occurrence and nature of AE or SAE
- The participant or the participant's general practitioner requests that the participant be withdrawn from the study
- The participant refuses to comply with the requirements of the protocol
- The participant requires a change to their maintenance inhaled corticosteroid dose during the study.

6.5. Disclosure, Publication and Confidentiality

Confidentiality of participants will be maintained. Patient identity will be limited to authorised staff working on this study. Participants will be assigned a unique participant identification code. All data collected for the purposes of this study will be kept a separate folder and participants will not be identified from these folders. Any reports and/or publication arising from this study will only report average results and no identifiable individual data will be presented. During statistical data analysis the database will be stored in a password protected computer file. All data for the study will be retained on file by the principal investigators at the University of Newcastle, in a locked data storage site for a period of 15 years.

6.6. Informed Consent, Ethical Review, and Regulatory Considerations

Each participant will have the opportunity to have all their queries answered by the investigators prior to study commencement and will not be coerced into signing the Consent Form. The informed consent process will be documented by the participant's dated signature on the Consent Form, which will be signed and dated by the investigator. The participant will receive a copy of the signed Consent Form and the Participant Information Statement.

The study will not commence until full approval has been granted by the Hunter New England Human Research Ethics Committee and the study has been registered with the ANZCTR. After obtaining full ethics approval, a Clinical Trials Notification (CTN) form will be lodged with the Department of Health and Ageing Therapeutic Goods Administration (TGA) for the use of the investigational product in this study. This study will be conducted in accordance with the ethical principles stated in the Declaration of Helsinki or the applicable guidelines of the Good Clinical Practice, which ever represents the greater protection of the individual.

7. Timeline

	2018									2019									
	04	05	06	07	08	09	10	11	12	01	02	03	04	05	06	07	08	09	10	11
Ethics/Safety Approval																				
Recruitment																				
Study visits																				
Laboratory Analyses																				
Statistical Analyses																				
Final Study Report																				
Manuscript delivered to AHNA																				

8. References

1. Masoli M, Fabian D, Holt S, Beasley R. Global initiative for asthma (GINA) program. The global burden of asthma: executive summary of the GINA dissemination committee report. Allergy. 2004;59(5):469-78.
2. Australian Bureau of Statistics. Asthma in Australia: a Snapshot, 2004–05.; 2007.
3. Australian Institute of Health and Welfare: Australian Centre for Asthma Monitoring. Burden of disease due to asthma in Australia 2003. Cat. no. ACM 16. Canberra: AIHW.; 2009.
4. Huang S-L, Lin K-C, Pan W-H. Dietary factors associated with physician-diagnosed asthma and allergic rhinitis in teenagers: analyses of the first Nutrition and Health Survey in Taiwan. Clin Exp Allergy. 2001;31(2):259-64.
5. Wickens K, Barry D, Friezema A, Rhodius R, Bone N, Purdie G, et al. Fast foods – are they a risk factor for asthma? Allergy. 2005;60(12):1537-41.
6. Hijazi N, Abalkhail B, Seaton A. Diet and childhood asthma in a society in transition: a study in urban and rural Saudi Arabia. Thorax. 2000;55(9):775-9.
7. Reicks M, Jonnalagadda S, Albertson AM, Joshi N. Total dietary fiber intakes in the US population are related to whole grain consumption: results from the National Health and Nutrition Examination Survey 2009 to 2010. Nutr Res. 2014;34(3):226-34.
8. Australian Bureau of Statistics. 4364.0.55.007 - Australian Health Survey: Nutrition First Results – Food and Nutrients, 2011-12 [Available from: http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4364.0.55.0072011-12?OpenDocument.
9. Roberfroid MB. Introducing inulin-type fructans. Br J Nutr. 2005;93 (Suppl 1):S13–25.
10. Eswaran S, Muir J, Chey WD. Fiber and functional gastrointestinal disorders. Am J Gastroenterol. 2013;108(5):718-27.
11. Timm DA, Stewart ML, Hospattankar A, Slavin JL. Wheat dextrin, psyllium, and inulin produce distinct fermentation patterns, gas volumes, and short-chain fatty acid profiles in vitro. J Med Food. 2010;13(4):961-6.
12. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341(6145):569-73.
13. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med. 2014;20:159-66.
14. Halnes I, Baines KJ, Berthon BS, MacDonald-Wicks LK, Gibson PG, Wood LG. Soluble fibre meal challenge reduces airway inflammation and expression of GPR43 and GPR41 in asthma. Nutrients 2017;9:57.
15. Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, et al. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. Nat Commun. 2015;6:7320.
16. Lomax AR, Calder PC. Prebiotics, immune function, infection and inflammation: a review of the evidence. Br J Nutr. 2009;101(5):633-58.
17. Vulevic J, Juric A, Tzortzis G, Gibson GR. A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. J Nutr. 2013;143(3):324-31.
18. Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. Int J Food Sci Nutr. 2014;65(1):117-23.
19. Dehghan P, Pourghassem Gargari B, Asghari Jafar-abadi M. Oligofructose-enriched inulin improves some inflammatory markers and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized controlled clinical trial. Nutrition. 2014;30(4):418–23.

20. Vulevic J, Drakoularakou A, Yaqoob P, Tzortzis G, Gibson GR. Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. Am J Clin Nutr. 2008;88(5):1438-46.
21. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. Respir Med. 2005;99(5):553-8.
22. Juniper EF, O'Byrne Pm Fau - Guyatt GH, Guyatt Gh Fau - Ferrie PJ, Ferrie Pj Fau - King DR, King DR. Development and validation of a questionnaire to measure asthma control. (0903-1936 (Print)).
23. Svedlund J, Sjödin I, Dotevall G. GSRS—A clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci. 1988;33(2):129–34.
24. Juniper EF, Guyatt GH, Cox Fm, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. Eur Respir J. 1999;14(1):32-8.
25. Ware J, Snow K, Kosinski M, Gandek B. SF-36® Health Survey Manual and Interpretation Guide. Boston, MA: The Health Institute, New England Medical Center; 1993.
26. Baines KJ, Simpson JL, Wood LG, Scott RJ, Fibbens NL, Powell H, et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. J Allergy Clin Immunol. 2014;133(4):997-1007.

9. Appendix

Appendix I. Participant Information and Consent Form

9.1. Appendix I: Participant Information and Consent Form

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Participant Information and Consent Form
SOLUBLE FIBRE FOR RESPIRATORY HEALTH STUDY

Invitation

You are invited to participate in a clinical intervention in adults with asthma, examining the effects of a soluble fibre supplement on asthma control and airway inflammation. The study is a clinical trial which will take 16 weeks to complete and involves taking an oral soluble fibre supplement and undergoing medical testing. This study is being conducted by Professor Lisa Wood, Professor Peter Wark, Dr Netsanet Negewo, Dr Bronwyn Berthon, Dr Rebecca McLoughlin and Ms Cherry Thompson from the Hunter Medical Research Institute and The University of Newcastle. Funding for this study has been provided by the Australian Health and Nutrition Association Limited trading as Sanitarium Health & Wellbeing Company. Before you decide whether or not you wish to participate in this study, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish.

1. 'What is the purpose of this study?'

In this study, we will examine whether a soluble fibre supplement is effective in improving asthma control and reducing airway inflammation in adults with poorly controlled asthma. Soluble fibre comes from plant based foods (fruits, vegetables, grains) and is not digested until it reaches the bowel. When it reaches the bowel, the bacteria that live there break it down into small molecules, which are known to be beneficial for our immune system. We are also testing the dose of the supplement that is needed to gain benefits and the timing of dosing that is most effective. There are four treatments in this study including; three treatments of the soluble fibre supplement (with different doses and timing of dosages) and a placebo treatment. In this study, we hope to gain further insight into the possible health benefits of soluble fibre in adults with asthma.

2. What does this study involve?"

This study is a clinical trial which takes place over 16 weeks, with an initial visit (V0) plus 8 study visits to the HMRI clinic. Each study visit (visits 1-8) will take approximately 2.5 hours. The study involves 4 x 2-week phases. During each phase, you will be asked to take a different supplement, twice daily for 14 days. Your asthma will be assessed at HMRI before and after each phase. Between each phase of the study there will be a 2 week period where you won't be taking any supplements. This break allows for the effects of the previous study supplement to be eliminated. During this break you will need to consume a fibre-controlled diet (described below).

3. 'Why have I been invited to participate in this study?'

This study may be suitable for you if you have a diagnosis of asthma and are over the age of 18 years. At the time of the assessment your asthma needs to have been stable for one month prior. This study is not suitable for you if you:

- Are currently smoking
- Have respiratory conditions other than asthma
- Have chronic or excessive alcohol consumption
- Are pregnant or breastfeeding
- Are unable or unwilling to modify your diet
- Do not adhere to your prescribed asthma medications
- Have had unexplained weight loss (>5% body weight) in the past 6 months
- Have current diagnosis of diabetes or active gastrointestinal disease
- Have difficulty digesting galactose (galactosemia) or ragweed allergy
- Are on maintenance therapy with systemic corticosteroid, immunosuppressive or antibiotic drugs
- Have used fibre, prebiotic or probiotic supplements within the previous 4 weeks or are unable or unwilling to limit your intake of soluble fibre for the duration of the study. If appropriate to do so, participants who currently use supplements, which are not being taken for a health condition, could potentially become eligible if they cease using their supplements 4 weeks prior to commencing the study.

4. 'What if I don't want to take part in this study, or if I want to withdraw later?'

Participation in this study is voluntary. It is completely up to you whether or not you participate. Whatever your decision, it will not affect your relationship with the HMRI, University of Newcastle or Hunter New England Health staff.

If you decide to withdraw from the study, you have the option of withdrawing all data relating to you and have any samples that have been taken destroyed. An exception to this is in the case of an adverse event, or a serious adverse event, where the data needs to be retained for regulatory reporting.

The researchers may withdraw a participant if it is considered in the participant's best interest or it is appropriate to do so for another reason. If this happens, the researchers will explain why and advise you about any follow-up procedures or alternative arrangements as appropriate.

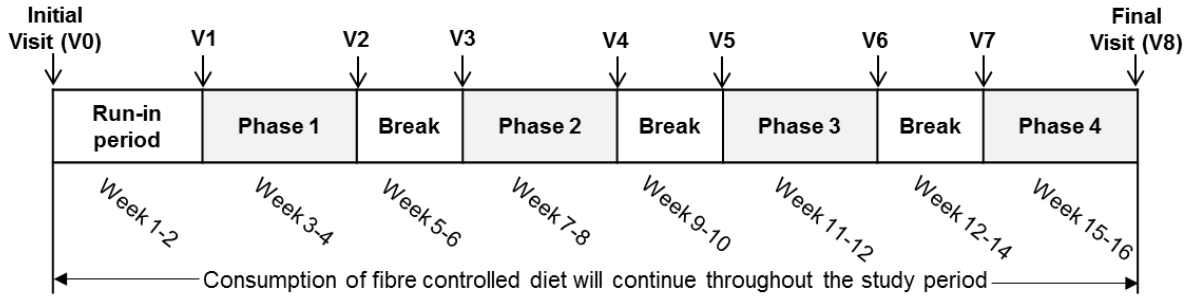
5. "What is the supplement that I will be asked to take?"

The soluble fibre we are using is an oligosaccharide blend. Oligosaccharides are sugar molecules that are joined together.in chains of varying lengths. Oligosaccharides are naturally found in foods such as fruits, vegetables and whole grains, while the placebo is made from the starch in whole grains. Both oligosaccharides and starch are also added to some food products as an ingredient. The soluble fibre supplement (and placebo) is in a powder form and will be packaged into sachets. During each phase of the study you will need to mix a powder sachet into 250mL water (room temperature) and consume every morning and evening. The sachets will contain:

	Morning	Evening
1. High dose (once per day)	12g soluble fibre	6g placebo
2. High dose (twice per day)	6g soluble fibre 6g placebo	6g soluble fibre
3. Low dose	6g soluble fibre 6g placebo	6g placebo
4. Placebo	12g placebo	6g placebo

The placebo will look exactly the same as the high and low doses of soluble fibre, but does not contain any active ingredients. The order that you receive each of the supplements will be randomly decided (like tossing a coin). This study is double blinded, which means that neither you nor the study staff will know which supplement you are taking at what time. At the end of the study we will look at which supplement you took during each phase of the study, then compare the results to work out whether the supplements made a difference to your asthma. Please note that you will be supplied with the study supplements required for this study.

Study visit timeline



6. "What happens at the Initial visit?"

Prior to the initial visit you will have been provided with the participant information and consent form, either by post or email, and given time to consider whether or not you would like to participate. You will have also been contacted by telephone to have the study explained to you, answer any questions you may have and asked preliminary questions about the suitability of the study for you. If you agree to participate in the study, you will be invited to come into the HMRI clinic for an initial face to face visit (V0). If we determined on the telephone screen that you will need to undergo tests that would require you to withhold your usual asthma medications prior to the initial visit, we will ask you to send your signed consent form via reply paid mail or email. Otherwise, you will be asked to sign the form during the initial visit. The initial visit will take a maximum of 1.5 hours. Tests you will undergo at this visit include:

- A brief medical history;
- A brief medication usage history;
- Measurement of your height, weight, blood pressure and lung function using spirometry;
- Questionnaire on asthma control, asthma exacerbations and dietary intake;
- Allergy skin prick test, which only needs to be completed on one occasion. In this test a small amount of fluid is put on your skin, then tiny pricks are made on the skin at this location (this doesn't break the skin and is not painful). If you are allergic to the fluid, a small itchy lump will occur. This only lasts for an hour or so and if it is annoying we can give you some cream, which will take the itch away.
- Saline challenge will be undertaken only if there is no previous evidence of asthma diagnosis (see below for a description of the tests). Alternatively, you may be provided with a peak flow meter to take home and record your peak expiratory flow twice daily for a period of at least one week, by exhaling forcefully into a small hand held device.

If following the initial visit it is determined that this study is not suitable for you, we will advise you of the results and, with your permission, any abnormal results from your routine clinical tests will be forwarded to your GP for follow up. If this study is suitable for you, we will organise a mutually convenient time for you to begin the study.

7. "What do I need to do to prepare for the study visits?"

- You will need to fast overnight and in the morning for 12 hours before each study visit. During this fasting period, you are able to drink water as desired. The longest you will be asked to fast is 12 hours. If you feel you will be unable to do this, please notify the study staff.
- We will ask you to withhold your asthma medications for 6-24 hours, depending on which medications you use. However, if you feel that your symptoms worsen during this time, you should use your normal medications, and then come to your visit at the HMRI clinic as planned.

8. "What tests will be performed at each visit (1-8)?"

- **Blood Test** - At each visit approximately 27mL (less than 1.5 tablespoon) of blood will be taken from a vein in your forearm after a 12 hour overnight fast, to measure your levels of inflammation and plasma short chain fatty acids.
- **Spirometry**- Your lung function will be measured by blowing into a spirometer, a machine that measures the amount of air expelled from your lungs. You will be asked to blow into the spirometer until your lungs are empty (approximately 6 seconds).
- **Saline Challenge** - You will be asked to inhale a mist of salty water delivered by a nebuliser. You will be asked to do this for 30 seconds, 1 minute, 2 minutes, and three lots of 4 minutes. A breathing test will be done at the end of each period. This is a routine lung test. During the test we will ask you to cough to produce a sample of sputum, which we will measure for inflammation. The test will be stopped at your request or if your breathing test worsens and you will be given Ventolin if you develop any problems with your breathing. Ventolin is a medication that immediately relieves constriction of the airways and is inhaled by mouth through a spacer device.
- **Exhaled nitric oxide –** (eNO) You will be asked to do a breathing test to measure the inflammation in your airways. This is a simple test and you will be asked to breathe in and then out using a mouthpiece.
- **Blood Pressure**– Your blood pressure will be measured using an automatic blood pressure monitor.
- **Questionnaires** – During your visit you will be asked to complete questionnaires related to your asthma control, quality of life, diet, medication use and gastrointestinal symptoms. These questionnaires will take between 5-15 minutes to complete.
- **Stool sample collection** – We will provide you with a stool collection kit to collect a stool sample before each visit, which you will freeze until you come in for your visit.

9. "What else will I be asked to do during the study?"

- Two weeks prior to the start of the study, and for the duration of the study, we would like you to change your diet to include no more than 2 serves of fruits and vegetables per day and consume ½ cup of bran-based cereal per day to avoid constipation.
- We would also like you to avoid other soluble fibre-rich foods including oats, oat bran, beans, seeds and sources of probiotics such as yoghurt and fermented milk drinks. A dietitian will give you advice on how to do this prior to starting the study.
- You will also be provided with 8 standardised evening meals, which you will be asked to consume the night before each of your scheduled visits. This will consist of a frozen pasta meal (purchased from a supermarket and made in Australia) that you will heat according to the instructions on the package. The standardised meal is to replace your usual evening meal. On the following morning, you will attend the clinic at HMRI for medical testing (visits 1-8).

- You will be supplied with a study diary, to record each day whether you have taken the supplement and your asthma medications and to record any unusual symptoms or additional medications used during the study period. This will take about 1 minute of your time each day.

10. 'Are there risks to me in taking part in this study?'

- The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. Please advise the research team if you normally feel dizzy or faint when you have blood collected.
- The saline challenge test can cause difficulty breathing, coughing, some discomfort in your chest and wheezing. This is brief and responds promptly to reliever medications such as Ventolin.
- If you are pregnant, intending to become pregnant or breastfeeding, you cannot participate in this study. If at any time you think you may have become pregnant, it is important to let the researchers know immediately.
- The study supplements used in this trial contain only nutrients that are normally obtained from the diet, in doses approved for use in food. Some individuals may experience mild symptoms of flatulence or bloating for 12-24 hours after consuming each dose.

11. 'What happens if I suffer injury or complications as a result of the study?'

If you suffer any injuries or complications as a result of this study you should contact the study coordinator as soon as possible, who will assist you in arranging appropriate medical treatment.

12. 'How will my confidentiality be protected?'

Only the study investigators will know whether or not you are participating in this study. Any identifiable information that is collected about you in connection with this study will remain confidential and will be disclosed only with your permission, or except as required by law. Only the study investigators will have access to your details and results, which will be held securely at the Hunter Medical Research Institute.

13. 'What happens with the results?'

Your results including breathing tests will be available to be sent to your general practitioner, at the end of the study at your request. The results of the study will also be available to you at the completion of the study; however you should be aware that the study may take over a year to complete. For all participants in the study we would like to access and record the visits in your medical records. This will involve our staff accessing your medical record and recording the results of your visit in your patient notes. Blood, sputum and faecal samples collected in this study will be stored securely and may be used in the future for further research, only if you agree and the research has been approved by the Human Research Ethics Committee. We plan to discuss/publish the results of the study. In any publication, information will be provided in such a way that you cannot be identified. Development of any products with commercial applications that result from the research will be owned by the funding body, and neither the researchers nor the participants will have rights to any financial benefits.

14. Costs

Participation in this study will not cost you anything nor will you be paid. Parking will not cost you anything and a parking space will be reserved for you prior to each visit. The study

supplements required as part of this study will be provided to you at no cost. We will also provide you with a meal at each visit following the blood test because you will have been fasting. Participants will be reimbursed for travel expenses associated with participation in this study.

15. 'What should I do if I want to discuss this study further before I decide?'

When you have read this information, one of the named researchers will discuss any queries you may have with you. If you would like to know more at any stage, please do not hesitate to contact any of the other investigators on the numbers listed.

Prof Lisa Wood - Chief Investigator	Tel: 02 4042 0147
Prof Peter Wark - Co-investigator	Tel: 02 4042 0110
Dr Bronwyn Berthon - Study Coordinator	Tel: 02 4042 0116

16. 'Who should I contact if I have concerns about the conduct of this study?'

This study has been approved by the Hunter New England Human Research Ethics Committee, reference number 2018/ETH00114. If you have concerns or complaints about the conduct of this study, you should contact:

Dr Nicole Gerrand, PhD
Manager, Research Ethics and Governance Office
Hunter New England Local Health District
Locked Bag 1, HRMC NSW 2310
Tel: (02) 4921 4950
Email: nicole.gerrand@health.nsw.gov.au

The Manager is the person nominated to receive complaints from research participants. You will need to quote reference number 2018/ETH00114.

Thank you for taking the time to consider this study.

If you wish to take part in it, please sign the attached consent form.

This information sheet is for you to keep.

Prof Lisa Wood
School of Biomedical Sciences & Pharmacy
Hunter Medical Research Institute
Lot 1 Kookaburra Circuit
New Lambton Heights NSW 2305
Ph: 02 40420147 Fax: 02 40420046
Email: Lisa.Wood@newcastle.edu.au

Participant Consent Form (Participant Copy)

SOLUBLE FIBRE FOR RESPIRATORY HEALTH STUDY

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the information statement, a copy of which I have retained.

I understand I can withdraw from the project at any time and do not have to give any reason for withdrawing.

I consent to-

1) Completing the tests involved in the study
2) Completing questionnaires to obtain research data
3) Allowing research personnel access to my medical record and to record attendance and results in my file

☐ I consent to secure storage of blood, sputum and faecal samples collected in this study to be used in future research, subject to approval by the Hunter New England Human Research Ethics Committee (tick box if you agree)

I understand that my personal information will remain confidential to the researchers.

I have had the opportunity to have questions answered to my satisfaction.

Name _____

Signature _____ **Date** _____

I have informed the above person about this research and am sure that they understand both the content of the Information statement and the additional information I have provided.

_____

Investigator/Delegate Name (printed)

Prof Lisa Wood
School of Biomedical Sciences & Pharmacy
Hunter Medical Research Institute
Lot 1 Kookaburra Circuit
New Lambton Heights NSW 2305
Ph: 02 40420147 Fax: 02 40420046
Email: Lisa.Wood@newcastle.edu.au

Participant Consent Form (Researcher Copy)

SOLUBLE FIBRE FOR RESPIRATORY HEALTH STUDY

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the information statement, a copy of which I have retained.

I understand I can withdraw from the project at any time and do not have to give any reason for withdrawing.

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Name _____

Signature _____ **Date** _____

I have informed the above person about this research and am sure that they understand both the content of the Information statement and the additional information I have provided.

_____

Investigator/Delegate Name (printed)