DIETARY FIBRE AS A MODULATOR OF AIRWAY INFLAMMATION IN ASTHMA (FAIM STUDY)

RESEARCH PROTOCOL

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Sponsor:
John Hunter Hospital Charitable Trust Grant

PROTOCOL VERSION CONTROL

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Glossary of Terms

ACQ	Asthma control questionnaire
AHR	Airway hyper responsiveness
ANZCTR	Australian New Zealand Clinical Trials Registry
CRP	C-reactive protein
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GCP	Good clinical practice
GINA	Global Initiative for Asthma
GPR41/43	Free fatty acid receptors (G-protein coupled receptors)
GSRS	Gastrointestinal Symptom Rating Scale
HAT	Histone acetyl transferase
HDAC	Histone deacetylase
HMRI	Hunter Medical Research Institute
HNEH	Hunter New England Health
HREC	Human Research Ethics Committee
IL	Interleukin
NFκB	Nuclear factor kappa B
OCS	Oral corticosteroids
SCFA	Short chain fatty acid
TNFα	Tumour necrosis factor alpha

SYNOPSIS

Title: Dietary fibre as a modulator of airway inflammation in asthma (FAIM study)

Sponsor: John Hunter Hospital Charitable Trust

Primary Outcome: Plasma short chain fatty acids (SCFA)

Secondary Outcomes: Airway inflammation (sputum cell counts, IL-8, FeNO), systemic inflammation (plasma CRP and TNFα), faecal SCFA and microbiota, lung function, asthma control, molecular mechanisms.

Study Duration: March 2015 – March 2016

Study Population: n=17

Doctor diagnosed stable asthma

Aged 18+ years

Males and Females

Never or ex-smokers

Non-pregnant/breastfeeding

Study Design: This study is a double-blind, randomised, placebo-controlled, 3-way crossover trial including 17 clinically stable asthmatic adults to examine the effect of soluble fibre/probiotic supplements on plasma short chain fatty acids (SCFA), airway and systemic inflammation and to study the mechanisms by which soluble fibre exerts these effects. Subjects will complete 3 x 7 day interventions, with a 2 week run in period and 2 week washout periods between interventions (intervention order randomly allocated).

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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 and systemic inflammation in clinically stable asthmatic adults. The soluble fibre inulin, will be delivered at a dose previously shown to modulate the microbiome, reduce inflammation and be well tolerated. The duration of intervention (7 days) reflects the time required to achieve maximum improvements in gut microflora and the washout period (2 weeks) is adequate to allow the microflora to return to baseline composition.

3. Hypothesis

The low soluble fibre content of westernised diets inhibits the growth of beneficial commensal bacteria and reduces circulating short-chain fatty acids (SCFA) levels, contributing to asthma development and progression. Increasing SCFA levels will improve gut microbiota composition and reduce inflammation in asthma, via inhibition of histone deacetylases and activation of free fatty acid receptors (GPR41/43).

4. Aims

In clinically stable adults with asthma, we aim to examine the effects of soluble fibre and soluble fibre+probiotic treatment on:

1. Plasma SCFAs

2. Airway inflammation, systemic inflammation, lung function, asthma control, and faecal SCFA and microbiota

3. Molecular mechanisms including total histone deacetylase (HDAC) and histone acetyltransferase (HAT) activity in peripheral blood mononuclear cells (PBMCs), gene expression of HDAC1-11 and G protein coupled receptor (GPR)41/ 43 in PBMCs and sputum cells.

5. Background and Preliminary studies

Asthma affects approximately 300 million people worldwide [1] and in Australia, asthma prevalence is ~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. Insoluble fibres, such as cellulose, have an important role in the gastrointestinal tract as they provide bulk for the production of faeces and elimination of waste. As insoluble fibres are indigestible, they are biologically inert. Conversely, soluble fibres, such as fructans (eg inulin), pectin, gums and psyllium, are partially fermented by commensal bacteria in the colon, providing the substrate for production of physiologically active by-products, including the short chain fatty acids (SCFA): acetate, propionate and butyrate. Butyrate is the major energy source for colonocytes, propionate is primarily absorbed by the liver, while acetate is the primary SCFA that enters the circulation [9]. Indeed, individuals consuming a high fibre diet have high blood levels of acetate [10]. SFCA that enter the circulation have the capacity to influence cells within peripheral tissues. SCFA have attracted much attention in recent years due to their anti-inflammatory properties.

The gut microbiome and SCFA production:

The quantity and rate of SCFA production depends on the species and amounts of microflora present in the colon, as well as the dietary components that are available as substrates for fermentation. The human colon contains a large and diverse microbiome, including species such as Bifidobacterium and Lactobacillus that confer beneficial effects to the host, including promotion of gut integrity, antagonism against pathogens and immune modulation [11]. Many host factors influence the composition of the gut microbiome, for example obesity; the proportion of beneficial Bacteroidetes bacteria is decreased in obese compared to lean individuals [12]. SCFA production can be increased by boosting the amount of healthy bacteria with the use of probiotics. This involves the direct delivery of live bacteria to the host, either in supplemental form or via functional foods. Another effective strategy for enhancing SCFA production is the use of prebiotics, which are nutrients that promote the growth of healthy bacteria. Some types of soluble fibre, such as inulin and oligosaccharides, act as prebiotics, and have the advantage of being able to increase SCFA levels in two ways: firstly by stimulating the growth of beneficial commensal bacteria which digest soluble fibre and secondly by increasing the amount of substrate available for digestion by these bacteria. Significant and maximal changes in the microflora occur within just one week of prebiotic use [13].

Dietary fibre and inflammation:

In observational studies, an inverse relationship between fibre intake and systemic inflammation (serum CRP and IL-6) has been reported [14,15]. Our pilot data demonstrate that dietary fibre is also inversely associated with airway inflammation in asthma (Figure 1) [16].

Figure 1: In a study of 137 stable asthmatics and 65 healthy controls, we reported that dietary fibre intake was lower in severe asthmatics vs controls (~5g/day). In multiple regression models adjusted for energy intake, age, gender and BMI, fibre intake was inversely associated with airway eosinophils (Fig 1A) and positively associated with all lung function measures, including FEV1 %predicted (Fig 1B), FVC %predicted and FEV1/FVC [16]

Fig 1A:

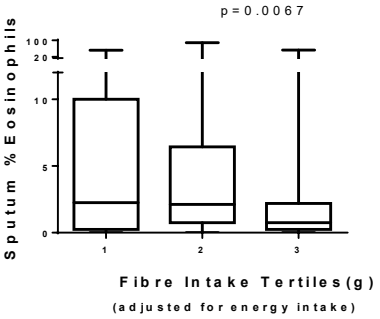
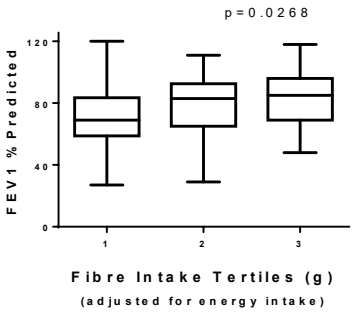


Fig 1B:



Anti-inflammatory mechanisms of SCFA:

Evidence suggests that SCFA have anti-inflammatory effects due to regulation of various molecular signalling pathways (Figure 2):

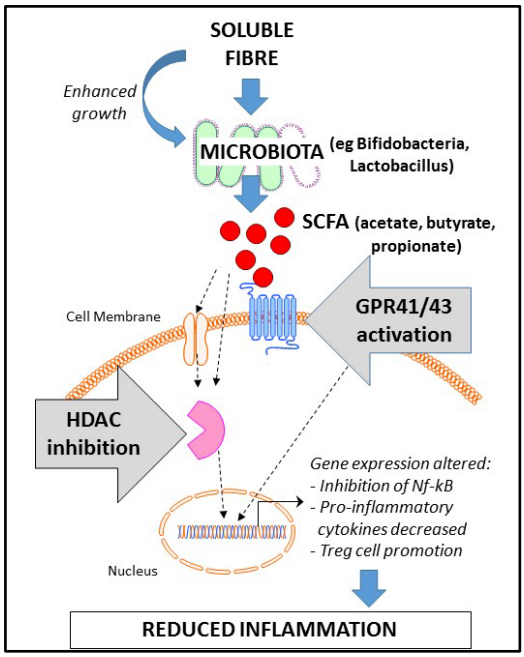


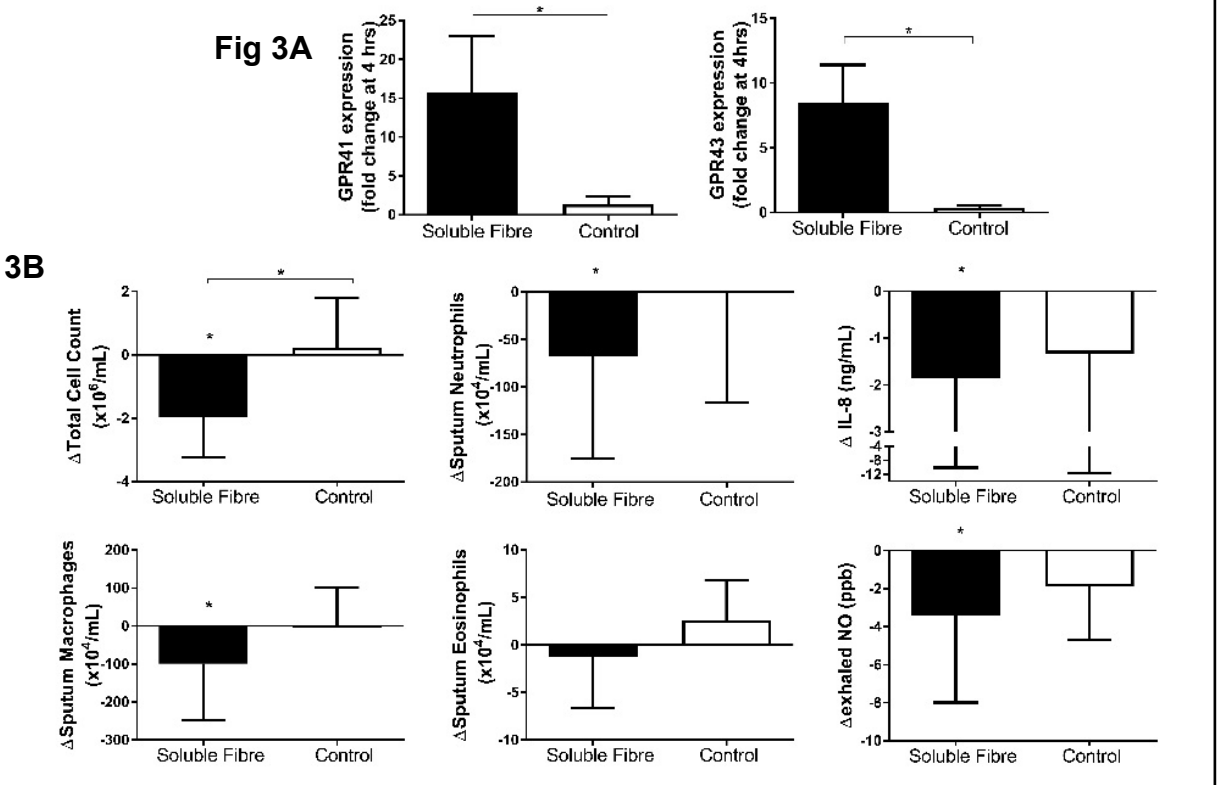
Figure 2

i. Free fatty acid receptor activation: Soluble fibre-derived SCFAs can bind to the endogenous receptors, free fatty acid receptor 2 (FFAR2) also known as G-protein-coupled receptor 43 (GPR43) and free fatty acid receptor 3 (FFAR3) also known as GPR41. GPR43 expression is highly enriched in immune cells, as well as being expressed in cells of the gastrointestinal tract and adipocytes, and is preferentially activated by acetate and propionate. GPR41 is primarily expressed in the gastrointestinal tract and is preferentially activated by propionate and butyrate [17].

SCFAs have anti-inflammatory effects in immune cells via GPR43 activity. A recent study in mice showed that SCFA supplementation led to an increase in the size and function of the colonic T regulatory cell (cTreg) pool [18], which was dependent on GPR43 activity. As Treg function has been shown to be impaired in allergic airways disease in mice [19], this is of particular relevance to asthma. Studies have also shown that GPR43/41 stimulation by SCFAs is necessary for the resolution of airway inflammation in animal studies. In an allergic airway inflammation model, GPR43-deficient mice showed more severe inflammation, with

increased inflammatory cell numbers in the lung lining fluid and higher levels of eosinophil peroxidase activity and inflammatory cells in lung tissue [20]. In another study in mice with allergic airways disease [21], a fibre-rich diet changed the composition of the gut microbiota, by increasing proportions of the Bacteroidaceae and Bifidobacteriaceae families, which are potent fermenters of fibre into SCFA. Indeed, increased circulating SCFA levels were observed and airway inflammation was attenuated, with dendritic cells having an impaired ability to activate Th2 effector cells in the lung [21]. Consequently, in mice fed either a high fibre diet or directly administered with acetate or propionate in their drinking water, airway inflammation could not be sustained; after an allergen challenge, cellular infiltration (eosinophils), IL-4, IL-5, IL-13 and IL-17A levels were reduced in the lungs and airway hyperresponsiveness improved. The effects were dependent on GPR41, but not GPR43. These animal models suggest soluble fibre intake modulates airway inflammation, via mechanisms involving the free fatty acid receptors GPR41/43. 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 (Figure 3) [22].

Figure 3: Single dose of soluble fibre [22]: Asthmatic adults (n=29) consumed a single dose of soluble fibre (3.5g inulin) or control. In subjects who consumed inulin, GPR41 & GPR43 gene expression were upregulated in sputum cells at 4hrs (Fig 3A). Sputum total cells, neutrophils, macrophages, IL-8 and exhaled nitric oxide (eNO) decreased at 4 hrs (Fig 3B).



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 [23].

Clinical Supplementation trials to boost SCFA and reduce inflammation:

In humans, most intervention studies using SCFA have been designed to improve inflammation and symptoms in ulcerative colitis patients. Most have directly delivered butyrate by enema, or used butyrate tablets with slow release coating to release butyrate into the colon [10]. To impact diseases of the peripheral organs, the goal is to improve circulating SCFA levels. In animal studies this has been done by directly delivering massive doses of oral SCFA. However in humans, oral delivery of SCFA is not optimal, as >80% of acetate delivered orally is oxidised, with plasma acetate levels only remaining elevated for ~60 min [24]. An alternative approach is supplementation with prebiotic soluble fibres, which successfully increase circulating SCFA levels [25], modulate the microbiome and modify aspects of the immune system [26]. 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)[26]. Inulin and/or oligosaccharide supplementation (5.5-10 g/day for 8-12 weeks) has been shown to reduce serum CRP, TNFα and IL-6 in overweight subjects and Type 2 diabetics [27-29]. In a double-blind, placebo controlled trial in 44 elderly subjects, using 5.5g oligosaccharides/day for 10wks[11], 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 TNFα in whole cell cultures stimulated by LPS[11]. In the airways, human data describing the effects of prebiotics is lacking, but animal studies show that oligosaccharides attenuate airway eosinophilic inflammation and reduce IL-4 and IL-5 in lung tissue [30,31]. Our pilot data show that in asthmatics, a single dose of soluble fibre (in a pre-/ probiotic combination) modified systemic and airway inflammation (Figure 3).

6. Research Plan

6.1 Study Design

A double-blind, randomised, placebo-controlled three-way crossover trial including clinically stable asthmatic adults (n=17). The effect of the soluble fibre and soluble fibre/probiotic treatments on plasma short chain fatty acid (SCFA) production, airway inflammation, systemic inflammation, lung function, asthma control, faecal SCFA and microbiota and the molecular mechanisms responsible will be examined. The trial will be conducted in accordance with Good Clinical Practice (GCP) standards and will be approved by 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

Exclusion criteria:

- Respiratory conditions other than asthma
- Asthma exacerbation or respiratory tract infection (OCS or antibiotics) in the past month
- Current smoking (last 6 months)
- Any use of antibiotics in the past month
- Unable or unwilling to modify diet
- Pregnancy or breastfeeding
- Diagnosed bowel disorders or intestinal disorders
- Nutritional, fibre or probiotic supplement or laxative use within the previous 4 weeks (this includes any vitamin or minerals, sports supplements, meal replacements, probiotics and fibre preparations)
- Current use of any medication known to significantly influence inflammation (e.g. corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDS));
- Subject has a clinically important medical illness (including serious psychological disorders) likely to interfere with management or participation in the study.
- Participation in any other interventional research study in the last 4 weeks

Randomisation

All participants will receive both treatments and the placebo. Treatment order will be randomly allocated via unique randomisation codes using a 3x3 latin square method [32] by an

independent statistician. The randomisation service will be managed by an independent statistician at the Hunter Medical Research Institute.

Adherence

Adherence to the study intervention will be monitored at each visit by sachet/pill countback and 24-hr food recall (to check background diet). Participants will also record supplements consumed 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

6.2 Intervention

Study treatment arms

1. Inulin (6 grams, twice daily) / Placebo capsule (once daily)
2. Inulin+Probiotic (6 grams, twice daily) / Probiotic capsule (once daily)
3. Placebo (Maltodextrin powder) (6 grams, twice daily) / Placebo capsule (once daily)

Study supplements

Inulin - Frutafit® CLR (Sensus, The Netherlands)

Probiotic – Multi-strain probiotic capsule containing Lactobacillus acidophilus LA-5 (7.5 billion colony forming units (CFU)), Lactobacillus rhamnosus GG (8.75 billion CFU) and Bifidobacterium animalis subspecies lactis BB-12 (8.75 billion CFU) (Caruso's Natural Health, Sydney, NSW, Australia)

The inulin/placebo powder will be provided in individual dose sachets. Participants will be instructed to mix one whole sachet with liquid, e.g. water, milk, juice or cordial (not soft drink), until smooth and drink immediately, each morning and night before food. Participants will be asked to take one capsule (probiotic/placebo) before food, in the morning only.

6.3 Procedures

Participant Recruitment

Participants will be recruited via the Hunter Medical Research Institute volunteer database and HMRI newsletter. Other recruitment sources will include media release and placing of a recruitment flyer in the HMRI building and John Hunter Hospital. Initially, interested potential participants will be contacted by telephone and an eligibility/screening form will be completed. If the participant is eligible at this stage, they will be invited to attend the HMRI building for a screening visit where informed consent will be obtained and eligibility confirmed.

Study Visits

Participants will be screened for study eligibility by telephone prior to study commencement, and will then attend a screening visit where they will have a brief medical examination, dietary questionnaire, have their blood pressure, weight and height measured. If no abnormalities or exclusion criteria are identified, they will be entered into the study. They will commence the study background diet for 2 weeks, then attend the HMRI clinic on 6 occasions, before and after each 7 day intervention (inulin, inulin+probiotic and placebo), which will be separated by 2 weeks (Figure 4). During each intervention phase, participants will be telephoned for motivational purposes, to record compliance with the intervention, the study diet, and to establish whether any adverse effects have been experienced.

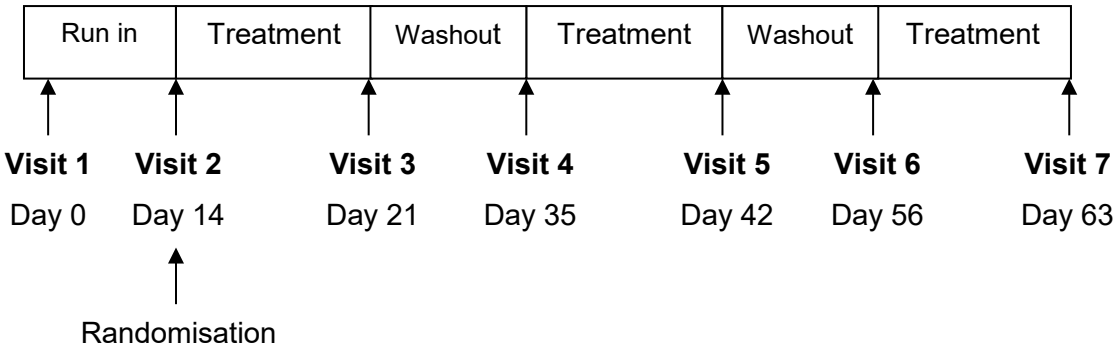


Figure 4. Study Schema

Screening Visit (Visit 1):

The following assessments and procedures will be performed:

- Written informed consent will be obtained.
- Medical history
- Medications: asthma and concomitant (following screening, participants will be advised to consult study staff prior to taking any new medications, including over the counter medications).
- Height and weight without shoes
- Smoking history
- Allergy history
- Exacerbation questionnaire
- Blood pressure
- Study diet information sheet & instructions for faecal sample collection
- 24 hr food recall questionnaire
- Spirometry and saline challenge (if there is no evidence of asthma diagnosis)

Visits 2-7:

Participants will be instructed to withhold their asthma medications (short acting β2-agonists

and anti-cholinergics for 6 hours, long-acting β2-agonists, combination inhalers and long acting theophyllines for 24 hours, and short acting theophyllines for 12 hours) and fast for 12 hours prior to each visit.

The following assessments and procedures will be performed:

- Venous blood collection
- Clinical asthma evaluation
- Dietary intake assessment
- Gastrointestinal Symptom Rating Scale
- Blood pressure
- Exhaled nitric oxide
- Spirometry, saline challenge and combined sputum induction
- Faecal sample collection

Study diet

For 2 weeks prior to the first visit and for the duration of the study, participants will be instructed by a study dietitian to consume a fibre-controlled 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. Participants will be instructed to consume ½ cup of bran-based cereal (45g serve contains 1.5g soluble and 11.8g insoluble fibre) daily to ensure adequate total fibre intake. Subjects will also be instructed to avoid sources of probiotics such as yoghurt and fermented milk drinks and sources of inulin such as asparagus and artichokes. We will explain to subjects that they can consume all other foods including 2 serves of fruit or vegetables as usual.

6.4 Outcome Measures

Primary: Plasma SCFA

Secondary: Airway inflammation (sputum cell counts, IL-8, FeNO), systemic inflammation (plasma CRP and TNFα), faecal SCFA and microbiota, lung function, asthma control, molecular mechanisms.

6.5 Clinical Assessment

1. Venous blood collection

Blood will be collected by the research assistant, in the clinical trials centre on level 4 of the HMRI building according to the venepuncture SOP (GCP009). Blood collection will be terminated if the participant experiences any adverse reactions or wishes to terminate the procedure. The blood to be collected (27ml at visit 2-7) is less than 1% of total blood volume, and thus represents no hemodynamic risk to participants.

2. Clinical Asthma Evaluation

At visit 2, asthma will be classified according to the Global Initiative for Asthma (GINA) guidelines as intermittent, mild persistent, moderate persistent or severe persistent, based on symptoms such as episodic wheezing, breathlessness, cough and chest tightness [33].

At visits 2-7, the validated Juniper Asthma Control Questionnaire (ACQ) [34] will be administered 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 [35]. 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.

3. Dietary Intake

At each visit a 24-hour dietary recall will be administered by a dietitian to assess adherence to the study diet. Participants will be asked to recall the amount and types of food and beverages that they consumed in the previous 24hrs.

4. Gastrointestinal Symptoms

To assess changes in gastrointestinal symptoms throughout the study, the validated Gastrointestinal Symptom Rating Scale (GSRS) questionnaire will be completed at each study visit [36]. 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.

5. 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 [37].

6. Exhaled nitric oxide

The fraction of exhaled nitric oxide (FeNO) will be measured at each visit according to the

ATS/ERS criteria [38] using Ecomedics chemiluminescent detector unit (CLD 88sp FENO, Ecomedics, Switzerland), and reported in parts per billion (ppb). Ecomedics measures FeNO from the exhaled breath, which can be used as a marker of eosinophilic airway inflammation. 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. The average of three technically acceptable attempts will be calculated. FeNO is always to be measured prior to spirometry as forced breathing manoeuvres may alter FeNO results.

7. Spirometry, saline challenge and combined 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 theophyllines for 24 hours, and short acting theophyllines 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 (FEV_1) 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 FEV_1 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 FEV_1 falls ≥15% again during the test, or if the participant declines to continue.

8. Faecal sample collection

Faeces samples will be collected by participants at home using the faeces collection kit (stool collection potty, sterile specimen jar, gloves, specimen bag) provided and stored in the home freezer at -20°C until the next clinic visit. Samples will be brought into the clinic using the esky with ice brick provided, then stored at -80°C.

6.6 Laboratory methods

6.6.1 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.

6.6.2 Blood samples

(a) Processing

Blood will be collected into EDTA tubes, centrifuged at 20˚C, 3000 rpm for 10 minutes then plasma will be selected and stored at -80˚C. Remaining blood cells will be used for isolation of PBMCs using the Ficoll-Paque PLUS (GE healthcare, Sydney, Australia) density gradient method [43], as per the manufacturer's recommendations. Cell counts and viability tests with trypan blue staining were performed. Isolated PBMCs will be stored in buffer RLT for RNA

extraction and gene expression analysis. Nuclear proteins will be extracted from PBMCs using the Active Motif Nuclear Extract Kit (Active Motif, Carlsbad, CA, USA) and quantified by the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA), as per the manufacturers protocol.

(b) Plasma SCFA

SCFAs will be measured in plasma by gas chromatography (GC). Plasma samples (200 uL), containing heptanoic acid (internal standard, 100 nmol) will be extracted with ether (3 mL). The ether layer will be transferred to a clean tube containing 0.2 M sodium hydroxide (50 uL). 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 (30 uL). 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.

(c) Total HDAC enzyme activity assay

Total HDAC enzyme activity will be measured in PBMC nuclear protein extracts using the Active Motif fluorescent HDAC Assay Kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's recommendations. Briefly, the substrate is incubated with 3ug of total nuclear protein extracts and incubated for 60mins. Following incubation, developer solution is added and fluorescence measured at 360nm excitation and 460nm emission (FLUOstar Optima, BMG Labtech, Durham, NC). All samples and standards will be measured in duplicate. A deacetylated HDAC standard curve will be used to quantify HDAC activity (uM/ug).

(d) Total HAT enzyme activity assay

Measurement of total HAT enzyme activity in PBMC nuclear protein extracts will be performed using the Active Motif fluorescent HAT Assay Kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's recommendations. Briefly, 0.5ug of total nuclear protein extracts is incubated with acetyl-CoA and histone H3 substrate peptide for 20min. Developer solution is then added to react with the free sulfhydryl groups on the CoA-SH, producing a fluorescent reading of acetyltransferase activity. Fluorescence will be measured at 360 nm excitation and 460 nm emission (FLUOstar Optima, BMG Labtech, Durham, NC, USA). All samples and standards are to be measured in duplicate. A standard curve of β-mercaptoethanol will be used to quantify HAT activity (uM/ug).

(e) HDAC subtype protein assays

To determine protein levels of select HDAC subtypes (HDAC2 and HDAC7) in nuclear protein extracts, EpiQuik colorimetric assay kits (Epigentek) will be used as per the manufacturer's instructions. These colorimetric assays work as an ELISA-like reaction, and utilise similar principles and procedures. HDAC2/7 proteins present in the experimental nuclear protein

bind to the unique HDAC affinity substrate that is stably coated on the strip wells. The bound HDAC2/7 is then recognised with a high-affinity HDAC2/7-specific antibody, and the amount of HDAC2/7 is colorimetrically quantified through an ELISA-like reaction following the addition of a secondary detection antibody. The amount of HDAC2/7 is proportional to the intensity of the colour development.

6.6.3 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.

(b) Faecal SCFA

Faecal SCFA concentrations will be determined at Flinders Analytical (Flinders University, Australia) by flame ionisation detection on an Agilent Technologies 7890A gas chromatograph fitted with a flame-ionisation detector using nitrogen as the carrier gas [44]. Approximately 200 µl of unbuffered distilled water is added to 100 mg of faecal sample. Supernatant is collected after centrifugation at 13,000 x g for 10 mins. A 500 µl aliquot of the faecal supernatant is acidified to below pH 2.0 with 85% orthophosphoric acid, and 11 mM 4-methylvalerate was used as internal standard. Samples (1 µl) are separated on a SCE Analytical BP21 column (15 m x 0.25 mm I.D., with a 0.25 µm film thickness). Acetate, propionate, n-butyrate, iso-butyrate, n-valerate and iso-valerate will be quantified using a standard curve with 4-methyl valerate as an internal standard.

(c) Faecal microbiota

Extraction and quantification of bacterial DNA from faecal samples, and sequencing of the V4 hypervariable region of the bacterial 16S rRNA gene, will be performed as described previously [45]. Quantitative PCR assays will be used to assess levels of bacterial species as described previously [46]. Levels are normalised to total bacterial load, and differences resulting from treatment expressed as fold change [46]. Faith's phylogenetic diversity is calculated using QIIME (version 1.8.0, [Caporaso, 2010]) and taxa richness, Shannon-Weiner diversity, and Simpson's evenness calculated using PRIMER (version 6, PRIMER-E Ltd, Plymouth, UK). Bray Curtis similarity is calculated using the beta_diversity.py QIIME script. Principal coordinate analysis will be used to visualize sample clustering based on Bray Curtis similarity distances. Distance from centroid is calculated using PRIMER. Permutational multivariate analysis of variance (PERMANOVA) was used to test the null hypothesis of no difference amongst a priori-defined groups using PERMANOVA + add-on package for PRIMER, computed using unrestricted permutation of raw data with 9,999 random permutations at a significance level of 0.01. Level of microbiota change resulting from treatment will be assessed based on Bray Curtis similarity distance between paired pre-

treatment and post-treatment samples.

6.6.4 Inflammatory mediator analysis:

Plasma and sputum markers will be assessed by commercial ELISA: CRP, IL-6, IL-8, TNFα (R&D). Plasma CRP will be analysed using a high-sensitivity commercial ELISA assay (MP-Biomedicals, Santa Ana, California, USA) as per the manufacturer's instructions. The sensitivity for this assay is 5ug/L. Plasma IL-6 will be analysed using a high-sensitivity commercial ELISA assay (R&D Systems, Minneapolis MN USA) as per the manufacturer's instructions. The sensitivity for this assay is 0.7pg/ml. Plasma TNF-α will be analysed using a high-sensitivity commercial ELISA assay (R&D Systems, Minneapolis MN USA) as per the manufacturer's instructions. The sensitivity for this assay is 5.5pg/ml. All samples will be tested in duplicate.

6.6.5 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 is then 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 HDAC subtypes (1-11), GPR43 and GPR41 are combined with cDNA Taqman gene expression master mix as per manufacturer's instructions in duplicate real-time PCR reactions (7500 Real Time PCR System: Applied Biosystems). Analysis is performed on the change in cycle threshold (ΔCt) between the target gene compared with the housekeeping gene 18S rRNA, calculated using 2-$\Delta\Delta$Ct relative to 18S and the mean of the baseline value [47].

6.6 Statistical Analysis

Analysis will be performed with STATA 15 (StataCorp, College Station, Texas, USA) and reported as mean ± standard deviation or median [interquartile range]. Within group comparisons of outcomes compared to baseline will be assessed using the student t-test or Wilcoxon signed-rank tests. Per protocol analysis of mean difference in outcomes between treatments will be tested using mixed model regression, accounting for repeated measures. An independent ANOVA of period totals by treatment sequence will be used to test the assumption of no carry over effects. Associations will be assessed using Spearman's correlations. Significance will be accepted if $p<0.05$.

6.7 Sample size calculation

Based on previous studies [25], we hypothesise that we would observe a change in plasma acetate of ~1SD. With n=15 subjects, we would have 90% power to detect a difference between groups, using alpha=0.025 (to avoid type 1 error when comparing 3 groups).

Allowing for 10% dropouts, we aim to recruit n=17 subjects.

7. Safety and ethical considerations

10.1. 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 questioned by study personnel at each visit and during each phone call regarding any adverse symptoms related to consuming the study supplement.

Some clinical assessments performed during this research can be associated with adverse effects. 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.

Any adverse/abnormal results prior to or during the study will be reported to the CI and to the participants' GP (with the participants permission) to ensure appropriate follow up, with any severe adverse events to be reported to the HNEHREC within 72 hours (see 'Reporting of Adverse Events' section).

The side effects of having blood collected may include bleeding or bruising at the insertion site and possible dizziness and/or fainting. The saline challenge and sputum induction 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 supplement 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.

10.2. 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 study supplement. 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 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 study supplement 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.

10.3. 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 investigators and study coordinators for monitoring purposes.

10.4. 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.

10.5. Disclosure, Publication and Confidentiality

Confidentiality of participants will be maintained. Participant 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 in 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.

10.6. 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.

8. Timeline

	2015										2016			
	Feb	Mar	Apr	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Ethics & Safety Approval														
Recruitment & Data Collection														
Laboratory Analysis														
Manuscript Preparation & Submission														

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10. Appendix

Appendix I. Participant Information and Consent Form

Appendix II: Asthma Control Questionnaire

Appendix III: Gastrointestinal Symptom Rating Scale

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Participant Information and Consent Form

Dietary fibre as a modulator of airway inflammation in asthma

Invitation

You are invited to participate in a research study examining if a soluble fibre and probiotic supplement can help to reduce inflammation in adults with asthma. This study is being conducted by Associate Professor Lisa Wood, Dr Katherine Baines, Dr Bronwyn Berthon and Professor Peter Gibson from the Hunter Medical Research Institute and The University of Newcastle and is part of a larger programme looking at diet and asthma. This study is being undertaken as part requirement for a PhD at the University of Newcastle by Rebecca Zapirain, under the supervision of Associate Professor Lisa Wood and Doctor Katherine Baines.

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?'

We will examine whether a soluble fibre and probiotic supplement are effective in increasing markers in the blood that may help to reduce airway inflammation. Soluble fibre comes from plant based foods and absorbs water in the intestine like a sponge. Probiotics are a source of live bacteria which boost numbers of beneficial bacteria in the body. In the large bowel soluble fibre provides nourishment to, and is fermented by, healthy bacteria to produce anti-inflammatory compounds. We hope to gain further insight into how soluble fibre may be useful in adults with asthma.

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

This study may be suitable for you if you are aged 18+ years old.

This study is not suitable for you if you are: a current smoker, are unable or unwilling to limit your diet to 2 serves of fruits and vegetables per day and consume All Bran daily for the duration of the study, currently taking cholesterol lowering medication, pregnant or breastfeeding.

3. '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 staff caring for you.

If you decide to withdraw from the study, you have the option of withdrawing all data relating to you and have all 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.

4. 'What does this study involve?'

If you agree to participate in this study you will be asked to sign the Participant Consent Form. We will also need to check that the study is suitable for you and to do this we will invite you to come into the clinic for a screening visit, which will take approximately 30 minutes. This visit will include:

- A brief medical history; current medications
- Measurement of your height, weight and blood pressure;
- Questionnaire on asthma exacerbations and dietary intake
- Spirometry and a saline challenge may be undertaken if there is no evidence of asthma diagnosis

If following the screening visit it is that that this study is not suitable for you, we will advise you of the results and, with your permission, will forward the information to your GP for follow up. If this study is suitable, we will organise a mutually convenient time for you to begin the study.

The study takes place over 9 weeks. 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 All Bran each day. We would also like you to avoid some foods such as yoghurt and fibre supplements. A dietitian will give you advice on how to do this prior to starting the study.

Two weeks after you have started the study diet, you will attend the clinic at HMRI for your first study visit (visit 1). Each study visit will take approximately 1.5 hours.

The study involves three phases where you will be asked to take the study medication for 7 days for each phase, with assessment at HMRI before and after each phase. Between each phase of the study will be a 2 week period to allow for the effects of the study medication to be eliminated.

Study timeline:

Start Study Diet	Phase One	Break	Phase Two	Break	Phase Three
Week 1-2	Week 3	Week 4-5	Week 6	Week 7-8	Week 9

The study medication that you will receive for each phase of the study will include a combination of a single serve powder sachet that is mixed with liquid and taken twice daily and a capsule that is taken once daily. These will contain a soluble fibre powder and a placebo capsule, a soluble fibre powder and a probiotic capsule or a placebo powder and placebo capsule.

The placebo will look exactly the same as the soluble fibre and probiotic supplement, but does not contain any active ingredients. You will receive each of these supplement regimes and the placebo, to take for a period of 7 days each. The order that you receive them in will be randomly decided (like tossing a coin). This study is blinded, this means that you will not know which regime you are on at what time. At the end of the study we will look at which supplement regime you took in each phase of the study then compare the results to work out whether the supplements made a difference.

Please note that you will be supplied with the study medication required for this study. You will also be supplied with a study diary, to indicate each day whether you have taken the

medication, 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.

Prior to coming into the clinic, you will need to fast for 12 hours before each visit, and 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. 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.

Visits 1-6 (for details on each test see below):

- Blood test
- Spirometry
- Saline Challenge
- Exhaled Nitric Oxide test
- Blood pressure
- Questionnaires
- Stool sample collection

5. 'What does each of the tests involve?'

- **Blood Test** - At each visit approximately 20mL (1 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 gene expression.
- **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 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.

6. '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 medication 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 supplements used in this trial are available over the counter and we are asking you to take them in doses within the ranges approved by the Therapeutic Goods Administration. When soluble fibre supplements are taken the most common side effect is mild flatulence. Less common side effects can include gastrointestinal pain or discomfort, diarrhoea, nausea, and stomach pressure with a sensation of fullness. In some people use of the probiotic supplement may cause cramps or pain in the stomach area, constipation, diarrhoea, mucus in the stool, bloated stomach area, and discomfort in the upper stomach area or flatulence. However these symptoms are generally mild and will disappear when you stop taking the supplement. It is important to notify the study staff if and when any of these symptoms occur, straight away.

7. '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.

8. '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. You will be notified by letter if this occurs. Only the study investigators will have access to your details and results that will be held securely at the Hunter Medical Research Institute.

9. '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. 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. 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. Samples collected in this study will be stored securely and may be used in further research only if you agree and the research has been approved by the Human Research Ethics Committee.

10. 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 capsules required as part of this study will be provided to you at no cost. We will also provide you with a light snack at the end of each visit because you will have been fasting.

11. '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 it and any queries you may have with you. If you would like to know more at any stage, please do not hesitate to contact her or any of the other investigators on the numbers listed.

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HMRI, Lot 1 Kookaburra Circuit
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Tel: 02 4042 0147

Dr Katherine Baines
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Professor Peter Gibson
Senior Staff Specialist and Conjoint Professor
School of Medicine & Public Health, University of Newcastle
HMRI, Lot 1 Kookaburra Circuit
New Lambton Heights NSW 2305
Tel: 02 4042 0143

12. '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 15/03/18/3.03 If you have concerns or complaints about the conduct of this study you should contact:

Dr Nicole Gerrand, PhD
Manager, Research Ethics and Governance
Hunter New England Local Health Network
Locked Bag 1, NEW LAMBTON, NSW. 2305
Tel: (02) 4921 4950
Fax: (02) 4921 4818
Email: Nicole.Gerrand@hnehealth.nsw.gov.au

The Manager is the person nominated to receive complaints from research participants. You will need to quote reference number 15/03/18/3.03.

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)

Dietary fibre as a modulator of airway inflammation in asthma

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

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

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

I consent to-

1) Completing the tests involved in the study
2) Completing questionnaires to obtain research data
3) A copy of my results being sent to my General Practitioner
4) Allowing research personnel access to my medical record and to record attendance and results in my medical record

I consent to secure storage of samples collected in this study to be used in future research, subject to approval by the Hunter New England Human Research Ethics Committee.
YES ☐ NO ☐

I understand that my personal information will be maintained in confidence by 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)

Dietary fibre as a modulator of airway inflammation in asthma

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

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

I understand I can withdraw from the study 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) A copy of my results being sent to my General Practitioner
4) Allowing research personnel access to my medical record and to record attendance and results in my medical record

I consent to secure storage of samples collected in this study to be used in future research, subject to approval by the Hunter New England Human Research Ethics Committee.
YES ☐ NO ☐

I understand that my personal information will maintained in confidence by 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)

10.2. Appendix II: Asthma Control Questionnaire

1. On average, during the past week, how often were you woken by your asthma during the night?
0 Never
1 Hardly ever
2 A few times
3 Several times
4 Many times
5 A great many times
6Unable to sleep because of asthma

2. On average, during the past week, how bad were your asthma symptoms when you wake up in the morning?
0 No symptoms
1 Very mild symptoms
2 Mild symptoms
3 Moderate symptoms
4 Quite severe symptoms
5 Severe symptoms
6 Very severe symptoms

3. In general, during the past week, how limited were you in your activities because of your asthma?
0 Not limited at all
1 Very slightly limited
2 Slightly limited
3 Moderately limited
4 Very limited
5 Extremely limited
6 Totally limited

4. In general, during the past week, how much shortness of breath did you experience because of your asthma?
0 None
1 Very little
2 A little
3 A moderate amount
4 Quite a lot
5 A great deal
6 A very great deal

5. In general, during the past week, how much of the time did you wheeze?
0 Not at all
1 Hardly any of the time
2 A little of the time
3 A moderate amount of the time
4 A lot of the time
5 Most of the time
6 All the time

6. On average, during the past week, how many puffs of short-acting bronchodilator (eg Ventolin) have you used each day?
0 None
1 1-2 puffs most days
2 3-4 puffs most days
3 5-8 puffs most days
4 9-12 puffs most days
5 13-16 puffs most days
6 More than 16 puffs most days

To be completed by a member of the clinic staff

7. FEV_1 pre-bronchodilator …….
FEV_1 predicted……...
FEV_1 % predicted……
(Record actual values on the dotted lines and score the FEV_1 % predicted in the next column)

0 > 95% predicted
1 95-90%
2 89 –80%
3 79-70%
4 69-60%
5 59-50%
6 <50% predicted

Score Q1-7 = _____ ACQ(7) (Q1-7 score/7) = _____
Score Q1-6= _____ ACQ(6) (Q1-6 score/6)= _____

10.3. Appendix III: Gastrointestinal Symptom Rating Scale

Response scale

(1) No discomfort at all.
(2) Slight discomfort.
(3) Mild discomfort.
(4) Moderate discomfort.
(5) Moderately severe discomfort.
(6) Severe discomfort.
(7) Very severe discomfort.

(1) Have you been bothered by stomach ache or pain during the past week? (Stomach ache refers to all kinds of aches or pains in your stomach or belly.)	
(2) Have you been bothered by heartburn during the past week? (By heartburn we mean a burning pain or discomfort behind the breastbone in your chest.)	
(3) Have you been bothered by acid reflux during the past week? (By acid reflux we mean regurgitation or flow of sour or bitter fluid into your mouth.)	
(4) Have you been bothered by hunger pains in the stomach or belly during the past week? (This hollow feeling in the stomach is associated with the need to eat between meals.)	
(5) Have you been bothered by nausea during the past week? (By nausea we mean a feeling of wanting to be sick.)	
(6) Have you been bothered by rumbling in your stomach or belly during the past week? (Rumbling refers to vibrations or noise in the stomach.)	
(7) Has your stomach felt bloated during the past week? (Feeling bloated refers to swelling in the stomach or belly.)	
(8) Have you been bothered by burping during the past week? (Burping refers to bringing up air or gas through the mouth.)	
(9) Have you been bothered by passing gas or flatus during the past week? (Passing gas or flatus refers to the release of air or gas from the bowel.)	
(10) Have you been bothered by constipation during the past week? (Constipation refers to a reduced ability to empty the bowels.)	
(11) Have you been bothered by diarrhoea during the past week? (Diarrhoea refers to frequent loose or watery stools.)	
(12) Have you ever been bothered by loose stools during the past week? (If your stools have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being loose.)	
(13) Have you been bothered by hard stools during the past week? (If your stools have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being hard.)	
(14) Have you been bothered by an urgent need to have a bowel movement during the past week? (This urgent need to open your bowels makes you rush to the toilet.)	
(15) When going to the toilet during the past week, have you had the feeling of not completely emptying your bowels? (The feeling that after finishing a bowel movement, there is still more stool that needs to be passed).	