**The role of gluten and fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) in Functional Dyspspsia: A potential crossover with non-coeliac gluten sensitivity**

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**OVERVIEW**

This phase of the project seeks to evaluate the link between two gastrointestinal conditions, functional dyspepsia (FD) and non-coeliac gluten or wheat sensitivity (NCG/WS), and will follow on from phase 1 of the study.

*Functional dyspepsia*Functional dyspepsia refers to troublesome upper gastrointestinal symptoms which are thought to arise from the gastroduodenal region, comprising early satiety, postprandial fullness, and epigastric pain or epigastric burning (1). Population-based studies suggest about 10% of Australians have functional dyspepsia, with a slight female predominance (2-4). Functional dyspepsia often negatively impacts on quality of life and work productivity (5, 6). Traditionally eating smaller regular low fat meals is the advice offered, as the stomach and duodenum can process such meals more easily (a high fat intake slows gastric emptying) (7) and gastric distension is minimised. Wheat may induce typical dyspepsia symptoms and wheat elimination may provide relief in some cases although strong empirical evidence is lacking (8). Theoretically a low carbohydrate (low FODMAP) diet, an established therapy for IBS, may help by reducing upper intestinal distension but there is no empirical evidence in functional dyspepsia (8). Recently a unifying disease model has been proposed for functional dyspepsia (3). Either an infection, microbiome alteration or a food allergen such as wheat induces increased duodenal permeability and duodenal eosinophilia with or without increased mast cells, activating a Th2 mucosal immune response. Local duodeno-gastric reflex responses to low grade inflammation alter gastroduodenal function including, in a subset, impaired fundic relaxation. Circulating cytokines such as TNF-alpha may lead to systemic and central nervous system symptoms such as anxiety. The concepts are all supported by experimental evidence, and the model represents a paradigm shift with profound treatment implications if correct. In addition to dietary modification, treatment options include acid suppression, pro-kinetic agents, fundic relaxors such as cisapride, antibiotics such as rifaximin, tri cyclic anti-depressants and psychological therapy. *Non-coeliac wheat or gluten sensitivity*

A significant proportion of the general population without a diagnosis of CD avoids gluten or wheat in the diet, with several large cohort studies from around the world reporting a prevalence of self-reported wheat sensitivity (SRWS) between 4.3 and 13%. Non-celiac gluten or wheat sensitivity (NCG/WS) is a clinical syndrome which has attracted increasing international attention in recent years. It is characterized by adverse gastrointestinal (GI) or extra-intestinal symptoms related to the ingestion gluten or wheat containing food, which cannot be explained by either CD (diagnosed with a combination of serological testing and duodenal biopsies) or wheat allergy (diagnosed with IgE mediated immune- allergy tests) (9). The existence of this syndrome has been supported through several randomized double-blind placebo-controlled dietary crossover trials (9-12), and in the absence of a validated biomarker, current consensus criteria requires a blinded placebo controlled crossover challenge of gluten in order to make the diagnosis (13). Only a small number of patients (16-22%) who self-report wheat sensitivity, or respond to a gluten free diet, will ultimately fulfil criteria for NCG/WS after strict double blind, randomized, placebo controlled dietary challenge (8, 14). This method of diagnosis also has a high nocebo response rate of 40% (14). Furthermore, it is unclear whether it is gluten, or other compounds contained in wheat based foods, such as FODMAPS or amylase trypsin inhibitors, are responsible for generation of symptoms (15, 16). Both SRWS and NCG/WS have been shown to be associated with functional gastrointestinal disorders, in particular the irritable bowel syndrome (IBS), with dietary trials demonstrating that gluten or wheat may be responsible for symptom generation in a subgroup of patients with this disorder (9, 10, 17). Whilst the immunology and pathophysiology of CD is relatively well understood, much less is known about NCWS. It is hypothesized that the innate immune system plays a role in the pathogenesis of NCWS with increased expression of toll- like receptors seen in patients with NCWS (18). Further observations regarding the pathogenesis have included increased intestinal permeability (12, 19, 20), interferon gamma expression (21), epithelial cell damage (22), and duodenal eosinophilia (9) (Table 4). However, many studies examined patients in whom a strict diagnosis of NCWS had not been made by DBRPCC . Despite these early reports, there are still no clearly accepted pathological hallmarks of the disease, nor are there any serological tests or associated enzyme deficiencies, making diagnosis of this syndrome difficult and reliant on the cumbersome dietary trial (8, 22, 23).

*The overlap between functional dyspepsia and non coeliac wheat or gluten sensitivity*

Whilst the link between NCG/WS and IBS has been established in a number of studies ((17, 24-27)), less is known about the role of wheat and gluten in functional dyspepsia (FD). Only one study to date has explicitly investigated the overlap between FD and NCGS in a pooled cohort of patients IBS and FD and suspected wheat intolerance (10). It found that of the specific symptoms which showed a significant and specific association with gluten ingestion, 2 of the 4 most responsive to the blinded challenge were symptoms associated with FD rather than IBS (post prandial fullness (p=0.01), and early satiety (p=0.03)) (10). Notably duodenal eosinophilia has been observed in a major subset with postprandial distress syndrome (28). Duodenal eosinophilia has also been demonstrated in NCWS (9). It is conceivable that one of the underlying explanations for this finding in FD is food intolerance and wheat may play a role (28).

Our hypothesis is that there is a subset of patients with functional dyspepsia for whom dietary triggers such as gluten and FODMAPS are responsible for their symptoms. We suspect that there is an overlap between the two conditions of functional dyspepsia and non- coeliac wheat or gluten sensitivity.

**AIMS**

1. To determine whether gluten or FODMAPs are responsible for the generation of gastrointestinal and extra-intestinal symptoms in functional dyspepsia using a randomized, double blind, placebo controlled, dietary crossover trial
2. To determine the clinical features associated with diagnosis of diet responsive functional dyspepsia, including the specific symptoms which FODMAP and gluten generate
3. To determine whether duodenal eosinophilia and lymphocytic duodenosis are markers of gluten or FODMAP sensitivity in patients with functional dyspepsia
4. To determine whether there is a specific immunological signature associated with FODMAP and gluten responsive functional dyspepsia, compared with diet unresponsive functional dyspepsia and healthy controls
5. To evaluate the effect of a gluten free diet on the microbiome of patients with FD

**PRELIMINARY DATA**

Results from a large population based survey in the Hunter region has established a link between functional dyspepsia and self-reported wheat sensitivity supporting our hypothesis. A total of 3825 people (mean age 58.4 years, age range 18-100 years and 47.5% males) randomly selected from the Australian population returned a mail survey (Digestive Health & Wellbeing Survey, response rate = 45%) which contained questions on wheat avoidance, GI symptoms, demographic, medical and lifestyle factors. We defined self-reported wheat sensitivity as people who reported gastrointestinal symptoms on ingestion of wheat based foods, but did not suffer from coeliac disease, inflammatory bowel disease or bowel cancer. FGIDs (FD and irritable bowel syndrome) by Rome III. The prevalence of self-reported wheat sensitivity in this cohort was 13.7% (95% CI 12.5-14.9%). Only 11% of these had received a doctor diagnosis of wheat or gluten intolerance. The prevalence of CD was 1.2% (95% 0.8-1.6). The most commonly reported GI symptoms associated with self-reported wheat sensitivity included abdominal pain, bloating, and abdominal distention. In a multivariate analysis, a diagnosis or self-reported wheat sensitivity was significantly associated with the irritable bowel syndrome and functional dyspepsia, female gender, younger age, and food allergy. Doctor diagnosed CD was associated with several GI symptoms, as well as a diagnosis of FD.

**RESEARCH PLAN AND METHODOLOGY**

The study will be conducted in 3 phases

***Phase 1***: Patient recruitment and initial clinical evaluation; overlaps with original phase 1 of the study

*Phase 2: Budesonide trial (separate, unrelated part of the study)*

***Phase 3.1***: Dietary trial

***Phase 3.2***: Nested case control study of diet responsive functional dyspepsia (gluten and/ or FODMAP) with diet unresponsive functional dyspepsia and healthy controls; clinical, pathological, immunological and microbiological characteristics

**Patient recruitment:**
Patients will be recruited through various sources as per phase 1 of the study. Suitable patients who consent to phase 3 of the study and who meet the eligibility criteria outlined below will be enrolled into the dietary trial. We will aim to recruit 50 patients with functional dyspepsia diagnosed according to the internationally accepted Rome III criteria. Broad inclusion criteria are listed below.

**Inclusion criteria:**

1. Adults aged >18 years
2. Males and females
3. Functional dyspepsia; diagnosed by Rome III criteria
4. Were consuming a normal gluten and normal FODMAP containing diet for prior to their scheduled endoscopy and serum sample collection (as determined by the dietary evaluation using food frequency questionnaires and 24 hour recall questionnaire collected in phase 1)

**Exclusion criteria:**

1. Inflammatory bowel disease
2. Coeliac disease; negative TTG and/or absence of villous atrophy D2 biopsy at endoscopy
3. Current or previous gastrointestinal malignancy
4. Peptic ulcer disease
5. Systemic inflammatory condition
6. Diabetes mellitus
7. Wheat allergy; positive IgE specific serum test
8. Food avoidance as determined by dietary evaluation in phase 1 of the study
9. Immunosuppression
10. Active infection
11. Pregnant patients

**Initial clinical work up:**

In addition to the information already collected in phase 1 of the study, the following information will be collected from all patients prior to the dietary trial if not already available from the medical record:

1. Serum sample for;
	1. FBC, CRP, ESR,
	2. tTG IgA, total IgA
	3. HLA DQ2/8 status
	4. anti-gliadin antibodies
	5. wheat specific IgE
	6. T cell subtype analysis

**Already available from phase 1** and relevant to this project will include;

* Demographic and medical information
* Gastrointestinal symptoms from the phase 1 questionnaire (including nepean dyspepsia index, GI outpatient questionnaire, Rome III diagnostic questionnaire, IBS severity scoring system, hospital anxiety and depression scale, medication history)
* Dietary history
* Endoscopic and serum samples for immunological analysis
* Stool sample for microbiome analysis

**PHASE 2: DIETARY TRIAL**

For those who meet the inclusion and exclusion criteria will enter the dietary trial phase of the study. The dietary trial is currently the only diagnostic test available for the diagnosis of non-coeliac gluten sensitivity, and this protocol is in accordance with the current international consensus guidelines (13). An overview of this process is shown in Figures 1 and 2 below.

**Figure 2.** Summary of dietary challenge



**Figure 2.** Flow diagram for dietary trial

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The **main outcomes** of the dietary trial (PHASE 3.1) are listed below:

***Primary outcome***: Response of dyspeptic symptoms to dietary removal and subsequent re-challenge with gluten and FODMAPs

***Secondary outcomes***: Response of other gastrointestinal symptoms and extra-intestinal symptoms to dietary removal and subsequent challenge with FODMAPS and gluten

**Intervention steps**; (see Figure 1)

1. Dietitian or trained physician review at enrolment to educate patient regarding a gluten free, low FODMAP diet (visit 1, HMRI))
2. Symptom assessment at baseline using the Nepean Dyspepsia Index, IBS SSS, outpatient GI questionnaire and wheat sensitivity questionnaire (appendix 1)
3. Participants to follow this gluten free, low FODMAP diet for 4 weeks
4. Symptom assessment will be done weekly during the run in diet using the wheat sensitivity questionnaire
5. Compliance to be reviewed after 4 weeks using GFD scoring tool (see figure 3) (29) and using a food diary for the adherence to the FODMAP component of the diet (no validated tool for use here) (Visit 2, HMRI)

**Figure 3:** Validated tool for assessing GFD compliance



1. **For those whose dyspeptic symptoms respond to the run in diet,** defined as a greater than 30% response in dyspeptic symptoms as measured by the wheat sensitivity questionnaire at visit 2(13);
	1. Repeat serum testing of ESR and CRP
	2. Repeat serum testing for immunological analysis as detailed in phase 1
	3. Repeat stool sample for microbiome analysis
2. These subjects will then proceed to move on to the dietary challenge stage: Double blind randomized placebo controlled crossover gluten and wheat challenge (Figure 1). Cooked gluten and FODMAP will be administered in the form of a museli bar (see section below, “food preparation”)
	1. FODMAP depleted gluten 15 g/day (commercially available)
	2. High FODMAP, gluten free
	3. Placebo 10-15g/day (no FODMAP, no gluten)
3. Patient provided with labelled study food to be taken home and stored in the freezer for the duration of the trial (visit 2)
4. Each challenge will be for 1 week, with 1 week washout between each challenge (6 weeks total). The order in which participants receive the study food will be randomized.
5. Participants will receive a phone call during each dietary challenge week (ie. fortnightly) to ensure compliance and ascertain presence of adverse effects
6. Symptom assessment will be done daily throughout trial using the specific wheat sensitivity questionnaire developed for the study in a mobile app format (Qualtrix) or paper based questionnaire
7. Visit at the end of the trial (Visit 3, week 7-8) to discuss the response to the trial (gluten/ FODMAP sensitivity)

***Confirmation of food sensitivity***

In those with a positive response to gluten or FODMAP, they may be challenged again with the causative food along with placebo to confirm the presence of a food sensitivity, to overcome the high nocebo response previously reported in trials of this type (See figure 2).

**Study food preparation measures:**

Food will be prepared in the University of Newcastle study kitchen by an accredited person.

Food will be labelled and vacuum packed and stored in a freezer for 3 months at a time. Participants will be instructed to store the study food in their home freezer during the trial.

The food will be prepared according to the following recipe:

High FODMAP low gluten             Low FODMAP high gluten            Low FODMAP low gluten

1 cup golden syrup (340g)            1 cup golden syrup                          1 cup golden syrup

½ cup coconut oil (105g)                ½ cup coconut oil                             ½ cup coconut oil

2 cups rice puffs                               2 cups rice puffs                               2 cups rice puffs

1 cup rice flour                                  1 ½ cups gluten flour (190g)         1 ½ cups rice flour

1 cup cranberries                             1 cup cranberries                             1 cup cranberries

¼ cup coconut                                   ¼ cup coconut                                   ¼ cup coconut

2 teaspoons ground cinnamon   2 teaspoons ground cinnamon   2 teaspoons ground cinnamon

½ cup inulin (?? amount)

**PHASE 3.2: NESTED CASE CONTROL STUDY**

**Characterisation of the pathological and immunological features of wheat sensitive and FODMAP sensitive FD**

We anticipate that phase 3.1 of the study will yield four subsets of participants;

1. Gluten responsive functional dyspepsia (ie. non-coeliac gluten sensitivity)
2. FODMAP responsive functional dyspepsia
3. Gluten and FODMAP *responsive* functional dyspepsia
4. Gluten and FODMAP *unresponsive* functional dyspepsia

Using these four groups, we will perform a nested case control study evaluating the clinical, pathological, immunological and microbiological profiles of these four groups using the information collected in phase 1 and phase 3.1 of the study. Control groups collected in phase 1 of the study will also be used here. The specific features that will be compared across the 5 groups are listed below:

1. Clinical features
* Demographic information
* Medical comorbidities
* FD subtype
* Specific dyspeptic symptoms that affect the participant
* Other gastrointestinal symptoms (such as bloating, abdominal pain)
* Diet responsive extraintestinal symptoms such as fatigue, headache and anxiety
1. Pathological features
* Duodenal eosinophilia
* Lymphocytic duodenosis
1. Immunological
* T cell subtype analysis
* Gut homing T cells in biopsy samples
1. Microbiological
* Stool sample

**PHASE 3.3: EXPLANT CULTURE WITH GLUTEN AND FODMAP EXTRACT**

Explant cultures have been used to investigate immune activation in the duodenum as detailed in phase 1 of the study. In addition to the analyses detailed in phase 1, we will also assess if duodenal immune activation is responsive to a dietary trigger by culturing the cells in the presence of gliadin extract.

**PHASE 3.4 IMMUNOLOGICAL AND MICROBIOLOGICAL RESPONSE TO THE LOW FODMAP, GLUTEN FREE DIET**

Finally, we will compare the immunological profile (through serum and biopsy analysis) and microbiological profile (through 16s RNA analysis of stool samples) collected in phase 1 and phase 3.1 of the study (that is, before and 4 weeks after commencing the low FODMAP, gluten-free diet) in order to evaluate the change in the intestinal microbiome and immune system in response to a gluten free, low FODMAP diet.

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**Appendix 1**: Draft Wheat Sensitivity Questionnaire

**Wheat Sensitivity Questionnaire**

**Date completed: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. **Were you able to consume the study food today?**

[] Yes [] No []Partially [] Not applicable (run in/ washout diet)

1. **The following questions refer to specific symptoms you may have experienced today. For each, please begin by indicating whether or not you experienced this symptom, and if so, you will be asked further questions.**

* 1. **Early satiety or post prandial fullness**
		1. [] Yes [] No (move on to question 2b)
		2. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptom start after ingestion of the study food?

[] Yes [] No (move to part iv)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did this symptom last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your normal daily activities?

[] Yes [] No

* 1. **Epigastric pain or epigastric burning**
		1. [] Yes [] No (move on to question 2b)
		2. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptoms start after ingestion of the study food?

[] Yes [] No (move to part iii)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did it last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your daily activities? [] Yes [] No
	1. **Lower abdominal pain**
		1. [] Yes [] No (move on to question 2b)
		2. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptoms start after ingestion of the study food?

[] Yes [] No (move to part iii)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did it last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your daily activities? [] Yes [] No
	1. **Loose stools**
		1. [] Yes [] No (move on to question 2b)
		2. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptoms start after ingestion of the study food?

[] Yes [] No (move to part iii)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did it last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your daily activities? [] Yes [] No
	1. **Bloating**
		1. [] Yes [] No (move on to question 2b)
		2. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptoms start after ingestion of the study food?

[] Yes [] No (move to part iii)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did it last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your daily activities? [] Yes [] No
	1. **Lack of wellbeing**
		1. [] Yes [] No (move on to question 2b)
		2. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
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 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptoms start after ingestion of the study food?

[] Yes [] No (move to part iii)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did it last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your daily activities? [] Yes [] No
	1. **Headache**
		1. [] Yes [] No (move on to question 2b)
		2. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
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 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptoms start after ingestion of the study food?

[] Yes [] No (move to part iii)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did it last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your daily activities? [] Yes [] No
	1. **Tiredness (more than usual, or limiting)**
	[] Yes [] No (move on to question 2b)
		1. Please rate this symptom in terms of its severity (0 being no symptom at all, and 10 being the most intense)

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |

 0 1 2 3 4 5 6 7 8 9 10

* + 1. Did this symptoms start after ingestion of the study food?

[] Yes [] No (move to part iii)

* + - 1. if so how long after?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. How long did it last?

\_\_\_\_\_\_\_\_\_ minutes

* + 1. Did this symptom prevent you from performing your daily activities? [] Yes [] No
		2. Did this symptom prevent you from performing your daily activities? [] Yes [] No
1. **Would you say you had adequate relief today from your upper abdominal symptoms?**

[] Yes [] No