ASSESSMENT OF GLUTEN-FREE DIETS IN AN AUSTRALIAN POPULATION WITH COELIAC DISEASE AND THEIR IMPACT ON SYMPTOMS, MUCOSAL, NUTRITIONAL AND METABOLIC PARAMETERS.

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DECLARATION OF ORIGINALITY

The work described in this thesis was carried out by the author, except where otherwise stated. None of the material has been previously presented for the purpose of obtaining any other degree.

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Thankyou to The Coeliac Society of NSW, the experience of Graham and Cheryl Price and those with coeliac disease who have spent much time giving me insight into the difficulties they face when purchasing and consuming gluten-free foods. The frustrations of aspects of the gluten-free diet are illustrated in the poem below by Shelagh Ford, Mt Nelson, Tasmania *(The Australian Coeliac, 1999)*.

Optimism

Well I thought I'd make something to eat.
Something baked, something nice, something sweet.
So I pulled out a book
To have a good look
As to how to whip up such a treat.
The recipes weren't gluten-free
but that wasn't a problem for me.
I'd swap and I'd change
Amounts rearrange
And the cake would be lovely with tea.

Well, the book called for "self raising flour"
And "180^0 for an hour".
Swap ounces for pounds,
Change the flours around,
Add some cream that's a tiny bit sour.

I added some soy and some rice
(The pea flour was not very nice).
A cupful or two
Some soda stirred through
And this cake'll puff up in a trice.

An egg substitute had me vexed,
'cos the book said "an egg goes in next"
but the fridge, it was bare.
Not an egg was in there,
And this problem had me quite perplexed.

"Leave it out" was the pathway I chose.
It's only for taste, I supposed.
I'll double the spice
And make it taste nice
And I really feel I'm home and hosed.

Swap the marg for some oil, lined the tin with some foil.
Icing sugar, I hastened to add.
Then some fruit and some wine.
Reduce cooking time
And this cake'll be the best that we've had.

Left my brain wave to cook whilst I finished my book
And relaxed with some music on low.
It smelt really yum
And my rumbling tum
Had my taste buds all ready to go.

For afternoon tea, the children and me
Would tuck into a super delight.
We'd laugh and we'd talk
Take the dog for a walk
It all felt so "homey" and right.

But when they came home, they found me alone
With my eyes all puffy and sore.
The cake was all flat
The bottom was black
And the centre was really quite raw.

They patted my knee, and comforted me,
Soothing the stresses and strains.
But, I've learnt that the key
To great gluten-free
Is to mix on one leg when it rains.

ABSTRACT

Coeliac disease (CD) is a gastrointestinal disease caused by a hypersensitivity to gluten in genetically predisposed individuals. A gluten-free diet (GFD) is expected to relieve symptoms and reverse the characteristic villous atrophy. Persistent symptoms and ongoing mucosal pathology, observed in a subset of people with CD, are usually attributed to non-compliance with the GFD.

Patients adhering to a GFD rarely eat no gluten at all. According to the international *Codex Alimentarius* standards, a food labelled 'gluten free' can contain up to 0.3g/100g of protein from a gluten containing grain. On a GFD based on these standards, patients have been found to ingest 5-100mg (average 34mg) per day of gluten. In addition to these small amounts, many patients also have occasional inadvertent exposure to foods containing larger amounts of gluten. Australia followed the *Codex* labelling standards until 1995; since then, foods can only be labelled 'gluten-free' if they contain no detectable (<0.003%) gluten (NDG).

To determine whether persistent symptoms in some coeliac patients on a GFD were due to ingestion of small amounts of gluten allowed in foods labelled 'gluten-free', or to other non-gluten food intolerances, 39 symptomatic subjects were recruited. Of 22 adhering to a *Codex-GFD* at the outset, symptoms resolved completely in 5 and improved significantly in 10 after changing to a *NDG-GFD* for $\geq$ 3 months. Of 26 patients who undertook a diagnostic elimination diet to investigate possible non-gluten food intolerances, 24 improved significantly and 19 completed a challenge protocol to identify the food substances responsible for symptoms.

To investigate the effects of ingestion of small amounts of gluten on the small bowel mucosa, nutritional status and bone mineral density (BMD), 48 coeliac patients were recruited and asked to follow a *NDG-GFD* for a 2-year period. Their small bowel biopsies, BMD, nutritional parameters and dietary intake were examined at entry, 12 months and 2 years. The *NDG-GFD* was found to be nutritionally adequate and no subject was malnourished. Twenty-two patients had an abnormal small bowel biopsy at some point in the study period, 5 of which were abnormal throughout. This was unrelated to gluten intake, and had no detrimental effect on symptoms, nutritional status or BMD. Long-term avoidance of the small amounts of gluten permitted on a *Codex-GFD* did not result in improvement in the small bowel pathology in those with an abnormal biopsy at the outset.

Conclusions:

(1) Persistent symptoms experienced by some patients with CD may be due to the consumption of small amounts of gluten that are permitted on a *Codex-GFD*. Such individuals may be regarded as belonging to a 'more sensitive' subgroup.

(2) Non-gluten food intolerances can cause symptoms in patients who remain symptomatic despite careful adherence to a *NDG-GFD*.

(3) The *NDG-GFD* is nutritionally adequate.

(4) Small bowel mucosal abnormalities can persist in some patients independent of symptoms and regardless of adherence to a *NDG-GFD*.

(5) Nutritional status and BMD are not adversely affected in the longer term (2 years) by the persistence of villous atrophy in coeliac patients adhering to a *NDG-GFD*.

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PUBLICATIONS

1. Coeliac Disease: Changing clinical spectrum and food labelling laws. Selby WS and Faulkner-Hogg KB. Current Therapeutics, 1998;June;31-37

2. Dietary analysis in symptomatic patients with coeliac disease on a gluten-free diet: The role of trace amounts of gluten and non-gluten food intolerances. Faulkner-Hogg KB, Selby WS, Loblay RH. Scand J Gastroenterol 1999;34:784-789.

3. Persistent mucosal abnormalities in coeliac disease are not related to the ingestion of trace amounts of gluten. Selby WS, Painter D, Collins A, Faulkner-Hogg KB, Loblay RH. Scand J Gastroenterol 1999;34:909-914.

LIST OF ABBREVIATIONS

Ab	Abnormal
AGA	Anti-gliadin antibody
AGAA	Anti-gliadin antibody A
AGAG	Anti-gliadin antibody G
ANZFA	Australia, New Zealand Food Authority
ATD	Autoimmune Thyroid Disease
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toluene
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body-Mass Index (kg/m^2)
CD	Coeliac Disease
CHO	Carbohydrate
Codex	Codex Alimentarius
Codex-GFD	Codex-gluten free diet
CSIRO	Commonwealth and Scientific Industrial Research Organisation
df	degrees of freedom
DH	Dermatitis Herpetiformis
DHF	Digestive Health Foundation
DXA	Dual energy X-ray Absorptiometry
ELISA	Enzyme Linked Immunosorbent Assay
EMA	Endomysial Antibody
EMAA	IgA anti-endomysial antibody
EMAG	IgG anti-endomysial antibody
ESPGAN	European Society for Paediatric Gastroenterology and Nutrition
F	Female
FAO	Food and Agricultural Organization
FSANZ	Food Standards Australia, New Zealand
GFD	Gluten-Free Diet
HLA	Human Leukocyte Antigen allele

HRT	Hormone Replacement Therapy
IBS	Irritable Bowel Syndrome
IEL	Intraepithelial lymphocyte
IgA	Immunoglobulin A
IgG	Immunoglobulin G
Inc.	Incorporated
kg	kilogram
kJ	kilojoule
L2	Lumbar 2
L4	Lumbar 4
m	metres
mg	milligrams
M	Male
MAC	Mid-upper arm circumference
MAMC	Mid-upper arm muscle circumference
Mg	Magnesium
MHC	Major Histocompatibility Complex
MSG	Monosodium glutamate
N	Normal
NDG	No Detectable Gluten
NDG-GFD	No detectable gluten-gluten free diet
NFA	National Food Authority
NNS	National Nutrition Survey
NS	Not significant
NSW	New South Wales
penia	osteopenia
PVA	Partial Villous Atrophy
RDI	Recommended Dietary Intake
RPAH	Royal Prince Alfred Hospital, Sydney, Australia
S	subject
SD	Standard deviation
Stom.	Stomach
STVA	Sub Total Villous Atrophy
TCR	T-cell receptor

TS	Triceps skinfold
tTG	tissue transglutaminase
TVA	Total Villous Atrophy
USDA	United States Department of Agriculture
VA	Villous atrophy
V/C	Villous / Crypt ratio
WHO	World Health Organization
#	Number

DEFINITIONS

Codex Alimentarius food standard for the production of gluten-free foods allows 3g/kg of protein from gluten containing grains to be incorporated into foods labelled and sold as gluten-free.

Australian Food Standard (1995) allow foods to be labelled as 'gluten-free' only if they contain no detectable gluten *(<0.003g/100g)*, oats or malt.

Codex-GFD: based on the Codex Alimentarius standard, allowing the regular (6 or more times per year) inclusion of food ingredients containing up to 0.3% protein from gluten-containing grains (principally wheat starch and malt).

NDG-GFD (No Detectable Gluten-GFD): based on the Australian Food Labelling Standard (1995), containing no detectable gluten (measured by ELISA with lower limit of detection <0.003%), malt or oats.

Overt gluten-containing diet: A diet which regularly (6 or more times per year) includes foods made with wheat flour (e.g. bread, pasta, cakes, biscuits, ice-cream cones), rye, barley or oats.

Codex-permitted gluten: This term describes ingredients which contain small amounts of gluten, up to a maximum of 0.3g per 100g (0.3%), as permitted in foods labelled "gluten-free" under the Codex Alimentarius food standard. E.g. wheat starch, which can be the main ingredient in many gluten-free foods & malt.

Trace gluten: This refers to ingredients which contain barely detectable levels of gluten (<0.01%), such as maltodextrin and thickeners. These ingredients are usually found in small quantities in foods.

Overt gluten: This describes intakes of gluten from grains or ingredients that should always be avoided by people with coeliac disease. Examples of these are wheat and rye flour, wheaten bread, biscuits and pasta.

Classification of Gluten Intakes used in the longitudinal study

A = ≤ 0.01mg gluten / year

B = gluten intake 0.01 - 10mg gluten / year

C = gluten intake 10 - 1000mg gluten / year

D = gluten intake >1000mg gluten / year

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INTRODUCTION

At the outset, the present study of dietary aspects of coeliac disease was prompted by the observation that non-gluten food intolerances, rather than inadvertent gluten ingestion, proved to be the cause of continuing gastrointestinal symptoms in several symptomatic patients with coeliac disease referred to the Allergy Unit at Royal Prince Alfred Hospital in the early 1990s. The initial intention was to examine the role of non-gluten food intolerances more thoroughly by studying a larger group of subjects with coeliac disease who had persistent symptoms despite strict adherence to a gluten-free diet (GFD).

At that time, many patients believed that the minor amounts of gluten, present in foods that were then allowed on a GFD, were responsible for their ongoing symptoms. The advent of better testing methods *(Skerritt et al, 1991)* for gluten in foods, along with lobbying from members of the Coeliac Society of Australia, successfully resulted in the National Food Authority (NFA)[1] making more stringent the *Australian Food Standards* governing the labelling of foods as 'gluten-free'. In 1995, the *Food Standards* were revised such that foods could only be labelled as 'gluten-free' if they contained no detectable gluten (NDG) as defined by a highly sensitive ELISA immunoassay. Until that time the food standards in Australia followed the less stringent international *CODEX Alimentarius* guidelines, based on a crude protein nitrogen assay.

[1] Subsequently ANZFA (Australian & New Zealand Food Authority), and now FSANZ (Food Standards Australia & New Zealand).

These changes in the food standards provided an opportunity to test the additional hypothesis that the minor amounts of gluten allowed on a gluten-free diet could be responsible for symptoms, mucosal pathology and/or metabolic consequences in a broader, unselected group of patients with coeliac disease.

BACKGROUND

'Gluten-free' food labelling

The *Codex Alimentarius* is a set of international food standards, drawn up by a committee of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), which includes compositional guidelines for the labelling of foods, including those labelled as 'gluten-free'. Each country has a set of food standards that may either be adopted directly from the *Codex*, or may be varied according to local circumstances. Although most follow the *Codex* guidelines broadly, there are variations in the 'gluten-free' labelling standards between Canada (the most stringent), the United Kingdom, Europe, the USA, Australia and New Zealand.

Until 1995, the Australian and New Zealand 'gluten-free' food standards followed the *Codex* guidelines, allowing commercial foods with ingredients derived from gluten containing grains to be labelled 'gluten-free' provided they contained less than 3g/kg (0.3%) of protein from those grains. This criterion was based on the Kjeldahl method of protein nitrogen estimation, and allowed such products to contain up to 0.4-0.6 g of gluten per kg of starch derived from wheat, barley or rye *(Skerritt & Hill, 1991; Hekkens, 1991)*. In 1995, ANZFA amended the food standards such that foods could only be labelled 'gluten-free' if they contained no detectable gluten as defined by a sensitive and specific ELISA immunoassay, the lower detection limit of which was 0.03g/kg (0.003%). Oats and malt should also be excluded.

'Gluten-free' Diets

As outlined above, foods labelled as 'gluten-free' rarely contain no gluten at all. Thus, a 'gluten-free' diet as routinely recommended by doctors and dietitians for patients with coeliac disease will inevitably include foods containing trace amounts of gluten, even if labelled as 'gluten-free'. Indeed, *Kaukinen et al, (1999)* found that amongst 25 patients adhering strictly to a Codex defined GFD (see below) there was a mean intake of 34 mg of gluten per day, with a range of 5-150 mg/d.

At the time the initial study began, conventional wisdom was that these small amounts of gluten were not harmful to patients with coeliac disease, and they were generally accepted as allowable on a GFD. However, the possibility that there might be a subgroup of more 'sensitive' coeliac patients, reactive to trace amounts of gluten, had not previously been systematically examined.

In order to determine whether some patients can develop symptoms, biopsy abnormalities and/or metabolic consequences from the trace amounts of gluten in a GFD, advantage was taken of the changes introduced by the revised ANZFA 'gluten-free' labelling standard in 1995. To help characterise the stringency of the GFD being followed by subjects entering these studies, two categories were defined:

1. *Codex-GFD:* a GFD where subjects are consuming foods permitted to be labelled as 'gluten-free' according to the *Codex* standard. Such foods have ingredients that contain up to 0.3% protein from gluten containing grains, for example wheat starch, malt extract, malt, starch and modified starch.

2. *No Detectable Gluten-GFD (NDG-GFD*): a GFD where subjects are consuming foods permitted to be labelled as 'gluten-free' according to the revised food standard introduced by ANZFA in 1995. Such foods have ingredients which contain less than 0.003% gluten. Wheat starch and malt could not be consumed on this more stringent GFD, but maize cornflour, modified maize starch, glucose syrup and caramel colour are acceptable ingredients.
3. *Overt gluten-containing diet:* a diet which regularly includes foods made with wheat flour (e.g. bread, pasta, cakes, biscuits, and ice - cream cones), rye, barley or oats.

To place these amounts in perspective, a person on a normal, unrestricted diet would be expected to consume roughly 10-15 g of gluten per day. By comparison, a person with coeliac disease adhering to a *Codex-GFD* would be expected to consume about 100-fold less (range of 5-150 mg/d; *Kaukinen et al, 1999*) and a person adhering to a *NDG-GFD* would be consuming a further 100-fold less again.

In order to describe the quantities of gluten eaten by subjects throughout the study a number of terms have been introduced:

- Codex-permitted gluten: This term describes ingredients which contain small amounts of gluten, up to a maximum of 0.3g per 100g (0.3%), as permitted in foods labelled "gluten-free" under the Codex Alimentarius food standard. E.g. wheat starch, which can be the main ingredient in many gluten-free foods & malt.

- Trace gluten: This refers to ingredients which contain barely detectable levels of gluten (<0.01%), such as maltodextrin and thickeners. These ingredients are usually found in small quantities in foods.

- Overt gluten: This describes intakes of gluten from grains or ingredients that should always be avoided by people with coeliac disease. Examples of these are wheat and rye flour, wheaten bread, biscuits and pasta.

Dietary Adherence ('compliance')

In the present studies, any subject on a *Codex-GFD* at entry was switched to a *NDG-GFD* after all the initial assessments had been carried out. Throughout these studies, all but one of the subjects were adherent to the *NDG-GFD*. It is important to emphasise that ingestion of these small amounts of gluten by coeliac patients adhering to a *Codex-GFD* or a *NDG-GFD* is not an indication of non-adherence ('non-compliance'). In practice, occasional inadvertent ingestion of gluten-containing foods is unavoidable for all but the most obsessive coeliac patient. Amongst the subjects in these studies, documented inadvertent ingestion of gluten was infrequent, and was calculated by extrapolation to be the equivalent of a mean daily intake of 3-4 mg per day — well below the amounts ingested in fully adherent patients on a *Codex-GFD* (*Kaukinen et al, 1999*).

The GFD referred to in most of the published literature on coeliac disease is the *Codex-GFD* rather than the more stringent *NDG-GFD*. Few studies have documented the actual amounts of gluten ingested, and none have been as exhaustive as the present one in documenting all possible sources of trace gluten ingestion.

EVOLUTION OF THE STUDIES IN THIS THESIS

Initially, coeliac patients with continuing symptoms despite adherence to a GFD were recruited to determine whether non-gluten food intolerances could be responsible. The study protocol required them to undergo full dietetic and medical evaluation (including baseline small bowel biopsy). Before undergoing dietary testing for non-gluten food intolerances, those patients following a *Codex-GFD* were asked to change to the more stringent *NDG-GFD* for 3 months. Quite unexpectedly, several patients reported partial or complete relief of symptoms after making this change, raising the possibility that there is individual variation in sensitivity to trace amounts of gluten in the coeliac population.

A questionnaire survey of members of the Coeliac Society of NSW was therefore conducted to determine whether there was a relationship between ongoing symptom frequency/severity and the stringency of dietary gluten exclusion. This was done in 1995, before the changes to the *Australian Food Standards* were implemented, with the assistance of a Master of Nutrition and Dietetics student, Michelle Stuart.

Surprisingly, also, in the initial study cohort half the patients were found to have small bowel mucosal abnormalities on biopsy. This did not appear to be related to the stringency of their GFD at entry into the study, and did not change in the short term (3 months) in those who changed to the *NDG-GFD*.

A second study was therefore initiated with a larger group of patients, not selected on the basis of ongoing symptoms, incorporating a longer period of observation. Recruitment began in 1996, and participants were monitored carefully over 2 years for dietary gluten intake,

symptoms, small bowel pathology, and relevant nutritional, immunological and metabolic parameters (including bone mineral density).

The basic hypothesis underlying this second study was that over time, more stringent gluten avoidance would reduce the occurrence of symptoms as well as small bowel mucosal abnormalities, with improvement in nutritional parameters and bone mineral density. The nutritional adequacy of the *NDG-GFD* was also analysed in this group of patients and was compared with data from the 1995 Australian National Nutrition Survey.

CHAPTER 1

1

COELIAC DISEASE

Background and literature review

DEFINITION

The term “coeliac disease” is derived from the Greek word “koiliako”’ which means “suffering in the bowels”. The translated text of Aretaeus of Cappodocia, a Greek physician of the 2nd Century AD who is often credited with the first description of coeliac disease, states; “if the stomach be irretentive of the food and it pass through undigested and crude, and nothing ascends into the body, we call such persons Coeliacs” (*Tizard, 1973).*

Today coeliac disease is one of the most common chronic gastrointestinal diseases among Caucasians. It occurs in genetically pre-disposed individuals. The age of onset is variable and there is a slight female predominance *(AmGA, 2001-B).* Known previously as non-tropical sprue and Gee-Herter’s disease, it is also known as gluten-sensitive enteropathy, and coeliac sprue *(Van Berge-Henegouwen & Mulder, 1993).*

In coeliac disease, ***gluten***, a sub-fraction of wheat protein, leads to immune damage to the small intestinal mucosa *(Marsh, 1992).* This also happens with the homologous proteins in rye (secalin), barley (hordein), triticale (a hybrid of wheat and rye) and possibly oats. Although oats have traditionally been excluded in a GFD this is currently under review since there is evidence that the homologous protein (avenin) is not cross-reactive in coeliac patients *(Shan et al, 2002).* In untreated coeliac disease the small bowel mucosa becomes inflamed with

infiltration of lymphocytes into the lamina propria and loss of the normal villous structure. This 'flat' histological appearance of the small bowel in untreated coeliac disease is called ***villous atrophy*** (VA). Varying degrees of severity are described as 'partial VA' (PVA), 'sub-total VA' (STVA) or 'total VA' (TVA) (Figure 1.1). This mucosal damage is reversed, either totally or partially, by withdrawal of gluten from the diet.

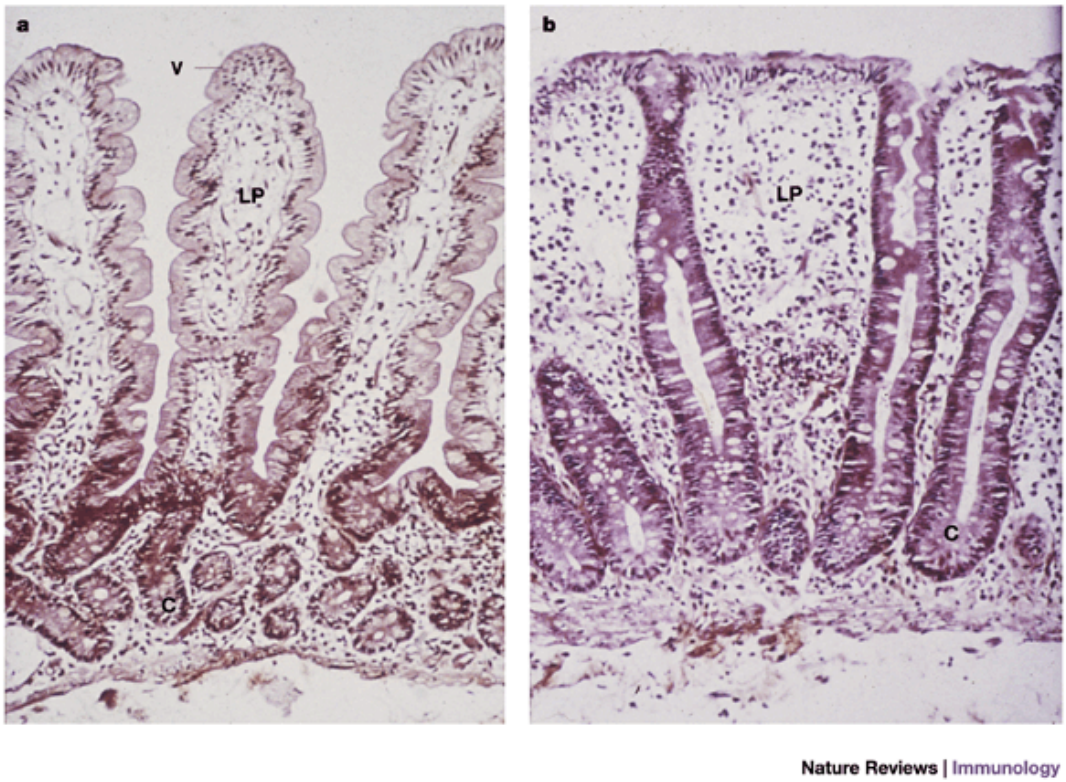

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Figure 1.1: Histology of normal and abnormal villi in the small bowel. (a) Normal villi (v) height with lamina propria (LP) extending from the crypt into the villi. (b) Characteristic total villous atrophy of undiagnosed coeliac disease.

PREVALENCE

Coeliac disease is predominantly a disorder of Caucasians. Its prevalence varies geographically but a worldwide average is estimated to be 1:266 *(Fasano & Catassi, 2001)*. In Europe, serological screening studies have estimated the prevalence to be 1 in 250-300

(*Catassi et al, 1994 & Ascher et al, 1994)* of the general population. A greater prevalence is seen in Northern Ireland with estimates of 1 in 122 (*Johnston et al, 1997*). Although adult coeliac disease has generally been considered to be rare in the USA, a recent study in Baltimore using antiendomysial antibody screening, revealed a prevalence of 1 in 250 (*Not et al, 1998*).

In Australia, the frequency of clinical coeliac disease was estimated to be about 1 in 2000 individuals *(Selby, 2001).* However, as with studies in other countries, a recent serological study in Western Australia has revealed a much higher prevalence, approximately 1:250, than previously believed (*Hovell et al, 2001*). This suggests that a significant proportion of individuals have either milder presentations or clinically silent forms of the disease. There are also a number of associated disorders where the prevalence of coeliac disease is much higher, for example, insulin-dependent diabetes and thyroid disease. These are discussed later.

GLUTEN and GLIADIN

The word ***gluten*** comes from the Latin word meaning glue. Gluten is defined as 'the cohesive mass that remains when dough is washed to remove starch granules' (*Marsh, 1992)*. It is found in the protein portion of the cereal grains wheat, rye, barley and oats as well as in hybrid grains such as triticale and in many ingredients derived from these grains. Since the development of agriculture, most societies have had a staple grain or cereal (for example, wheat, barley, rye, rice, millet or corn) featuring prominently in the daily diet. In modern times, wheat has outstripped all other grains in popularity and consumption, largely because the elastic properties of wheat gluten permit the baking of leavened bread. Carbon-dioxide, produced from yeast fermentation, is trapped in the dough by the rubbery gluten-starch matrix.

When baked or heated, the gluten sets, leaving an aerated, porous product. The binding properties of gluten also provide wheat flour, or isolated gluten flour, with many other functions in the preparation of domestic and commercial food items. Mild forms of processing and heating do not appear to alter the ability of gluten to provoke small bowel mucosal damage in patients with coeliac disease. *(Marsh, 1992; Anderson et al, 2000).*

Cereals are grasses (Gramineae) containing starchy grains that can be used as food. They can be divided into four major groups, largely on the basis of the chemical structure of the storage proteins in the seeds (*Colot et al, 1989 in Marsh, 1992*), Figure 1.2.

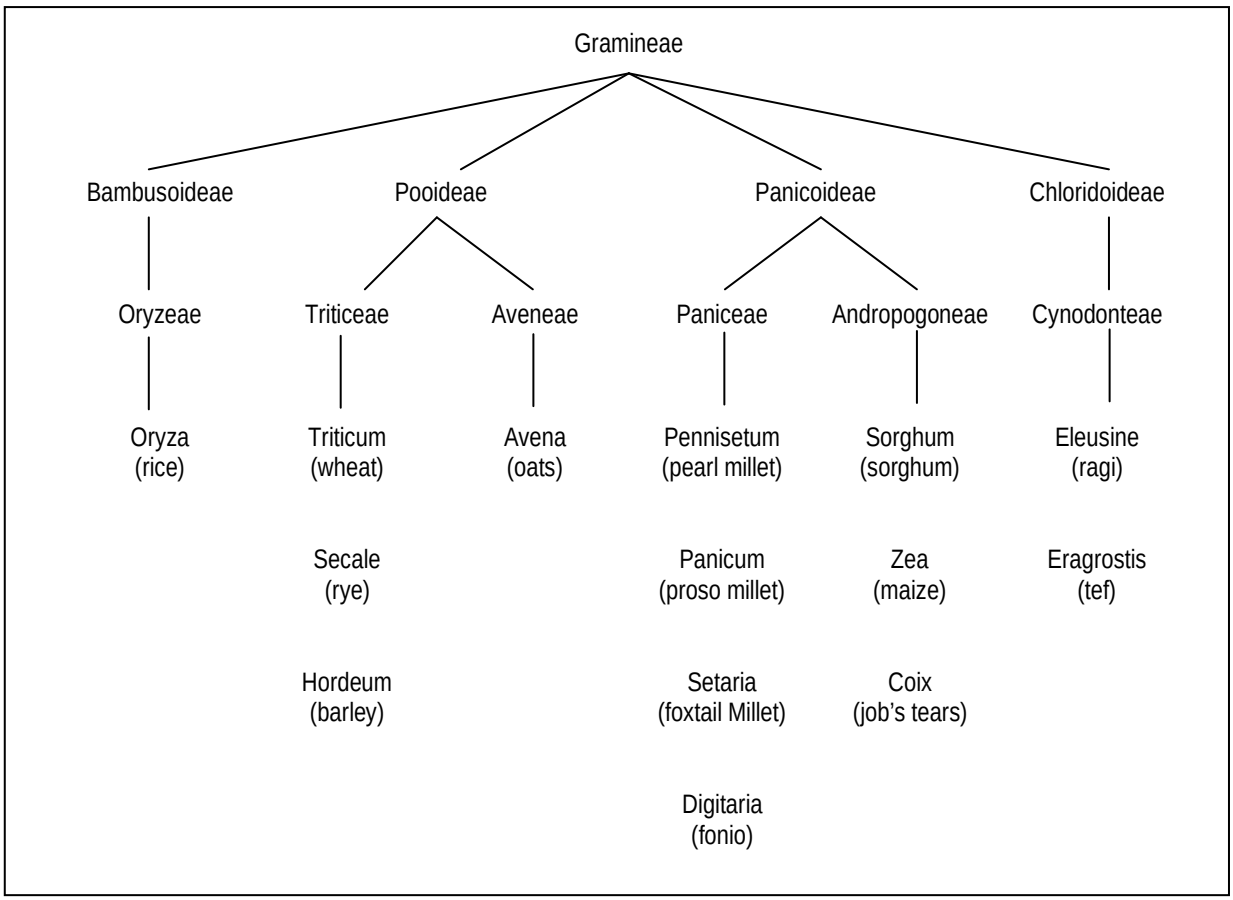

Figure 1.2: The taxonomic relationship of cereal grains *(Colot et al, 1989 in Marsh 1992).*

The main subfamilies are Bambusoideae, Panicoideae, Chloridiodeae and Pooideae. The Pooideae subfamily can be divided into 2 tribes. The first, Triticeae, contains the major temperate cereals wheat, rye and barley, while the second, Aveneae, contains oats. The major tropical cereals, maize, sorghum and most millets (small seeded cereals) are found in the subfamily Panicoideae. Rice belongs to a structurally different subfamily, Bambusoideae, and is not closely related to the other cereals. It is only the Pooideae that are implicated in coeliac disease.

Wheat protein can be separated into 4 major fractions (Figure 1.3):- ***albumins*** (soluble in water), ***globulins*** (soluble in 10% NaCl, but insoluble in water), ***prolamins*** (soluble in alcohol) and ***glutelins*** (the insoluble remainder) *(Ciclitira & Ellis, 1991)*.

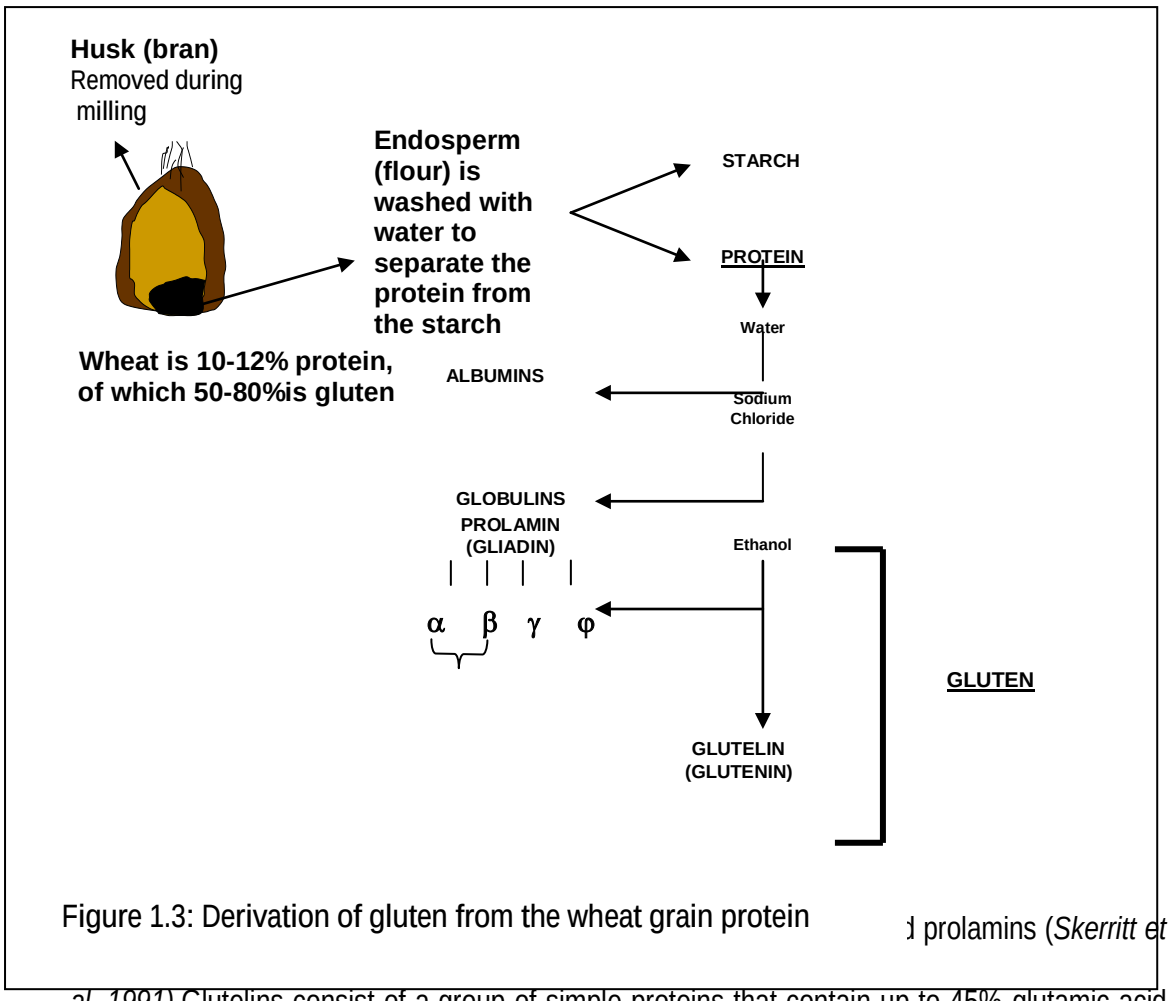

Figure 1.3: Derivation of gluten from the wheat grain protein

d prolamins (*Skerritt et al, 1991).*Glutelins consist of a group of simple proteins that contain up to 45% glutamic acid

and are generally insoluble in water, salt solutions and dilute ethanol but at extreme pH values they do become soluble (*Osborne, 1924, in Marsh, 1992)*.

The exact chemistry varies among the different grains, leading to each type of glutelin being assigned a specific name. The best known glutelins are ***glutenin*** in wheat, ***hordenin*** in barley and ***orycenin*** in rice. Historically the glutelins have not been considered damaging in coeliac disease, however a recent study suggests that the wheat glutenins harbour at least two T-cell stimulatory peptide sequences *(Vaistij et al, 2002).*

Prolamins are soluble in 50-90% alcohol (*Ciclitira & Ellis, 1991*). Their name reflects the high content of the amino acid proline and of amide nitrogen, derived from the amino acid glutamine, the proportions of which vary among the grains *(Godkin & Jewell, 1998)*. As a result, the prolamin fraction from each of the gluten containing grains is also given a specific name (Table 1.1). The proportion of prolamin in the protein fraction of each grain also varies.

GRAIN	PROLAMIN	% of prolamin in the protein
Wheat	gliadin	40-50
Rye	secalin	30-50
Barley	hordein	35-55
Oats	avenin	5-15
Maize	zein	75-80
Rice	orzenin	5-15

Adapted from *Moulton (1959), Shewry and Tatham (1990) and Thompson (1997)*

Table 1.1: Prolamin content of the different grains.

Gliadins are sub-fractions of prolamin and can be classified according to their relative mobility in either starch or polyacrylamide gel electrophoresis. The subfractions are named alpha,

beta, gamma and omega in decreasing order of electrophoretic mobility (*Ciclitira & Ellis, 1991).* Subsequent amino acid sequencing (*Skerritt et al, 1991)* has shown that there is little difference between the alpha and beta gliadins so they are generally considered part of the same group.

The ability of a grain to elicit mucosal damage depends upon the amino acid sequences that make up the structure of the prolamins and the amount present in each of the cereals *(Godkin & Jewell, 1998*). Even though the rye and barley prolamins are not highly homologous to the alpha and beta gliadins of wheat, they still appear to damage the small bowel mucosa of people with coeliac disease *(Kreis et al, 1985)*. Although avenins, the prolamins present in oats, also have some amino acid sequence homology with the prolamins of wheat, rye and barley, they do not appear to produce mucosal damage. The prolamins of rice (orzenin) do not show significant homology with the prolamins in wheat, rye, barley or oats *(Marsh, 1992)*. The zeins of maize are also unrelated to the prolamins of the Triticeae (*Marsh, 1992*). The prolamins of sorghum, millets and Job's tears are not as well studied, but the available information indicates that they have similar prolamins to those found in maize *(Marsh, 1992*). This explains why these grains do not play any role in coeliac disease and are suitable as wheat substitutes in a GFD.

OATS

Historically, there has always been reservation about the validity of excluding oats from the coeliac diet. Willem-Karel Dicke, who originally discovered the role of gluten in coeliac disease in 1953 thought oats should be excluded from the GFD. However four other groups throughout the 1950's and 1960's advocated acceptance of oats in the GFD *(Sheldon 1955; Hansted 1956; Moulton 1959; Rubin et al, 1962).* These uncertainties arose because of:

- the lack of a means to test the pathogenicity ("toxicity") of oats (a position rectified with the advent of the small bowel biopsy and serum antibody tests)
- the lack of knowledge of regarding amino acid sequences responsible for mucosal damage
- the lack of molecular studies of oats (being addressed in recent years) including differences in cereal chemistry between oats and wheat.

As shown in Figure 1.2, Pooideae is the taxonomic family for wheat, rye, barley and oats. This can be divided into two subgroups:

- 'triticeae': which contains wheat, rye and barley
- 'aveneae': which contains oats.

This reflects the fact that there is a structural difference between the wheat, rye and barley when compared with the oat plant. Avenins in oats constitute only 5-15 % of the total seed protein content, significantly less than in wheat, barley and rye (Table 1.1). Even if oat avenins are damaging to the gut in patients with coeliac disease it is likely to require a much larger intake of oats to bring about an equivalent pathological effect in terms of grams of protein ingested.

The cereal chemistry of oats gives further evidence that it may not be damaging to coeliacs.

The amino acid sequences, 'proline-serine-glutamine-glutamine' or 'glutamine-glutamine-glutamine-proline', are found commonly in the prolamins that cause mucosal damage in coeliac disease. One molecule of wheat prolamin (α-gliadin) contains 5 such sequences; prolamins of barley (β-1-hordein) and oats (avenin) contain 2 each and maize (α-zein) contains none *(de Ritis et al, 1988)*. Now that the immunogenic epitope on A-gliadin and other proteins have been discovered, it remains to be seen if these are found in avenin. *Shan et al*

(2002) report that oat avenin does not contain homologues of an undigested 33 amino acid sequence found in gliadin, secalin and hordein which is deamidated by tTG.

It remains unclear whether oats can be safely consumed by people with coeliac disease. *Troncone et al* concluded in 1996 that "oat prolamins are able to activate the T-cell mediated mucosal immune response in the coeliac jejunum, and represent a warning against the inclusion of oats in the diet of coeliac patients." However, today the results of many small short-term clinical and histological studies seem to indicate that moderate amounts of oat, (50g per day), can be consumed by adults with coeliac disease and dermatitis herpetiformis without mucosal changes or development of symptoms *(Janatuinen et al, 1995: Reunala et al, 1998; Hardman et al, 1999)*. Half this quantity of oats can be consumed daily by children with similar results *(Hoffenberg et al, 2000)*. These authors cautioned that the short-term study results should not be extrapolated to the safety of oats in the long-term. A subsequent 5-year study concluded that it is safe to include oats in a GFD (*Janatuinen et al, 2002).* These authors reported that the Finnish and United Kingdom Coeliac Societies have recently modified their dietary guidelines recently to include a statement that "moderate amounts of oats can be consumed by most coeliac patients without risk". The American Gastroenterological Association (AmGA) medical position statement says that oats may be permitted but then goes on to caution that the majority of commercially available oat flours were contaminated with wheat gluten *(AmGA-1, 2001*). Sweden has also developed a national policy which allows moderate amounts of oats to be returned to the diet of people with coeliac disease *(Hallert et al, 1999).*

PATHOGENESIS

A number of theories have been proposed over the years to explain the origins of coeliac disease. The current view is that the pathogenesis of coeliac disease involves interactions between genetic, immunological and environmental factors (*Ciclitira, 1999, Chpt 74*).

Environmental factors

The ingestion of gluten and the immunogenic sites on gliadin are very important in the development of small bowel pathology characteristic of coeliac disease *(Anderson et al, 2000; Vader et al 2002; Schuppan & Hahn, 2002).* While delayed introduction of gluten into the diet does not prevent the ultimate development of coeliac disease, breastfeeding has been reported to delay the age of onset of symptoms and to decrease the ultimate risk of developing coeliac disease (*Auricchio et al, 1983; Stevens et al, 1988; Persson, 2002; Nash, 2003).* In identical twins only 75% are concordant for coeliac disease (*Polanco et al, 1981).* The 25% discordance suggests that environmental factors other than gluten may be involved *(Kagnoff, 1992; Holtmeier et al, 1997; Hernell et al, 2001)*. These environmental triggers could include intercurrent infections, or major stresses on metabolism by surgery, trauma or pregnancy *(Marsh, 1992; Anderson et al, 2000; Persson, 2002)*

Genetic Factors

There is a roughly a 10% increase in prevalence of coeliac disease amongst first-degree relatives *(Stokes et al, 1976)*. The high concordance of coeliac disease amongst twins is also indicative of genetic links (*Polanco et al, 1981; Greco et al, 2002)*. More recent twin studies report discordance where long term follow-up has shown that the healthy twin went on to develop coeliac disease *(AmGA-2, 2001).*

For nearly 30 years it has been recognized that individuals with certain human leukocyte antigen alleles (HLA) have a greater prevalence of coeliac disease. Three clusters of genes encoding HLA antigens (Class I, II, III) together form the major histocompatibility gene complex (MHC) on the short arm of chromosome 6 *(Korman et al, 1985)*. Class II genes can be divided into 3 sub-regions called HLA-DP, HLA-DQ and HLA-DR *(Kagnoff, 1990)*. Coeliac disease has a strong association with HLA-DQ2 and with HLA-DR3 *(Godkin & Jewell, 1998; AmGA-2, 2001).* The HLA-DR3 association is thought to be secondary, as a result of its linkage disequilibrium with HLA-DQ2 *(AmGA-2, 2001)*. In Southern Europe and Israel, HLA-DQ8, in association with HLA-DR4, is found more commonly in those with coeliac disease *(AmGA-2, 2001; Godkin & Jewell, 1998)*. Although HLA-DQ2 is present in 90% or more of Caucasians who have coeliac disease (*Kagnoff, 1990; Sollid & Thorsby, 1993*), it should also be noted that approximately 25% of Northern Europeans carry DQ2, the vast majority of whom never develop coeliac disease *(AmGA-2, 2001*).

Susceptibility to coeliac disease in families is largely determined by possession of certain HLA-DQ genes. However not all people with these genes develop coeliac disease, suggesting that other non-HLA genes may also be involved *(AmGA-2, 2001).*

Immunological factors

The discovery that tissue transglutaminase (tTG) is the antigen of endomysial antibody A (EMA) in coeliac disease was a major breakthrough in understanding the pathogenesis *(Dieterich et al, 1997)*. Tissue transglutaminase is a widespread intercellular enzyme that is capable of catalysing bonds between glutamine and lysine residues. It is synthesised in small amounts by mononuclear cells, fibroblasts and endothelial cells that reside in the gut and is

stored in these subepithelial cells in an inactive form (*Schuppan et al, 1998; Molberg et al, 1998;Schuppan & Hahn, 2002*). Mechanical or inflammatory stress releases the enzyme.

The prolamins that are damaging in coeliac disease are unique because they are rich in proline and glutamine *(Shan et al, 2002)*, for which tTG has a strong affinity *(Schuppan & Hahn, 2002)*. *Anderson et al, (2000)* concluded that the dominant A-gliadin peptide 57-73 (QLQPFPQPELPYPQPQS) is recognized by tTG, although only the glutamine residue in position 65 is deamidated to ultimately allow the gliadin molecule to bind to the HLA-DQ2 molecule. *Shan et al (2002*) found that wheat gliadin, rye secalin and barley hordein contain homologues of a 33 amino acid sequence that is not broken down by any of the digestive or brush border enzymes and went on to show that it is the positioning of the prolines, the second most abundant amino acid in gluten, with respect to the glutamines that determines which glutamines will react with the tTG *(Vader et al, 2002)*. It is now recognised that multiple sites are deamidated to allow binding to the HLA-DQ2 and/or DQ8 molecules *(Vader et al,2002; Schuppan & Hahn, 2002).*

The process that eventually leads to the destruction of the villous structure begins when gliadin comes in contact with tTG (Figure 1.4a). The tTG deamidates selected glutamine residues, converting them to negatively charged glutamic acid, a necessary condition for binding to HLA-DQ2 and DQ8 molecules. This activates CD4+ inflammatory T-cells which produce mucosal damage and stimulate the production of antibodies to gluten and tTG *(Mowat, 2000; Shan et al, 2002; Schuppan & Hahn, 2002; Farrell & Kelly 2002).* Target sites have now been found in the other immunogenic prolamins, secalin and hordein, but homologous sites have not been identified in oats or other “non-toxic” grains such as rice and maize (*Shan et al, 2002*).

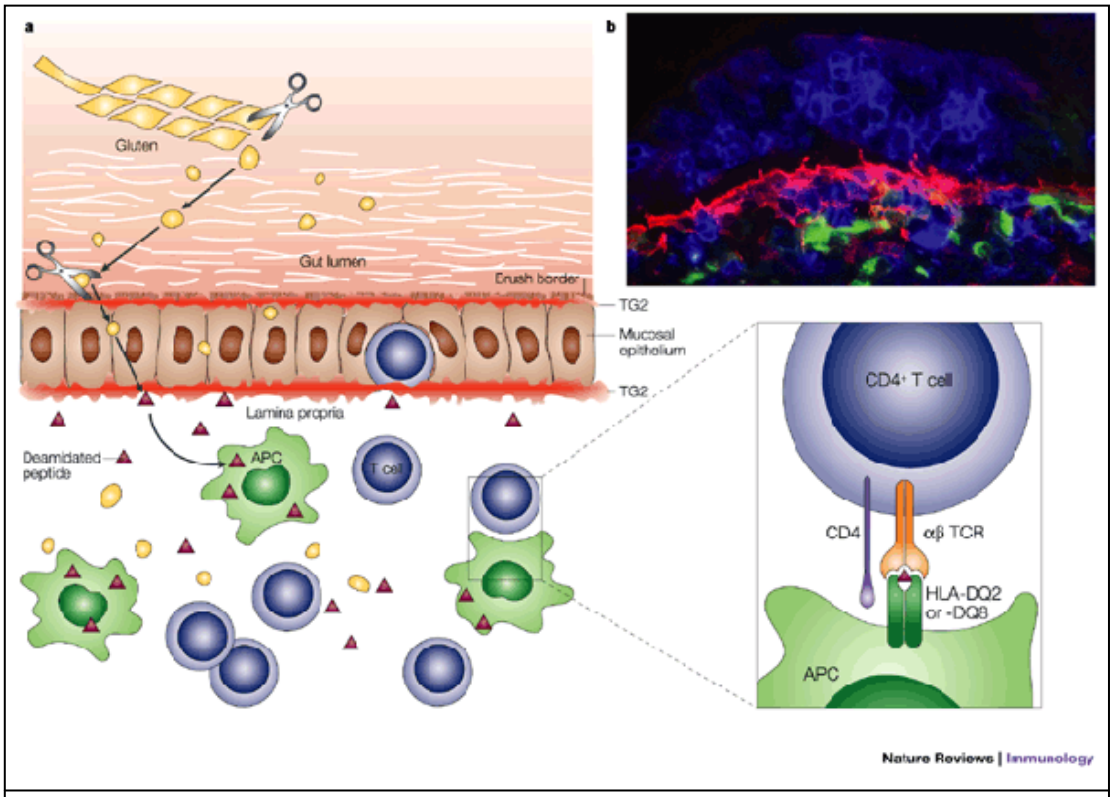

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Figure 1.4: Factors leading up to the development of coeliac disease. (a) depicts the process described above. TG2=tTG; ▲ = De-amidated gliadin & tTG complex; APC=antigen presenting cell; (b) Immunofluorescence staining of the mucosa in coeliac disease where tTG is pink, HLA-DQ is green and T-cells are purple.

Numerous antibodies are produced in response to the damage caused by T-cells in the presence of gluten in the small intestine. Antibodies to gliadin (anti-gliadin antibody, AGA) are produced locally in the intestinal mucosa when gluten containing grains are ingested by people with coeliac disease. These antibodies are detected in the sera of the majority of people with untreated coeliac disease (but may not appear in those who are IgA deficient). AGA can also be detected in patients with cow's milk intolerance, IgA nephropathy, parasite infections, other gastrointestinal diseases, (e.g. Crohn's disease, eosinophilic enteritis, tropical sprue) and in some apparently normal people. Since their specificity is low, AGA is not a good

marker of disease and may reflect only the increased permeability of the intestinal mucosa to certain food proteins (*AmGA-2, 2001; Fasano & Catassi, 2001*).

When damage occurs to the villi, antibodies are also produced against the endomysium. This is a connective tissue structure that lies between the myofibrils in the gastrointestinal tract *(AmGA-2, 2001).* Measurements of this antibody has higher predictive value than the anti-gliadin antibodies but may still be negative in those with IgA deficiency. It is also not reliable in children under age 2 *(Fasano & Catassi, 2001).*

In 1997 Dieterich et al, was able to show that endomysial antibodies were directed at tTG. Anti-tTG antibodies are now utilized as a serological screening test for coeliac disease.

IMMUNOASSAYS FOR GLUTEN

Given the growing use of gluten containing products throughout the food industry and the requirement of gluten-free diets, the need arose to develop a simple method to measure the gluten content in the foods. As the gliadins are readily heat-denatured, many tests available before 1990 were not reliable for quantitation of gluten once the gluten protein had been heated or processed. *Skerritt & Hill, (1991)* devised a new ELISA (enzyme linked immunosorbent assay) test based on monoclonal antibodies to cross-reactive heat-stable omega-gliadins. The antibodies selected for the assay bound to proteins that were not denatured by heat. Two tests were developed *(Skerritt et al, 1991)*. The first, the “gluten Lab-Test”, was designed for use by industry to test uncooked, cooked and processed foods. The second, the “Rapid Gluten Test Kit” was designed to provide semi-quantitative results for use in the home by people with coeliac disease who were unsure of the gluten content of a poorly labelled manufactured product or a restaurant meal.

The monoclonal antibodies used bind with equal strength to the prolamins from wheat, rye, triticale and barley, but not to the prolamins from rice and maize. The food sample is prepared according to the manufacturers instructions and is then incubated with the monoclonal antibody before being incubated with an enzyme conjugated antibody. A chromogenic substrate is added to the complex and the colour response compared with a gliadin standard. The published sensitivity of the assay is 0.001% gluten *(Skerritt et al, 1991)* and the limit of the detection of the assay is 3ng gliadin. However the manufacturers of gluten-free food use a lower limit of detection of 0.003% gluten produced in kits supplied by Medical Innovations and used by ANZFA in the new food standard that came into effect in 1995. Similar monoclonal antibody tests have been developed in the United Kingdom *(Freedman et al, 1987; Ciclitira & Ellis, 1991).*

In 1994, when ANZFA announced the new "no detectable gluten" standard for the labelling of foods as "gluten-free", this was to be defined by the lowest limit of detection of the best test available when the food was produced. At that time the lower limit was <0.003% *(Medical Innovations)*. Since consistent results can now be obtained at levels of 0.001- 0.002% gluten, as of January 2003 foods can only be labelled "gluten-free" in Australia and New Zealand, if they contain less than 0.001% gluten and no oats or malt *(FSANZ, 2002).* Central to the testing method is the ability of the ELISA test to detect the presence of gliadins. As a result, assays of foods consisting largely of other prolamins, such as malt (hordein) and oats (avenin) may underestimate the total gluten content of the food and for this reason any food containing malt or oats cannot be labelled gluten-free, even though the ELISA test may be negative (*FSANZ, 2002*).

DIAGNOSIS

Small Bowel Biopsy:

Coeliac disease is defined in terms of the small bowel's morphological response to the presence or absence of gluten, demonstrated by small intestinal biopsy. Biopsy specimens are generally obtained from the duodenum by endoscopy but may also be taken from the jejunum with a special purpose biopsy capsule or very recently by enteroscopy.

The guidelines of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN), for the diagnosis of coeliac disease require evidence of villous atrophy while eating gluten, with improvement or normalization of villous architecture on a gluten-free diet, *(Mäki & Collin, 1997)*. The second biopsy is usually performed 6 months after beginning a gluten-free diet.

Serological screening tests:

Although there are now a number of serological screening tests available, none can substitute for histological diagnosis.

Antigliadin Antibody

Until recently, the most widely used serological markers were IgG and IgA anti-gliadin antibodies (AGA). The sensitivity and specificity of IgG AGA are too low to make this test of diagnostic value, (Table 1.2). False negative IgA AGA can occur because of the association between coeliac disease and IgA deficiency (3-5%). The highest AGA levels are found in untreated subjects, with a progressive fall during the early months of dietary gluten exclusion (*Marsh, 1992, Chpt 3,p. 60)*.

Serological Test	*Sensitivity (%)	*Specificity (%)
IgA AGA	75 - 90	82 - 95
IgG AGA	69 - 85	73 - 90
IgA AEA immunofluorescence assay	85 - 98	97 - 100
tTG (ELISA, guinea pig tissue)	95 - 98	94 - 95
tTG (Dot blot, human tissue)	93	99
* The sensitivity and specificity vary greatly with the different laboratories. *Adapted from Farrell & Kelly, 2002*.		

Table 1.2: Reported sensitivities and specificities of laboratory tests.

Anti-endomysial and anti-tTG Antibodies

The Anti-endomysial antibody (EMA) assay was developed in the 1990's and has greater diagnostic sensitivity and specificity (>95%) for coeliac disease than AGA (*Dahele & Ghosh, 2000).* Although IgA and IgG EMA tests have been developed, the former is generally preferred. *Rostami et al, (1999-b)* report a correlation between EMA and villous atrophy and the positive response rates of the EMA blood tests. In each subject who had total villous atrophy (TVA), EMA was positive. EMA was positive in 70% of those with sub total villous atrophy (STVA) and in 31% with partial villous atrophy (PVA).

It is now known that the antigen recognized by the anti-endomysial antibody is tTG *(Dieterich et al, 1997).* The antiendomysial antibody test is an immunofluorescence assays and are qualitative, subject to the interpretation of the microscopist. IgG and IgA antibodies to tTG are measured by a quantitative ELISA method. The sensitivity and specificity of the assay is slightly less than for EMA, but is improved if human rather than guinea pig tTG is used (Table 1.2) (*Troncone et al, 1999; Wong et al, 2002; Farrell & Kelly, 2002).*

These serological tests can be used to screen people who are at increased risk of coeliac disease such as first-degree relatives, those with Type I diabetes or other autoimmune disorders, or one of the less common associations with coeliac disease. A positive antibody test alone is insufficient for the diagnosis of coeliac disease and must be followed by a demonstration of an abnormal small bowel biopsy before a GFD is instituted. Histological improvement demonstrated subsequently (usually 6-12 months later) confirms the diagnosis.

There is no place for an empirical trial of a gluten free diet to diagnose coeliac disease. Patients with non-coeliac gluten intolerance and/or irritable bowel syndrome often improve symptomatically when wheat is excluded from their diet. Many such patients will subsequently want to know if they have coeliac disease or not. Since serological markers become negative and the small bowel mucosa returns to normal in coeliac patients on a GFD, accurate diagnosis requires a period of gluten challenge which many patients find difficult because of the return of symptoms.

CLINICAL FEATURES

Coeliac disease was once thought to be a childhood disease that was out-grown. The classical description of such children included features of malabsorption (of virtually all nutrients), weight loss or failure to thrive, abdominal cramping, bloating, flatus, nausea, vomiting, muscle wasting, diarrhoea, steatorrhoea, weakness and bruising *(Digestive Health Foundation, 2000).* Characteristically the children had a very distended abdomen and wasted buttocks.

Coeliac disease was also subsequently recognised in adults who presented with the classic symptoms of malabsorption (often with the depletion of key nutrients such as iron, and folate),

weight loss, peripheral oedema, diarrhoea, abdominal cramp, bloating, flatus, nausea and vomiting.

In recent years, the clinical picture of coeliac disease has changed. This classic group of symptoms is relatively uncommon. It has become evident that minor, non-specific complaints such as fatigue, loose stools, recurrent mouth ulcers and flatulence can be a result of underlying coeliac disease. Unexplained anaemia, secondary to iron and/or folate deficiency, is another common presentation. In adults and children, features can include: delayed menarche; recurrent miscarriages; infertility in either gender; enamel defects of permanent teeth; ataxia; mood disturbances; general malaise; failure to thrive or meet growth potential; and/or sub-optimal school performance *(Farrell & Kelly, 2002; Fraser & Ciclitira, 2001)*.

Coeliac disease is associated with a number of immunological and non-immunological disorders which may be the dominant clinical presentation (discussed below). This diversity of presentations of coeliac disease can lead to delayed or misdiagnosis in up to 60% of adults.

THE SPECTRUM OF COELIAC DISEASE

Coeliac disease can begin at any age, presumably due to the interaction between genetic factors and chance encounters with environmental stimuli. It is assumed that, once triggered, continued exposure to gluten leads to progressive mucosal damage and, eventually, clinical manifestations.

It has become evident that there are varying degrees of mucosal abnormality, short of full-blown villous atrophy. Moreover, mucosal changes may or may not be associated with clinical symptoms. With the advent of serological screening tests many patients with asymptomatic

coeliac disease are being detected in population surveys *(McNicholl et al, 1975; Ciclitira & Hall, 1990; Collin & Mäki, 1994; Visakorpi & Mäki, 1994; Marsh, 1995; Ferguson, 1995).*

This has led to the proposal that several coeliac disease categories can be recognized: the so-called “coeliac iceberg”.

1. Silent coeliac disease
2. Clinical coeliac disease
3. Potential and Latent coeliac disease

Silent Coeliac Disease

This term applies to a “patient with manifest intestinal mucosal changes typical of CD, which can be restored to normal with a gluten-free diet, but who has no florid clinical symptoms” *(Visakorpi, 1994: Mäki & Collin, 1997).* Most patients falling into this category have been detected using antibody screening, for example, in family members of affected subjects or in type 1 diabetes mellitus. The size of this group will depend on awareness of the possibility of coeliac disease and the availability of antibody testing. Although the term implies absence of clinical problems, in many individuals this is not the case. Symptoms may be subtle, for example, tiredness, poor school performance or behavioural problems in children. Iron deficiency or reduced bone mineral density can be other manifestations (*Fasano & Catassi, 2001)*. Many of those with silent coeliac disease who were supposedly asymptomatic on screening, often report improvement in their overall well-being after starting a GFD (*Fasano & Catassi, 2001).*

Clinical Coeliac Disease

This term applies to the clinically symptomatic patient who has typical villous atrophy with clinical improvement and histological normalization on a gluten-free diet. *(Mäki & Collin, 1997)*.

Potential and Latent Coeliac Disease

Several case reports in the literature have documented the development of CD in patients who were known to have previously normal mucosa *(McConnell & Whitewell, 1975; Egan-Mitchel et al, 1981; Mäki et al, 1990; Mäki et al, 1991 a, b).* The terms 'potential' and 'latent' have been used in the past to describe such individuals, as well as asymptomatic people suspected to be at risk of developing classical CD lesions due to the presence of AGA antibodies and increased IEL counts in the absence of other mucosal abnormalities *(Ferguson & Murray, 1971; Egan-Mitchel et al, 1981; Mäki et al, 1991-b; Collin et al, 1993; Ferguson et al, 1993; Ferguson, 1995)*. More recently, the concept of a ***coeliac disease trait*** has emerged with the advent of more specific serological tests, along with HLA-DQ2, DQ8 or DR3 genotyping *(Fasano, 2003).*

ASSOCIATED DISORDERS

The emphasis on the malignant consequences of coeliac disease has often overshadowed the non-malignant complications, even though some of these can significantly affect quality of life.

Dermatitis Herpetiformis

Dermatitis Herpetiformis (DH) is a gluten sensitive, blistering skin disease that is strongly associated with coeliac disease and is sometimes referred to as "Coeliac disease of the skin". It is characterized by eruptions of itchy blisters typically around the elbows, knees and buttocks. Although DH can occur without underlying coeliac disease, this is very uncommon, particularly if subtle mucosal changes such as increased IEL counts are considered. Less than 10% of patients with DH have gastrointestinal symptoms, even though all of them may have coeliac disease *(Reunala, 1998)*. Thus, DH is an example of gastroenterologically 'silent', but dermatologically active coeliac disease.

The diagnosis of DH is made by finding granular IgA deposits in the dermal papillae on skin biopsy. It is generally recommended that patients with DH start a GFD. This will usually result in improvement in the rash, although it may take up to 2 years for the rash to be controlled. The rash can return within about 12 weeks if gluten is re-introduced into the regular diet.

Dapsone (a sulphonamide) usually is given to help control the rash in the short term. In those who improve with a GFD, Dapsone can usually be stopped after about 2 years, with the GFD continuing for life.

Type 1 Diabetes Mellitus

This disorder is strongly associated with coeliac disease, both in adults and children with a frequency of 1:25 – 1:50 or 2-5%of the type 1 diabetes mellitus population *(Gadd et al, 1992; Poececco & Ventura, 1995; Westman et al, 1999)*. This increased risk is because of the common HLA DR3 – DQ2 haplotype *(Sategna-Guidetti et al, 1994; Sjoberg et al, 1998; Cronin et al, 1997).* Both conditions are usually diagnosed before the age of 30. Diabetes usually develops first. Many of these patients have no overt manifestations of coeliac disease. However delayed growth, non-specific symptoms and iron deficiency are more common than expected and have often been overlooked *(Cronin et al, 1997; Sategna-Guidetti et al, 1994*). It is now recommended that all newly diagnosed patients with type 1 diabetes mellitus be screened for coeliac disease once a year for at least 2 years after the diagnosis of diabetes.

Autoimmune Thyroid disease

Autoimmune Thyroid Disease (ATD) is also common in patients with coeliac disease again because the diseases share a common HLA genetic background *(Catassi et al, 1994; Velluzzi*

et al, 1998; Valentino et al, 1999; Cuoco et al, 1999; Larizza et al, 2001). The reported frequency varies but is estimated by one group to be approximately 1:200 *(Velluzzi et al, 1998)*. ATD occurs in 14-30% of patients with coeliac disease *(Velluzzi et al, 1998).*

Whether a GFD improves thyroid function and reduces antibody titres is debatable (*Valentino et al, 1999; Larizza et al, 2001)*. It has been suggested that if coeliac disease can be diagnosed and the GFD instigated before the age of 10, then this may prevent the onset of other autoimmune disorders *(Ventura et al, 1999)*.

Down's Syndrome

Coeliac disease is 20-43 times more prevalent in Down's Syndrome than in the normal population *(Similä & Kokkonen, 1990; Hilhorst et al, 1993; Gale et al, 1997)*. A number of reasons have been postulated to try to explain this finding. Down's Syndrome children are usually weaned early which has been shown to lead to a 5-fold increase in coeliac disease (*Greco et al, 1988*). Children with Down's Syndrome have a variety of immunological disturbances and show evidence of premature aging and associated loss of T-cell function *(Gale et al, 1997).* The frequency of gastro-intestinal infections amongst Down's Syndrome children who reside in institutions is also increased and this may interfere with the integrity of the small bowel (*Gale et al, 1997)*.

The frequency of false positive AGA is said to be high in Down's Syndrome, so anti-endomysial or anti-tTG antibodies are more appropriate screening tests and should be repeated more than once (*Gale et al, 1997)*.

Neurological Disorders

Neurological syndromes are a recognized but unusual complication of coeliac disease that can occur in about 8-10% of patients (*Pellecchia et al, 1999)*. The neurological syndromes can include ataxia, peripheral neuropathy, dementia, epilepsy and myopathy *(Hadjivassiliou et al, 1996; Pellecchia et al, 1999).* Although earlier reports described these disorders in symptomatic coeliacs, the majority are now diagnosed through screening tests.

Ataxia is the most common neurological manifestation of coeliac disease *(Hadjivassiliou et al, 1998)*. The term "gluten ataxia" has been used to describe it. It is mostly found when patients presenting with idiopathic ataxia are screened using AGA or EMA. It is not clear whether a gluten-free diet can reverse the ataxia, with some authors reporting improvement and some not (*Bhatia et al, 1995; Hadjivassiliou et al, 1996; Beversdor et al, 1996; Hadjivassiliou et al, 1998; Pellecchia et al, 1999)*[2]*.* As malabsorption is not widely seen in this group, treatment with vitamin and mineral supplements appears to be ineffectual as well *(Mauro et al, 1991; Battisti et al, 1996; Muller et al, 1996; Pellecchia et al, 1999).*

The pathogenesis of these neurological disorders is unknown. The villous atrophy in these patients does not always recover on a GFD leading to the supposition that perhaps it is this persistently abnormal mucosa that underlies the development of neurological disorders *Muller et al, 1996)*. Alternatively anti-gliadin anti-bodies may react against cross-reacting antigens in the brain and spinal cord *(Hadjivassiliou et al, 1998; Pellecchia et al, 1999).*

[2] One such patient seen at the RPAH Allergy Unit has had a dramatic improvement on a GFD and relapses within 24 hours after challenge or inadvertent exposure.

COMPLICATIONS

Anaemia

When iron deficiency anaemia is unresponsive to oral iron, it is highly suggestive of gluten-induced malabsorption. This sign that CD may be present in an individual is gaining recognition. Many years ago the malabsorption of iron in coeliac disease was studied in terms of its haem and non-haem states. *Rubin, (1960)* postulated that the non-haem iron (Fe^{++}) was maximally absorbed in the duodenum and the upper jejunum, where the small intestine is thought to be more damaged. Therefore malabsorption of this type of iron was probably related to the extent of the small bowel mucosal damage. *Anand et al, (1977)* have also reported that the absorption of non-haem iron is reduced in people with untreated coeliac disease. However they describe that the absorption of haemoglobin iron remains the same in the treated and untreated state because the haemoglobin is likely to be in an absorbable form when it reaches the lower more normal small bowel mucosa in both the untreated and treated coeliac. A number of studies have now shown that this iron deficiency improves once a gluten-free diet is instigated *(Rubin, 1960; Anand et al, 1977; Björkman et al, 1985; Collins et al, 1986).*

Low Bone Mineral Density

An individual's normal bone mineral density (BMD) is determined by multiple genetic and environmental factors with estimates of heredity accounting for up to 70 percent of the variance in bone density in women and men *(Slemenda et al, 1991; Riggs, 1997)*. It is now well described that osteoporosis and osteopenia are found in the majority of patients with undiagnosed coeliac disease *(Marsh, 1994; Valdimarsson et al, 1994; Walters et al, 1995; Pistorius et al, 1995; Mora, 1999)*. The frequency of unrecognised coeliac disease is also

increased in a population attending an osteoporosis clinic *(McFarlane et al, 1992; Butcher et, 1992; Mather et al, 2001; Meyer et al, 2001).* In one study, approximately 50% of women and men had an osteoporosis diagnosis (*McFarlane et al, 1992).* Reduced BMD is also found in children (*Bayer et al, 1998).* Reduced bone mass may be more common in males than females, at any age *(Bayer et al, 1998; Meyer et al, 2001)* although this has not been a universal finding (*McFarlane et al, 1992; Mora, 1999).*

Malignancy

Patients with untreated coeliac disease are at increased risk of malignancies, in particular cancers of the mouth, oesophagus and especially small bowel lymphoma. Up to 1 in 10 will be affected *(Holmes et al, 1989; Corrao et al, 2001).* The frequency of lymphoma is also increased in DH *(Collin et al, 1996). Holmes et al, (1989)* found that the risk returns to that of the general population on a GFD, although for lymphoma this took 5 years or more. This study also showed that the risk remained elevated in those taking reduced gluten, in which people irregularly ate normal gluten-containing foods, providing evidence that coeliacs should remain on a gluten-free diet for life, despite the presence or absence of symptoms.

It is worth emphasizing that at the time of Holmes' study, *Codex Alimentarius* food labelling guidelines were in use in the United Kingdom. As a result malt and wheat starch would have been eaten by people on a 'gluten-free diet'. This suggests that the risk of developing malignancy is not increased when on a GFD containing wheat starch and malt.

DIETARY MANAGEMENT OF COELIAC DISEASE.

Samuel Gee was the first to suggest that the cure for coeliac disease might be found in diet *(Gee, 1888).* In 1924 Sidney Haas reported that his banana diet was the cure *(Haas, 1924)*. Many additional dietary interventions were tried during this first half of the twentieth century. Examples included a carbohydrate diet of fruit plus a puree of potatoes or tomatoes, a beefsteak diet, a milk diet (2-2.5 l/day) or a fruit only diet *(Van Berge-Henegouwen & Mulder, 1993)*. In the 1930's a Dutch Paediatrician, Willem-Karel Dicke, began experiments with wheat-free diets, although the generally recommended treatment until after World War II was a fat free diet, used to prevent the complications of steatorrhoea *(Marsh, 1992)*. Throughout this period Dicke noted that when the children in his ward were fed wheat starch, there was an improvement in their symptoms, but it was not until the 1950's that it became generally accepted that clinical improvement in coeliac disease correlated with the removal of gluten from the diet.

The Gluten-Free Diet:

Before the introduction of the GFD the published mortality rates for people with coeliac disease were between 10-30% *(AmGA, 2001).* With the introduction of the GFD these rates fell dramatically to 0.4% in one publication *(Sheldon, 1969).*

Although it is often incorrectly assumed to be a straightforward subject, the question of what constitutes a gluten-free diet remains highly controversial worldwide. A series of investigations beginning in the 1950's concluded that wheat, rye, barley and oats were harmful to people with coeliac disease *(Dicke, 1950; Dicke et al, 1953; van de Kramer et al, 1953)*. Rice flour, maize (corn) flour, buckwheat flour, potatoes and wheat starch were considered gluten free and acceptable for use in the diet *(Dicke, 1950; Dicke et al, 1953; van de Kramer et al, 1953).* Triticale (a hybrid of wheat and rye) is also included in the harmful grains (*Anand et al, 1978)*.

While not eating products made with wheat, triticale, rye and barley may seem to be simple, following a GFD has become more complicated because over the last twenty years what needs to be avoided has evolved to include not only the parent gluten-containing grains, but also the many ingredients that have been derived from them. Examples include wheat starch, wheat-derived thickeners (1400-1450), malt (from barley) and beer, to name a few. The advent of more sensitive testing methods for gluten has shown that residual amounts of gluten remain detectable in these ingredients.

Recent studies suggest that oats may not be damaging in coeliac disease (as discussed above). Even if not, there is still concern that oats may have been grown, transported, stored or processed in situations where cross-contamination from wheat, barley and rye might have occurred. A five-year follow-up study in Finland (*Janatuinen et al, 2002*) has concluded that oats are acceptable for consumption by the majority of people with coeliac disease. However they state that oats in Finland are not contaminated and the results cannot necessarily be applied worldwide. In Australia it is still recommended that oats be avoided because of contamination. Australia's largest oat producer has estimated that the wheat contamination of their oats is 0.004-0.005% *(Personal communication, Mr. Frank Lee, Goodman & Fielder, Uncle Toby's Oats, Oct 2002).* The recent addition of oats back into the diet of people with coeliac disease in other countries, adds another level of complexity to the definition of a GFD.

Wheat Starch and Residual Gluten

The experiments with wheat starch were first performed by Dicke in children, who were found to recover well *(Dicke, 1950; Dicke et al, 1953; van de Kramer et al, 1953)*. Wheat starch is the carbohydrate component of the wheat grain (Figure 1.5). To extract this, the grain is first soaked for a number of days. The soaked grains are then drained and dried to extract the starch. Although the original use for wheat starch was to stiffen clothing, today it is used by the

food industry mainly as a thickening agent for pie fillings and sauces, gravies, pancake mixes, biscuits, wafers, baby foods, custard powders, ice-cream, in baking powder and as a carrier and filler in meat products *(Rogers, 1990).*

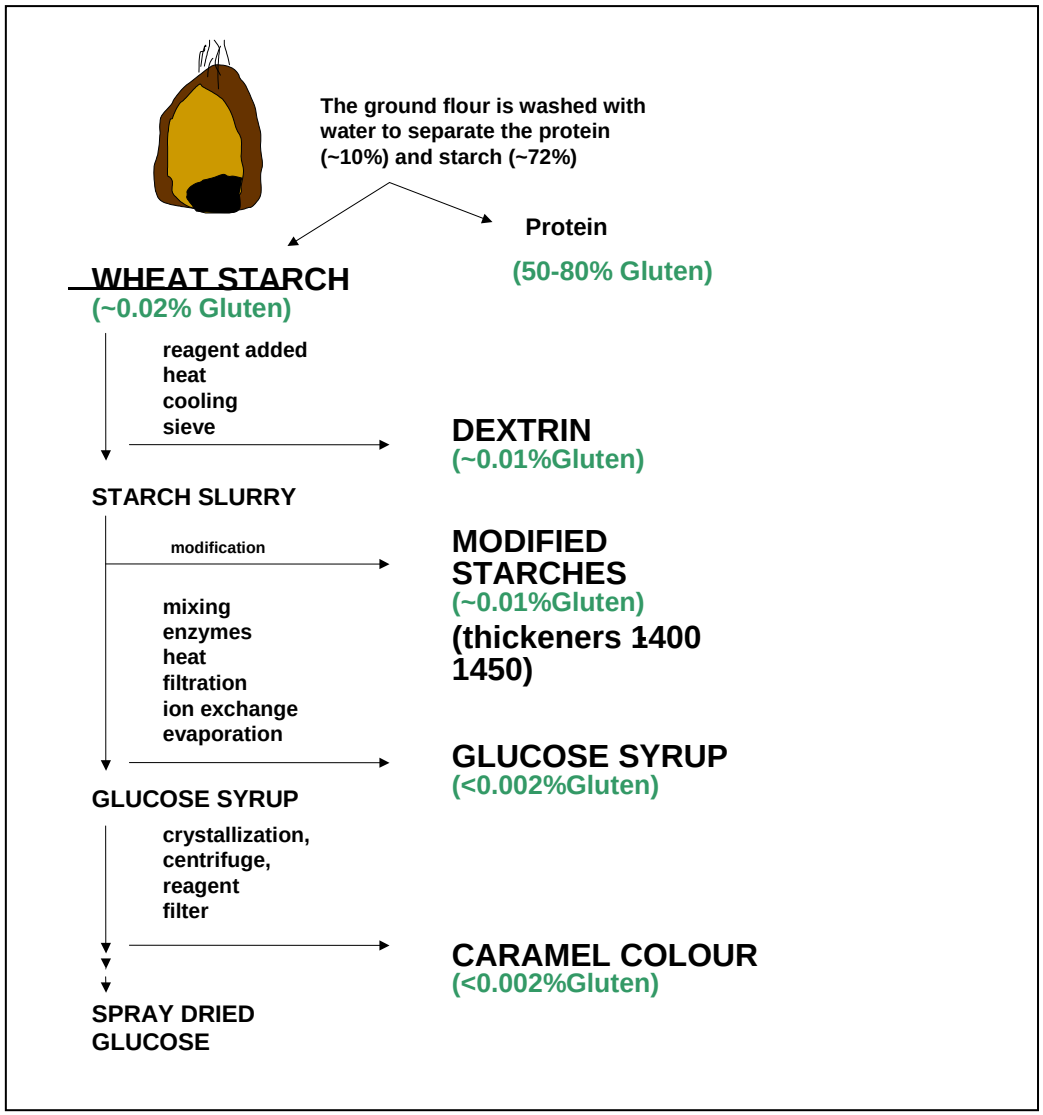


Figure 1.5: Approximate gluten content of ingredients derived from wheat. (Values for derivatives of wheat starch obtained from communications with Starch Australia, as mentioned above).

This extraction process does not remove the entire protein component of the grain. The development of sensitive immuno-assays for gluten have made possible the detection of the residual amounts of gluten in wheat starch. A great deal of current research is devoted to the question of "how much gluten in a coeliac's diet is too much gluten"? The quantity of gluten

remaining in wheat starch is largely dependent on how thoroughly the wheat flour has been washed beforehand. A Danish study has reported up to 1% gluten remaining in wheat starch *(Mulder et al, 1993)*. *Skerritt et al, (1987)* stated that, in Australia, "industrial ***wheat starch*** is separated by a thorough washing process, which yields a product that contains 0.05% nitrogen…almost of all of the protein nitrogen is from non-starch granule associated proteins". It has been estimated by the Australian wheat starch producers, in 1996, that approximately 0.02% of gluten remains in the end product *(personal communication; Mr. Brian Nicholson & Ms. Jenny Smit)*. As wheat starch is refined into its component carbohydrates for various industrial and food uses, additional gluten is removed during production (Figure 1.5). As a result many of these products contain less gluten than can be detected using currently available assays.

Wheat is not the only source of the ingredients shown in Figure 1.5. However, in Australia, wheat is the predominant grain grown and is usually the cheaper source of starch from which many other ingredients, used in the food industry, can be produced. Maize, tapioca or rice (which do not contain gluten) are the predominant grains in other countries.

The term ***cornflour*** is confusing and warrants mention, as the starch can be derived primarily from either wheat or maize. The former contains gluten (small amounts of which are retained in the extraction process), whereas the latter is gluten-free. Since the introduction of the new *Australian Food Standard*, in 2003, this source must now be indicated on the product label.

Malt

Malt is derived predominantly from barley. Grains of barley (or sometimes wheat, rye or rice) are soaked in water to soften and germinate. The purpose of this is to activate certain enzymes that are useful in baking, brewing and in the production of distilled alcoholic drinks.

After the emergence of the sprouts, the germination is carefully stopped so as to retain the desired enzyme activity. The industrial value of the malt depends on the enzymatic activity being retained. During germination the grain starch is converted to sugar, which when kiln dried is called 'malt' *(Rogers, 1990)*. The malt is then milled to break open the grain and release the sugar syrup, which, when dried under vacuum, produces malt extract. Malt extract, containing small quantities of hordein (*Skerritt & Hill, 1991),* is mainly used for brewing beer, distilling spirits, flavouring milk drinks and as a sweetener coating many packaged breakfast cereals. Malt flour is stone or roller-milled wheat or barley malt and is used as an improver in the baking industry *(Rogers, 1990)*. It is generally understood that when the term malt is used alone (without indicating the grain source) it refers to malt from barley. ***Rice malt*** is occasionally seen as an ingredient and it is gluten-free.

The inclusion of malt in the GFD remains controversial. While dietary recommendations for coeliac patients in Europe and the United Kingdom include this ingredient, it is not always recommended in Canada, the United States or Australia *(AmGA-1, 2001; FSANZ).* Malt was one of the ingredients removed in Australia after the change in Food Standards in 1995 because of the likelihood of small amounts of hordein that may go undetected by the commercial gliadin-based test kits.

Gluten fillers

Non-meat proteins such as gluten are also widely used in processed foods because they prevent the loss of fat or water and so improve the cooking yield, they act as a binder in meat to increases the ease with which meat can be sliced; they enhance the stability of oil/water emulsions in food; and they save on ingredient costs by maintaining the protein content of a meat product. Wheat flour and poor quality wheat starch are used frequently as a thickening agent in soups and desserts. Gluten can also be present in products such as pharmaceuticals

and confectionery *(Skerritt & Hill, 1991)*. Foods or products containing these fillers also should be avoided in a GFD.

WHAT IS A GLUTEN-FREE DIET?

Labelling Foods Gluten-Free

The term *'Gluten-Free Diet'* implies that all of the foods eaten contain zero gluten. This has always been a misconception. Even though they are labelled gluten-free, commercial foods in most countries often contain small amounts of gluten, depending on the specific food standards governing food labelling in the country where the food is manufactured *(see Introduction)*.

The latest report from the *Codex Alimentarius* Commission states that a food can be labelled as gluten free if "any mixture of foods that do not contain prolamins and foods that have been rendered "gluten-free", does not contain a gluten level exceeding 200ppm (0.02%) (*Joint FAO/WHO Food Standards Program, 2000)*.

The consequence of this standard is that small amounts of gluten will be consumed by coeliacs on a GFD. The dietary advice given to coeliac patients by both doctors and dietitians fell in line with the labelling standards, hence it is not an unreasonable assumption that the foods and ingredients allowed in the "gluten-free diet" are synonymous with the "Codex" guidelines. This is important to remember when reviewing the literature as it can be assumed that almost all of the gluten-free diets referred to will contain these small trace amounts of gluten, unless otherwise specified.

The question has since arisen as to whether these trace amounts of gluten should be included in a GFD, or whether in fact any gluten should be allowed. This has produced much controversy. In 1988 the WHO/FAO proposed that a limit of 1mg prolamin per 100g dry matter be set for food to be labelled as gluten-free (*Hekkens, 1991*). This issue has still not been resolved by the *Codex Alimentarius* Commission, but is still under consideration. The uncertainty about gluten allowances is reflected in the varying Food Standards, that now exist in different countries, which control the labelling of gluten-free food items.

Australia was one of the first to move away from *Codex* and adopt a more stringent standard for food gluten-free labelling and production, after the invention of a method that was able to detect the presence of gluten in food. In March of 1995 the *Australian Food Standards* changed, such that final commercial food products could no longer contain detectable gluten, oats or malt, if they were to be labelled as gluten-free *(ANZFA, 1995).* "No detectable gluten" was defined as ≤ 0.003%, as determined by the best available test at the time (*ANZFA, 1995; Skerritt et al, 1990; Medical Innovations)*. The lower limits of detection in use from December 20th 2002 has been set at ≤ 0.001-0.002%. This may make adherence to a GFD more difficult as manufacturers may be less able to meet these more stringent requirements and some previously tolerated products may no longer be labelled 'gluten-free'.

The United Kingdom and much of Europe still adhere to the *Codex* standards. The national guidelines adopted in Canada in 1997 state that gluten-free foods may not contain any wheat, oats, barley, rye or triticale or any parts thereof [3]. In North America the use of wheat starch has been formally discouraged in all coeliacs and complete avoidance is recommended in those with non-responsive coeliac disease *(Campbell, 1992; AmGA-1, 2002)*. The detailed

[3] www.inspection.gc.ca/english/bureau/labeti/guide/7-0-0ae.shtm#7-15-7 (accessed 20/2/03)

advice given to patients about what constitutes a 'GFD' will follow the food standard used in each particular country.

One problem with the current ELISA immuno-assay for gluten is that it cannot reliably detect secalin and hordein. This means that tests on foods containing these grains (such as beer and malt-containing breakfast cereals) will almost certainly underestimate the prolamine content. This also makes it difficult to determine the exact amount of gluten eaten by a coeliac from these non-wheat grains *(Skerritt et al, 1990).* Avenin is not detectable at all with the assay and as a result, no oat-containing food can be labelled as gluten-free under the current *Australian Food Standard*.

Other Grains To Avoid

There can be confusion when products based on gluten-containing grains are given another name. Coeliacs need to be aware of these. ***Atta flour***, made from the grains of wheat, is traditionally used to make chapattis, but today is also used for cakes, biscuits and some pre-packaged mixes. ***Couscous*** is not a grain or seed from a plant, but rather a commercial product made from other flours. Once made with millet, it is now made with wheat (*Rogers, 1990*). ***Graham flour*** is whole grain wheat flour named after an American Presbyterian Minister, Sylvester Graham. ***Semolina*** is the coarsely milled inner endosperm of wheat. ***Triticale*** is a hybrid of wheat and rye. ***Burghul, durum and spelt*** are other varieties of wheat with various uses throughout the food industry. ***Wheatgerm and wheat*** bran are other products of the wheat grain that must be avoided in coeliac disease.

Rye (or pumpernickel) and ***barley*** have been subjected to some degree of testing and classified as unacceptable in a gluten-free diet *(Marsh, 1992). Ellis et al, (1990)* has been able

to show by means of ELISA assay that both *malt and beer*, produced from barley, contains sufficient hordein to be avoided by people with coeliac disease.

Grains To Allow In The Gluten-Free Diet

Many gluten-free grains are available for use as part of a GFD. Their nomenclature can also be confusing, particularly if the term “wheat” or “flour” is part of the name.

Buckwheat, also known as kasha, duck-wheat, Indian wheat, piroshki or budweizen, is frequently, and incorrectly, referred to as a cereal grass because the seed of the buckwheat is used as a source of flour in the same way as many grains. Buckwheat is actually related to rhubarb and has a high lysine content making it a useful adjunct grain in the vegetarian diet (*Skerrit, 1986*). The alcohol-soluble buckwheat proteins bear little similarity to the wheat alcohol soluble prolamins (*Skerrit, 1986)*, so is suitable for inclusion in a GFD. *Millet*, also known as bulrush millet, finger millet, ragi, kaffir corn, guinea corn, teff, adlay and Job’s tears, refers to a group of grasses with many small seeds on each ear of grain. It is mistakenly called a grain because the seeds are often ground to produce a flour for cooking flat breads in poorer countries It is also used as a porridge and for brewing beer. In commercial food cooking this flour gives texture and flavour to products (*Rogers, 1990). Quinoa* is an annual herb that is a native of Peru. The seed resembles millet. *Amaranth* is an ancient Aztec cereal that is small and millet-like in shape. It is very rich in protein and is recently being marketed worldwide. *Sorghum*, also known as cholam, jowar or juar, is a member of the grass or millet family. *Arrowroot* can come from two plant sources. Both plants produce ground tubers which are mixed with water and then put through a screen to remove the fibrous matter. The starch particles then settle out so that they can be removed and dried. *Besan*, gram flour or chickpea flour is made from grinding dried chickpeas (a legume). *Carob*, also known as algarroba,

locust bean, locust pod and St. John's bread, comes from drying the fleshy bean pods of the carob tree (or locust tree). *Hops* is a common member of the mulberry family. *Rice* has many uses in food production. It can be boiled or steamed as an accompaniment to meat and vegetable dishes. The grain can be ground to a flour for use in baked goods. It can also be fermented to produce Sake wine and vinegar *(Rogers, 1990)*. *Glutinous rice* refers to the property of these grains to become sweet and sticky when boiled, as for the sweetener Amazake. *Sago,* also known as pearl sago, are small balls of starch prepared from the inner trunk of various kinds of palm trees. The *soya* bean, also known as soy or kinako, is a legume which is cleaned, cracked and hulled before being broken into chips, reduced to flakes and grits and then ground to a flour. *Tapioca*, also known as cassava, mandioca, manioc or yucca, comes from cassava roots which are washed, peeled and ground with water before being passed through a screen where the starch is allowed to settle and dry out. All these products are acceptable for use by people with coeliac disease.

BENEFITS OF A LIFE-LONG GLUTEN-FREE DIET

When Dicke first introduced the gluten-free diet, coeliac disease was thought to be only a childhood disorder. Once the child was able to tolerate gluten, usually in their early teens, gluten was re-introduced to the diet. Adult coeliac disease was later recognized. A study in Switzerland *(Shmerling & Frankx, 1986)* observed that 20% of the children recruited during 23 years, reintroduced gluten to their diet and were subsequently re-diagnosed with coeliac disease 2-15 years later. *Holmes et al, (1989)* were the first to suggest that a gluten-free diet should be life-long in all patients based on their study of malignant complications.

Maintenance of the gluten-free diet will produce a return to health and minimize the long-term risk factors associated with the coeliac disease. The aims of the gluten-free diet are:

1. reduce symptoms
2. lead recovery of the small intestinal mucosa,
3. reduce the risk of developing malignancies of the gastro-intestinal tract
4. reduce the bone mineral loss associated with coeliac disease
5. improve nutritional status

Symptom Expression

Although most coeliacs will have resolution of their symptoms when they start a GFD and will remain so while they are adherent to it, nevertheless, there are still some whose symptoms continue despite gluten restriction. These people are usually suspected of poor compliance or of having inadvertent ingestion of gluten. However, careful clinical and dietary assessment of this group indicate that is not the presence of gluten in their food that is responsible. Anecdotal observations and small studies suggest that gluten in wheat starch could be responsible for some of this, but no large formal studies were done at the time that this thesis was begun in 1994 *(Ciclitira et al, 1984-a, b & 1985-a, b; Scotta et al, 1982).*

Histological Abnormalities

A gluten-free diet usually reverses the villous atrophy. The improvement starts distally and spreads proximally *(Schwartz et al, 1968).* It has been stated that “clinical improvement correlates better with the length of histologically improved intestine than with the severity of the lesion in the proximal intestine” (*Sleisnger & Fordtran, 1989)*. This explains the common observation that a clinical response to gluten withdrawal may precede the histological resolution by many months. Eventually, even the proximal biopsy specimens revert to normal in over 50% of patients maintained on a gluten-free diet. Most of the remaining patients show

significant improvement but may have minor degrees of persistent villous atrophy In a few patients, the villous atrophy does not change in the proximal small bowel despite a definite clinical response to prolonged gluten withdrawal.

Since these observations were made 30-40 years ago, it is likely that the gluten-free diet contained both wheat starch and malt as well as other sources of small amounts of gluten. Whether any persistent villous atrophy was due to these Codex-permitted amounts of gluten is uncertain (*Ciclitira et al, 1985-a)*.

Scotta et al, (1982), have shown the recovery of villi to normal in an 8 year-old boy after the removal from the diet the small amount of gluten ingested weekly in the form of a Holy Communion wafer.

Ciclitira et al, (1984-a), found no mucosal damage after the ingestion of a wheat starch product, containing 7mg gluten / 100g of flour, for 1 week, or a year later, when 10 adults consumed 2.4 – 4.8mg gluten per day for 6 weeks *(Ciclitira et al, 1985-a). Catassi et al, (1993)* concluded that after a four-week challenge in children with 100mg of gliadin (200mg gluten or 2.5g wheat flour) per day, a minimal increase in IEL count was seen in only a few subjects. In contrast, when 500mg of gliadin (1g gluten) per day was given there was a definite increase in IEL count and reduction in villous height. The latter quantity is well above that expected in a Codex-GFD. A similar study, also using the equivalent of up to 500g gliadin showed an increase in the number of IEL in the crypts but no change in mucosal architecture (*Auricchio & Troncone, 1991).* Despite lack of biopsy damage they advocate a strict GFD devoid of foods containing these small amounts of gliadin. A longer-term study detected no difference in small

bowel findings between subjects on a wheat starch containing diet and a normal non-coeliac control group *(Edjerhamn et al, 1988 p294-297).*

Improved Nutritional Status

In addition to the clinical improvement on a GFD, deficiencies of iron, folic acid, ferritin, calcium, zinc and vitamin B_6 can be reversed.

Anand et al, (1977), demonstrated improvement in the absorption of Ferrous (Fe++) iron in those consuming a gluten-free diet. Interestingly the absorption of Haemoglobin iron was not dependent on the integrity of the small bowel mucosa. Ferritin levels also improve once gluten has been removed from the diet *(Souroujon et al, 1982).* Indeed this group suggested that ferritin levels are the easiest laboratory measure to determine treated from non-treated coeliacs and that it could be used to assess the response to treatment with a gluten-free diet. Low levels of vitamin $B_{6\ are}$ found prior to beginning a GFD *(Reinken et al 1976)*. This was rectified once the patients established a GFD. The normalization of calcium absorption, after instigation of a gluten-free diet has also been shown (*Schwartz et al, 1968; Molteni et al, 1995)*.

The proximal small bowel is purported to be the main site of absorption for zinc (*Davies, 1980),* however some zinc is also believed to be absorbed distally *(Andersson et al, 1976; Antonson et al, 1979). Crofton et al, (1983)* has been able to show that zinc absorption improves when gluten is removed from the diet.

CHAPTER 2

2

CONTINUING SYMPTOMS

Continuing symptoms in coeliac disease from the ingestion of trace amounts of gluten found in some gluten-free diets and non-gluten food intolerances.

INTRODUCTION

Adherence to a GFD is generally believed to result in the disappearance of symptoms and the resolution of the histological abnormality in coeliac disease. However, while most people become symptom-free on their diet, there is a proportion in whom this is not the case. Doctors, dietitians and Coeliac Society support groups hear frequent anecdotal accounts of continued symptoms despite adherence to the GFD.

In many patients, the cause of these continuing symptoms is unclear. Although persistent symptoms can be caused by conditions unrelated to coeliac disease (e.g. lactose intolerance) inadvertent or intentional ingestion of gluten is most often blamed, even when the patient denies eating foods containing gluten.

The question of whether continuing symptoms could be caused by the minor amounts of gluten found in a *Codex-GFD* remains uncertain and it is possible that individual differences exist in symptomatic and mucosal responsiveness to gluten *(Ciclitira et al, 1985-a; Kumar et*

al, 1985; Montgomery et al, 1988; Thornquist et al, 1993). The results of a survey, describing the prevalence of continuing symptoms in people with coeliac disease, will be introduced in this chapter.

The work described here was prompted by anecdotal observations in several patients with coeliac disease, referred to the Allergy Unit at Royal Prince Alfred Hospital for dietary investigation, that co-existing non-gluten food intolerances were responsible for continuing gastrointestinal symptoms. This had been assessed by using the same elimination diet and challenge protocol that is used for people who present with irritable bowel syndrome *(Loblay & Swain, 1986).* The aim of the present study was to examine prospectively the role of such non-gluten food intolerances in a larger group of subjects with coeliac disease who were still experiencing symptoms despite adherence to a carefully documented GFD.

METHODS

Subject Recruitment

Volunteers were sought from The Coeliac Society of New South Wales, Inc. A brief letter describing the study and asking for volunteers was provided to the Society for distribution to its members. Recruitment began in April 1994 and was completed in September 1995. Eligible subjects were required to have persistent bowel symptoms despite consuming a GFD. Specific symptoms that were sought included diarrhoea, urgency, bloating, abdominal pain, and/or flatulence.

Volunteers of both sexes, aged between 18 and 75, were initially screened by phone. The diagnosis of coeliac disease must have been made by the standard criteria of villous atrophy

demonstrated on small bowel biopsy, with improvement on a follow-up biopsy after a period of at least 6 months on a gluten-free diet. Those with dermatitis herpetiformis alone, pregnant or lactating women were excluded. Fifty individuals fulfilled the entry requirements and attended an interview at the RPAH Allergy Unit where the study was discussed further, and informed consent obtained.

Study Protocol

An initial medical history and examination was performed in the Allergy Unit by Dr. Loblay in order to confirm each volunteer's eligibility for the study. Each person was asked to bring, where possible, copies of their diagnostic biopsy results. If these were not available, attempts were made to obtain copies of the biopsy reports from their general practitioner or gastroenterologist. The study protocol is outlined in Figure 2.1.

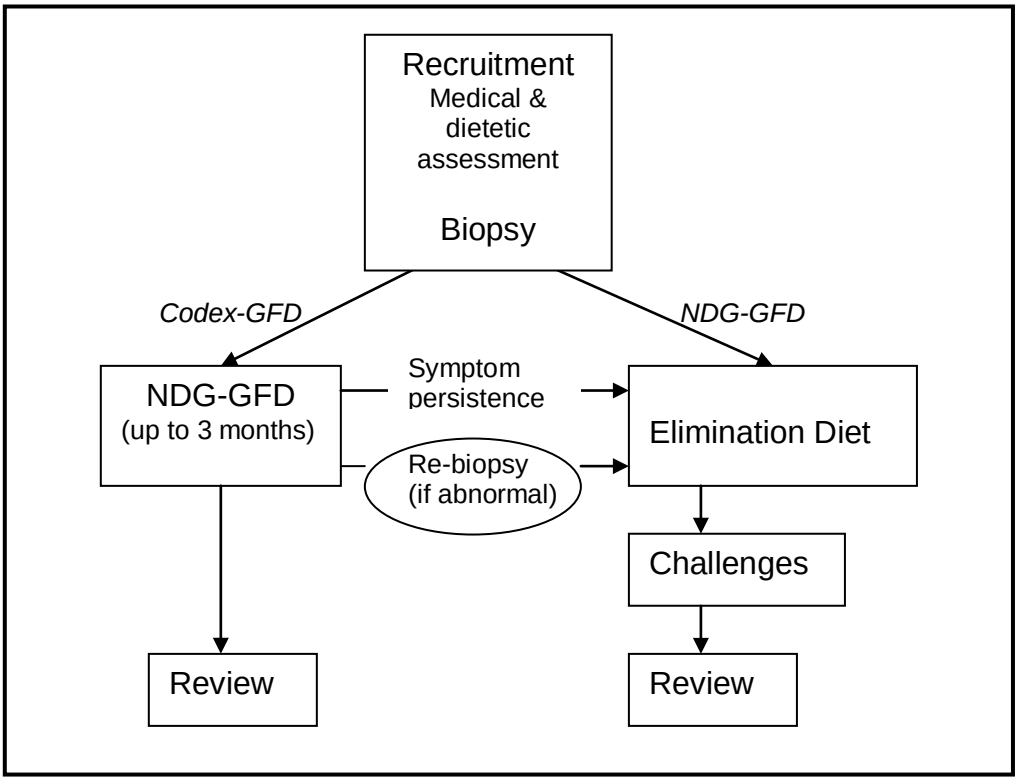


Figure 2.1: Study protocol for symptomatic patients.

Inclusion and exclusion criteria are shown in Tables 2.1 and 2.2 and the clinical information obtained at the initial medical assessment is summarised in Table 2.3.

- Males & females aged 18-75 years
- Documented definitive diagnosis of coeliac disease
- Persistent or recurrent gastrointestinal symptoms despite adherence to a GFD
- General clinical condition otherwise stable with no significant weight loss during the previous 12 months
- Absence of other significant concurrent disease

Table 2.1: Inclusion criteria

- Dermatitis herpetiformis alone
- Other significant concurrent disease (cardiac, respiratory, renal, haematological, neurological, or malignancy). Patients with stable diabetes mellitus were not necessarily excluded, but were evaluated on an individual basis
- Anticoagulant therapy (including low-dose aspirin prescribed for prophylaxis of thromboembolism)
- Advice from the patient's family practitioner or gastroenterologist indicating that enrolment is contraindicated
- Patients with a history of aspirin-induced anaphylactoid attacks were not necessarily excluded. However, aspirin was removed from the challenge battery in such cases
- Moderate or severe asthma
- Pregnant or lactating women

Table 2.2: Exclusion criteria

Coeliac disease	• Year of diagnosis of coeliac disease • History of symptoms during childhood • Continuing symptoms since diagnosis • History of disease diagnosis / other diagnoses before coeliac disease • Length of time to be diagnosed with coeliac disease • Weight history • Number of small bowel biopsies • Were they referred to a dietitian after coeliac disease was diagnosed • Length of time on Gluten-free diet before feeling well • Family history of coeliac disease
General Health	• Diabetes history (personal and family) • Heart disease history (personal and family) • Lung and airways history (personal and family): asthma, hay fever or sinus problems • Headache / migraine frequency • Liver disease history (personal and family) • Renal disease history (personal and family) • Blood pressure history • Blood iron levels
Skin problems	• Dermatitis Herpetiformis occurrence • Eczema • Hives • Angioedema
Gastro-intestinal problems	• Mouth ulcer occurrence • Nausea history • Stomach pain and cramp • Bloating • Bowel movement type
Hormonal and or reproductive history	• Age periods began and period history • Childbirth / conception history • Menopause • Other when relevant
Food avoidances other than gluten	• Reason and the symptom the subject felt the food provoked • Allergic history • Family history of food avoidances • Any smell aversions
Living and working environment	• Energy levels • Stress levels • Current levels of exercise
Drug intake	• Medication intake • Smoking history and current smoking status • Alcohol history and current intake levels

Table 2.3: Specific information obtained at the initial interview and examination.

'Gluten-free' Diet Categories

Before the study began it was anticipated that the subjects to be enrolled would have varying levels of trace and Codex-permitted gluten in their gluten-free diets since, when recruitment commenced in 1994, Australia was following gluten-free labelling standards based on the *Codex Alimentarius* guidelines. Although a new Australian standard for the labelling of gluten-free foods had been proposed, it did not make an impact on the supermarket shelves until mid-1995. As the initial aim of the study was to look for non-gluten food intolerances that could provoke continuing symptoms in people with coeliac disease, it was considered possible that ingestion of minor amounts of gluten found in foods labelled 'gluten-free' under the *Codex Alimentarius* standards, might confound the results of the study. Indeed, some of the subjects had already excluded wheat starch, malt, and any other ingredient which could have contained trace amounts of gluten from their diet in the belief that these might have been responsible for their persistent symptoms. These subjects avoided buying many of the products labelled 'gluten-free' on the supermarket shelves for this reason, and were cooking much of what they ate from basic ingredients.

In order to help characterise the diet that each participant was following, three categories of diet were defined based on the frequency of consumption of foods containing different amounts of gluten:

- *Codex-GFD*: based on the Codex Alimentarius standard, allowing the regular inclusion of food ingredients containing up to 0.3% protein from gluten-containing grains (principally wheat starch and malt).

- *NDG-GFD* (No Detectable Gluten-GFD): based on the Australian Food Labelling Standard (1995), containing no detectable gluten (measured by ELISA with lower limit of detection <0.003%), malt or oats.
- *Overt gluten-containing diet:* A diet which regularly includes foods made with wheat flour (e.g. bread, pasta, cakes, biscuits, ice-cream cones), rye, barley or oats.

For the purpose of the studies presented here in this thesis, a person was considered to be regularly ingesting either overt gluten ingredients or Codex-permitted gluten ingredients, if they consumed 6 or more of these ingredients during the year. This was an arbitrary figure reflecting the dietary strictness desired by the researchers.

Dietary Assessment Tools

All subjects underwent a detailed dietary assessment by the author, after their medical interview and examination. This dietary interview served a dual purpose: the subject was informed about what was involved with the elimination diet and challenge procedure; and the subject's knowledge about the presence of small amounts of gluten in gluten-free foods was assessed, in order to help in explaining what was required in the *NDG-GFD*. Once this interview was completed and the subject's suitability for the study was confirmed, written, informed consent was obtained (Appendix 1).

Before determining which dietary category the subject fell into, each was given a *Food Brand Questionnaire* and a *One-week Food and Symptom Diary* to complete and return to the author/dietitian at the time of their biopsy (usually 2 weeks later). These documents are described below.

Food Brand Questionnaire

At the time the study began no existing questionnaire designed to assess a person's adherence to a gluten-free diet could be found. Such a questionnaire was therefore designed, by the author, aiming to elicit as much information as possible about all sources of gluten that could be ingested by a coeliac (Appendix 2). Many of the questions asked for information about the brands of food chosen for cooking at home, including commercially-prepared bread and cake mixes, cereals, sauces and individual ingredients such as flours, keeping in mind that not all foods chosen would necessarily be labelled "gluten-free" according to either the *Codex* or new *Australian Food Standards*. There were also questions designed to determine which foods were chosen as snacks from take-away shops, which foods were eaten in restaurants or at friends' houses, and what steps were taken to determine whether these foods were gluten-free or not. Questions were also aimed at finding out what medications, tablets, powders or syrups were being ingested, because these sources of gluten are often overlooked. If there was uncertainty as to the composition of any product in the diet, the relevant food and/or pharmaceutical companies were contacted to ascertain (where possible) the presence of gluten, wheat starch or malt. In addition the questionnaire asked whether any gluten-containing product had been consumed within the three months prior to the subject's first study biopsy.

One Week Food and Symptom Diary

This diary was also designed by the author in order to collect information about the continuing symptoms each subject was experiencing (Appendix 3). It recorded all the foods, beverages and medications that were consumed during the consecutive days of a single week. Food intakes were not expected to be weighed or quantified. However, the general food choices made and the degree to which patients were adhering to a GFD could be assessed from this.

The appropriate manufacturer was approached if an item's gluten content required clarification. This food diary was also used to confirm the findings of the questionnaire and to identify additional food items (usually snacks) that were not a frequent component of the subject's diet and therefore may not have been reported in the questionnaire. As the study progressed this diary was kept on a daily basis to assess dietary adherence to the requirements of both the GFD and the elimination diet.

Subjects also kept a record of the symptoms they experienced, during the week they kept the food diary, and graded them as *mild, moderate, marked* or *severe*.

Once the two documents described above had been returned, each subject's GFD was categorised. This was done in conjunction with the information obtained at the initial dietary interview.

Antibody Testing

Serum was obtained at the initial attendance from each subject for antigliadin antibody testing. Both IgA and IgG antibody was assayed by means of ELISA. Endomysial antibody assays were not available at the commencement of the study.

Small Bowel Biopsies

All subjects underwent endoscopic biopsy of the distal duodenum at RPAH within 2 weeks of entry into the study, with the exception of one in whom a follow-up biopsy had been performed by their own gastroenterologist within one month of enrolment (this was used as the study entry biopsy in that patient). In two patients, a second biopsy had not been performed after the

diagnosis of coeliac disease, and the study entry biopsy served as their follow-up diagnostic biopsy.

Histopathology

A routine report was issued for each biopsy taken. Each specimen was reviewed at the end of the study by a single pathologist, Dr. Dorothy Painter, and also by the collaborating gastroenterologist, Associate Professor Warwick Selby. These reviews were conducted blind, i.e. without the reviewer knowing the identity or diet category of the patient. The slides were coded and mixed with a group of normal non-coeliac control biopsies, also coded. An overall assessment was made as to whether each biopsy was normal or showed villous atrophy. The villous/crypt ratio (V/C) was also assessed and used to categorise the biopsy as either normal (V/C $\geq$ 2:1), partial villous atrophy (1-2:1), subtotal villous atrophy (< 1: 1) or total villous atrophy. Inter-observer agreement was high, but if there was any discrepancy consensus was reached by agreement between these two observers, without knowledge of the subjects' gluten intake category.

Study groups

When the dietary analyses were completed and the result of the small bowel biopsy known each subject was assigned to one of three groups (Figure 2.1):

Group 1: Those adherent to a *NDG-GFD* diet at entry into the study. They were commenced on the elimination diet, as outlined below, regardless of whether their small bowel biopsy was normal or abnormal.

Group 2: Those adherent to a *Codex-GFD* in whom small bowel biopsy was normal. They were asked to follow a *NDG-GFD* for a period of 3 - 12 weeks. If they became largely or completely symptom-free during the first 3 weeks, and remained so for 3 months, they did not proceed to the next dietary phase and their participation in the study ended. If significant symptoms persisted beyond 3 weeks then they commenced the elimination diet.

Group 3: Those adherent to a *Codex-GFD* in whom small bowel biopsy was abnormal. They were advised to follow a *NDG-GFD* for a period of 3 months, followed by a second small bowel biopsy. Regardless of this biopsy result, if symptoms persisted, they proceeded to the elimination diet. If symptoms had resolved they were considered to have completed the study.

Throughout the study all subjects kept daily records of food intake and symptoms using the *Food and Symptom Diary* described above.

Elimination Diet

The elimination diet was modified from that described previously *(Gibson & Clancy, 1978*). This excludes foods containing natural salicylates, amines, and/or glutamate, as well as those with added colourings, preservatives, monosodium glutamate (MSG) and lactose. Based on clinical experience in patients with coeliac disease, dairy products, soy and millet-containing foods were also excluded.

Subjects were given two information booklets developed by the RPAH Allergy Unit to help people with food intolerance understand the nature of the problem, the elimination diet, and the challenge protocols.

The first booklet ("*The Simplified Elimination Diet",* Appendix 4) contains practical information required by patients who are undergoing investigation of possible food intolerance:

- Details about which fresh fruit, vegetables, grains, meats, beverages, snacks and condiments can be consumed on the elimination diet, and which are to be avoided.
- A list of food additives, by number, that need to be avoided.
- An outline of how to implement the food chemical challenge protocols when this phase of the process is reached.
- Information on how to make sure the new diet is balanced.
- Examples of foods that can be enjoyed for meals and snacks.

The second booklet ("*Salicylates, Amines & Glutamate", Appendix 5)* contains:

- Lists of the natural chemicals contained within a variety of foods.
- The approximate levels (low, moderate, high and very high) of the natural chemical found in a variety of food.
- The list of food additive numbers that may produce symptoms in some people.

Subjects were also provided with *"Friendly Food*", (*Swain et al, 1991)* a book produced by the RPAH Allergy Unit providing suitable recipes, food preparation hints, and shopping lists for patients with food intolerances.

Subjects were instructed to follow the elimination diet for a period of between 2-6 weeks initially. Those who experienced a significant improvement in symptoms during this time (≥50% as perceived by the individual) commenced the challenge phase. Those who failed to improve significantly after 6 weeks, were instructed to resume their usual gluten-free diet and were considered to have completed the study.

Challenges

Subjects remained on the elimination diet throughout the challenge phase. Initially, open challenges were conducted with milk, soy and millet, as follows:

Milk Challenge: 1-3 cups of milk per day for 3 days. If tolerated they could then add cream cheese and plain or vanilla varieties of yoghurt and ice-cream for the rest of the week.

Soy Challenge: 1-3 cups soymilk per day for 3 days. If tolerated then for the rest of the week products that use soy flour or soybeans could be consumed. If subjects did not like soymilk then soy flour and soybean products could be eaten as an alternative from the first day.

Millet Challenge: 1-2 bowls of puffed millet to be eaten per day for 3 days. If tolerated, other millet flour products could then be introduced for the rest of the week if desired.

Any of these foods that were tolerated without recurrence of symptoms were reintroduced into the diet before further challenges were undertaken.

The remainder of the challenges were conducted double-blind. Test substances and placebos were dispensed in identical opaque capsules which were coded and administered in an arbitrary order. The following substances were used: acetylsalicylic acid, phenylethylamine and tyramine, MSG, sodium propionate, sodium nitrate/nitrite, antioxidants (BHA and BHT),

preservatives (sodium benzoate, sorbate and metabisulphite), colourings (tartrazine and erythrosine) and lactose. Placebo capsules contained potato starch and sucrose. Following each challenge, a symptom-free period of three days was required before proceeding to the next challenge. Challenge dosages outlined in Appendix 6.

Each subject was instructed to keep a daily food, medication and symptom diary throughout the elimination diet and challenge period. A challenge was considered positive if it provoked a recurrence of symptoms. These symptoms were rated by the subjects as mild, moderate, marked or severe. After completion of the double-blind challenge battery each subject was reviewed and reactions were recorded before the challenge code was broken.

Data was entered into Minitab database for analysis. The study was approved by the Ethics Review Committee of the Central Sydney Area Health Service (RPAH Zone).

Diet and Symptom Survey

A cross-sectional survey *(Stuart et al, 1997-b)* of the Coeliac Society of NSW was initiated by the author in 1995 to survey data from a large community group and not a clinically selected group of Australian coeliacs. Since there is such a strong theoretical link between gluten exclusion and symptom improvement, the degree of gluten restriction of the Coeliac Society members' diets was to be ascertained. It was thought that the change in the Australian Food Standard in 1995, which governs the labelling and gluten content of foods labelled gluten-free, was an opportune time to enquire about the dietary habits and symptom perceptions of this selected group. On the whole it was anticipated that the group would be following the *Codex-GFD* containing wheat starch and malt as this was the Australian labelling standard until 1995.

Questionnaires were sent to all members of the Coeliac Society of New South Wales endeavouring to quantify the presence, severity and frequency of a range of common symptoms, before and after the diagnosis of coeliac disease. The same questionnaire (Appendix 2) as is presented in this chapter, was sent. A *Symptom Record Sheet* (Appendix 7) was developed for this mail out and altered for use in the work presented in Chapter 5.

RESULTS

Fifty volunteers fulfilled the entry requirements and attended the initial interview. Eight decided not to proceed due to perceived difficulties in adhering to the elimination diet. Two others became pregnant, and one subject withdrew after having been diagnosed with carcinoma of the bladder. This left 39 subjects - 37 females and 2 males. The median age of the subjects was 42 years (range 19-75 years). The median age at diagnosis of coeliac disease was 37 years (range 3-62 years) with a median duration of disease of 6 years (range 1-29 years).

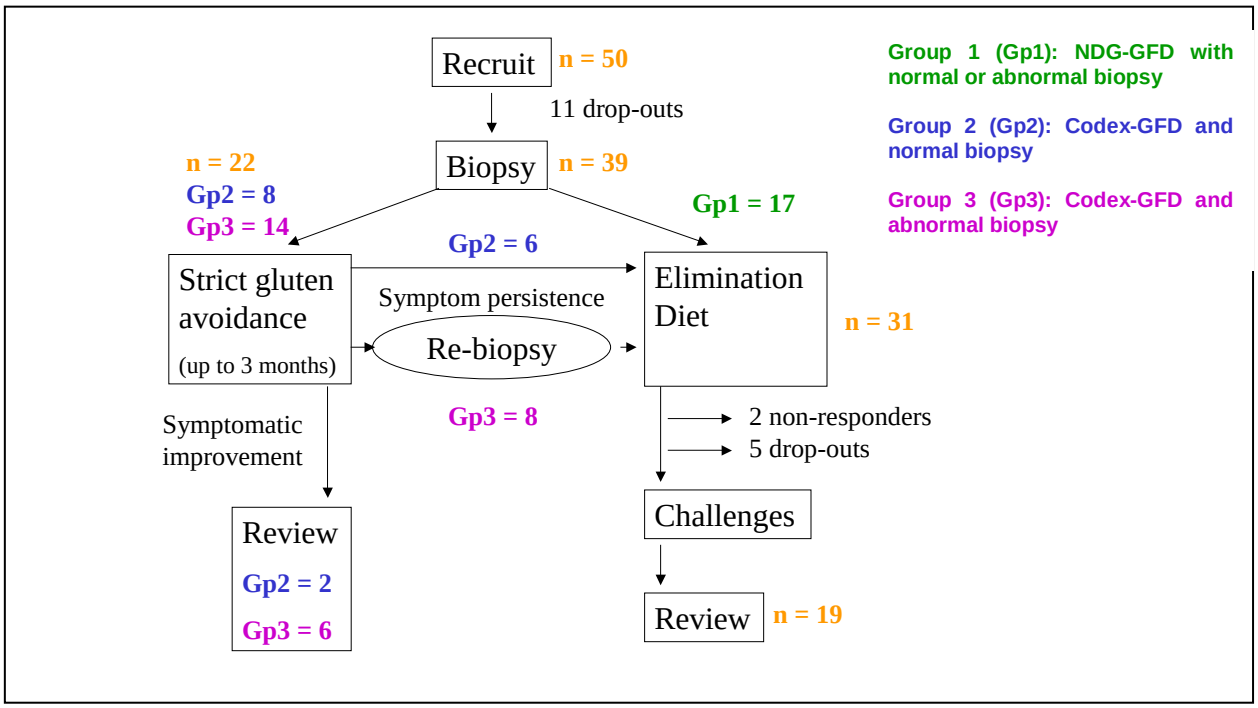


Figure 2.2: Summary flowchart of study participation

The assessment categories and progress flowchart for these 39 subjects is shown in Figure 2.2. Of this group, 25 (64%) had an abnormal biopsy at study entry. Seventeen were already on a *NDG-GFD* and commenced the elimination diet directly (Group 1). In 7 of these the duodenal biopsy was normal whereas in 10 there were varying degrees of villous atrophy. A repeat biopsy was not required as a part of the study.

Twenty-two subjects were adhering to a *Codex-GFD* at entry to the study. The entry biopsy was normal in 8 (Group 2) and abnormal in 14 (Group 3). These subjects were instructed to eliminate all foods that contained Codex-permitted gluten from their diet (principally wheat starch based bread mixes and malt-containing breakfast cereals). Five of the 22 reported a complete resolution of symptoms after this dietary change, and were considered to have completed the study. In 3 others there was a significant reduction in symptoms, to the extent that they did not consider it necessary to continue in the study. Repeat small bowel biopsy was not performed. Overall, a clinically significant response was obtained in 8 of the 22 subjects (36%) merely by removal of Codex-permitted amounts of gluten from the diet.

Fourteen subjects in Groups 2 and 3 experienced little or no symptomatic benefit after switching to a *NDG-GFD* for three months. These subjects progressed to the elimination diet phase of the study. In 8 of these 14, the entry biopsy showed villous atrophy and a further biopsy was performed before beginning the elimination diet. In only two was there a return to normal, whereas in 2 others the histological appearances had deteriorated.

Eighteen (58%) of the 31 subjects who began the elimination diet had an abnormal biopsy at entry. Of these 31 subjects, 17 were from Group I and the 14 remaining subjects were from Groups II and III. Of these, 24 (77%) experienced a greater than 50% reduction in their

symptoms and proceeded to the open food challenge phase. Five others withdrew due to the restrictions imposed by the elimination diet, and it was therefore not possible to adequately assess their response. In the remaining two subjects there was no symptomatic improvement despite adherence to the elimination diet for 6 weeks.

The 24 subjects who improved proceeded to open food challenges. The results are shown in Table 2.4. Twelve (50%) reacted to soy and 11 (46%) to milk. Nine of the 22 (41%) challenged with millet had a positive response. The results of the double-blind food chemical challenges are also shown in Table 2.4. Five subjects withdrew at this stage for family reasons and/or due to difficulties in managing the diet. One elected not to undergo challenge with lactose.

CHALLENGE	NUMBER OF SUBJECTS	NUMBER POSITIVE	PERCENT
Soy*	24	12	50
Millet*	22	9	41
Milk*	24	11	46
Amine	19	11	58
Salicylate	20	10	50
Glutamate	19	9	47
Propionic acid	19	9	47
Anti-oxidant	19	8	42
Preservative	19	8	42
Colours	19	8	42
Lactose	18	6	33
Nitrate	19	5	26
Sucrose	19	4	21
Potato starch	19	3	16

Table 2.4: Number of positive responses to whole food and chemical challenges.
**These were open whole food challenges. All others were double-blind challenges with encapsulated food substances.

Overall, the most frequent challenge reactions were to amines (58%), salicylate (50%) and soy (50%). Placebo reactions to starch and sucrose occurred in 3 and 4 individuals, respectively. Each of the 24 subjects responded to at least 2 challenges, the mean number being 5 (range 2-9) (Figure 2.3).

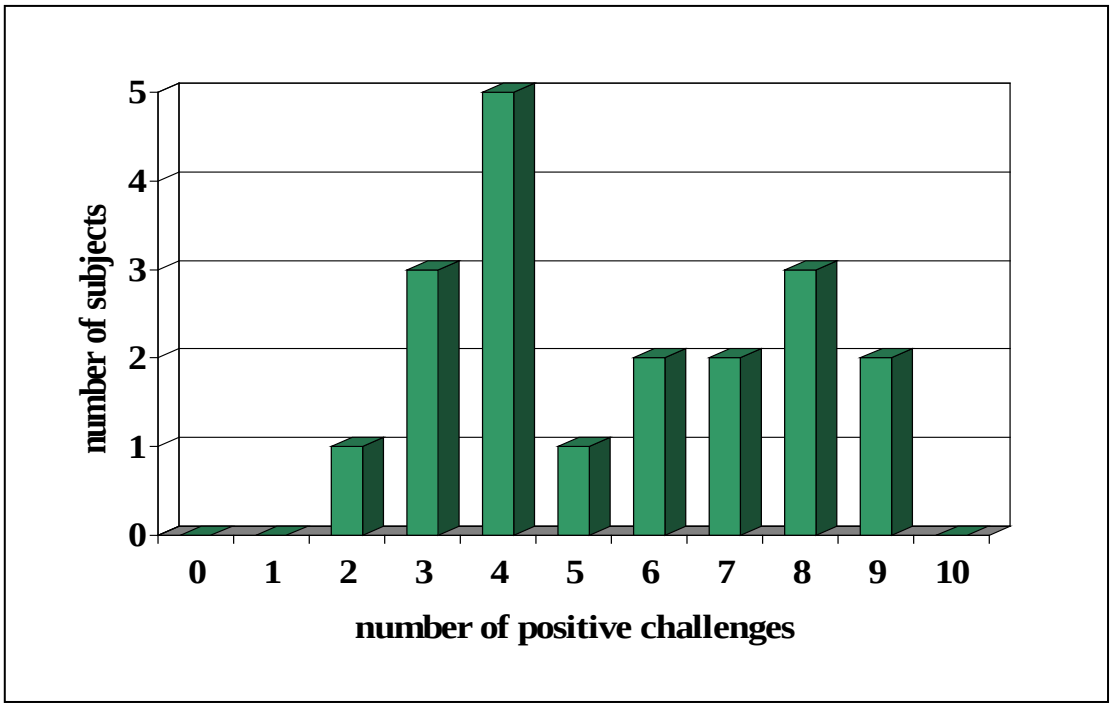


Figure 2.3: Number of positive reactions per person completing the challenge procedure (n=19)

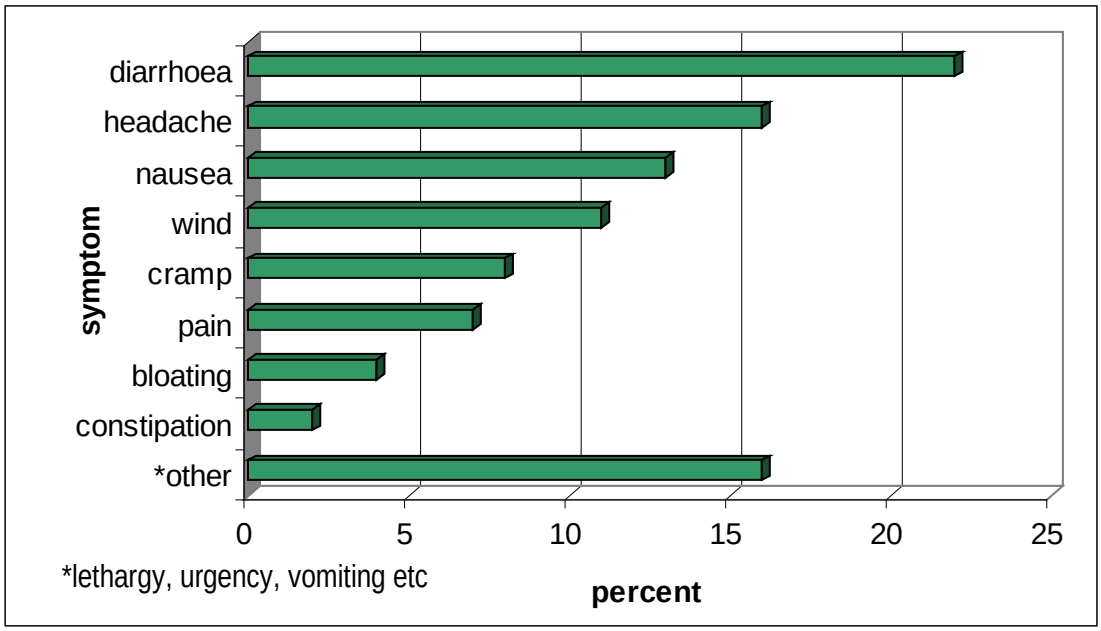


Figure 2.4: Symptoms elicited by whole foods and food chemicals

Diarrhoea was the most common symptom provoked by the challenges, followed by headache, nausea and flatulence (Figure 2.4). As expected, milk and lactose most frequently provoked flatulence, diarrhoea and abdominal cramps. It is interesting to note that of the 11 subjects who reacted to milk, only 4 had a positive lactose challenge. This is most likely to be due to differing lactose doses ingested in the two challenges (~25 grams in the open challenge vs. 500 mg in the encapsulated double-blind challenge). Each of the double-blind chemical challenges was capable of provoking one or more gastrointestinal symptoms in susceptible individuals, but some also provoked headache and/or lethargy.

Although patients are normally allowed to eat millet and soy on the elimination diet routinely used for investigation of food intolerance at the RPAH Allergy Unit, these foods were excluded from the diet in the present study because of anecdotal reports from coeliacs of symptoms triggered by soy or millet. Interestingly, although soy and millet are gluten-free, the open challenges with these foods provoked symptoms in approximately 50% of patients (Table 2.4). Soy provoked flatulence, bloating and abdominal pain, as well as headache. Millet provoked nausea, bloating and headache.

Diet and Symptom Survey

The collation of the data from the questionnaire mail out was performed by Michelle Stuart, a Master of Nutrition and Dietetic student and supervised by Kim Faulkner-Hogg, Dr. Robert Loblay and Associate Professor Warwick Selby. Of the 1672 questionnaires sent, 965 were returned. Only 18.2% of the respondent group were classified as following a *NDG-GFD*, while 71.9% were found to be following a *Codex-GFD* and 5.9% were eating usual gluten foods. Four percent were unable to be classified. Of the 71.9% following the *Codex-GFD*, 73% believed that they were following a strict gluten-free diet.

As expected, there was a great reduction in symptoms after the onset of some form of gluten restriction following the diagnosis of coeliac disease. Even though there was a significant decrease in both severity and frequency of symptoms, across all diet categories, 69% of respondents were still experiencing gastrointestinal symptoms despite the gluten restriction. While 39% regarded these symptoms to be mild, at least 18% reported the occurrence of gastrointestinal symptoms, at least daily, after diagnosis (and GFD) and in 10% the symptoms were regarded as severe *(Stuart et al, 1997-a)*.

DISCUSSION

There is considerable variation in the clinical presentation of coeliac disease, ranging from asymptomatic patients (detected either by serological screening) or because of iron deficiency anaemia and/or full blown malabsorption *(Trier, 1991)*. It may also be recognised incidentally in association with a number of other disorders, including dermatitis herpetiformis, IgA deficiency, insulin-dependent diabetes mellitus, osteoporosis and (uncommonly) cryptogenic neurological illness *(Trier, 1991; Halsted, 1996; Hadjivassiliou et al, 1996)*. Nevertheless, the majority will have gastrointestinal symptoms prior to diagnosis while they are consuming a normal, gluten-containing diet.

It is expected that these symptoms will resolve after the patient commences a gluten-free diet, provided they adhere to it. However, this appears not to be the case in all. How frequently symptoms persist is not clear. *Fine et al, (1997)* reported persistent diarrhoea in 17% of 78 patients with treated coeliac disease. In the *Diet and Symptom Survey* conducted through The Coeliac Society of NSW Inc., it was found that 18% of 965 questionnaire respondents reported the occurrence of gastrointestinal symptoms, at least daily, after diagnosis and in 10% the

symptoms were regarded as severe. Ninety percent were on either a *Codex-GFD* or *NDG-GFD* (*Stuart et al, 1997-b).*

The results of the studies described in this chapter demonstrate that there are two principal causes for persistent symptoms in patients with coeliac disease who are adherent to a GFD:

1. the Codex-permitted amounts of gluten ingested in wheat starch or malt based foods (which are allowed in a *Codex-GFD*), and
2. non-gluten food intolerances.

(i) Trace / Residual gluten

The occurrence of symptoms caused by the Codex-permitted gluten present in wheat starch-based products was first described by *Ciclitira et al, (1984-a)*. They challenged 10 subjects on a wheatstarch-based "gluten-free diet" and found that symptoms developed in four within a six week period. In the present study, conducted from 1994-1996, of 22 patients with persistent symptoms who were adherent to a *Codex-GFD*, over one-third experienced complete or substantial improvement when they switched to a *NDG-GFD*. This suggests strongly that the persistent symptoms were due to the minor amounts of gluten allowable on a *Codex-GFD.*

In 1997, *Chartrand et al, (1997)* in a Canadian study described the same phenomenon. They were able to provoke a variety of gastrointestinal symptoms in 11 of 17 subjects, none of whom had ever consumed wheat starch previously to the study challenge. These symptoms resolved within 10 days to 3 weeks of discontinuing the wheat starch bread used in the challenge. In two of 3 patients with dermatitis herpetiformis there was a relapse of skin lesions during the challenge. They also included a control group of coeliac subjects who had regularly been consuming wheat starch bread for an average of 6 years, but who had no symptoms. All

of these observations indicate that varying degrees of intolerance to trace amounts of gluten occur in patients with coeliac disease.

The question of whether all coeliacs should follow a *NDG-GFD* or whether the Codex-permitted amounts of gluten found in the *Codex-GFD* (principally as wheat starch and malt) can be allowed for some or all patients remains controversial *(Maki & Collin, 1997).* Certainly, there is now little doubt that such products can provoke symptoms in a subgroup of more sensitive patients.

Perhaps until this issue is resolved, patients with coeliac disease should be advised to consume a gluten-free diet that excludes products based on wheat starch and malt. This has been made easier in countries such as Canada (*Chartrand et al, 1997)*, where the Food Standards[4] already prohibit the labelling of foods as 'gluten-free' if they are based on any part of a gluten-containing food, as well as in Australia, where foods can only be labelled 'gluten-free' if they contain no detectable gluten, no oats or malt. However, since the declaration of the presence of gluten in food products is not yet mandatory, patients still need to be educated to read the ingredient lists on food labels, looking principally for the presence of wheat starch and malt. Examples where this may be important include "cornflour" (which can be derived from either wheat or maize grains) and many of the commercial rice and corn breakfast cereals (which are dusted with malt extract to enhance the flavour). The new *Australian Food Standards* introduced by FSANZ in December 2002 require that by December 2004, all packaged foods with ingredients derived from a gluten-containing grain must list the relevant grain on the label. This should make avoidance of trace amounts of gluten easier for patients who are at the sensitive end of the clinical spectrum.

[4] www.inspection.gc.ca/english/bureau/labeti/guide/7-0-0ae.shtm#7-15-7 (accessed 20/2/03)

(ii) Food Intolerances

Although patients who remain symptomatic on a 'gluten-free' diet are often suspected of knowingly or inadvertently ingesting gluten, careful dietary analysis in the present study showed that this was not the case. The findings indicate that intolerance to other foods or food chemicals should be considered. Twenty-four of the 31 patients (77%) had a significant improvement in their symptoms on the elimination diet, and each of those who completed the challenge phase experienced reactions to multiple food substances.

The complex dietary manipulations undertaken in this study presented problems for some subjects. Twelve failed to complete the elimination diet and challenge phase. This was largely because of difficulty with dietary adherence and the associated social restrictions it imposed. However, compliance was very good amongst those patients in whom there was relief of chronic distressing symptoms.

The pattern of non-gluten intolerances documented here amongst persistently symptomatic coeliac patients is very similar to that seen in patients presenting to the RPAH Allergy Unit with irritable bowel syndrome (IBS). Previous studies of this patient group have shown that 43% of 159 patients presenting with IBS became symptom-free on a strictly supervised elimination diet (*Loblay & Swain, 1986).* Of these, over 90% reacted to double-blind challenges with one or more of the following: glutamate (72%), salicylate (69%), nitrate (64%), preservatives (63%), amines (55%), tartrazine (53%), antioxidants (48%), and lactose (26%). Almost all patients reacted to more than one substance, and the pattern of intolerances was highly idiosyncratic.

Several other studies have examined the relationship between food intolerance and IBS. *Alun Jones et al, (1982)* described provocation of symptoms in 14 of 21 patients, confirmed by double-blind challenge in six. Similarly, *Nanda et al (1989)*, reported symptomatic improvement in 48% of 189 patients treated by dietary exclusion. Seventy-three of 91 patients in their study were able to identify one or more specific food intolerances. In a multicentre study, *Stefanini et al, (1995)* documented improvement in 60% of 209 patients using an elimination diet.

The present study strongly suggests that there exists a subgroup of patients with coeliac disease who have a coexisting irritable bowel-like syndrome with symptoms exacerbated by a range of non-gluten food substances. Indeed, the coexistence of IBS with other gastrointestinal disorders, including coeliac disease, has been documented previously *(Fine et al, 1997; Isgar et al, 1983).*

The implication of these observations is that if coeliac patients continue to experience symptoms on a GFD, and removal of trace amounts of gluten does not lead to improvement, consideration should be given to the possibility of co-existing non-gluten food intolerances.

Diet and Small Bowel Biopsy Changes

Whether or not there is an adverse effect on the small bowel mucosa of coeliac patients from the trace amounts of gluten ingested on a *Codex-GFD* has been unclear. In their challenge of seven patients with wheat starch bread for 1 week, *Ciclitira et al, (1984-a)* found no change in jejunal morphology. Likewise, *Edjerhamn et al, (1988)* detected no difference in biopsy findings between subjects on a wheat starch-containing GFD and a normal non-coeliac control group. In the present study, pathological changes in the small bowel at study entry were

unrelated to the ingestion of trace amounts of gluten. Moreover, there was no significant change in mucosal appearance 3 months after switching from a *Codex-GFD* to a NDG-*GFD* in those where the initial biopsy was abnormal.

These biopsy findings dispel two commonly held misconceptions. Firstly, many workers believe that symptoms are only present in people with coeliac disease when the small bowel mucosa is damaged. Secondly, although persistent villous atrophy is generally attributed to ingestion of gluten, either inadvertent or intentional, this was not borne out in the present study.

The findings described in this study related to a 3 month period of time in a life long disease. What effects trace amounts of gluten could have over a longer period has not been adequately studied. The information presented in the next few chapters examines the relationship between gluten-free diet type, metabolic parameters and small bowel morphology over a two-year period.

CHAPTER 3

3

LONGITUDINAL STUDY: GENERAL METHODS

A two-year follow-up study of the mucosal, metabolic and nutritional effects of the NDG-GFD in people with coeliac disease – METHODOLOGY

INTRODUCTION

The short term study of a group of coeliac subjects described in Chapter 2 showed that in approximately one-third of symptomatic coeliacs on a *Codex-GFD,* the removal of the small amounts of gluten found in their diet led to significant improvement or complete resolution of symptoms. No relationship between these amounts of gluten and mucosal abnormalities could be demonstrated, and a number of patients still had villous atrophy on small bowel biopsy which did not resolve after changing to a *NDG-GFD* for 3 months. This raised the question of whether a longer period of more stringent gluten avoidance would lead to histological resolution. It was also unclear whether any persistence of villous atrophy, despite adherence to a GFD, could be associated with long term problems such as nutritional deficiency or metabolic abnormalities for example osteopenia or osteoporosis. This uncertainty prompted the subsequent longer-term study of a group of coeliacs over a two year period. The aim was

to determine whether adherence to a *NDG-GFD* would improve parameters such as bone mineral density, other metabolic markers and small bowel architecture.

Volunteers were asked to adopt and maintain a *NDG-GFD* for the 2-year study period. As well as addressing the questions above, the nutritional status of each subject, the nutritional adequacy of their GFD and their symptoms were recorded prospectively.

This chapter describes the dietary assessment methodology used to study the above issues. Specific methods are described in the relevant chapters where the results are shown.

STUDY PROTOCOL

Members of The Coeliac Society of NSW were again recruited via a flyer in their quarterly magazine. Recruitment took place between June 1996 and May 1997. The collection of data was concluded in June 1999.

The entry criteria were:

- age between 18 and 75 years
- biopsy proven coeliac disease for at least 2 years
- adherence to a GFD (by their own assessment)
- no other major illness.

The form of GFD that they were following at the time of entry into the study was not an entry criterion nor was the presence or absence of symptoms. The outline of this longer-term study is shown in Figure 3.1, below.

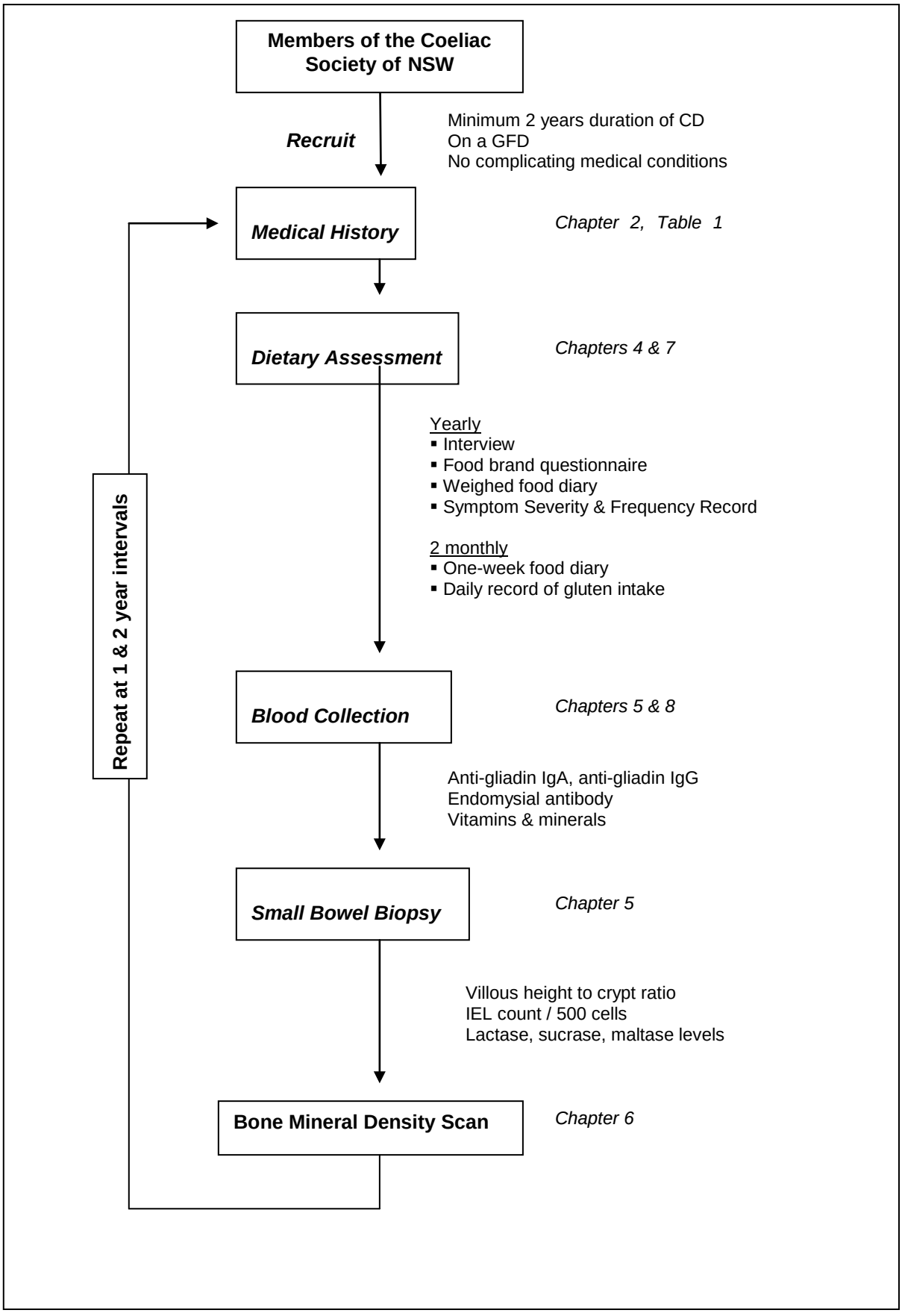


Figure 3.1: Flowchart of the longer-term study

Appointments were made for the first 50 telephone respondents. Forty eight attended the initial interview (38 females, 10 males).

Four subjects dropped out during the first year of the study, 3 females and 1 male, and did not attend for any follow-up after the initial data collection. Their results are not included in the analysis. This left 44 subjects in the study, 35 females and 9 males.

The average age of the group was 47.9. years (median 49 years; range 20-72 years). The average age at diagnosis of coeliac disease was 38.8 years (median 42.5 years; range 6 months-70 years). The mean duration of coeliac disease was 9.3 years (median 5 years; range 2-45 years). At the time of recruitment 38 people were on a *NDG-GFD.* This had been for an average of 1.8 years (range 1-10 years). Six subjects were on a *Codex-GFD* for an average of 6.1 years (range 1-24 years).

The study was approved by the Ethics Review Committee of Central Sydney Area Health Authority (RPAH Zone). All subjects gave written informed consent.

DIETARY ASSESSMENT TOOLS

Experience with the short-term study showed that it was difficult for subjects to keep a daily food, drink and medication diary for long periods of time. It was therefore apparent that the long-term study required the development of additional tools for data collection, particularly for dietary assessment, in order to confirm dietary adherence and determine gluten intake as carefully as is possible during the two-year period. These dietary tools aimed to collect as much information about each subject's diet as was possible, without the need for them to record every item that they ate for two years. Some of the previously used diet assessment forms (Chapter 2) were included (*Food Brand Questionnaire* and *One-week Food Intake*

Diary) with minor modifications where necessary. In addition, new tools were developed to make it easier for subjects to complete the information needed to gauge their gluten intake over the longer duration of the study.

The tools used are described below. They are all shown in appendices at the end of the thesis.

1. Food Brand Questionnaire: (Appendix 1)

This questionnaire is the same as used in Chapter 2.

2. One-week Food Intake Diary: (Appendix 8)

The one-week food diary remained essentially the same as the one described previously. However, the daily record of symptoms was no longer required so this part was removed from the diary.

3. Information Sheet: (Appendix 9)

A one-page sheet was distributed to each subject explaining how to complete the *One-Week Food Intake Diary* and the *Food Brand Questionnaire*. It also explained how to fill in the *Symptom Questionnaire*, which is discussed later (Chapter 5).

4. Supplement Record Sheet: (Appendix 10)

It was clear from some of the answers in the *Food Brand Questionnaire* that a number of subjects were taking a variety of vitamins, minerals, medications and natural medicines. There was insufficient space in the *Food Brand Questionnaire* to note them all, so the *Supplement Record Sheet* was designed and distributed initially only to those who required more space than the Questionnaire allowed. It was later used by all subjects when information was

required about the quantity of their vitamin and mineral supplement intake for the nutritional analyses. This is discussed further in Chapters 7 & 8.

Each subject was asked to list the medications, vitamins, minerals and natural medicines that they were taking. In addition, information about the manufacturer was requested so that they could be contacted to ascertain whether or not the preparation was gluten-free according to the *NDG-GFD* definition.

5. Gluten Intake Record Diary: (Appendix 11)

Although ideally there would have been no dietary mishaps at all during the study, it was realised that this was unlikely to be the case. To replace the need to keep a food intake diary for every day of the study, the gluten intake diary was devised for the purpose of recording any known or suspected gluten intake when it occurred, for whatever reason.

At the initial interview the author discussed with each subject the ingredients which could be included in the *NDG-GFD* and which were to be avoided. The latter included those products usually found in a *Codex-GFD*, such as wheaten cornflour/starch, malt, malt extract, unidentified starch sources, wheat-derived hydrolysed vegetable protein and unidentified thickeners. Glucose syrup, maltodextrin and caramel colour were considered to be gluten-free, even if derived from wheat.

A table outlining the foods and ingredients that were allowed and those that were to be avoided, was given to each subject (Table 3.1).

AVOID	AVOID	ALLOW	ALLOW
1	2	3	4
Wheat Rye Barley Oats Semolina Couscous Triticale Pasta Noodle Crumbs Wheat vermicelli Farina Burghul Spelt Durum Hydrolysed Vegetable Grain	Cornflour Wheaten cornflour Wheat starch Malt Malt extract Malt vinegar Starch Modified starch	Dextrin * Pre-gel starch * Stock * Thickeners * 1400-1450) Dextrose* Glucose syrup Glucose powder Glucose Caramel colour Maltodextrin Beverage whitener Glucono-delta-lactone Hydrolysed Vegetable Protein*	Maize cornflour Corn starch Modified maize starch Rice Corn / maize Soy Buckwheat Millet Sorghum Arrowroot Sago Tapioca Besan (chickpea) Cornmeal Rapeseed Dhal Seeds Psyllium Quinoa Lupin Amaranth Anti-caking agents Cider / wine vinegar
Normal Diet			
	Codex Alimentarius (< 0.3% protein from gluten containing grains)		
		GLUTEN-FREE (< 0.003% gluten) Australian Food Standard (1995)	
			Non-gluten derived

* These ingredients can be derived from wheat, maize or tapioca sources. Wheat derived sources contain very small amounts of detectable gluten and should not be eaten.

Table 3.1: Foods to either avoid or include in the GFD.

The foods listed in column 1 are gluten-containing foods found in a normal diet and which all subjects were instructed to avoid. Oats were included in this list. The foods and ingredients

found in column 2 are acceptable ingredients when labelled as 'gluten-free' under the *Codex Alimentarius* guidelines, and would be consumed in a *Codex-GFD* but not a *NDG-GFD.* Subjects were therefore advised to avoid these ingredients during the study because of the trace amounts of gluten found in them. The products in column 3 fell into 2 groups: Those without the asterisk contain no detectable gluten (even if derived from wheat) and were included in the study diet. Those with the asterisk required the subject to make enquiries about gluten content with the food manufacturer before consumption. The foods in column 4 are naturally gluten-free and can be eaten freely in any GFD.

Each subject was asked to write down in the *Gluten Intake Record Diary* all instances when they had eaten any of the foods or ingredients which were not permitted. These diaries were collected every 2 months.

6. Food Ingredient Check List: (Appendix 12)

As the study progressed there were several subjects who failed to return the *Weighed Food Diary*, *One-Week Food Diary* or *Gluten Intake Record Diary*. In an attempt to collect at least some information about their diet, a *Food Ingredient Check List* was devised. These subjects were asked to complete this at the time of their study visit. This list was used to determine whether they were aware of the ingredients that they should avoid, if they knew the products they had eaten which could contain these ingredients, and to see how frequently they recalled eating any of the ingredients listed in columns 1 and 2. It was accepted that information collected in this manner could not be accurately compared with that obtained from other subjects using the other food assessment tools. The results using this *Food Ingredient Check List* were therefore only included in the analysis when appropriate.

7. Weighed Food Diary: (Appendix 13)

On entry, as well as before the one- and two-year follow-up visits, each subject was asked to record, weigh &/or measure all of the food and drink consumed over four consecutive days, one of which was a weekend day and three week days. This *Weighed Food Diary* was developed with the assistance of the dietetic staff at the Human Nutrition Department, University of Sydney. Existing examples of validated, three-day, weighed food diaries were modified to create a diary suitable for this project. The main alterations included:

- The addition of a fourth day, so that, overall, food intakes would be recorded from each day of the week avoiding any individual daily bias.
- Increase in the space provided to record the information and creation of a booklet-style presentation package.
- Alterations to the information and examples given, to suit a gluten-free diet.

The subjects could choose whether they recorded Sunday, Monday, and Tuesday, Wednesday, or Wednesday, Thursday, Friday, Saturday. Preferably, each food was to be recorded by weight in the diary booklet, but measurements of cups, millilitres and centimetres were acceptable if the weight could not be obtained. A separate booklet was supplied to record the recipes used during this period (Appendix 14).

Height, weight, age and approximate exercise levels were recorded at each appointment. These details were required by the nutritional database to enable calculation of the Recommended Dietary Intake (RDI, previously known as the recommended daily intake) for each of the subjects.

Subjects were asked to complete the diary within one month of receiving it and return it in a reply-paid envelope. At the first and second year follow-up visits, the *Supplement Record*

Sheet (Appendix 10) was added to the *Weighed Food Diary* package in order to enable the tablet vitamin and mineral intakes to be calculated and added to the values obtained from the food diary.

8. Recipe and Food Grid Booklet (Appendix 14)

This recipe record booklet was compiled as an adjunct to the weighed food diary. This enabled assessment of nutrients in a gluten-free recipe which was not available in the computer programme used for nutrient analysis, which relies predominantly on wheat-based recipes for foods such as cakes, pastas, breads and biscuits. The recipes submitted by the subjects for breads, cakes, muffins, dressings, etc., were then used to compile a gluten-free reference database for use when other subjects could not supply like-recipes.

For each recipe, the ingredients used, their quantity and the cooking method were recorded. The latter was important when, for example, a boiled egg was eaten rather than a fried egg (where the fat contents are different). They were also asked to describe and weigh a serve of the final product, eg, a cup of fried rice or an eighth of a piece of cake, and record how many serves they ate.

The *Food Grid* is a standard dietetics tool for estimating the size of a food and hence its approximate weight and nutrient content. Pages drawn-up in a 1cm grid format were supplied so that a rough 2- or 3-dimensional drawing could be made of any unusually shaped food (eg a bacon rasher) that was unable to be weighed. This is particularly useful for meals eaten in a restaurant. The dietitian could then study the drawing and assign an appropriate weight to the food.

The information obtained from the *Weighed Food Diary* and from the *Recipe and Food Grid Booklet* was used to analyse nutrient intake at various times during the study. The methods used and the results are described in Chapters 7 and 8 and are not discussed further here.

DIETARY ASSESSMENT

Each subject underwent a detailed dietary interview by the author at their initial appointment. They were asked to follow a *NDG-GFD* for the two years of the study, regardless of the type of GFD that they were on at entry. This interview covered the following relevant issues that would help in their understanding of what was involved, using the dietary tools described above as an aid:

- A discussion of the change in the *Australian Food Standard* for 'gluten-free' food labelling after March 1995
- A history of intake of wheat starch and malt based products, if relevant, to gain and idea of when the subject made ingredient changes to their diet.
- The nature of the dietary restrictions required for the study.
- A history of their dairy intake from childhood (necessary for the bone mineral study, described in Chapter 6).

Unlike the first study, the *One Week Food Diary*, the *Food Brand Questionnaire* and the *Symptom Frequency and Severity Record Sheet* (described later) with the accompanying *Information Sheet*, were sent to each volunteer after they had accepted over the phone. The completed forms were brought with them to the initial interview and were used in conjunction with their ingredient recall history to determine their GFD category at entry, as described in Chapter 2.

SYMPTOM ASSESSMENT

Symptom Questionnaire: (Appendix 15)

The *Symptom Questionnaire* asked about the type, frequency and severity of any symptoms a subject was experiencing at the time that the form was being completed. It was completed at entry and at each of the yearly follow-up visits. Specific symptoms such as diarrhoea, pain and lethargy were listed, but space was also provided for inclusion of other non-listed symptoms. Symptoms were graded by the subjects according to both their severity and frequency as shown below. This was a more precisely defined grading system than the one used in the short term study described in Chapter 2.

Frequency :The subject was asked to rate the frequency of each symptom experienced according to the following scale:

0	never
1	less than once per month
2	monthly
3	weekly
4	daily.

Severity: Assessment of severity was based on the following guidelines:

1	*Mild*	Aware of the symptom, but it is easily tolerated.
2	*Moderate*	Enough to cause interference with daily life or usual activities
3	*Severe*	Incapacitating, with inability to work or to take part in usual activities.

An information sheet was sent out with this questionnaire to assist in its completion. Both this and the *Symptom Questionnaire* are shown in Appendix 15.

DATA ANALYSIS

Data were entered and analysed in Microsoft Excel. The analysis was performed with the advice and assistance of Associate Professor Sing Kai Lo, University of Sydney.

CHAPTER 4

4

LONGITUDINAL STUDY: dietary adherence & gluten intake

An estimate of the inadvertent gluten intake in the "gluten-free" diets of people with coeliac disease.

INTRODUCTION

Following a gluten-free diet is not simply a matter of only eating foods that are labelled 'gluten-free'. Even though the *Food Standards* permit manufacturers to label products as 'gluten-free', they are not required to do so. People with coeliac disease consume many commercial foods which they believe are free of gluten even though they are not labelled 'gluten-free'. This makes it very important to educate people with coeliac disease to read food labels. However, labels can be misleading and do not always indicate that there is a complete absence of gluten in a particular product. For example, under the *Food Standards* in force during the study, if "cornflour" was listed as an ingredient this could have come from either wheat starch or maize. The same situation arose with other ingredients such as thickeners and hydrolysed vegetable protein. People with coeliac disease also do their own cooking at home; eat at friend's houses; consume fast foods and snacks; and eat out in restaurants. Foods consumed in these circumstances are potential sources of inadvertent gluten ingestion, which at times, are beyond the control of the coeliac individual.

The current chapter deals with the issue of how much gluten is eaten by subjects adhering to a gluten-free diet, recognizing that total gluten avoidance is generally not possible in practice and that inadvertent and other incidental ingestion can occur as part of daily living. Quantitative information about the amounts of gluten ingested in these circumstances has not been documented previously, but may be of considerable importance in understanding variations in small bowel morphology, bone mineral density and nutritional status amongst patients with coeliac disease.

METHODS

Forty-four subjects were enrolled into the study between June 1996 and May 1997 (see Chapter 3). The flow chart for this part of the study is shown in Figure 4.1.

DIETARY ASSESSMENT

The tools used to assess gluten intake are described in Chapter 3. They are listed below:

1. *Food Brand Questionnaire* (Appendix 1)
2. *One-week Food Intake Diary* (Appendix 8)
3. *Information Sheet* (Appendix 9)
4. *Supplement Record Sheet* (Appendix 10)
5. *Gluten Intake Record Di*ary (Appendix 11)
6. *Food Ingredient Check List* (Appendix 12)

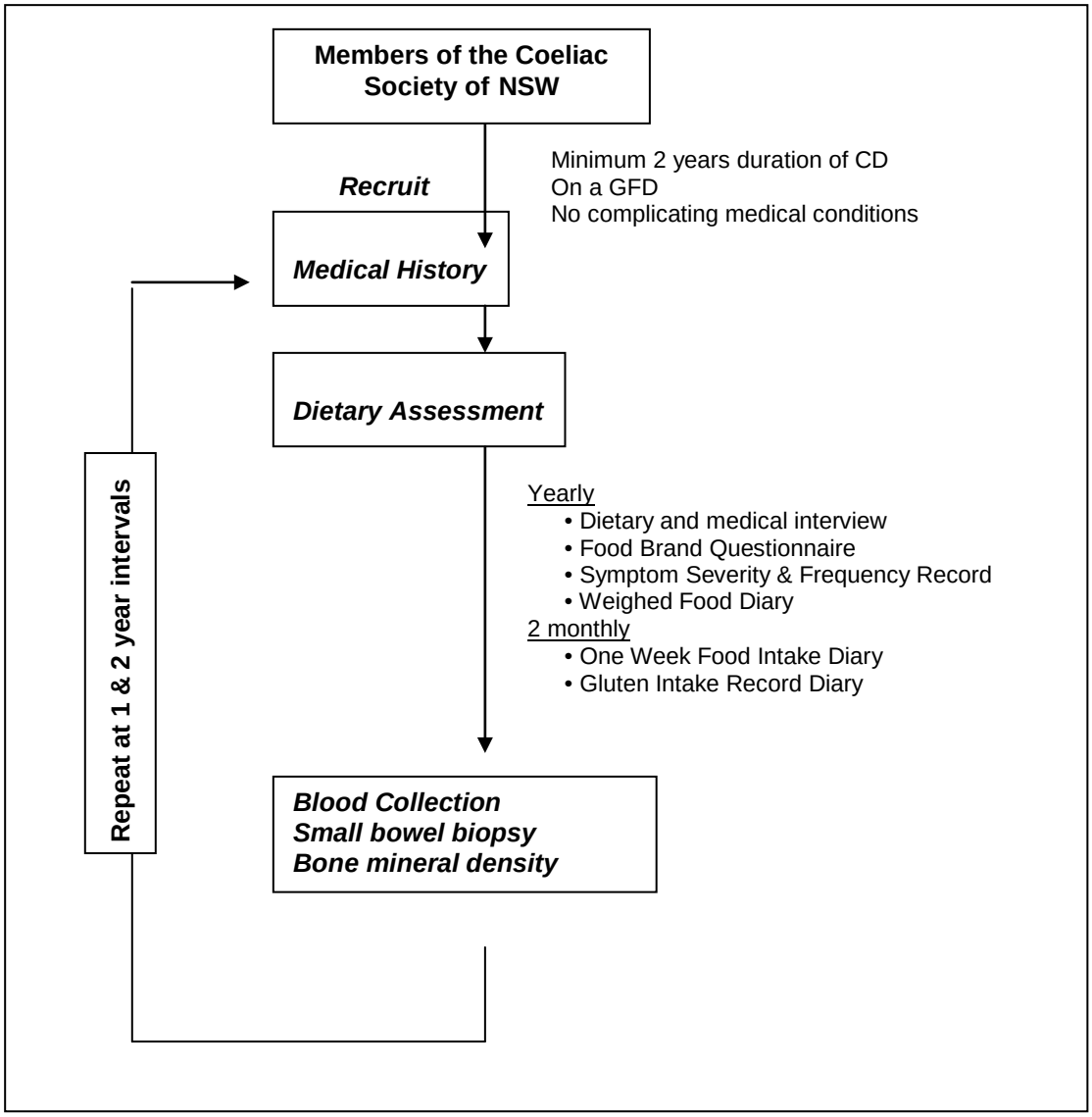


Figure 4.1: Flowchart of the longer-term study

Determining the frequency and quantity of gluten ingested

Throughout the two-year study period a *Gluten Intake Record Diary* and a *One Week Food Intake Diary* were sent by mail at 2-monthly intervals. These diaries were used to gather information about the frequency and approximate quantity of any gluten ingested during the study period. The frequency of gluten ingestion refers to the number of times per year a subject ate gluten at any one sitting.

However, assessment of the quantity of gluten eaten was much more difficult. The amount could vary from the Codex-permitted gluten found in wheat starch, to that in a single mouthful of a wheaten-pasta lasagne, or a meal containing 12-crumbed king prawns. Estimation of yearly gluten intake for any given subject was only possible if the gluten content of the foods they were eating could be calculated.

In some instances, the person with coeliac disease did not personally prepare foods that may have contained gluten. To estimate the gluten intake on these occasions a generic recipe was found for the food in question. The quantity of any wheat flour (or other gluten grains/flour) eaten was then determined from the amount consumed and the subject's gluten intake calculated. Where the precise amount of food eaten was not recorded (e.g. 1 slice of cake), the Australian SERVE Nutritional database *(SERVE Nutritional Management System for Microsoft™ Windows®)* was used to obtain the weight of a standard serving size of the food. This computer package utilises an Access database incorporating published tables of analyses of commonly consumed Australian foods *(NUTTAB, 1992)*. The SERVE Nutritional Management System for Microsoft™ Windows® was developed by Mike and Hazel Williams and is available throughout Australia. Although very few gluten-free grains and gluten-free commercial products had been included in the NUTTAB tables, only the nutritional analyses of gluten-containing foods and standard serving sizes were necessary for this part of the study. Additions of gluten-free foods to the SERVE database were made from the nutritional analysis of each subject's gluten free diet. An in-depth explanation of this is presented in Chapter 7.

Where possible, the author contacted commercial food manufacturers, confectionery manufacturers and pharmaceutical companies, to obtain ingredient information for the relevant product to estimate its gluten content. Where this information was not available from the

manufacturer, the gluten content of the commercial food was estimated from a standard recipe. It was considered that these uncertainties were likely to be of minor significance. It was impossible to trace potential contaminants of all food items eaten in restaurants, so unless the gluten ingredient was definitely established, these occurrences have been recorded only as 'possible' intakes of gluten and not included in the calculations of gluten eaten per year. Often the suspicion of gluten was raised because the subject felt unwell after the meal even though they had requested the meal be gluten-free and had been assured there were no gluten-containing ingredients.

Although most subjects returned all or the majority of their diaries, this was not the case for every one. For those who did not return any diaries the alternative *Food Ingredient Check List* was given at the one- and/or 2-year visits. However, the data were not reliable enough to quantitate their yearly gluten intake, so their results were not included in any of the analyses.

Once the quantity of any gluten-containing food or ingredient that had been eaten was determined, it was necessary to calculate the actual gluten, and hence gliadin, content. This was done in the following ways:

Gluten content of wheat

This was based on figures obtained from *Catassi et al (1993).* According to that report:

2.5g wheat flour ≅ 200mg gluten ≅ 100mg gliadin.

Recipes were obtained for the various wheat products consumed. Examples included cakes, breads, sweet biscuits, crackers, pasta, ice-cream cones, Eucharist wafers, sauces and batters. The weight of flour in the recipe was ascertained and the gluten content of the amount eaten was calculated.

The situation with wheat starch is not as clear as with wheat flour. The gluten content may vary depending on the washing process of the flour (Chapter 2). Even though experts at CSIRO claim that well washed and processed wheat starch contains no detectable gluten (personal communication, Dr. Colin Wrigley), this is not always the case in practice. Nevertheless, Australian wheat starch is reasonably consistent in its gluten content.

Australian wheat starch and icing mixture:

Personal communications with Starch Australia (Mr. Brian Nicholson & Ms. Jenny Smit), one of Australia's leading starch producers, indicates that their wheat starch contains approximately 0.02% gluten (0.02g/100g) when tested with the *Medical Innovations* test kit. These manufacturers believe that wheat starch from other companies in Australia would be similar. This figure has therefore been used as being representative of the gluten content of Australian-produced wheat starch.

Icing mixture (a combination of pure icing sugar and wheaten cornstarch), produced by the same company, has an upper limit of 5% wheat starch. This figure was used in all icing mixture calculations.

Wheat starch not produced in Australia:

A Danish team demonstrated that wheat starch could contain a substantial amount of gluten, with levels of 0.1g per 100g of starch or more *(Mulder et al, 1993).* A representative figure for wheat starch produced in other countries could not be obtained. If all the allowable protein in a food labelled as 'gluten-free' under the *Codex* guidelines is assumed to be gluten, then the gluten content of wheat starch would be 0.3g per 100g. It was decided to use an average of the *Codex* figure (0.3g/100g) and the Australian wheat starch figure (0.02g/100g), i.e. 0.16g

gluten/100g for wheat starch produced outside of Australia, in calculations of gluten content for those participants who travelled overseas (usually Europe) during the 2-year study period.

Gluten content of other grains

Wheat is a well studied grain and is the main source of gluten in the Western diet. Other grains have not received as much attention as wheat and their prolamin contents are not known. It is therefore necessary to make some assumptions when calculating their gluten content.

Rye:

The SERVE nutritional analysis program indicated that 100g of rye flour contains 12.8g of protein. Since 30-50% of rye protein is secalin, a figure of 40% was used and assuming that secalin is equipotent (gram for gram) in causing coeliac pathology, the 'equivalent' gluten content was calculated as follows:

100g of rye flour ≅ 5.12g secalin prolamin = 5.12g gluten equivalent

Barley:

The SERVE program indicated a protein content of 8.6g per 100g of raw barley. Barley protein has a prolamin (hordein) content of 35-45%. As with rye, an average of 40% was used in the calculations of equivalent gluten content:

100g barley ≅ 3.44g of hordein prolamin = 3.44g gluten equivalent

Malt:

Ellis et al (1990) have been able to show that malt, derived from germinated barley, contains gluten in the form of the prolamin hordein. The exact gluten content is very difficult to estimate and depends largely on how the malting process was performed. The anti-gliadin antibodies in

the ELISA for gluten are not very specific for hordein, so, in general, the gluten content of malt is thought to be underestimated. However, for the purposes of this study the following estimate was obtained from the *USDA Nutritional Database:* 100g of barley malt contains 10.28g protein. The assumption was made that the hordein quantity of barley malt protein is the same as the hordein quantity of the barley protein from which it was derived.

100g barley malt ≅ 4.11g (40% of 10.28g) malt hordein prolamin

= 4.11g gluten equivalent

Oats:

None of the subjects in this study ate oats during the two-year period and hence there was no contribution to their gluten intake from this grain.

Calculating yearly gluten intake

From the gluten-containing foods recorded in the 2-monthly *Gluten Intake Record Diary*, cross-checked with the *One-week Food Intake Diary*, a table of gluten content of foods eaten was made for each subject. From this, the amount of gluten ingested annually by each subject could be calculated. The results were expressed as mg/year.

RESULTS

For reasons beyond the control of the researchers, the data collected for some subjects was incomplete. Table 4.1 below summarizes why, unless otherwise stated, the data of certain subjects were excluded from the results at certain time points in this thesis. The results from these subjects were also removed from all 3 years of data when longitudinal analyses were performed. This is indicated in the relevant Chapter(s).

SUBJECTS EXCLUDED			REASON
ENTRY	YEAR 1	YEAR 2	
5, 6, 14, 46		24, 47	Dropped out
33	24, 33, 47	9, 16, 18, 33	Insufficient dietary information recorded
	17	17	No biopsy
	1		Travelling
	23	23	Gluten-containing diet (non-compliance with the protocol)

Table 4.1: Subjects excluded from the data pool.

Of the 6 diaries sent to each participant per annum, an average of 4.6 (mode 5) diaries were returned in the first year and an average of 4.9 (mode 6) were returned during the second year of the study. When an individual's diaries recorded instances of gluten ingestion in each diary, and all 6 had not been returned, their results were extrapolated for that year. In most, however, the consumption of gluten was only occasional and extrapolation was not conducted. Data from subjects who returned fewer than 3 diaries per year were not included in the group analysis (Table 4.1).

In the first year of the study, only 4 subjects recorded no intake of any foods containing gluten, either overtly or in the trace or Codex-permitted amounts allowed on a *Codex-GFD*, and 35

recorded definite or possible ingestion of trace, Codex-permitted and/or overt gluten on one or more occasions (Table 4.2). In the second year, 12 subjects had no recordable gluten ingestion, while 26 had ingested trace and/or overt amounts at least once.

	Year 1		Year 2	
	definite	possible	definite	possible
Zero gluten	4	-	12	-
Trace gluten only	10	2	7	-
Overt gluten only	10	-	10	-
Trace & overt gluten	10	3	7	2

Table 4.2 Incidental gluten ingestion (number of subjects)

Those who ingested overt gluten did so an average of 1.6 times in the first year (range 1 – 3) and 2.6 times (range 1 – 8 in the second year. [Subject 36, who took Holy Communion wafers (containing overt gluten) 53 times in the first year and 70 times in the second, was considered an 'outlier' and was excluded from this group analysis. Her gluten intake is plotted in Figure 4.2].

Those who ingested gluten from Codex-permitted sources did so an average of 2.4 times in the first year (range 1 - 6) and 4.2 times in the second year (range 1 – 21). [In Table 4.2, subjects 10 and 38 were excluded from the analysis of the first year results because they were taking wheat-starch containing essential medication (Oroxine) daily, but were included in the second year results since a gluten-free formulation had become available by then. Subjects 7 and 39 were excluded from the analysis of the second year results, since they were having Codex-permitted amounts of gluten on a daily or weekly basis for significant periods of time during that year. The gluten intakes of these subjects are shown in Figures 4.2 and 4.3].

Quantifying gluten ingestion

Both the frequency and quantity of gluten ingestion is required to obtain an overall picture of adherence to the gluten-free diet. The number of subjects in this study was too small to determine this. Moreover, the *NDG-GFD* and *Codex-GFD* categories alone did not describe the gluten intake in a measurable and comparable manner. In order to be able to look for correlations between gluten ingestion and biopsy findings, symptoms and/or bone mineral density changes, it was necessary to categorise the amount of gluten ingested annually in ways that would permit statistical analysis. The total amount of gluten ingested by each subject during the first and second year was estimated as outlined in the methods section above and plotted on a logarithmic scale (Figures 4.2 and 4.3). [In each of these figures, a gluten intake of 0 mg/year was plotted as 0.001 mg/year.]

Table 4.3 shows the new GFD categories that were established from the information shown in these logarithmic graphs:

Classification of Gluten Intakes		
A	=	≤ 0.01mg gluten / year
B	=	gluten intake 0.01 - 10mg gluten / year
C	=	gluten intake 10 - 1000mg gluten / year
D	=	gluten intake >1000mg gluten / year

Table 4.3: Revised gluten-free diet classifications

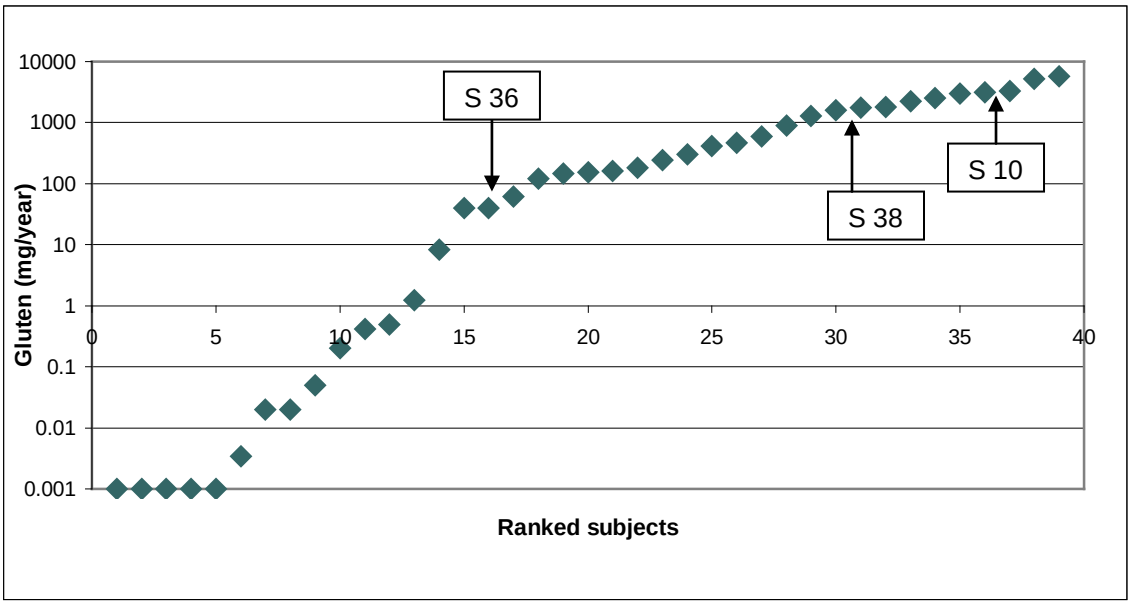


Figure 4.2: Log graph of the gluten (mg) quantities consumed during the first year.

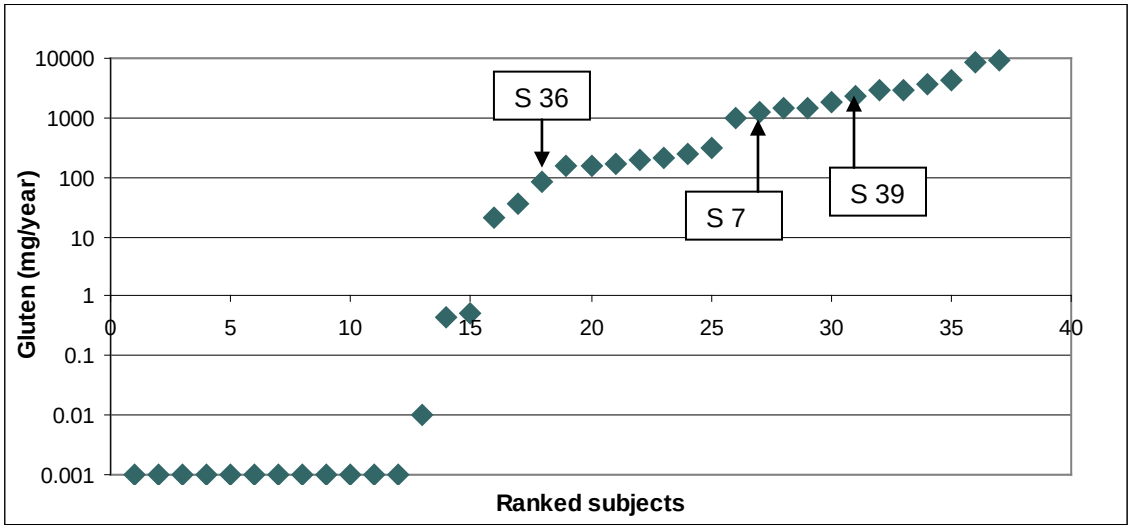


Figure 4.3: Log graph of the gluten (mg) quantities consumed during the second year.

The gluten intakes from each subject were now classified in terms of frequency and quantity. To illustrate variations in the pattern and quantity of gluten ingestion over the course of one year, data from four subjects (S-41,50,45,49 respectively) are shown below in Figure 4.4.

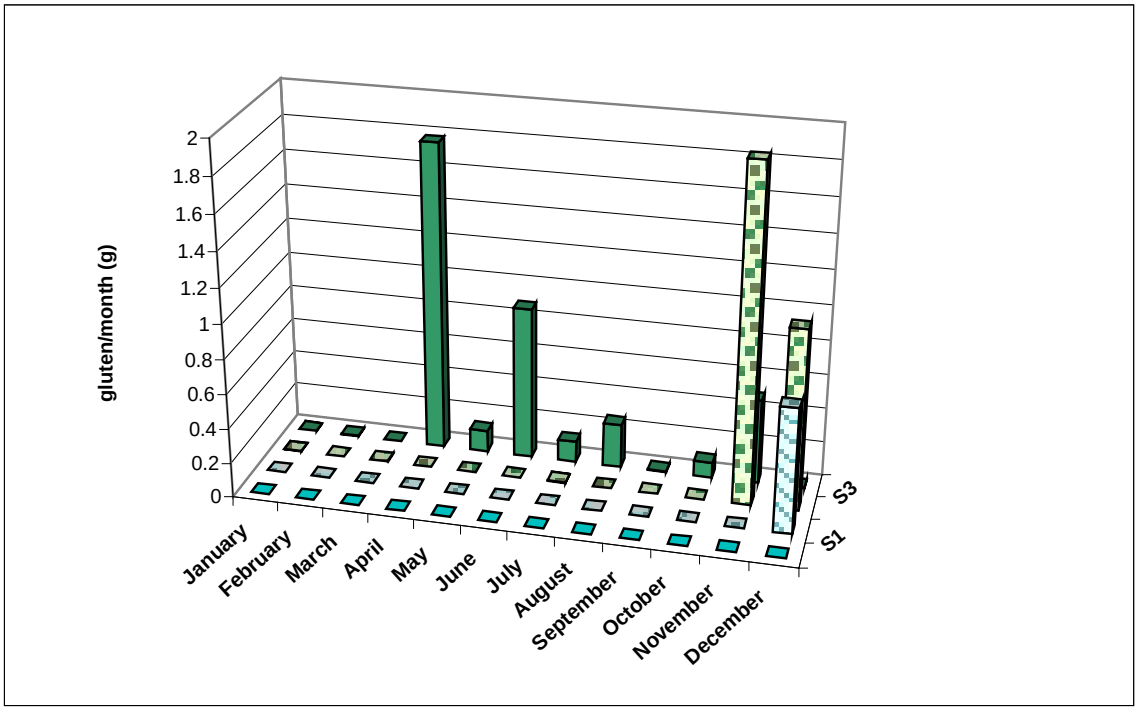


Figure 4.4: Varying accidental gluten intake ingestion patterns in 4 subjects.

The information in Tables 4.4 and 4.5 illustrates the interpretation difficulties of categorising individual gluten intake. These tables show that subject 23 was eating a gluten-containing diet and was omitted from the analyses. The next highest gluten intake during the first year (Figure 4.2) was by subject 20 who did not consume any Codex-permitted ingredient foods throughout the year but on two occasions ingested large amounts of gluten in a meal. Based on frequency alone, she was therefore categorized to the *NDG-GFD*. However, at Christmas she consumed a generous slice of wheaten Christmas pudding and on one other occasion during the year she ate 4-battered King prawns, hence her overall gluten intake was considered high. The person with the next highest gluten intake in year 1, subject 49, was initially classified as consuming a *Codex-GFD,* but recorded 6 instances of ingestion of Codex-permitted ingredients such as wheat starch and malt, as well as 3 instances of small quantities of overt gluten. The gluten eaten included of a slice of wheat flour bread, some wheaten breadcrumbs

and a small amount of wheaten pasta. Tables 4.4 and 4.5 Categorize gluten quantities consumed throughout each study year[5].

subj. #	GFD	Gluten (mg/year)	Diet Classific'n	# Gluten / year	# Codex-Gl / year
2	NDG	0.02	B	0	4
3	NDG	0.02	B	0	2
4	NDG	0.0034	A	0	1
7	NDG	300	C	0	3
8	NDG	2244	D	2	0
9	NDG	2520	D	2	possible
10	NDG(med)	3136.87	D	2	(250 med)
11	NDG	40	C	0	possible
12	NDG	468	C	3	1
13	NDG	888	C	3	0
15	NDG	0.2	B	0	1
16	NDG	180	C	0	4
17	NDG	0	A	0	0
18	NDG	0	A	0	0
19	NDG	1296	D	1	0
20	NDG	5720.8	D	1	0
22	NDG	154.2	C	1	1
23	GL-omitt	12012.2	D	12	possible
26	NDG	1.23	B	0	5
27	NDG	0	A	0	0
28	NDG	0.49	B	0	4
29	NDG	3264.99	D	1	possible
30	NDG	61	C	1	possible
31	NDG	0.42	B	0	1
32	NDG	40	C	1	0
34	NDG	160	C	1	0
35	NDG	8.4	B	1	0
36	NDG(ch)	148.32	C	1 (52ch)	0
37	NDG	242.4	C	2	0
38	NDG (med)	1760.204	D	1	(60 med)
39	NDG	1783.69	D	1	2
40	NDG	0.05	B	0	1
41	NDG	0	A	0	0
42	NDG	121	C	1	2
43	NDG	1610.72	D	1	2
44	NDG	0	A	0	possible
45	NDG	2962.18	D	3	1
48	NDG	595.3	C	2	2
49	C-GFD	5169.46	D	2	6
50	NDG	411	C	2	0

Table 4.4: Gluten intake during the first year of the study. [NDG=*NDG-GFD*, C-GFD=*Codex-GFD*, Gl= *overt gluten eaten 6 or more times*, ch-church, med-medicine]. (Subject 23 omitted from the analyses).

subj. #	GFD	gluten (mg/year)	diet Classific'n	# Gluten / year	# Codex-Gl / year
1	NDG	1840.3	D	1	0
2	NDG	0.01	A	0	1
3	NDG	0.45	B	0	3
4	NDG	0	A	0	0
7	C-GFD	1217.52	D	0	417
8	NDG	9441.6	D	2	possible
10	NDG	0	A	0	0
11	NDG	0	A	0	0
12	NDG	160.01	C	2	0
13	NDG	320	C	2	0
15	NDG	0	A	0	0
17	NDG	192.2	C	1	2
19	NDG	0	A	0	0
20	Gl	3811	D	7	1
22	NDG	1441.38	D	5	3
23	Gl-omitt	42305.5	D	15	1
26	NDG	0	A	0	0
27	NDG	0.5	B	0	1
28	NDG	0	A	0	0
29	NDG	4398	D	4	0
30	NDG	21	C	0	1
31	C-GFD	2906.82	D	2	10
32	NDG	0	A	0	0
34	NDG	160	C	1	0
35	NDG	247.4	C	1	1
36	NDG(ch)	81.2	C	1 (70ch)	0
37	NDG	966	C	0	5
38	NDG	0	A	0	0
39	C-GFD	2263.25	D	2	52
40	NDG	0	A	0	0
41	NDG	0	A	0	0
42	NDG	35.74	C	1	1
43	Gl	2933.3	D	6	0
44	NDG	216	C	1	0
45	NDG	166.5	C	1	0
48	NDG	0	A	0	0
49	Gl	8536.85	D	8	21
50	NDG	1440	D	1	possible

Table 4.5: Gluten intake during the second year of the study. [NDG=*NDG-GFD*, C-GFD=*Codex-GFD*, Gl= *overt gluten eaten 6 or more times*, ch-church]. (Subject 23 omitted from the analyses).

[5] Cross reference to subjects omitted from the analysis- Table 4.1

Frequency of ingestion was also found to be misleading when making clinical judgements about the quantity of gluten ingested. For example, even though subject 36 consumed the Holy Communion wafer 53 times during that year, her total gluten intake was only a little above 100mg for the whole year (Figure 4.2) since the rest of her diet was very free of gluten mishaps. It therefore seemed justified to keep her diet classification as "*NDG-GFD"*, instead of "gluten containing". The average amount of gluten ingested by the subjects in each of the years is summarized in Table 4.6.

	FIRST YEAR N=39			SECOND YEAR N=36		
	mg gluten / year	Range: mg gluten / year	mg gliadin / year	mg gluten / year	Range: mg gluten / year	mg gliadin / year
Average gluten intake per year	904.8	0-5721	452.4	1156.6	0-9441.6	578.3
Extrapolated average gluten intake per day	2.47	0-15.6	1.2	3.2	0-25.8	1.6

Table 4.6: Study participants average gluten intake each year.

Reasons for eating overt gluten

The main reasons reported for eating foods containing overt gluten, are tabulated in Table 4.7. Many subjects listed more than one reason. Most gluten ingestion occurred by mistake or when the person with coeliac disease was unable to control the ingredients in the meal.

Circumstance	Year One (n=40)	Final Year (n=37)
Starving/craving	6	6
Mistake	3	5
Sauces	1	1
Church	1	1
Restaurant/special occasion/friends house	4	4
Never ingested	21	17
Other	1	2
No answer	4	1

Table 4.7: Reported circumstances for eating overt gluten.

Few people in this study group ate out frequently, as shown below in Table 4.8.

Frequency	Year One (n=40)		Final Year (n=37)	
	Take-away	Restaurant	Take-away	Restaurant
Never	4	3	5	2
Rare or occasionally	12	8	12	10
Daily	1	0	1	0
Once per week	4	4	5	9
Several times per week	3	1	3	0
Once every 2 weeks	5	7	5	4
Monthly	6	13	5	11
No answer	5	4	1	1

Table 4.8: Reported frequency of eating take-away foods and meals at restaurants.

DISCUSSION

Diet Records

Since many participants found it difficult to maintain a daily food intake diary for extended periods in the short-term study (Chapter 2), it was considered that a detailed weekly food intake record filled out once every 2 months was an achievable alternative for the longitudinal study. As gluten was not expected to be consumed regularly, a *Gluten Intake Record Diary* was attached to the 2 monthly food diaries, in the hope of amassing more information about dietary mishaps than could be gleaned after one year of recall alone. This gluten intake record proved to be the single most valuable tool for gathering information about the various types of intermittent gluten ingestion.

Terminology

Adherence to a GFD is a relative concept. It can be defined as the degree to which the person's actual daily dietary restriction matches the 'ideal' professional recommendations. Deviations can be large or small, regular or episodic, frequent or infrequent, and can occur knowingly, inadvertently or unknowingly. *Compliance* is a similar concept, but the term has

paternalistic overtones, implying that any deviation from the recommended dietary restrictions (*non-compliance*) is due to the person's intentional rejection of professional advice. In general, the less judgemental term '*adherence'* is to be preferred.

Adherence

As outlined in the introductory chapter, it is important to emphasise that occasional inadvertent ingestion of gluten-containing foods by coeliac patients who are following a *Codex-GFD* or a *NDG-GFD* should not be regarded as an indication of non-adherence or non-compliance, since in practice, this is unavoidable for all but the most obsessive coeliac patient. Amongst the subjects in the present study, documented inadvertent ingestion of gluten was infrequent, and was well below the amounts ingested by patients adhering to a *Codex-GFD* (*Kaukinen et al, 1999*, see Table 4.9). Even if the total intake of gluten in these subjects, for whatever reason, is considered, it is still well below that which would be consumed in a *Codex-GFD*.

This raises questions about the degree of adherence that can reasonably be expected of participants in clinical studies of coeliac disease, or, for that matter, from patients following a GFD prescribed in routine clinical practice. In all likelihood, every medium or long-term clinical study of patients with coeliac disease is likely to involve patterns of intermittent gluten ingestion similar to those documented here.

There is no generally accepted definition of what constitutes 'adherence' to a GFD. In the present study patients were considered to be adherent to the *NDG-GFD* or *Codex-GFD* categories if they ingested trace or overt amounts of gluten, respectively, on fewer than 6 occasions per year. Of the four non-adherent subjects classified as eating an overt gluten-containing diet, only one was deliberately choosing to eat gluten-containing foods when

gluten-free choices were available (i.e. was non-compliant) and her results have been excluded.

Table 4.9 summarizes the extrapolated daily gluten intakes of subjects in other research studies which sought to examine the effects of small amounts of gluten/gliadin in the diet. From this it can be seen that the estimated intake of subjects in the present study are significantly lower than those documented by other researchers.

Reference	Study gluten/gliadin intakes	Average daily gluten intake mg / day
Kaukinen et al, 1999	Codex permitted GFD foods in children and adults	Average 34 [Range: 5-150mg gluten/d]
Ejderhamn et al, 1988	Codex permitted GFD foods in children	10-26
Auricchio & Troncone, 1991	Gluten challenge	≤1000
Catassi et al, 1993	Gliadin challenge	500mg gliadin (1000 mg gluten)
Catassi et al, 1993	Gliadin challenge	100mg gliadin (200mg gluten)
Present study: (Table 4.4)	Extrapolated daily average during the first year of this study	2.47
Present study: (Table 4.4)	Extrapolated daily average during the second year of this study	3.2

NB: from analyses of usual gluten weighed food diaries, a normal gluten containing diet has approximately 7-14g gluten per day (Author).

Table 4.9: Comparison of daily gluten quantities and those used as gluten challenges in research studies.

Gluten ingestion

In the present study the ingestion of both overt and 'Codex-permitted' gluten occurred more frequently when the food eaten was not produced in the home. Eating out for some was a regular occurrence, so small amounts of Codex-permitted gluten were ingested on a regular basis. [In fact this may have occurred more frequently than documented due to the likelihood

that Codex-permitted ingredients were present in sauces found in many restaurant dishes]. In most cases, the ingestion of overt gluten or small amounts of Codex-permitted ingredients tended to be intermittent. On average, subjects ingested gluten on two occasions per year (range1-8).

The extrapolation of the total amount of gluten consumed in a year, to that consumed in a day, was not really representative of the ingestion pattern of gluten in this study. For example, in one case gluten ingestion involved the occasional eating of two slices of wheaten bread, while in another it involved regular ingestion of Codex-permitted gluten found in wheat starch. It is possible that high-level intermittent ("bolus") gluten ingestion has a different effect on the small bowel mucosa than the same amount of gluten ingested at low levels, but more frequently.

Published studies

Some studies *(Ciclitira et al, 1985-a; Faulkner-Hogg et al, 1999)* have concluded that in the short term, ingestion of wheat starch does not affect the small bowel mucosa adversely, but may lead to symptom occurrence. *Kaukinen et al, (1999)* followed a group of subjects eating wheat-starch for 6-24 months and concluded that wheat-starch based gluten-free flour products did not induce mucosal damage, but may have been responsible for some symptoms. *Catassi et al, (1993)* found that, in the short term, minimal biopsy damage occurs from 100mg of gliadin ingested daily. In one case, *Scotta et al, (1982)* showed that the villi returned to normal in an 8 year-old boy after the removal from his diet of the small amount of gluten ingested weekly in the form of a Holy Communion wafer. Investigations by the author into the gluten content of a wheaten wafer have shown that one wafer contains approximately 1.16 mg gluten (0.58mg gliadin). The implication is that this boy is at the "sensitive" end of the coeliac spectrum. *Chartrand et al, (1997)* in Canada reported that 15 of 17 people with coeliac disease who had never eaten wheat starch, and who were asked to include it in their diet,

became symptomatic, either immediately or over the next 10 months, from the daily ingestion of ~1.5mg gluten (0.75mg gliadin) in the form of wheat starch. Biopsy findings were not reported. Of the 31 participants, 35% reacted strongly to the wheat starch, while 12 % had only mild symptoms, supporting the notion that there is a spectrum of sensitivity to gluten amongst patients with coeliac disease. Similarly, the present study (Chapter 2) demonstrated that while very small amounts of gluten found in wheat starch and malt can cause symptoms in some people with coeliac disease, it did not affect their small bowel mucosa in a 3-month period of time.

Remaining questions

How to assess the significance of intermittent ingestion of small amounts of gluten is a problem for researchers and clinicians. Two methods of categorizing patterns of gluten intake have been considered. One is based on the frequency of dietary lapses (Chapter 2), the other on the quantity of gluten eaten in a given period of time (this chapter). Neither method alone is satisfactory. Evaluation is complicated by the fact that trace, Codex-permitted or overt amounts of gluten can be consumed unknowingly in situations where the person with coeliac disease is not responsible for preparation of their own food.

Several questions remain unanswered:

- How important are occasional dietary aberrations, eg. when small amounts of gluten are unknowingly consumed?
- Is the amount ingested during occasional lapses important?
- Is regular ingestion of Codex-permitted ingredients (allowable on a *Codex-GFD*) harmful?
- How frequent must lapses be to have clinically significant effects?

- Is there a subpopulation of patients who are “more sensitive” to gluten (with regards to mucosal damage) and who therefore need to follow a more stringent GFD?

To better understand the influence of trace amounts of gluten on disease outcomes, their effect on the small bowel mucosa, serum antibodies and metabolic markers such as bone mineral density were examined. The results are outlined in the chapters that follow.

CHAPTER 5

5

MUCOSA AND DIET

The effect of prolonged removal of Codex-permitted gluten on biopsy outcomes in people with coeliac disease.

INTRODUCTION

The initial study described in this thesis showed that 64% of subjects with coeliac disease had villous atrophy at entry despite adherence to a gluten free diet. A relationship was observed between the Codex-permitted gluten found in a *Codex-GFD* and symptoms, but not between this gluten intake and histology. However, this was a short-term study, following mucosal outcomes for a period of only 3 months. It is possible that removal of these small quantities of gluten over a longer period could have a beneficial effect on small bowel mucosa.

The minimum amount of gluten that can damage the small intestine in coeliac disease is not clear. According to *Auricchio & Troncone (1991)*, a daily intake of 1g gluten (500mg gliadin) does not affect villous height but there was an increase in intraepithelial lymphocytes (IEL) numbers. *Catassi et al (1993)* found that approximately 100mg gliadin (200mg gluten) per day did not produce any change in villous height/crypt depth (V/C) ratio and little IEL infiltration. However, 500mg of gliadin (1g gluten) per day, for four weeks, resulted in a reduction in villous height as well as an increase in IEL number (*Catassi et al, 1993)*. They concluded that mucosal damage is likely to begin with the ingestion of approximately 100mg gliadin, or more, per day.

The change in the Australian gluten-free food labelling standard provided the opportunity to examine whether the small amounts of gluten being ingested in the subjects described in Chapter 4 would have any adverse effect on small bowel histology and whether a change in gluten intake, from the daily ingestion of trace gluten in a *Codex-GFD* to the very small gluten ingestions occurring in the *NDG-GFD*, would resolve any persistent mucosal damage.

METHODS:

Subjects

Subjects were recruited from The Coeliac Society of NSW. Forty-eight responded and underwent an initial interview. The flow chart for of the study is again shown in Figure 5.1. If dietary analysis was not available over the study period then subjects were excluded.

Dietary assessment

The gluten intake for each of the subjects was determined as described in Chapter 4.

Blood collection

At entry, serum was collected for measurement of IgA and IgG antigliadin antibodies (AGAA and AGAG respectively), endomysial antibody and total serum IgA, IgM and IgG. At the year one and year two follow-up visits, IgA and IgG Endomysial antibodies (EMAA and EMAG respectively) were also measured.

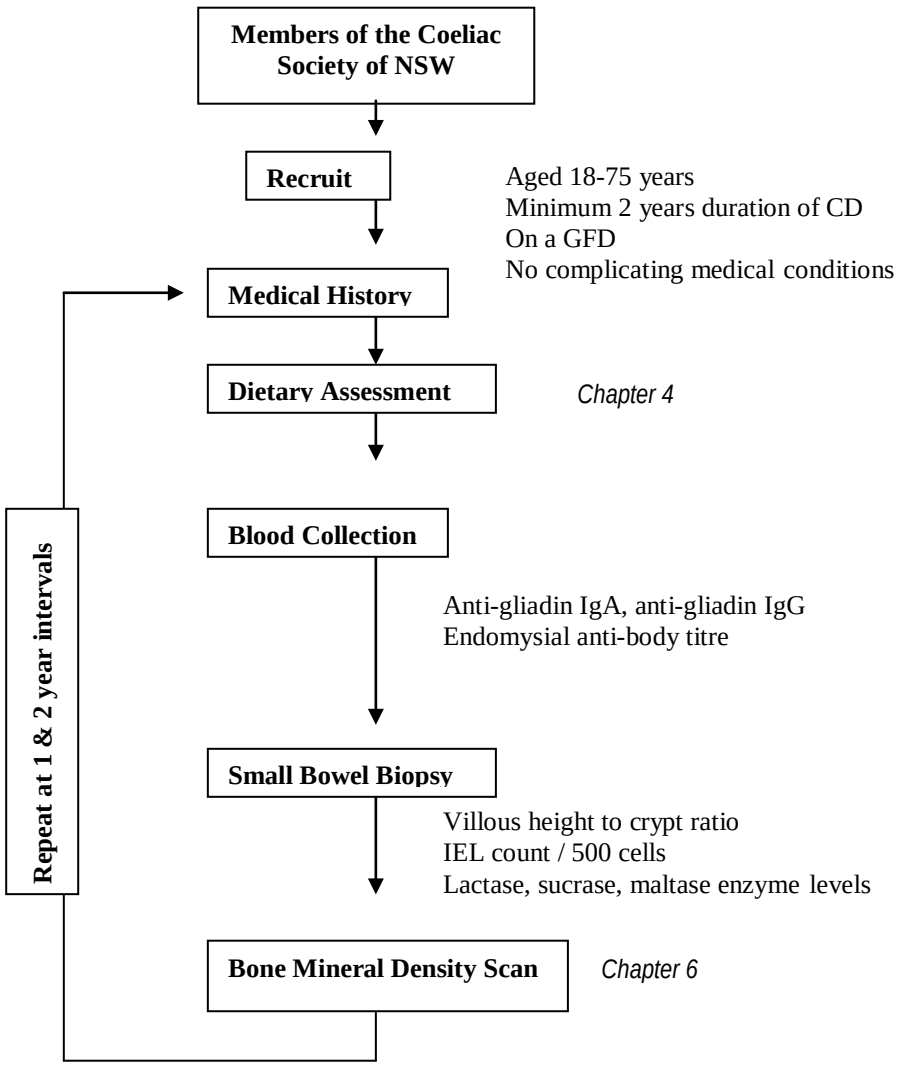


Figure 5.1: Flow chart of the longer-term study methodology

Small bowel biopsy

All subjects underwent endoscopic small bowel biopsy at entry. Most also had this repeated at the one- and two-year visits. Biopsy specimens were taken from the third part of the duodenum by upper gastrointestinal endoscopy. Coeliac and control biopsies were coded and examined without the knowledge of the underlying diagnosis. The histological findings were assessed independently, on 2 occasions, by a pathologist (Dr. D. Painter, Dept. of Anatomical Pathology, RPAH) and by a gastroenterologist (Associate Professor W. Selby). Inter-observer

agreement was high, but if there was any discrepancy consensus was reached by agreement between these two observers, without knowledge of the subjects' gluten intake category. For each specimen, V/C ratio, IEL count (per 500 cells) and activities of lactase, sucrase and maltase were assessed.

Intraepithelial Lymphocytes

Intraepithelial lymphocytes (IEL) were counted in each specimen and expressed as the number per 500 epithelial cells.

Disaccharidase activities

The activities of lactase, maltase and sucrase were determined by using the method of Dahlquist *(Dahlquist, 1968)*. Results are expressed as specific activity units (μmol substrate split/minute/gram protein at 37^0 C).

Symptom questionnaire (Appendix 15)

The *Symptom Questionnaire* asked about the type, frequency and severity of any symptoms a subject was experiencing at entry and at each of the yearly follow-up visits. This is presented more completely in Chapter 3.

Statistical Methods

Statistical analysis between the diet categories and biopsy outcomes were performed using the 2-sided Wilcoxson Rank Sum Test. The Wilcoxson Rank Sum Test was also used to compare the median annual gluten intake with both IgA and IgG antigliadin antibody tests.

RESULTS

Subjects

After the initial data collection 4 of the 48 subjects dropped out and their data has been excluded from the analysis. One other subject did not return the initial *Food Brand Questionnaire*, nor any subsequent food diaries over the next 2 years. From interview and the *Ingredient List* questionnaire, she was classified as being on a *Codex*-GFD, but there was insufficient dietary information to include her results for comparison in this part of the study. This left 43 subjects (34F; 9M) whose initial small bowel biopsies were available for analysis.

At the completion of the first year 41 subjects underwent repeat small bowel biopsy. Of the other subjects, one was travelling around Australia and was not available and the other declined follow-up biopsy but did provide dietary information. Of those who were biopsied, only 38 (32F, 6M) were included in the analysis. This was because two subjects, one of whom had developed a brain tumour, did not return sufficient dietary data. Both subsequently dropped out of the study. The other subject was excluded because she was openly eating gluten, which was a protocol violation.

At the end of the second year 40 subjects were available for biopsy. This included the subject who had been travelling at year one. The subject who had declined biopsy at year one did so again. Of those biopsied, three did not return sufficient dietary information. The subject eating gluten was excluded again. This left 36 (30F; 6M) for analysis.

Biopsy findings

Small bowel histology

At entry to the study, duodenal biopsy was normal in 29 subjects and abnormal in 14 (Table 5.1). By definition, V/C ratio was significantly lower in the abnormal biopsies than in the normal

ones. Ten were considered to have PVA (V/C ratio 1-2:1), 3 STVA (0 < V/C ratio < 1:1) and 1 had TVA (V/C ratio = 0). At year one, 32 biopsies were normal and 6 abnormal (PVA 4, STVA 2). The corresponding figures at the end of year 2 were 27 and 9, respectively, with PVA in 5, STVA in 3 and TVA in 1.

As expected, at each year IEL counts were greater, and lactase levels lower, in those with villous atrophy (VA) than in those without (Table 5.1). The activities of sucrase and maltase were similar to those of lactase (data not shown).

	Biopsy result	# of subjects	V/C ratio	IEL count	Lactase
Entry	Normal	29	2.8 (0.6)	97.5 (27.3)	19.5 (13.3)
	Abnormal	14	0.75 (0.4)	149.3 (64.9)	5.7 (6.3)
Year 1	Normal	32	2.7 (0.7)	92.9 (32.1)	21.1 (14.8)
	Abnormal	6	0.76 (0.4)	150 (70.0)	6.01 (5.1)
Year 2	Normal	27	2.6 (0.9)	85.5 (35.0)	18.5 (12.0)
	Abnormal	9	0.78 (0. 6)	172.7 (60.1)	3.4 (2.2)

Table 5.1: Biopsy Findings. Values are expressed as mean (SD).

Relationship between biopsy findings and GFD Category

On entry, five subjects were consuming a *Codex-GFD* and 38 were consuming a *NDG-GFD*. At year one, only a single subject was consuming what was considered to be a *Codex-GFD*. By the end of the second year this number was six subjects. At no time point was there any relationship between which of these two categories of gluten free diet was being consumed and either the presence of VA, IEL count or lactase activity (Table 5.2).

Biopsy result		# of subjects		V/C ratio mean (standard deviation)		IEL count mean (standard deviation)		Lactase mean (standard deviation)	
		NDG	CODEX +	NDG	CODEX +	NDG	CODEX +	NDG	CODEX +
Entry	N	26	3	2.79 (0.64)	3 (0.5)	97.4 (28.32)	98.3 (20.2)	17.45 (11.12)	37.06 (19.3)
	A	12	2	0.81 (0.36)	0.4 (0.14)	151.25 (70.13)	137.5 (17.67)	6.5 (6.47)	1.1 (1.55)
Year 1	N	31	1	2.55 (0.88)	2.5	93.22 (32.64)	85	21.15 (15.08)	22.1
Data*	A	6	0	0.78 (0.37)	-	150 (70.14)	-	6.02 (5.15)	-
Year 2	N	21	4 (1/4 GL)	2.55 (0.94)	2.93 (0.77)	91.9 (33.22)	78.75 (31.98)	17.76 (12.31)	24.67 (11.45)
Data*	A	7	1 (1/1 GL)	0.83 (0.61)	1	161.42 (48.88)	140	4.1 (1.99)	1.15 (2)

Table 5.2: Relationship between biopsy finding and gluten free diet.

Relationship between biopsy findings and estimated annual gluten intake

Although no relationship was found between persistent villous atrophy and overall category of gluten free diet, the estimation of annual gluten intake described in Chapter 4 allowed more detailed assessment of the effect of gluten on small bowel mucosa. At entry, information on gluten intake during the preceding three months was obtained retrospectively from the *Food Brand Questionnaire*. Allowing for this limitation, no difference in gluten consumption was found between those with normal biopsies and those with villous atrophy (Table 5.3). The same was true at years 1 and 2, during which time gluten intake was assessed prospectively.

		Gluten Intake (mg)	
Biopsy Finding	Entry*	Year 1	Year 2
Normal	0.8 (0-2237)	157 (0-5169)	160 (0-9442)
Villous atrophy	1.3 (0-2160)	234 (0-5720)[§]	36 (0-3881) [§§]

* Gluten intake for 3 months [§] p=0.36 normal vs VA [§§] p=0.75 normal vs VA

Table 5.3. The relationship between gluten intake and villous atrophy. Results are expressed as median and range. Statistical analysis was performed with the 2-sided Wilcoxson Rank Sum Test.

BIOPSY	ENTRY 3 months before biopsy		YEAR 1		YEAR 2	
	# gluten eaten/3mths	# Codex eaten/3mth	# gluten eaten/yr	# Codex eaten/yr	# gluten eaten/yr	# Codex eaten/yr
NORMAL BIOPSY		n=29	n=32		n=27	
number	10 / 29	7 / 29	20** / 32	16* / 32	14** / 27	11 / 27
median	1	2	1	2	1.5	3
range	1 - 2	1 – 90*	1 - 3	1 - 250	1 - 8	1 – 417***
ABNORMAL BIOPSY		n=14	n=6		n=9	
number	7 / 14	2 / 14	3 / 6	4 / 6	5 / 9	2 / 9
median	1	30.5	1	1.5	2	1
range	1 - 12	1 – 60*	1 - 3	1 - 4	1 - 7	1

Table 5.4. Summary of the frequency of gluten ingestion throughout the study, in subjects with normal and abnormal biopsy findings.

* These subjects had frequent wheat starch containing medication (90 & 60 times before Entry and 60 & 250 times during Year 1). ** 1 subject also had Holy Communion wafers 52 and 70 times in respective years. These were not included in the range. ***One subject ate daily, a cereal containing malt extract and often ate lollies that had wheat starch as an ingredient.

This lack of association at each time point was confirmed when the biopsy findings were studied in relation to the category of annualised gluten intake (A,B,C or D) as shown in Figure 5.2. Table 5.4 summarises the number of times subjects with both normal and abnormal mucosa, at each of the time points studied, ate food containing either overt gluten or Codex-gluten ingredients.

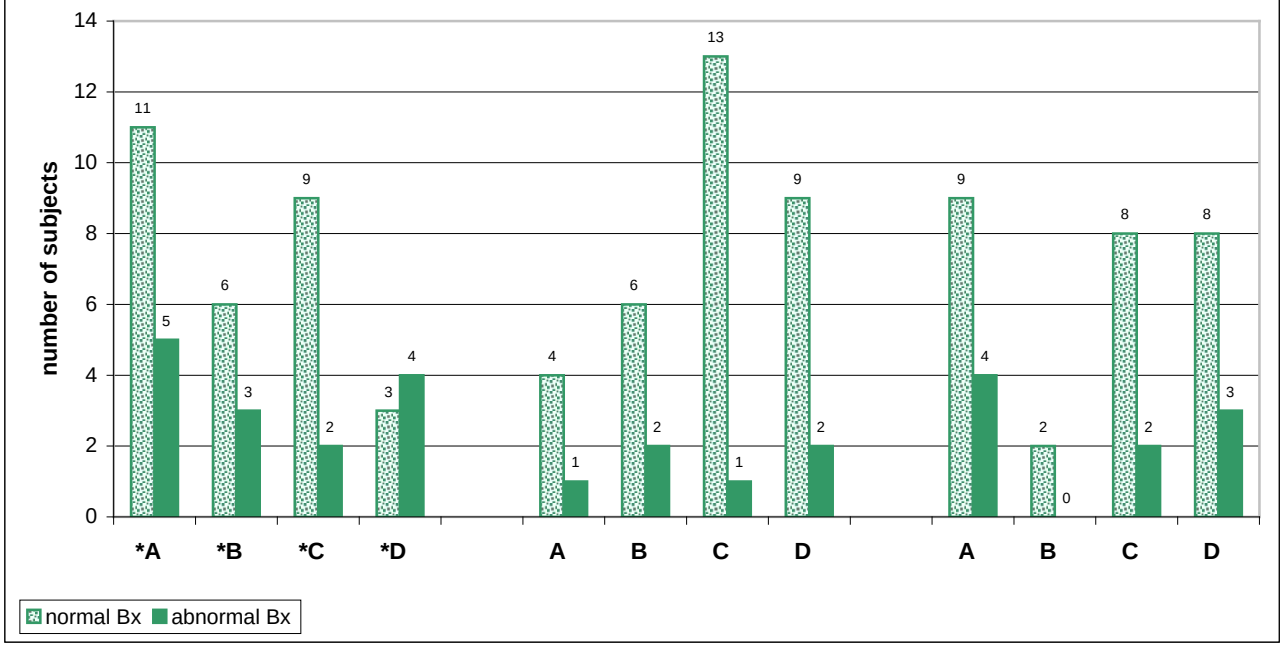
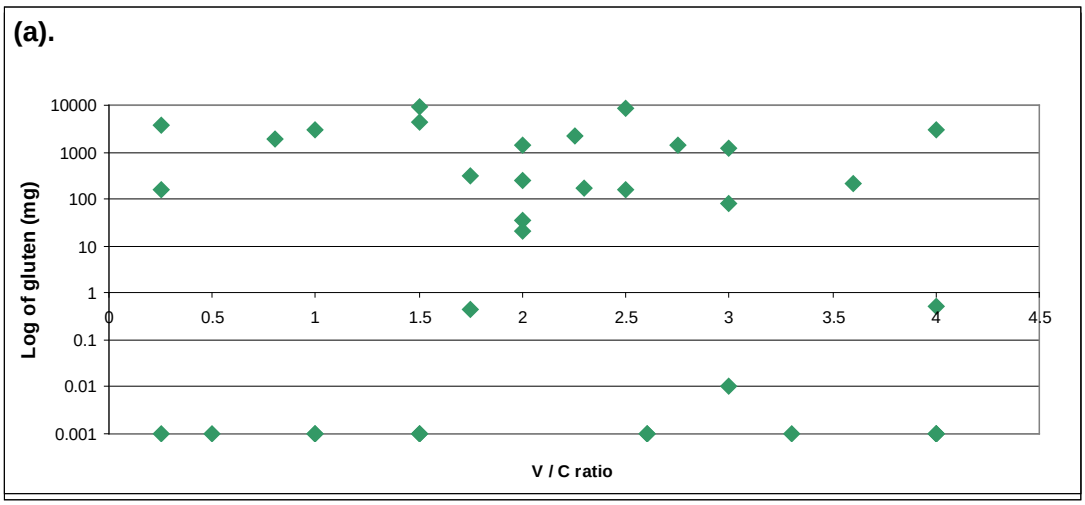
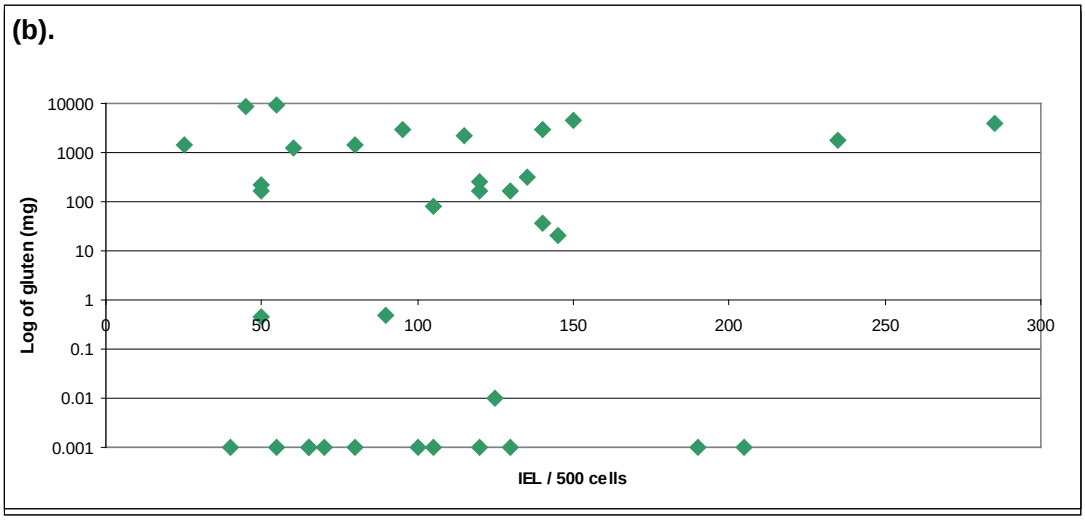


Figure 5.2: Comparison of biopsy outcome and yearly gluten intake at entry (left), Year one (middle) and Year 2 (right).

*Extrapolated values from the gluten intakes 3 months before the initial biopsy
A <= 0.01 mg gluten /year
B <= 0.01 <= 10 mg gluten/year
C >10<= 1000 mg gluten/year
D > 1000 mg gluten/year

IEL and lactase activities were also examined in relationship to annual gluten intake. As was the case with VA, no association was found at any time point. The values at year 2 are shown in Figures 5.3a-c.





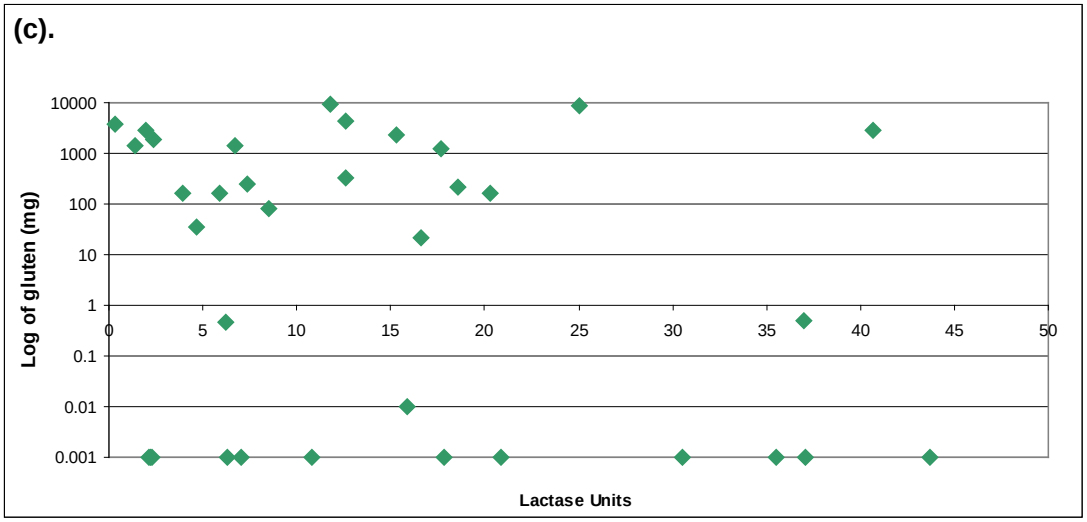


Figure 5.3. Relationship at year 2 between gluten intake and V/C ratio (a), IEL count (b) and lactase activity (c)

Variation in biopsy findings over time

There was some variation in biopsy findings over time. In 14 subjects the initial biopsy was abnormal. In 6 of these the mucosa was normal at 1 and 2 years. However, in 2 of these 6 the biopsy data were not included in the year 2 analysis because insufficient food diaries were completed to allow accurate assessment of gluten intake. In one of the 14, the biopsy was normal at 1 year but abnormal again at year 2. In 7 of the 14, villous atrophy was found throughout the study. However, in one of these, no biopsy was done at year one.

In 23 of the 29 subjects with normal biopsy on entry, the mucosa remained normal at each of the three time points. However findings from one of these subjects were not included in the year 2 analysis because she did not complete sufficient 2-monthly diaries. Three subjects had a normal biopsy initially which became abnormal, either at year 1 or year 2. In the first of these the biopsy was abnormal at year 1 (V/C = 3:2), but normal by year 2 (V/C = 3:1). She had fallen pregnant shortly after entry to the study and gave birth just before her biopsy at year 1. In the other two the biopsies were normal at year 1 but showed VA at year 2. Two other subjects dropped out of the study and the remaining subject, with a normal biopsy on entry, declined further biopsies.

There again did not appear to be any relationship between the gluten intakes, as assessed by annual diet category, and whether the biopsy appearances altered during the study. This was true overall as well as for each individual subject. This is shown in Table 5.5 for those who had an initial abnormal biopsy and Table 5.6 for those with an initial normal biopsy. As above, the 3 month gluten intakes recorded at entry have been extrapolated to yearly amounts.

Subject number	Entry		Year 1		Year 2	
	biopsy	diet	biopsy	diet	biopsy	diet
8	VA	D	N	D	N	D
9	VA	C	N	D	N	Insufficient data
10	VA	D	N	D	A	A
18	VA	A	N	A	N	Insufficient data
28	VA	B	N	B	N	A
36	VA	B	N	C	N	C
39	VA	C	N	D	N	D

Table 5.5 Diet category *vs.* biopsy findings in subjects with villous atrophy on entry.
(VA = villous atrophy; N = normal)

Subject number	Entry		Year 1		Year 2	
	biopsy	diet	biopsy	diet	biopsy	diet
2	N	C	A	B	N	A
26	N	A	N	B	A	A
42	N	C	N	C	A	C

Table 5.6 Diet *vs* biopsy result in subjects with normal mucosa on entry
(VA = villous atrophy; N = normal)

Serological Markers

Antigliadin Antibody (AGA)

IgA AGA was positive more often in subjects with villous atrophy than in those without at year 1 (3 of 6 *vs* 4 of 32; χ^2=7.02; p<0.01) and year 2 (3 of 9 *vs* 3 of 27; χ^2=3.97; p<0.05). For technical reasons, AGA was only available in 7 subjects on entry, but was positive in one of two with villous atrophy and only one of four with a normal biopsy. When the likelihood of a

positive AGA was compared with gluten intake, at either years one and two, there was no relationship seen (Table 5.7). This was seen whether the biopsy was normal or not.

	Median Annual Gluten Intake (range)	
	Year 1	Year 2
IgA AGA Negative	148 (0-5169)	58 (0-8537)
IgA AGA Positive	595 (0.02-5720)[§]	240 (0-9442)[§§]

[§] p=0.32; §§ p=0.33, Wilcoxon Rank Sum test

Table 5.7. IgA antigliadin antibody *vs* gluten intake.

Similar results were seen with IgG AGA (data not shown).

Endomysial Antibody (EMA)

On entry, EMA was undetectable in each of the 40 subjects in whom it was measured. It remained so in all but one subject, a 65 year old woman with partial villous atrophy on entry and subtotal villous atrophy on the following two biopsies. Although her diet was categorized as *NDG-GFD, NDG-GFD* and *gluten-containing* in respective years, her estimated annual gluten intake was in the upper range (5720mg gluten in year 1 and 3811mg gluten in year 2) of subjects in this study. By comparison, the subject excluded for openly eating gluten-containing foods had gluten intakes of 12012mg and 42305mg (respectively in year 1 and year 2) and she did not have raised EMA.

Symptoms, gluten intake and biopsy findings

The number of subjects who recorded at least one symptom in their diary at each of the time points of the study is shown in Figure 5.4. As can be seen, fatigue was the most common symptom, being experienced in over half at entry. Of the gastrointestinal symptoms, bloating, flatulence, abdominal pain and diarrhoea were also common. Symptoms were usually

multiple. Interestingly, constipation was also reported in approximately 15 subjects. Subjects were also asked to record those symptoms that they considered to be moderate or severe, according to the method described in Chapter 3. Fatigue was also the symptom that troubled subjects the most (Figure 5.5). Abdominal pain, diarrhoea and flatulence were again prominent.

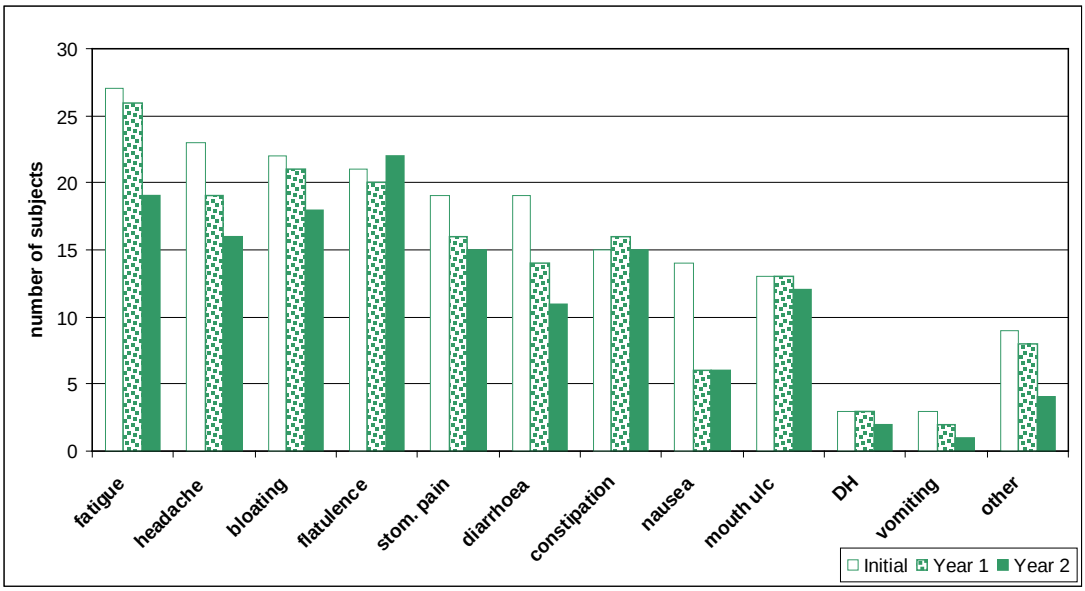


Figure 5.4: The number of subjects recording symptoms during the study.

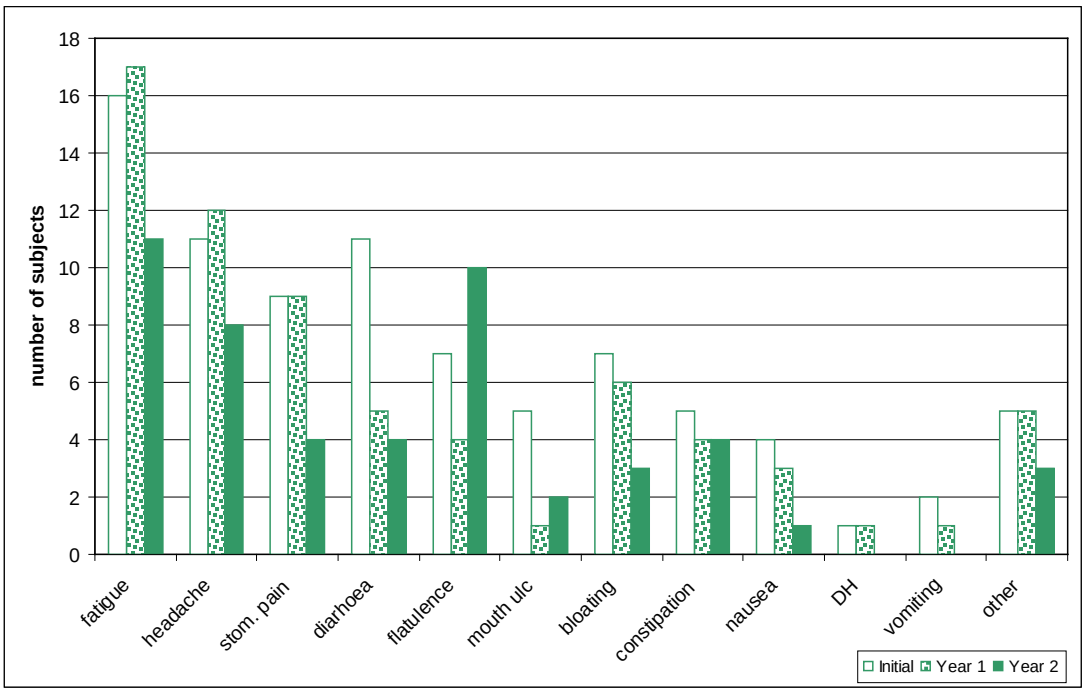


Figure 5.5: Subjects describing symptoms as moderate or severe during the study.

The relationship between both gluten intake and villous atrophy and the frequency with which each subject experienced the symptom and its severity was also examined. The findings for fatigue are shown in Figure 5.6. As expected, there was a correlation between its frequency and severity. However, there was no relationship to either the presence of villous atrophy (yellow triangles) or to the gluten intake (pink circles). The results for diarrhoea were the same, as seen in Figure 5.7, as it was for the other symptoms (data not shown).

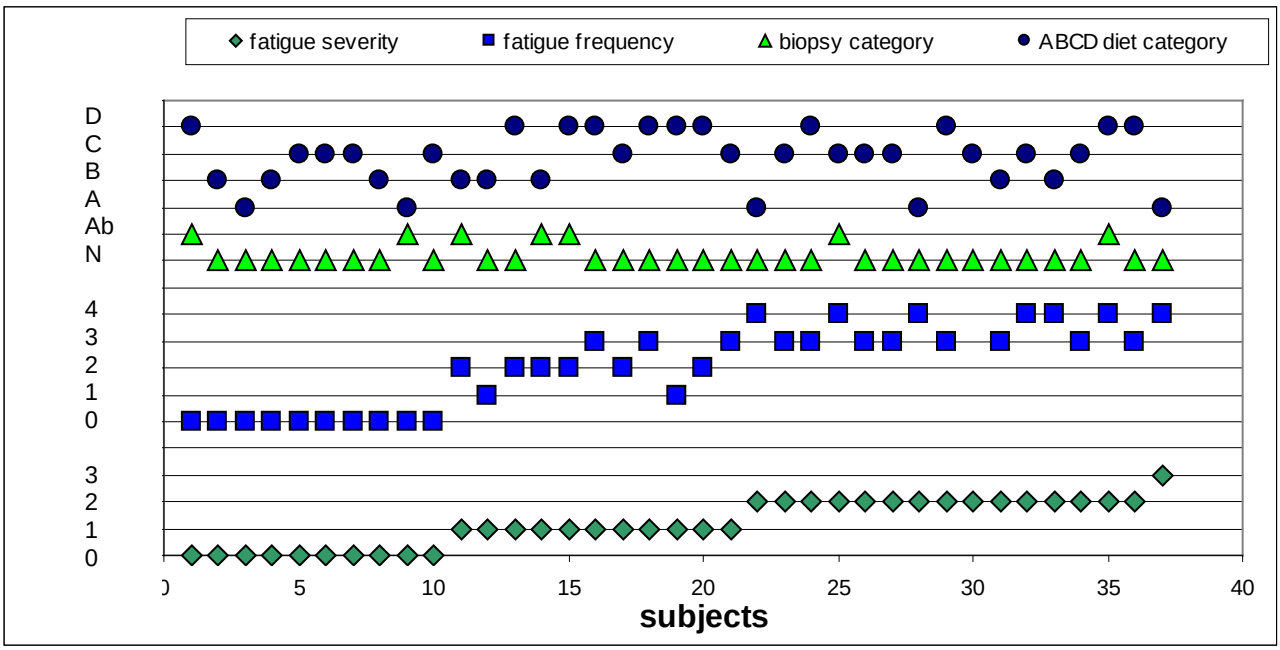


Figure 5.6: Relationship between frequency and severity of fatigue, biopsy findings and gluten intake in year 1 (ranked by the severity of the fatigue). Ab=abnormal biopsy; N=normal biopsy; severity and fatigue rating given in chapter 3.

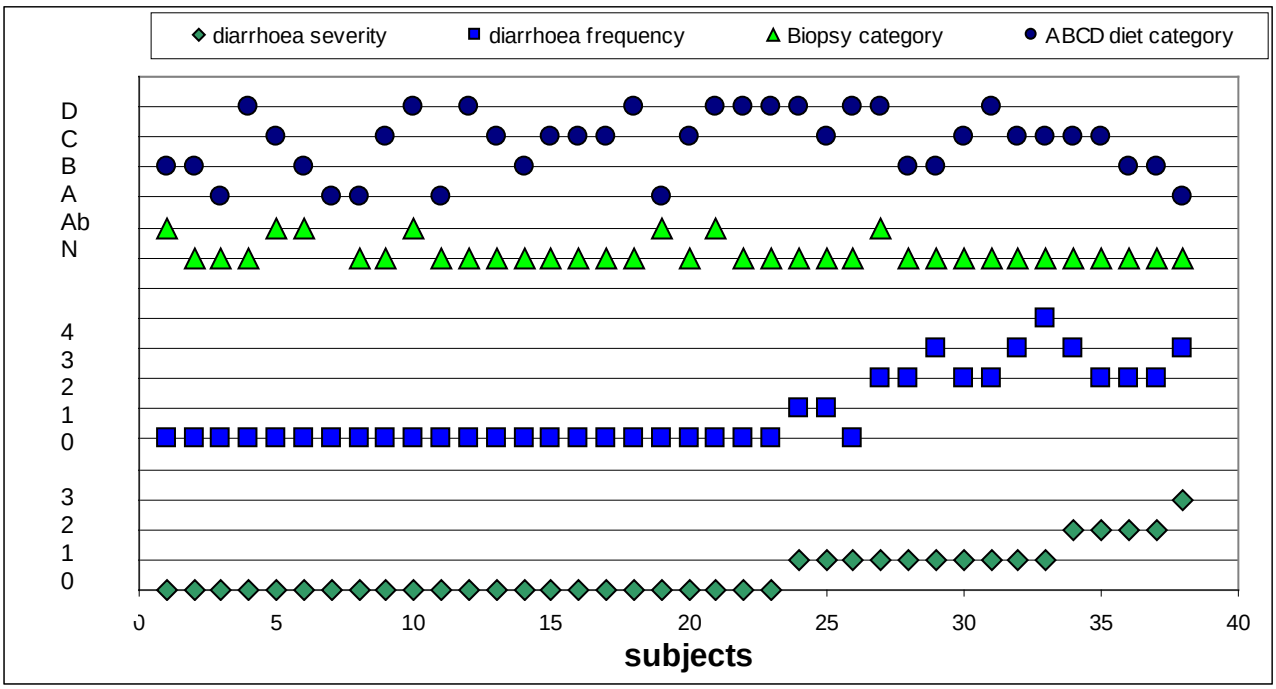


Figure 5.7: Relationship between frequency and severity of diarrhoea, biopsy findings and gluten intake in the first year (ranked by the severity of the diarrhoea). Ab=abnormal biopsy; N=normal biopsy; severity and fatigue rating given in chapter 3.

DISCUSSION

This is the first long-term analysis of the effects of small amounts of gluten on the small bowel mucosa in coeliac disease. The findings of this longitudinal study support those of the short-term investigation described in Chapter 2, namely that the persistent villous atrophy seen in some patients with coeliac disease is not related to the ingestion of the very small amounts of gluten found in a gluten free diet. Of the 43 subjects recruited, 14 had villous atrophy on entry despite compliance to either a *Codex-GFD* or the *NDG-GFD*. In some of these the mucosa returned to normal during the 2-year follow-up. However, neither at entry nor at one or two years was any correlation seen between the type of GFD (*Codex or NDG)* or estimated quantity of gluten eaten annually (category of gluten intake A, B, C or D). The findings for mucosal architecture were mirrored by IEL numbers and by disaccharidase activities.

All of the subjects reported on in this study were consuming a gluten free diet, which was a condition of entry. It must again be stressed that the amounts of gluten ingested by the subjects in this study were very small. Even the subject excluded from the analyses because she was eating gluten, was ingesting 10000mg of gluten per year. This intake represents an average of only approximately 30mg gluten (15mg gliadin) per day. *Kaukinen et al (1999)* reported that 34mg gluten (17mg gliadin) per day was the average gluten intake (range 5-150mg gluten), in his study, of those consuming products labelled under the *Codex Alimentarius* Food Standards, while *Ejderhamn et al, (1988)* reported that, in children, a GFD, defined by the *Codex Alimentarius*, contains approximately 10-26mg gluten (5-13 mg gliadin) per day. The proposed new European Community guidelines would allow up to 20 mg of gluten/100g in any product labelled gluten-free *(Ellis et al, 1998).* Over the course of a day those purchasing gluten free foods fulfilling these guidelines could eat significant amounts of gluten.

The gluten intakes for the rest of the subjects in this thesis were considerably less than this and, in all, well below the levels found in other studies quantifying the intakes of those regularly consuming foods labelled gluten-free by the *Codex Alimentarius*. The mean yearly intake of gluten during the first year of the current study was 904.8mg (R: 0-5721mg) and 1156.6mg (R: 0-9441.6mg) in the second year. This was not eaten on a regular basis, so a daily average does not necessarily reflect the actual exposure of the small intestinal mucosa to gluten at any one time. However, for comparison sake, the above figures correspond to an average daily intake of 2.5mg gluten in the first year and 3.2 mg in the second.

The levels consumed by the subjects in the current study are also less than those that have previously been considered to be the lower limit at which gluten can cause mucosal damage. As discussed earlier, *Catassi et al, (1993)* reported minimal villous damage or effect on IEL numbers following the ingestion of 100mg gliadin per day (≡200mg gluten) in children. *Ciclitira et al, (1985-a),* found no mucosal damage in 10 people after 6 weeks of ingesting a wheat starch-containing product (2.4-4.8mg gluten/day). In contrast *Scotta et al, (1982)* demonstrated recovery of villi to normal in an 8 year-old boy after removal of the small amount of gluten ingested weekly in the form of a Holy Communion wafer. The approximate gluten intake from one weekly Holy Communion wafer is only 1.2mg. This adds up to only 60mg gluten (30mg gliadin) per year. However, the subject would also have been consuming wheat starch products in his *Codex-GFD* and these were not assessed. One subject in our study ingested 1-2 Holy Communion wafers per week throughout the 2-year period. Other than this she maintained a very strict *NDG-GFD*. Although her initial biopsy was abnormal, her 2 follow-up biopsies were normal. Her annual gluten intake was estimated to be 148.3mg in the first year and 81.2mg gluten in the second year.

Ciclitira et al (1984-a), challenged 7 patients with wheat starch (2.4-4.8mg gluten/day) for one week, after it had previously been withdrawn for one week. In one subject there was a slight fall in V/C ratio following the challenge. In a subsequent study, using a six-week challenge, they were unable to detect any change in either epithelial cell height, V/C ratio or IEL counts *(Ciclitira et al, 1985-a)*. *Edjerhamn et al (1988)* were unable to demonstrate any differences in mean villous surface density or IEL counts between 11 patients on a gluten-free diet containing wheat starch (mean 16 mg/d gluten) and 7 normal controls, although at least two patients were outside the normal range. The results of this group of subjects reported here, support these observations and confirm that the small amount of gluten found in wheat starch and malt in products labelled gluten-free under the Codex-Alimentarius Food Standard, is not responsible for the villous atrophy, raised IEL counts and low lactase levels found in many of the subjects.

In this study, although there were a number of abnormal biopsies occurring in people eating more than 1000mg of gluten (500mg gliadin) per year there were others eating this amount with normal biopsies and still others with abnormal biopsies who were consuming much less than this or none at all, clearly showing a lack of relationship between gluten at these small amounts and persistent villous atrophy. This is not to be confused with the definite villous atrophy that occurs in coeliac disease when a gluten free diet is not followed. That each of the subjects in this study was adhering to their gluten free diet was confirmed by the detailed dietary evaluation, including interview and the various questionnaires and records that were used. *Mäki et al (1998)* concluded that small bowel biopsy is still the most sensitive method to monitor the effect of a GFD, even when only small transgressions of gluten ingestion have occurred. This may be true for the majority of patients when they knowingly eat gluten while on a gluten-free diet but our results clearly indicate that the mucosal abnormalities which may

persist in some patients while on the diet and are not due to the ingestion of extremely small intermittent amounts of gluten.

Testing for IgA AGA and for EMA has been proposed as a sensitive measure of gluten intake and hence both as an indication of adherence to a gluten free diet and of mucosal recovery. *Fotoulaki et al, (1999)* found that most subjects were still antibody-positive after one month on a GFD, 23-43% still positive by the end of the 3rd month and, at 6 and 9 months respectively, 17% and 10% were still positive. After 12 months, no patients in that study had detectable antibodies. They also found that antibody levels correlated with mucosal histology at diagnosis, on a GFD and after challenge. In the present study, IgA AGA was detectable in 7 subjects at year one, 3 of whom had villous atrophy and 4 who had normal biopsies. At year two the corresponding figures were 3 with villous atrophy and 3 with normal mucosa. While those with villous atrophy were significantly more likely to have a positive AGA there was no relationship with their estimated gluten intake. In fact, antibody was even found in a subject with no recordable gluten intake and a normal biopsy.

The best way to monitor a patient's compliance with their gluten free diet is for a careful dietary evaluation to be undertaken by an experienced dietitian who can take a thorough history and, if necessary, use some of the tools described here.

Rostami et al (1999-a,b) reported that positive EMA was seen mostly in patients with severe tissue damage. However, this was in patients not on a gluten free diet. They also found that only one of 33 coeliac disease patients, with persistent total villous atrophy despite following a gluten-free diet for more than one year, had a positive EMA. In the present study, EMA was also positive in only one subject, at the end of both the first and second years. She had

subtotal villous atrophy on both occasions and a gluten intake at the upper end of the range of the study (5720mg or 3811mg gluten). This was in less than 6 instances of detectable gluten ingestion throughout the first year and in 8 during the second year. This lady was well known to the researchers and there was no reason to doubt the reliability of her dietary data. No other subjects had detectable EMA, including those with abnormal mucosa and the subject excluded for regularly eating gluten.

These results confirm that both AGA and EMA become undetectable in the majority of coeliacs once they start a gluten free diet. However, a small proportion will still have a positive AGA but this is not because of the trace amounts of gluten found in their gluten free diet. If their adherence to the diet can be confirmed then it is possible that small bowel biopsy will show that they have persistent villous atrophy. What the significance of this is remains unclear, but is discussed below.

The results of the present study also indicate that IgA AGA cannot be used to assess the intake of very small amounts of gluten such as are found in a *NDG-GFD*. This also supports the findings of *Mäki et al (1998)*. Again, dietary assessment is more valuable. As a corollary, a positive AGA does not imply that the patient is withholding information regarding their diet, and surreptitiously eating gluten.

The frequency of symptoms in the patients in this study was not unexpected. This was found in our previous survey of The Coeliac Society of NSW *(Stuart et al, 1997-b*) and by *Fine et al (1997)*. Many of these subjects are believed to have irritable bowel syndrome although, in a minority, other causes such as lactase deficiency and pancreatic insufficiency may be responsible *(Fine et al, 1997*). The short term study in Chapter 2, in which symptomatic

coeliacs were sought, indicated that in some the persistent symptoms are due to the ingestion of the small amounts of Codex-permitted gluten contained in wheat starch and other foodstuffs found in a *Codex-GFD*. Two other studies have also described a variety of symptomatic responses in coeliacs consuming bread containing wheat starch *(Ciclitira et al, 1985-a; Chartrand et al, 1997)*. This occurs even in the absence of morphological damage (*Ciclitira et al, 1985-a; Thornquist et al, 1993)*. The two-year study described in this chapter showed that the frequency and severity of the symptoms was not related overall to any ingested gluten nor to the presence or absence of villous atrophy, the number of IEL or lactase activity. The difference in the relation to gluten intake compared with the short term study is most likely related to the fact that in the long term study symptomatic patients were not sought, and the symptoms were only found when the subjects filled in their questionnaires. Moreover, most patients were already on an *NDG-GFD*, which was not the case in the short term study.

Fatigue was the most commonly reported symptom. Although its severity and frequency varied over time from subject to subject, any improvement was unrelated to either the dietary category or any abnormalities on biopsy. The same was true of the other symptoms studied.

As in *Stuart et al's (1997-b)* study, these data may suggest that the less restrictive *Codex-GFD* could be followed in some people with CD, with similar symptom benefit to those following the *NDG-GFD.* It should be noted however that none of the subjects reported here consumed gluten amounts equivalent to a daily ingestion of 34mg/day which was reported to be an average gluten intake from a Codex Alimentarius GFD *(Kaukinen et al, 1999)*.

The study has not addressed the reasons why some people with coeliac disease choose to follow the gluten restrictions more rigidly than others, within the confines of their GFD choice.

Perhaps reducing the symptoms to a tolerable level is a motivating factor for some who are sensitive to the small amounts found in a *Codex-GFD*. In those in whom the symptoms are not too severe or troublesome the social restrictions of the *NDG-GFD* may be too difficult to cope with, so the *Codex-GFD* may suit their life-style better. Education is undoubtedly important as well. The subjects in this study were all members of The Coeliac Society of NSW. This organisation provides regular information on a GFD and available gluten-free products. Current issues and research into coeliac disease are also discussed. Patients can form their own opinions about what is suitable for them on the basis of this knowledge. The dietary instructions that patients receive from dietitians and other health professionals can also vary, particularly since there are questions about what is the "best" GFD, including those addressed in this thesis. Finally, since some forms of malignancy, particularly small bowel lymphoma (*Kumar et al, 1985; Holmes et al, 1989; Mazzacca, 1993,)* have been linked with non-compliance to a gluten-free diet, it is be possible that this is another reason why some coeliacs choose to be strict with their diet, independent of the presence of any symptoms. However, in the studies referred to above, the gluten-free diet followed by the majority of subjects would have been a *Codex-GFD* and adherence to this returned the malignancy risk to normal.

Two important questions arise as the result of these findings. Firstly, what is the reason behind the continuing villous atrophy despite adherence to a gluten-free diet? It is well recognized that complete return of the mucosa to normal is not always seen in coeliac disease after gluten withdrawal and it does not imply the presence of another disorder *(Selby et al, 1999; Faulkner-Hogg et al, 1999; Chapter 2)*. All of the subjects in this study had well documented coeliac disease. Like the group presented in Chapter 2, they did not have

refractory sprue, autoimmune enteropathy, ulcerative jejunitis, lymphoma or any other cause of persistent villous atrophy. These conditions are characterised by not just persistent villous atrophy but also by continuing presence of AGA and EMA as well as a progressive clinical deterioration with malabsorption and weight loss. Although symptoms were recorded by many patients upon specific questioning, they had few metabolic abnormalities and no evidence of malabsorption, as described in the following chapters.

Many of the mechanisms underlying the pathogenesis of coeliac disease have been elucidated but there remain unanswered questions. Evidence of an ongoing immunological response in treated coeliac disease, even in patients with a normal biopsy, can be seen in the increased numbers of IEL that may persist. These are predominantly of TCRγ/δ phenotype but may not be involved in the immune response to gliadin *(Kutlu et al, 1993).* Coeliac disease has many features consistent with an autoimmune process, including the major histocompatibility complex (MHC) class II gene association, the female preponderance and the presence of antiendomysial antibody, directed against tissue transglutaminase *(Feighery, 1998; Dieterich et al, 1997).* It is possible that once the immunological response to gliadin has been initiated in patients with coeliac disease it may continue in some even after gluten withdrawal, possibly stimulated by some other as yet unidentified autoantigen. The extent of the abnormality has not been studied. It is possible that it may be confined to the duodenum only, as recovery from coeliac disease progresses distally to proximally. A study to address this question is currently being established. This will also include studies of T-cell phenotypes, MHC class II genes, transglutaminase expression and other immunological parameters which have been found to be abnormal in patients with refractory sprue *(Verkarre et al, 2003).* One thing the present studies have shown is that persistent villous atrophy in otherwise well

coeliacs cannot be attributed to unadmitted or inadvertent gluten ingestion, as has been assumed in the past, at least once it has been confirmed the patient is eating a GFD.

The second question is whether the villous atrophy seen in these subjects has any metabolic or other clinical consequences. Reduced bone mineral density is seen in association with villous atrophy in untreated coeliac disease, or persists in those patients who fail to comply with their diet *(Valdimarsson et al, 1994).* However, bone mineral density has not been studied in subjects with villous atrophy on a well-documented long-term gluten-free diet. This is the subject of Chapter 6 of this thesis*. Holmes et al (1989)* demonstrated that the increased risk of malignancy in coeliac disease returns to that of the general population after adherence to a gluten-free diet (assumed to be *Codex-GFD*) for 5 years or more. That study did not examine mucosal histology but would undoubtedly have included patients with villous atrophy. Since the gluten-free diet being followed by this group would have been the *Codex-GFD*, which was and still is the standard in the United Kingdom, there is no reason to expect that persistent villous atrophy *per se* would be associated with a greater risk of malignancy provided the patient adheres to their diet.

Could asymptomatic patients with normal mucosa be able to ingest wheat starch and malt, which will make their gluten-free diet more varied and easier to comply with? This will depend not only on showing that the small amounts of gluten found in either the *Codex-GFD* or *NDG-GFD* do not cause mucosal damage but also that there are no metabolic consequences of their ingestion or of the villous atrophy. This is the subject of the following Chapters.

CHAPTER 6

6

METABOLIC OUTCOMES

Effects of trace amounts of gluten and of persistent villous atrophy on bone mineral density.

INTRODUCTION

It is now well recognized that osteopenia and osteoporosis are long-term complications of undiagnosed coeliac disease *(Valdimarsson et al, 1994; Walters et al, 1995; Pistorius et al, 1995)*. Approximately 40% of adults with coeliac disease have bone mineral density values more than 1 standard deviation below the age and sex standardised population mean *(McFarlane et al, 1992; Butcher et, 1992; Meyer et al, 2001)*. This is more than twice the proportion expected *(Walters, 1994).* Conversely, it has been suggested that the prevalence of coeliac disease among people with low bone mineral density (BMD) is increased by up to 10-fold *(Mather et al, 2001).*

This low BMD is considered to be a result of both impaired protein metabolism and impaired calcium absorption, secondary to the adverse effect of gluten on the small bowel mucosa. In untreated coeliac disease, the villous cells in the proximal small intestine are damaged and can no longer actively absorb calcium (*Walters, 1994).* In addition, free fatty acids are also not absorbed normally. The increased free fatty acids in the intestinal lumen bind calcium so that the quantity of calcium available for absorption is reduced. Malabsorption of vitamin D also occurs, and this may contribute to the development of bone loss *(Ng et al, 1992)*. Secondary

hyperparathyroidism from the decreased calcium uptake contributes to the further loss of bone mineral *(Walters, 1994).* Other general factors that can lead to bone mineral loss are decreased calcium intake due to lactose intolerance and prolonged avoidance of dairy products, amenorrhoea induced by a low body weight, and other factors such as race, heredity, environment and lifestyle, including lack of exercise, smoking habits and caffeine intake *(Wahlqvist, 2002, pp 369-375*). Once patients with coeliac disease start a gluten-free diet BMD improves, although it does not always return to ideal levels (*Corazza et al; 1996; McFarlane et al, 1996; Valdimarsson et al, 1996; Scotta et al, 1997; Mora et al, 1998; Meyer et al, 2001; Mora et al, 2001).*

At the time the studies presented in this thesis were commenced, it was not clear whether the persistence of villous atrophy was associated with greater BMD loss or if it hindered bone recovery. There were therefore two aims to the present study. The first was to examine baseline BMD in a group of coeliac subjects on a gluten free diet and to document any changes throughout the study period. The second was to correlate any changes in BMD with the findings on small bowel biopsy and with trace gluten intake while on a GFD. The following hypotheses were formulated:

- Subjects with persistently abnormal small bowel biopsies (despite following a GFD) would have lower BMD.
- The ingestion of Codex-permitted gluten in a *Codex-GFD* would lead to decreased BMD because the small bowel could be affected, resulting in malabsorption of the appropriate nutrients.
- The elimination of the trace amounts of gluten from the GFD diet *(NDG-GFD)* would improve villous atrophy and BMD.

This long-term study was instigated to look at the effects of trace amounts of gluten on the small bowel mucosa (Chapters 4 & 5) and in doing so allowed the opportunity to assess other metabolic parameters.

METHODS

Bone Mineral Density Scans

Bone mineral density was assessed by dual energy x-ray absorptiometry (DXA) *(Johnston et al, 1991)*. This is a low radiation technique that produces an accurate measurement of bone mass at selected sites of the skeleton. Osteopenia and osteoporosis can be defined as levels relative to the mean of a young population matched for gender. Comparisons are also made with an age, weight and gender matched population. Osteoporotic fractures are almost twice as likely with each standard deviation decrease relative to the mean *(Compston et al, 1987)*.

The DXA scans were performed at the Garvan Institute of Medical Research Institute, with the assistance of Professor John Eisman. All studies were performed with the same scanner to allow reproducibility over time in each individual subject. At each examination the following scans were performed:

- 2 scans of the spine: from lumbar (L) 2 to L4 vertebral bodies.
- 2 scans at the femoral neck.
- 1 total body scan

BMD was expressed in absolute terms (g/cm^2) as well as a T-score, comparing the mean BMD with a young sex-matched population, and a Z-score, which compares the mean BMD with age, gender and weight-matched controls, supplied by the instrument manufacturers.

The Z-score was calculated as follows:

$$\text{Z-score} = \frac{\text{Patient BMD} - \text{mean BMD of age, weight and gender-matched normals}}{\text{SD of BMD of age, weight and gender-matched normals}}$$

The T-score was calculated as follows:

$$\text{T-score} = \frac{\text{Patient BMD} - \text{mean BMD of young gender-matched normals}}{\text{SD of BMD of young gender-matched normals}}$$

Subjects BMD scores were classified twice. Initially they were compared with their own age, weight and gender matched controls as Z-scores: Given the normal distribution, approximately 17% of subjects could be expected to be at least 1 standard deviation (SD) below these controls and approximately 1 percent expected to be more than 2.5 standard deviations below these age and weight matched values.

Each subject was also compared with younger, gender matched controls in the following way:

Osteopenia: $-1 < \text{T-score} < -2.5$

Osteoporosis: $\text{T-score} \leq -2.5$

Subjects

Subjects were enrolled after the initial interview if they satisfied the criteria outlined in Chapter 3. The BMD scan was most often performed within 2 weeks of the study entry appointment and at subsequent 1 and 2-year follow-up appointments. Forty-four subjects, 35 females and 9 males, attended their entry BMD scan. One of the males had had a hip replacement and his data have not been included in these analyses. The data of 2 females were excluded because

one did not return the initial questionnaire allowing diet categorization and the other was classified as consuming a gluten containing diet. Of the 41 remaining subjects, one female was breast-feeding a newborn baby during the time of her first follow-up BMD appointment, but had ceased breast-feeding by the second follow-up appointment. Biopsy data are missing from one male subject who refused both follow-up biopsies. The dietary gluten quantitation data has been omitted in 2 subjects (males) in the first year and 3 subjects in the second year (females) for returning less than 3 of the 6 *Gluten Intake Diaries* during those years. These same 2 males dropped out before the second year data collection. One was diagnosed with a brain tumour and the other had new work responsibilities and was unable to commit to 2 days of testing.

Dietary Analysis

Gluten-free diet

The diets were initially classified as either as *Gluten-containing*, *Codex-GFD* or a *NDG-GFD* as previously described in Chapter 2. The trace gluten intake of each subject was then quantitated and categorised as A, B, C or D, as described in Chapter 4.

Calcium intake

At the initial interview subjects were asked to recall, as best they could, their intake of dairy foods and calcium supplements since childhood. This was obviously an approximation, since details on this were sparse for many of the subjects, given that the average age of the group was 48 years when they were recruited.

Participants' dietary calcium intake during the study was calculated at 3 time points; at entry and after 1 and 2 years in the study. This was done by analysing the nutritional content of all the foods, drinks and supplements ingested during 4 consecutive days, which were recorded

in a weighed food diary and compared with the recommended dietary intake *(NH & MRC, 1991)*. The methods and the full nutritional analysis are reported on in the following chapter.

Small Bowel Biopsy

Duodenal biopsies were obtained endoscopically and analysed as in Chapter 5. The histological findings are described in that chapter as related to gluten intake. The same subjects' results are used here for comparison with BMD.

Other Relevant Data

Cigarette Smoking:

At the initial appointment all subjects were asked if they were a current smoker or had smoked. Those who disclosed a history of smoking were asked to recall their smoking habits throughout their lifetime. They reported the approximate number of years that they had been smoking as well as the number of years that they had ceased smoking.

Menopause and Hormone Replacement Therapy:

Each of the female subjects gave a history of period, hormonal and fertility concerns. Those who had reached menopause were asked whether or not they were taking hormone replacement therapy with their doctor.

Nutritional Status:

Body Mass Index:

One measure of nutritional status is the body mass index (BMI; kg/m^2) which is used to estimate total body fatness and defines a scale for under weight, normal weight, overweight and obesity. The normal reference range for the calculation is 20-25. The body mass index (BMI) was calculated, at all 3 appointments, for each of the subjects. A BMI below 20 suggests inadequate nutrition. A score between 20 and 25 is considered appropriate to normal

weight. Scores greater than 25 and less than 30 are defined as overweight while those over 30 define obesity *(Wahlqvist, 2002).*

Skinfold Measurements

Skinfold measurements were taken from the subscapular and triceps region of the left side of the body. The methodology is presented more fully in Chapter 8. Mid-upper arm muscle circumference (MAMC) is a measure of body muscle mass and an indirect measure of total body protein *(Corazza et al, 1994).* MAMC is expressed as a percent of standard values, where less than 90% is considered to be indicative of mild malnutrition and less than 80% of moderate malnutrition *(Blackburn & Thornton, 1979).*

Serum Total Protein

Blood was drawn at years 1 and 2 only, for analyses of total serum protein and albumin, as a measure of total body protein status.

RESULTS

The average age of the 41 subjects was 48 years (median 49 years; range 20-65 years). The average age at diagnosis of coeliac disease was 38.5 years (median 43 years; range 6 months-63 years). The duration of coeliac disease was 9.6 years (median 5 years; range 2-59 years). At the time of recruitment 38 of these subjects were already following a *NDG-GFD.* This had been for an average of 1.9 years (range: 6 months-10 years). Three subjects were on a *Codex-GFD* for an average of 10.3 years (range: 3-24 years). All subjects were asked to follow a *NDG-GFD* for the 2-year study period.

The average BMI for the group studied in this thesis was 23.8 (range: 17.1-37; median 23.4). This was lower than the average BMI of the Australian population which was recorded as 26.3 (19.8-34.9, 5^{th}-95^{th} percentiles) by the 1995 National Nutrition Survey *(McLennan & Podger, 1998-a, p115)*. No one at any time in the study was regarded as being malnourished since measurements of MAMC were above 90% of standard (Chapter 8). All the serum total protein values were normal at years 1 and 2. Only one female recorded a low serum albumin in year 2. Although her BMI was 23, her total energy intake from food was low, with resultant low nutrient intakes. Twenty-nine subjects recorded a normal entry biopsy, 10 PVA, 1 STVA and 1 TVA.

The data presented in Table 6.1 summarize the entry characteristics of the study group when defined using their age, weight and gender matched controls (i.e. Z-score). The data show that none of the subjects had a BMD more than 2 SD below age and sex matched normals and only 11 (26%) had BMD more than 1 SD below age and sex matched controls. No one was smoking at the time the study was undertaken. Nine subjects who had previously smoked had ceased smoking 3-40 years before the entry data was collected. Of the 33 females, 15 were postmenopausal and 10 of those were taking hormone replacement therapy (HRT).

		Females	Females	Males	Males
		Z > -1 SD	Z < -1 SD	Z > -1 SD	Z < -1 SD
		26	7	4	4
Age; years	*mean*	49	42.2	53	46.7
	range	21-65	27-62	42-57	20-60
Age at diagnosis; years	*mean*	38.4	33.2	45.7	41.2
	range	0.5-63	3-53	32-55	17-54
Duration of CD; years	*mean*	13.2	9.2	7.4	5.7
	range	2-59	2-24	2.5-12	3-14
Smoking	*never*	19	6	3	4
	past	7	1	1	0
Ceased for; years	*number*		3	12	
	mean	20 (1*)			
	range	3-40			
	current	0	0	0	0
BMI; kg/m^2	*<20*	6	1	0	1
	20-25	12	5	2	0
	>25	8	1	2	3
HRT	*yes*	9	1	na	na
	no	17	6	na	na
Menopause	*pre*	10	5	na	na
	post	13	2	na	na
	changing	3	-	na	na

Table 6.1: Comparison of Entry data between people with BMD Z-score > -1 and those with BMD Z-score < -1. *no data in one subject

Of the 11 subjects with BMD (Z-score) values more than 1 SD below controls at entry, the bone mineral density in 2 (1F, 1M) normalized by the first year follow-up appointment and remained normal during the second year follow-up (Table 6.2). The gluten intake in this female was category C throughout, while the male subject had category A at entry and category B at years 1 and 2. Both male a female subjects had a normal biopsy throughout. The male subject had dietary calcium intakes greater than 100% RDI. The female subject was aged 40 at entry

and had previously had a hysterectomy. Her dietary calcium intakes were less than 100% RDI, though only at inadequate levels (45%RDI) in year 2.

Four other subjects with entry BMD Z-scores less than 1SD below controls, remained in this category at year 1, but had normal scores by year 2. These 4 females began the study with category A gluten intakes, which remained the same in 2, or rose to C or D by the end of the second year. Their small bowel mucosa was normal throughout, expect for 1 subject who had abnormal mucosa only at the final collection. The BMD Z-score outcome did not alter from 1 SD less than controls in 2 subjects. For the most part, these subjects were consuming gluten defined by categories A and B. Their biopsies were normal throughout except for one abnormal entry biopsy. Dietary calcium intake ranged from 77% to 183% of RDI (Table 6.2).

One male who had a constant gluten intake (C), a normal biopsy throughout, but varying dietary calcium intakes recorded BMD Z-scores less than 1 SD below controls at entry and year 2, but it was normal at year 1. At entry his calcium was very low (33% of RDI). This improved at year 1 (80% RDI) and again at year 2 (107% RDI). The BMD of one female also followed this pattern. Her gluten intake was constant (C) initially and during year 1, but was reduced (A) during the final year. Her biopsy was normal throughout and her calcium intake varied extensively and was not recorded in the final year. By the end of the study only one subject progressed to a BMD Z-score less than 2.5 SD below the controls. His dietary gluten was recorded as A, A and C in respective years. At entry he had a normal biopsy but he chose not to have further biopsies during the study. He had a history of lactose intolerance and his entry calcium intake was inadequate (48% RDI). With supplementation his calcium intake improved to 75% of RDI and 85% of RDI at the first and second year follow-ups (Table 6.2).

	ENTRY		YEAR 1		YEAR 2	
subj.#	Z BMD	Diet Category ^	Z BMD	Diet Category	Z BMD	Diet Category
3	Z < -1	A	N	B	N	B
4	Z < -1	A	Z < -1	A	N	A
7	N	C	Z < -1	C	Z < -1	D
11	Z < -1	C	N	C	Z < -1	A
13	Z < -1	C	N	C	N	C
17	Z < -1	A	Z < -1	A	Z < -2.5	C
19	Z < -1	A	Z < -1	D	Z < -1	A
24	N	A	Z < -1	*	**	**
26	Z < -1	A	Z < -1	B	N	A
28	Z < -1	B	Z < -1	B	Z < -1	A
34	Z < -1	A	Z < -1	C	N	C
37	Z < -1	C	N	C	Z < -1	C
40	N	A	N	B	Z < -1	A
42	N	B	N	C	Z < -1	C
49	N	D	Z < -1	D	Z < -1	D
50	Z < -1	A	Z < -1	C	N	D
subj.#	**Z BMD**	**BIOPSY**	**Z BMD**	**BIOPSY**	**Z BMD**	**BIOPSY**
3	Z < -1	N	N	N	N	N
4	Z < -1	N	Z < -1	N	N	N
7	N	N	Z < -1	N	Z < -1	N
11	Z < -1	N	N	N	Z < -1	N
13	Z < -1	N	N	N	N	N
17	Z < -1	N	Z < -1	*	Z < -2.5	*
19	Z < -1	N	Z < -1	N	Z < -1	N
24	N	N	Z < -1	N	**	**
26	Z < -1	N	Z < -1	N	N	PVA
28	Z < -1	PVA	Z < -1	N	Z < -1	N
34	Z < -1	N	Z < -1	N	N	N
37	Z < -1	N	N	N	Z < -1	N
40	N	N	N	N	Z < -1	N
42	N	N	N	N	Z < -1	N
49	N	N	Z < -1	N	Z < -1	A
50	Z < -1	N	Z < -1	N	N	N
subj.#	**Z BMD**	**Ca %RDI**	**Z BMD**	**Ca %RDI**	**Z BMD**	**Ca %RDI**
3	Z < -1	102	N	184	N	166
4	Z < -1	36	Z < -1	48.9	N	101
7	N	122	Z < -1	162	Z < -1	99
11	Z < -1	121	N	49	Z < -1	*
13	Z < -1	97	N	86	N	45
17	Z < -1	47	Z < -1	75	Z < -2.5	85
19	Z < -1	183	Z < -1	134	Z < -1	161
24	N	*	Z < -1	*	**	**
26	Z < -1	127	Z < -1	101	N	101
28	Z < -1	*	Z < -1	77	Z < -1	90
34	Z < -1	137	Z < -1	*	N	139
37	Z < -1	32	N	80	Z < -1	107
40	N	232	N	247	Z < -1	234
42	N	121	N	178	Z < -1	141
49	N	166	Z < -1	196	Z < -1	130
50	Z < -1	74	Z < -1	84	N	53

Table 6.2: Data on those with low BMD during the study period (Z-score)

* data not available; ** dropout; ^ extrapolated gluten values; -1 SD below controls; N=normal; Diet Categories ABCD.

There were 5 (4F) subjects who entered the study with normal BMD but recorded a BMD less than 1SD below age, gender and weight matched controls either at year 1 (2F, 1M), year 2 (2F), or both (1F). Three of these subjects completed the study with levels less then 1 SD below their control. Only the subject with 2 low scores was consuming category D gluten and had one abnormal biopsy at the end of year 2. The rest had normal biopsies throughout. All had adequate dietary calcium intakes. Weighed food diaries were not returned by one of these subjects, who later dropped out in year 2 (Table 6.2).

The entry data of this group is described below in terms of differences between those with low bone mineral density and those who had normal density for their age, weight and gender matched controls (Table 6.3).

Parameter BMD is compared with	t- value	X^2	p-value	df expected (tables used)	outcome
Age at appointment	1.3464		<0.2	39 (40)	Not significant
Age at diagnosis	0.5849		< 0.7	39 (40)	Not significant
Duration of diagnosed coeliac disease.	0.5266		<0.7	39 (40)	Not significant
Duration of diagnosed coeliac disease; transformed to Log	0.0174		>0.9	39 (40)	Not significant
Calcium intake (% of RDI)	0.7226		< 0.5	39 (40)	Not significant
Extrapolated gluten (mg/yr)	1.3297		< 0.2	39 (40)	Not significant
Extrapolated gluten (mg/yr); transformed to Log	1.5516		<0.2	39 (40)	Not significant
Male or female		2.71	< 0.1	1	Not Significant
Normal or abnormal biopsy		2.95	<0.1	1	Not significant
AB vs CD gluten intake		0.56	<0.5	1	Not significant

Table 6.3: Comparison of entry criterion between those with BMD Z-scores greater than –1 and those with BMD Z-scores less than –1.

The data in Table 6.3 show that there are no initial significant differences in any of the parameters measured between those who presented with low bone mineral scores and those who did not. The presence of low bone mineral density was not related to the age or gender of

the person, their age at diagnosis, the duration of their disease, the biopsy result, the amount of trace gluten being consumed or their overall dietary calcium intake. The values for duration of coeliac disease and gluten intake were also transformed to a Log value, before calculations, as they are most likely not normally distributed. Even in doing so there was still no significant relationship between these parameters and BMD outcomes. There was a slightly shorter time since diagnosis, 7.5 ± 1.7 vs 10.3 ± 2.9 years in the group with low BMD, although this does not reach statistical significance. Change in the bone mineral density over the two years of the study at the femoral head, lumbar spine and whole body are shown in Figures 6.1, 6.2 and 6.3 respectively. White columns represent the absolute bone density (g/cm^2) and the purple columns the Z-score. A column below the zero line indicates that the bone mass fell during the 2-year study period and one above indicates bone restoration.

Overall, there was a slight fall in BMD over time, however the change in the absolute bone density is small. While some gains can be seen in the femur head (6.2) and lumbar spine (6.2), the total body graph (6.3) shows that predominantly small losses in bone mineral density during the 2-year study period. The figures for Z-score comparisons are similar.

The change in the actual mineral bone mineral content (BMC) from entry to the end of the 2-year study are shown in Figure 6.4. Subjects 26, 34 and 50, who began the study with low BMD, completed the 2 years with an overall gain in bone mineral content and normal BMD (Z-score). Only 5 subjects had an overall positive bone mineral gain (mean: 0.054kg, SD: 0.05) during the 2 years. The remaining 36 recorded overall bone mineral losses (mean-0.095 kg, SD: 0.072).

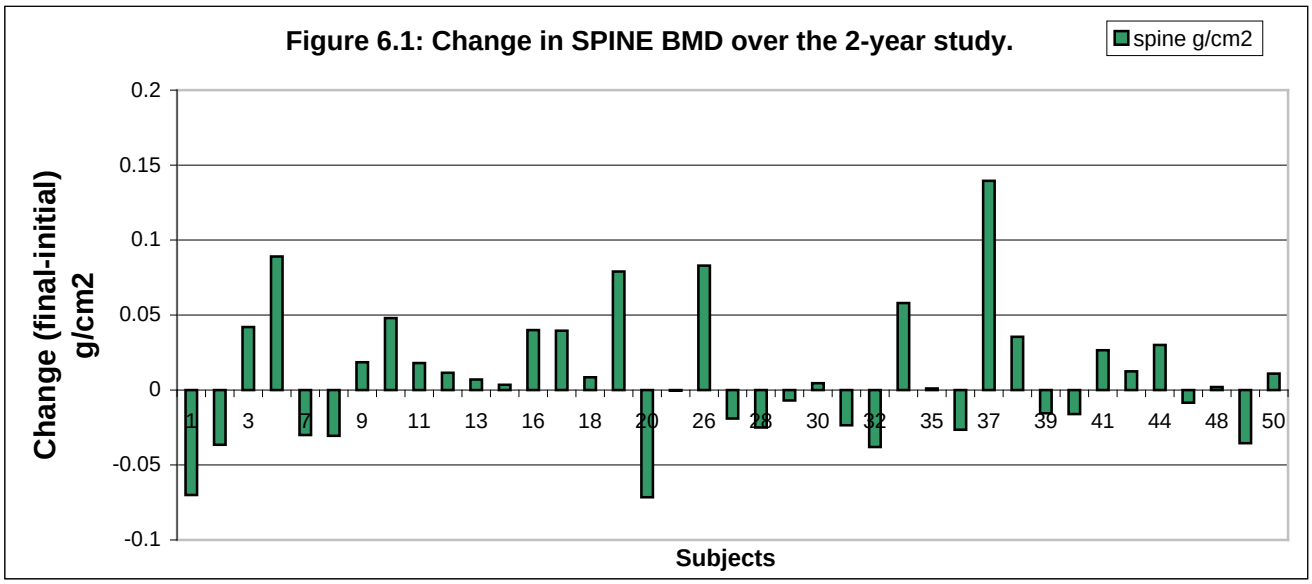


Figure 6.1: Change in SPINE BMD over the 2-year study.
spine g/cm2
Change (final-initial) g/cm2
0.2
0.15
0.1
0.05
0
-0.05
-0.1
1 3 7 9 11 13 16 18 20 26 28 30 32 35 37 39 41 44 48 50
Subjects

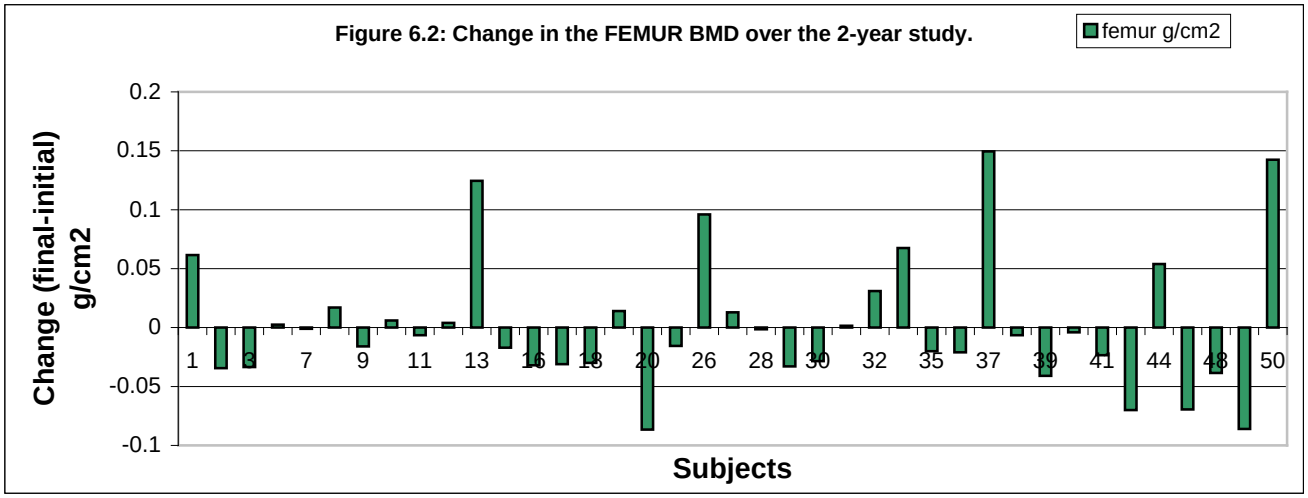


Figure 6.2: Change in the FEMUR BMD over the 2-year study.
femur g/cm2
Change (final-initial) g/cm2
0.2
0.15
0.1
0.05
0
-0.05
-0.1
1 3 7 9 11 13 16 18 20 26 28 30 32 35 37 39 41 44 48 50
Subjects

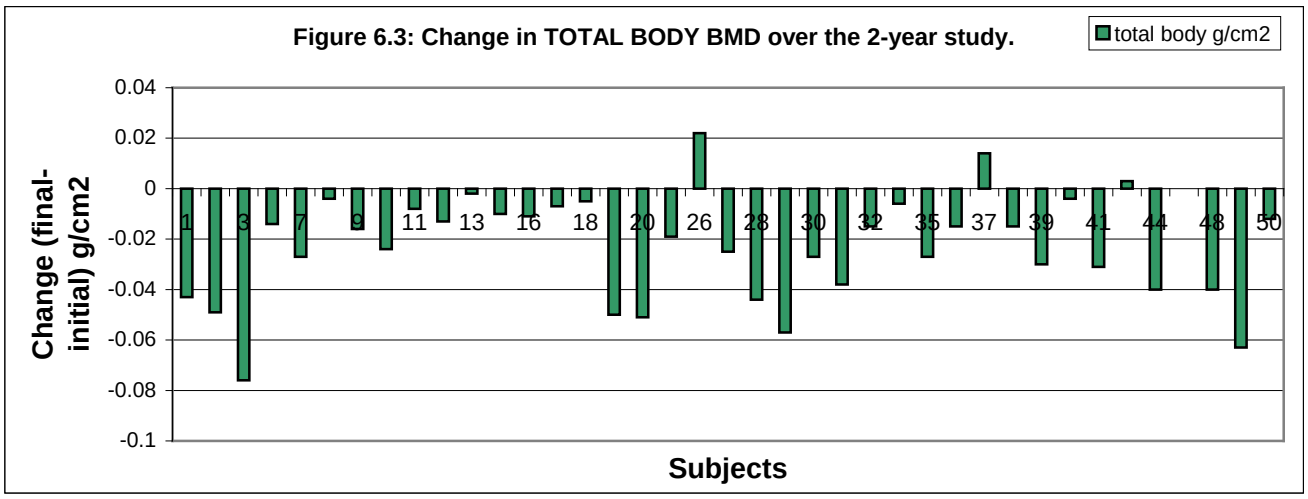


Figure 6.3: Change in TOTAL BODY BMD over the 2-year study.
total body g/cm2
Change (final-initial) g/cm2
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
-0.1
1 3 7 9 11 13 16 18 20 26 28 30 32 35 37 39 41 44 48 50
Subjects

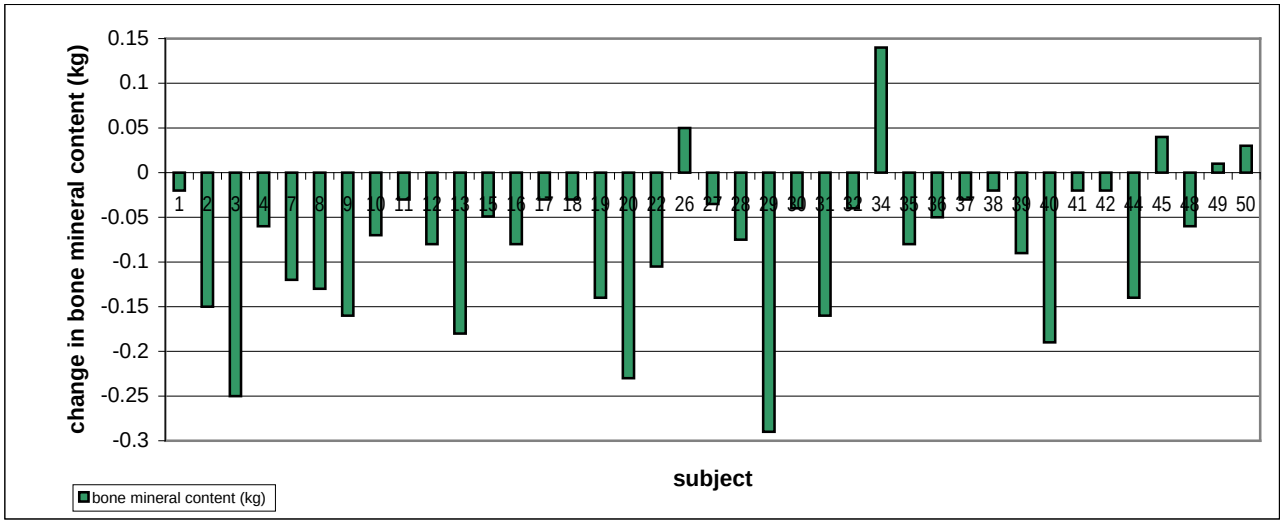


Figure 6.4: Change in the bone mineral content (kg) over time (final-initial)

Eight other subjects (S-3,4,11,13,17,28,37) who also began with BMD less than 1SD below matched controls for age, gender and weight, continued to lose BMC throughout the study. Of this group, subjects 3 and 13 showed the largest bone mineral losses, even though their BMD Z-scores normalized by the end of the 2-year study period. The small BMD loss seen in subject 17 resulted in his BMD Z-score being less than 2.5 SD below, at the end of the study, when compared to age, weight and gender matched controls. Despite these subtle differences there was no overall significant change in the bone mineral content during these 2 years ($p < 0.68$).

There were no significant group changes at any of the sites measured, during the 2-year study period, of the bone mineral density (Z-score and g/cm2) or bone mineral content (kg) by paired t-test.

A correlation test was then performed to determine whether the changes seen in Z-score and g/cm2 results, during the 2-year period, were related to the biopsy, trace gluten intake and/or dietary calcium intake results. The biopsies were classified as either normal or abnormal, the

quantitated gluten data was used (mg/year) and calcium was expressed as a percent of the recommended dietary intake (RDI).

There were significant correlations between the degree of small bowel villous atrophy and g/cm2 total body result (0<0.02) and the Z-score for the femur ($p<0.03$), spine ($p<0.001$) and total body ($p<0.001$). There was also a significant correlation between calcium intake as a percent of recommended intake and the Z-score for the femur ($p<0.001$) and spine ($p<0.01$). However there was no correlation at any of the sites measured between the BMD outcome and the amounts of trace gluten being consumed each year by the subjects. Although the BMC seemed to be significantly greater ($p<0.01$) when trace amounts of gluten were ingested this was probably influenced by the use of extrapolated gluten intakes at entry, which were often less than those recorded prospectively at years 1 and 2. When the extrapolated entry data were dropped from the calculation, there was no significant difference between the trace gluten intakes and the BMC outcomes ($p<0.2$).

Only 5 subjects had persistent abnormal mucosa throughout the study. The BMD results of one of these males have been omitted because he had a hip prothesis. The results of another female have been added to Table 6.4 as she had an abnormal biopsy at entry and year 2, but was travelling at year 1 so no data were collected. None of these subjects had low bone mineral density at any time during the study.

SUBJECT #	BONE MINERAL DENSITY OUTCOME Z-score		
	ENTRY	YEAR 1	YEAR2
1	Normal	No biopsy or BMD	Normal
12	Normal	Normal	Normal
15	Normal	Normal	Normal
20	Normal	Normal	Normal
41	Normal	Normal	Normal

Table 6.4: Z-score BMD outcomes in those subjects with persistent abnormal biopsies.

The outcomes of bone mineral density were also compared with young gender matched controls (T-score). At entry 20 (48%:17F, 3M) subjects were classified as normal, 18 (43%:14F, 4M) had osteopenia and 3 (7.3%:2F, 1M) had osteoporosis. Similarly to that presented above, the presence of low bone mineral density (T-score) at entry was not related to the gender or age of the person, their age at diagnosis, the duration of their disease, the biopsy result, the amount of trace gluten being consumed or their overall dietary calcium intake. The results for duration of disease and gluten intake were again transformed to Log values, however a significant relationship was still not found with these parameters.

Sixteen of the 20 subjects entering the study with normal BMD, completed the 2-year study with normal T-scores throughout. The T-score of 1 female decreased to osteopenia at both years 1 and year 2. One male subject dropped out in the final year. One of the remaining females progressed to osteopenia during the first year, but returned to normal by the second year, while the other female recorded osteopenia at year 2 only.

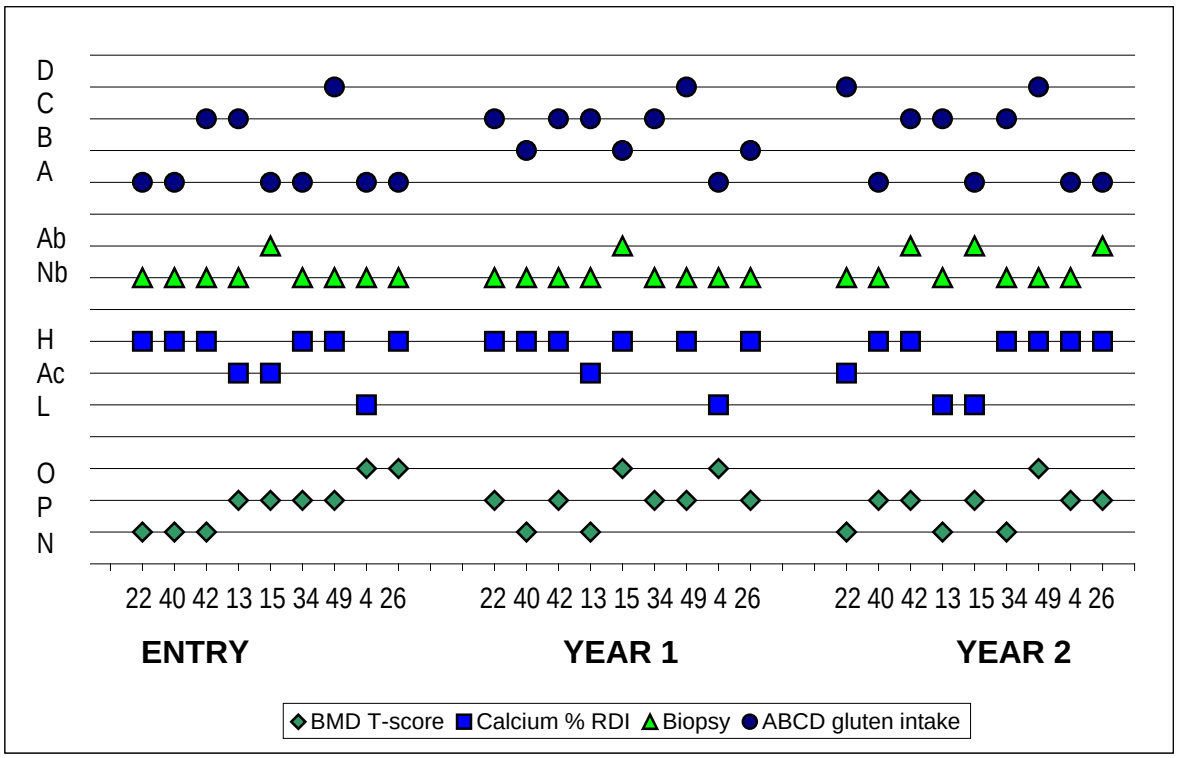
Fourteen of the initial 18 had constant osteopenic results during the 2 years when compared with their young gender matched controls. One male dropped out in the final year. The BMD

score in year 1 improved to normal in 1 female and remained normal at the second year collection. The BMD score improved to normal at year 2 in another female. One female subject recorded 2 osteopenic scores followed by osteoporosis at the final collection and she had normal mucosa throughout.

Although 3 subjects entered the study with osteoporosis only 1 male subject (BMI 19) had osteoporosis throughout. At entry he showed normal small bowel mucosa, but refused follow-up biopsies. He was lactose intolerant and had had many years on a dairy reduced diet. His dietary calcium intake at entry was inadequate (47.7% RDI) but this increased to adequate levels in subsequent years with the use of calcium supplements (75.5% & 85.5% RDI). In one of the 2 remaining females the BMD T-score improved to osteopenic levels at years 1 and 2. She had normal mucosa at entry and year 1, but showed PVA at year 2. Her gluten intake was very limited (A & B categories) and her calcium intake was consistently greater than 100% RDI. The BMD of the remaining postmenopausal female improved to osteopenic levels at year 2. Her gluten avoidance was one of the strictest in the study and her biopsy was normal throughout. Her dietary calcium intake was very low at entry and year 1, but improved at year 2 with the help of supplements (36.6, 48.9 and 101.9% RDI respectively)

Again the numbers in the group whose BMD changed, were too small to statistically determine factors that may have contributed to this outcome.

The within each subject parameters, that others have attributed to bone loss, and how these altered from entry to year 1 and year 2 are shown in Figure 6.5. The subjects are in the same order in each year (Subject 1 was away travelling during year 1). The graph has been sorted according to entry BMD results.



Ab=abnormal biopsy; Nb=normal biopsy; H=<100%RDI; Ac= <100%>67% RDI; L=<67% RDI; O=osteoporosis; P=osteopenia; N=normal BMD. Subject numbers are listed on the bottom row.

Figure 6.5: Comparison of parameters affecting BMD (T-score) within those subjects whose BMD changed over the 2-year study.

The within person variables plotted in Figure 6.5 do not seem to indicate that any of these factors shown are critically responsible for the alterations seen in the BMD (T-scores). The overall gluten intake in this small number does not seem to be an influencing factor. Although the present study had very few people (n=4) with persistent villous atrophy, the BMD losses were not more marked in this group (mean: –0.14, SD: 0.1) compared with the rest (mean: -0.15, SD: 0.09). Only 2 of the 4 subjects with persistently abnormal mucosa were considered to have reduced bone mineral density throughout when compared with their young gender matched controls. One female shown did not have data collected at year 1. The data group is too small to indicate whether the low bone mineral scores registered by the subjects were influenced by an abnormal mucosa due to persistent gluten ingestion.

The T-score BMD outcomes for the same subjects in Table 6.4, who had persistent abnormal biopsies during the 2-year study period are shown in Table 6.5.

SUBJECT #	BONE MINERAL DENSITY OUTCOME T-score		
	ENTRY	YEAR 1	YEAR2
1	osteopenia	No biopsy or BMD	Osteopenia
12	Normal	Normal	Normal
15	Osteopenia	Osteoporosis	Osteopenia
20	Osteopenia	Osteopenia	Osteopenia
41	Normal	Normal	Normal

Table 6.5: T-score BMD outcomes in those subjects with persistent abnormal biopsies

DISCUSSION

Comparison of this group of people with coeliac disease to their age, gender and weight matched controls showed that 7 females and 4 males had BMD scores less than 1 SD below their controls at entry. After one year, these low scores remained in 8 of these 11 subjects, normalized in 3 and a further 3 with normal BMD at entry recorded reduced BMD levels. At the second year follow-up the BMD normalized in a further 4 subjects, while 2 previously normal subjects recorded levels less than 1 SD below controls. The BMD was reduced to less than -2.5 SD below controls in 1 male.

The figures show that during the 2-year study period there was an over all decrease in the bone mineral content of nearly all study participants (although this was not significant), despite the adherence of most to the stricter *NDG-GFD*. This bone mineral loss was not more marked in those with continued biopsy damage compared with those who have normal villi or fluctuating biopsy results. Of interest is the observation that in almost all cases there was an

increase in spinal bone mineral density over the 2 years despite the trend toward reduced total body BMD.

Though institution of a GFD is associated with increases in BMD, bone mass is not always restored to normal levels in people with coeliac disease. In both children and adults, the institution of the GFD seems to be associated with increases in bone mass predominantly during the first year of the new dietary regime *(Mora et al, 2001; Mora et al, 1998; Scotta et al, 1997; Meyer et al, 2001; Valdimarsson et al, 1996; Corazzo et al, 1996; McFarlane et al, 1996)*. After this time the improvement seems to stabilize, still at suboptimal densities for many adults, regardless of continued compliance with their GFD. The remaining deficits in the bone mineral content could then be attributed to the coeliac disease which may possibly have interfered with the attainment of peak bone mass *(Meyer et al, 2001)*.

Meyer et al, (2001) found little statistical difference between BMD measurements of treated and untreated coeliacs. Others found that the BMD was significantly lower in treated adults with persistent villous atrophy, while the treated group with normal villi had BMD scores very similar to controls *(Valdimarsson et al, 1994; McFarlane et al, 1995)*. There were only 5 subjects in the group presented here who had persistent villous atrophy during the 2-year study period. The BMD results for one were omitted because he had a hip prothesis and the remaining 4 subjects had normal BMD Z-scores throughout. The only subject who progressed to a BMD score less than -2.5 SD below his age, gender and weight matched controls (Z-score) during the study, had a normal biopsy at entry, but he declined further biopsies at years 1 and 2.

It has been observed that more boys than girls had decreased bone mass and lower mean BMD scores *(Bayer et al, 1998)*. Others reported in their prevalence study that when compared to aged-matched controls more men, than either pre or postmenopausal women, had lower bone masses *(Meyer et, 2001)*. The were no significant differences when correlations were made with gender or age at study entry, age at diagnosis of coeliac disease, duration of coeliac disease, trace gluten intake, dietary calcium intake or presence of a normal or abnormal biopsy. This remained true when duration of coeliac disease and gluten intakes were transformed to Log figures, before analysis.

People who are undiagnosed or untreated during childhood and adolescence may never achieve peak bone mass. In those diagnosed before peak bone mass is reached, osteoporotic complications can be avoided if a gluten-free diet is properly pursued *(Mora et al, 1999; Mora et al, 1998)*. If coeliac disease is diagnosed after peak bone mass is reached, the ability of the GFD to restore normal bone mineralization remains uncertain *(Mora et al, 1999).* Some studies show no effect of the GFD in this group (*Pistorius et al, 1995)*. An American study suggests that the GFD improves bone mass especially during the first year of treatment and on average most people do not sustain further bone loss if the gluten-free diet is maintained *(Meyer et al, 2001)*. It is generally agreed that peak bone mass can be achieved if the coeliac disease is diagnosed, early enough to begin the GFD, before the end of puberty. However, subclinical disturbances of bone and mineral metabolism may persist and prevent complete restitution of low bone mass or osteoporosis. For example, levels of the intestinal calcium-binding protein calbindin-D9k, undetectable in biopsies of patients with active celiac disease, are reduced by approximately 75% even in treated patients with normal villous architecture" (*Staun & Jarnum, 1988).* Other authors have suggested, "the low BMD observed in coeliac disease may be related to the inflammatory process itself" *(Forneri et al, 1998).*

A diet questionnaire study conducted by *Meyer et al, (2001)* found that the BMD was lower in those who had reduced daily calcium intakes. This finding was not observed in this study. Many subjects with low daily food intakes of calcium neither supplemented their diet with mineral tablets or showed reduced bone densities. *Meyer et al, (2001)* found that the Americans taking calcium and vitamin D supplements had some of the lowest BMD scores. Our study only had 2-3 subjects classified with osteopenia (when compared with T-score values) and they were taking calcium supplements as reported by Meyer. This may be a reflection of this group as a whole who are trying all that can be offered to them in an effort to rectify or reverse the current status of their bones.

In order to loosely compare bone mineral density outcomes with historic calcium intakes, each subject was asked to recall, retrospectively, their dairy intakes during each decade of their life. Keeping in mind the obvious limitations of the methods, our results did not reflect the expected outcome that those with lower bone scores as adults had poorer calcium intakes during their teens when their peak bone mass is reached (data not shown).

The study does however show that the small quantities of gluten consumed are not responsible for continued small bowel damage (Chapter 5). Therefore the avoidance of these trace amounts of gluten, often eaten inadvertently by compliant coeliacs, may not contribute positively to bone gain.

It has been recommended that all patients with diagnosed coeliac disease undergo BMD testing, however the clinical significance of this reported reduction of bone mineral density in the treated coeliac disease is unclear. Two recent studies suggest that there is no overall increase in fracture risk in patients with coeliac disease compared with normal controls

(Vestergaard & Mosékilde, 2002; Thomason et al, 2003). The identification of fracture risk factors in coeliac disease have not been readily identified, but it has been suggested that "non compliance with the GFD, failure to respond fully to a GFD, glucocorticoid therapy, untreated hypogonadism, age, low body mass index and previous fragility fracture" are likely risk factors for fracture in this group *(Compston, 2003).*

The outcomes of a number of studies can be drawn upon to develop a treatment strategy to manage the bone loss in this group. Diagnosing coeliac disease prior to puberty helps the person reach their peak bone mass before losses begin to occur with age. Instigation and maintenance of a GFD is central to impeding the rate of bone loss by reversing the malabsorption principally of protein, calcium and vitamin D in this case. It is largely accepted now that the small bowel mucosa remains partially damaged in many treated coeliacs, so it has been suggested that calcium intakes of 1.5g per day be instituted in all middle-aged and older people with coeliac disease *(Heaney et al, 1977; Dawson-Hughes et al, 1990; Walters, 1994)*. Intakes of this magnitude would need to rely on the use of mineral supplements. *Marsh, (1994)* suggests that taking the 25-OH vitamin D form rather than the lipid-soluble calciferol form of vitamin D can help to remedy the imbalance. Removing all traces of gluten from the diet does not seem to improve mucosal outcomes beyond those in people consuming Codex-permitted ingredients in their GFD (Chapter 5). It is also suggested that intravenous therapy with intravenous potent bisphosphonates should be considered in those with osteoporosis and continued malabsorption *(Compston, 2003).* Other general therapies apply, for example, hormone replacement therapy, oral bisphosphonate therapy, weight bearing exercise, avoidance of caffeine and cigarettes and maintenance of normal weight.

CHAPTER 7

7

NUTRITIONAL ADEQUACY

Assessment of the nutritional adequacy of the NDG-gluten-free diet.

INTRODUCTION

A gluten-free diet has been the cornerstone of treatment for coeliac disease for the last 50 years since the role of gluten in this disorder was discovered. Initially the diet was only applied strictly during childhood years and a normal diet was resumed when symptoms appeared to settle. It was *Holmes et al, in 1989*, who presented the first evidence that the risk of gastro-intestinal cancers and lymphoma were higher in those who continued to eat gluten. Following this finding it was recommended that the gluten-free diet should be followed for life.

Adopting a GFD requires major changes to the grains and sources of starch that are ingested by coeliacs. The flavour, texture and recipe adjustments required by this does make it difficult for some people requiring a GFD to consume any quantity of the necessary grains. The immediate benefit to the nutritional state after a gluten-free diet has been initiated is reasonably well documented and occurs because of the improvement in mucosal architecture and recovery of normal absorption from the small intestine *(Schwartz et al, 1968; Reinken et al, 1976; Gawkrodger et al, 1988; Radzikowski et al, 1991; Corazza et al, 1994; Kemppainen et al, 1995)*. However, little has been written about the nutritional adequacy of the gluten-free diet itself.

A totally reliable and valid method for collecting detailed food intake data does not exist. Although 3 and 4 day food diaries are commonly used to determine average nutrient intakes it has been reported that 74-88 days in males and females respectively, are required to estimate, for example, individual calcium intakes within 10% of the true average, 95% of the time *(Bastiotis et al, 1987).* This group has published the required number of days needed to obtain this level of statistical accuracy for many of the vitamin and minerals commonly studied. However it is rare to have the time or resources to practically apply their findings. Food frequency questionnaires, food intake questionnaires, weighed food diaries, 24 hour recall, diet history taking and 7-day food diaries are just some methods used to acquire nutritional information from individuals or groups of people. The *Food Brand Questionnaire,* completed by all subjects, was not designed to elicit nutrient intakes. This questionnaire only asked if a food was consumed and how frequently it was consumed. To assign nutrient intake amounts to the food eaten, a quantitative dietary assessment method was required. Since quantitative methods are demanding and time consuming for both the subject and the analyser (*Gibson, 1990)*, a 4-day weighed food diary method was chosen to collect the detailed nutritional data. Intakes were recorded consecutively for 1 weekend day and 3-week days. Recordings lasting longer than this are not necessarily more accurate *(Livingstone et al, 1990)*.

Mineral Absorption

The damage to the small intestine in coeliac disease is believed to begin in the duodenum and to work its way progressively down the small intestine. Thus, while duodenal and jejunal abnormalities are generally universal, only about 10% of patients diagnosed with coeliac disease will have involvement of the terminal ileum. Similarly, the less severely damaged distal mucosa recovers more rapidly and completely than does the more severely damaged

proximal mucosa during treatment with a gluten-free diet *(Schwartz et al, 1968).* Calcium, iron, magnesium and zinc are absorbed predominantly in the duodenum *(Krause & Mahan, 1984)*. As such, their absorption will be particularly affected in coeliac disease and therefore may require special attention in the GFD. These micronutrients in a GFD will be the main focus of this chapter.

Thompson (2000) investigated the nutritional content of 86 manufactured gluten-free cereal products and compared them with the nutritional profile of usual gluten-containing cereal products. She concluded that folate, iron and dietary fibre contents were nearly always lower in the gluten-free cereal when compared with their gluten counterpart. In a previous study she also showed most gluten-free cereals provided lower levels of at least one of the vitamins, thiamin, riboflavin or niacin *(Thompson, 1999).* Neither of these studies looked at the adequacy of the gluten-free diet as a whole.

Collins et al (1986) assessed the gluten-free diets of coeliacs who had been on a GFD for one year. More than half of their subjects were consuming diets that met or exceeded the recommended daily allowance for nutrients, although they did not report on fibre. The intake of dietary nutrients most frequently found to be deficient were total energy, protein and iron. It has been reported that older men and women tend to underestimate their actual food intake and this may be why the total energy is often found to be lower than recommended *(Tomoyasu et al, 1999).* More recently a Swedish study investigated the vitamin intakes of people who had been consuming a GFD for approximately 10 years. They concluded that half of the adult coeliac patients showed signs of poor vitamin status (*Hallert et al, 2002)*. Little other information on adequacy of a gluten-free diet is available, and there is certainly no information relevant to an Australian population. The present study assessed the nutritional

adequacy of the diet of a group of Australian subjects with coeliac disease who had been adhering to their gluten-free regimen for an average of 9.3 years. Three weighed food diaries were completed and analysed, during a two-year period. The macronutrient data for total energy (kJ), protein, fat and carbohydrate are presented. The findings for a variety of micronutrients, particularly those absorbed in the upper small intestine - calcium, iron and magnesium - are also given. The gluten-free dietary analysis has been compared to the published general Australian nutrient data from the 1995 National Nutrition Survey *(McLennan & Podger, 1998-b).*

METHODS

The subjects used in this assessment of the nutritional adequacy of the GFD are described in Chapter 3. Briefly, 39 of the 48 recruited members of The Coeliac Society of NSW completed the weighed food diaries given to them at their initial appointment. The diaries were completed again after one year (n=38) and two years (n=39). Seasonal food variation occurred as the diaries were collected year round.

Weighed Food Diary

The *Weighed Food Diary and the Recipe and Food Grid Booklet* (Appendices 13 and 14) were used to assess the intake of each of the nutrients of interest. This was completed during the month after it was given out and returned in a reply-paid envelope.

Supplement Record Sheet

During the first and second year review, an additional *Supplement Record Sheet* (Appendix 10) was added to the *Weighed Food Diary* package to enable the non-food vitamin and mineral intakes to be compiled as well. The initial use of this sheet has been described in Chapter 4 when collecting information about the gluten content of ingested medications and supplements. After the first weighed food diary had been completed it was apparent that detailed information about the quantity and type of vitamin, mineral and other mixtures and supplements that may add nutritional value to the diet was missing. Consequently, this form was devised to fill this void and was given out at the first and second year follow-ups only. Specifically each subject was asked to list the medications, vitamins, minerals or other supplements that they were taking. Examples included echinacea, evening primrose oil, garlic and horseradish tablets, cod liver oil and St. John's wort. Only those formulae extolling nutritive claims were recorded in the nutrient analyses. Subjects were asked to record from the packages' ingredient panel what nutrients, and how much of them, was in each tablet &/or syrup that they were using. They were also asked to list how many tablets &/or syrups they took daily or, if not this frequently, how often they were taking these preparations. The manufacturers of the products that were listed on these record sheets were contacted to ascertain that the formulas ingested were gluten-free. The amounts of each vitamin and mineral calculated from this information were added to the nutrients determined from the *Weighed Food Diaries*. Therefore information could be accessed about the nutrients in the diet alone, or those in the diet plus supplements.

Analysing the weighed food diaries

The method of analysis of the *Weighed Food Diaries* is described in Chapter 3. On entry as well as at the one- and two-year follow-ups, each subject was asked to record, weigh &/or measure all of the food and drink consumed over four consecutive days, one of which was a

weekend day and three were week days. Each food was preferably to be recorded by weight in the diary booklet but measurements of cups, millilitres and centimetres were acceptable if the weight could not be obtained. A separate booklet was supplied to record the recipes used during this period (Appendix 14). Although some clarification of data was obtained by phone interview, on the whole, a standard serve size (obtained from the data-base) was used if the quantity eaten was unclear.

Height, weight, age and approximate exercise levels were recorded at each appointment. These details were required by the nutritional database, SERVE, to enable calculation of the Recommended Dietary Intake (RDI) for each of the individual subjects. For dietary fibre the recommended target intake was 30 g/day *(Better Health Commission, 1986).*

The Australian 'SERVE nutritional analyses software' *(SERVE Nutritional Management System for Microsoft™ Windows®)* was used to analyse the food diaries. The nutritional information in the SERVE Access database has been obtained from the published nutrition tables of the analyses of Australian food, NUTTAB *(NUTTAB, 1992)*. The SERVE Nutritional Management System for Microsoft™ Windows® was developed by Mike and Hazel Williams and is available throughout Australia.

Very few gluten free grains and gluten-free commercial products had been analysed in the NUTTAB tables, so additional gluten-free nutrient information was added to SERVE, from the USDA American food data base, currently available on the Internet [6] and the English McCance and Widdowson Food Composition tables, (*Paul & Southgate, 1978)*. The recipes used by subjects were entered into the computer and analysed using the generic values from

[6] *USDA data base web address:- http://www.nal.usda.gov/fnic/foodcomp/index.htm*

these databases. Some Australian food companies allowed their recipes to be analysed using the generic ingredient values available from this combined database. Occasionally a standard recipe was utilised, as the proprietary information could not be obtained. Some commercial food analyses were created by copying the nutritional data given by the company on the food label (incomplete for all nutrients), into an existing food analysis that was similar in composition to the used product. For example, the United States Department of Agriculture (USDA) database contained a nutrient analysis of a corn cake (puffed corn biscuit). This analysis was altered to include the energy, protein, fat, carbohydrate, fibre, and sodium etc, levels that were reported on the label by an Australian Manufacturer of corn cakes. The nutrients that were not analysed by the manufacturer will remain the same as reported in the USDA database. More recently, other databases have been developed which contain analyses of some gluten-free grains and products. However, these are also limited by the fact that few commercial gluten-free products have been analysed. Only common, widely consumed gluten-free food ingredients e.g. rice, boiled rice, polenta (cornmeal) and cornflour, tended to be assessed. By combining the NUTTAB data, the international databases, contacting a number of gluten-free food manufacturers and examining many gluten-free recipes it was possible to create a larger gluten-free database, in which fewer assumptions about the nutritional composition of ingredients and foods have been made.

The percentage of the RDI of the following nutrients was calculated for each subject: total energy (kJ), protein, niacin equivalents, riboflavin, thiamin, total retinol, vitamin C, iron, calcium, magnesium, phosphorous, potassium, sodium and zinc (NH & MRC, 1991). The percentage of target intake for dietary fibre was also calculated. The protein, fat, carbohydrate, alcohol and energy composition of the total diet was also assessed and expressed as a percentage of total energy intake. In general, it is recommended that a well balanced, or ideal

diet, should be made up of the following proportion of macronutrients. The protein intakes should be between 10-15% of the total energy intake (kilojoules) eaten, total fat intakes should be less than 30% of energy, total carbohydrates should be between 50-60% of the energy, and an alcohol intake of up to 3% of total energy is considered to be acceptable *(Wahlqvist, 2002, p533)*. Each RDI has a margin of safety built into the recommendation, therefore intakes less than 2/3 of the RDI (67%) are traditionally considered to be inadequate (*Briggs & Wahlqvist, 1984, p13).* The findings were also compared with data from the National Nutrition Survey.

National Nutrition Survey (NNS)

This is an Australia-wide food and nutritional intake survey undertaken between February 1995 and March 1996, by the Australian Bureau of Statistics and the Commonwealth Department of Health and Aged Care *(McLennan & Podger, 1998-a & b)*. A National sample of 13 858 people, consisting of adults aged 19 and over and children aged between 2 and 18 years, participated in the survey. The survey obtained 24-hour recall data for the previous day's intake of food, beverages and vitamin and mineral supplements. Data about height, weight, waist-to-hip circumference, blood pressure and other measures were also collected by a series of interviewers. The Australian Bureau of Statistics created their own data analysis package to analyse the information from the nutritional intakes recorded in the surveys. The results were published in 1998 by the Australian Bureau of Statistics and represent the usual Australian adult dietary intakes *(McLennan & Podger, 1998-b)*.

The NNS does not include analyses of the sodium intakes, nor information on derived and preformed niacin *(McLennan & Podger, 1998-b, p122).* Each person was asked whether or not they took specific vitamin or mineral dietary supplements, but this data was not included in

the published estimates of nutrient intakes *(McLennan & Podger, 1998-b).* Standard deviations were not calculated for the NNS data although standard errors are available for some data *(McLennan & Podger, 1998-b)*. The pooled intakes for all adults over the age of 19 have been used in this thesis as a comparison group that represents usual Australian dietary intakes.

Statistical Analyses

Comparative statistics have been calculated using the Microsoft Excel Database. Paired t-tests were used to determine whether the nutrient intakes of each subject varied significantly over time, from entry to the end of year 2. One sample t-tests were used to compare the mean nutrient intakes of the group with coeliac disease with those published in the National Nutrition Survey.

RESULTS

Initially, the data were analysed in two groups:

- All 42 subjects who completed at least one weighed food diary at any time point during the study. Thirty nine (32F:7M) had completed the initial *Weighed Food Diary*, 38 (31F;7M) at one year and 39 (32F:7M) at two years; and
- Only the 33 subjects who had completed all three weighed food diaries throughout the two-year study period.

The mean intakes for each of these subject groupings did not differ significantly and so the results presented in this chapter use the information obtained from the larger group.

The average age of the 42 subjects was 47.9 years (range 20-72 years; median 57 years). The mean age at diagnosis was 38.9 years (range 0.5-70 years; median 42 years) and duration of coeliac disease in 9.2 years (range 2-59 years; median 3 years). The average body mass index (BMI) was 23.7 (range 17.6-37; median 23.4). Eight of the subjects were classified as underweight (>20), with a BMI between 17.6 and 20. Twenty were in the normal range (>20<25), while 11 and 3 respectively were overweight (>25<30) or obese (<30).

The average total energy intake (kJ) was 93.2% of RDI at entry, 93.7% at year one and 96.3% at year two. The proportions of protein and fat that made up this energy intake were slightly higher than those regarded as ideal whereas carbohydrate (CHO) intake tended to be lower (Table 7.1). Alcohol consumption was low generally at less than 3% of the energy intake.

Nutrient	Mean percent of total kilojoules				
	Initial	Year 1	Final year	Mean of the years	Ideal*
Protein	17.2	18	17.9	17.7	10-15
Fat	34.3	34	33.2	33.7	<30
Carbohydrate	45.6	46	46.5	46.3	50-60
Alcohol	2.7	1.7	2.2	2.3	3

**(Wahlqvist, 2002, p533)*

Table 7.1: Mean protein, fat, CHO and alcohol intakes as a percent of the energy (kJ).

The mean nutrient intakes at each year from the diet alone are shown in Table 7.2. Figures 7.1 and 7.2 indicate the mean and ranges for the major macronutrients and micronutrients. None of the means fell below 67% of the RDI at any time point. In fact, the average intakes of protein, calcium, iron, magnesium, vitamin C, retinol equivalents, riboflavin, thiamin and niacin equivalents, sodium, potassium and phosphorous, were consistently above 100% of the RDI throughout the study period. Vitamin C was particularly high, more than 4 times the levels recommended by the standard. The mean energy and zinc intakes were regularly recorded between 90 and 100% of the RDI. The average fibre intake was well below 100% of the RDI (range: 71%-75%). There was little variation overall in any of the nutrients from entry to year one or year two. There was also no statistically significant within subject variation in nutrient intakes at each of these time points.

Although the mean intakes were adequate overall, this was not always the case for individual subjects. Seven subjects had a total energy intake of less than 67% of RDI (range: 32-66%). In only one of these was the intake low at more than one of the assessment points.

NUTRIENT	Percent of RDI : Food Intake Alone					
	Entry (N=39)		Year 1 (N=38)		Year 2 (N=39)	
	Mean	Range	Mean	Range	Mean	Range
Energy	93	46-158	93	31-142	96	51-150
Protein	175	82-309	177	68-291	185	83-280
Calcium	113	32-333	121	44-285	109	28-234
Iron	155	44-374	159	25-342	157	36-341
Magnesium	140	58-330	127	35-255	124	70-267
Zinc	97	51-169	99	24-195	94	51-151
Vitamin C	463	114-1489	463	39-1558	444	88-897
Thiamin	148	53-377	152	38-331	154	57 -300
Riboflavin	155	30-484	161	47-302	167	64-282
Niacin Equivalents	261	125-459	263	75-396	267	138-461
Total Retinol	136	43-311	169	46-376	146	32-331
Sodium	267	109-1169	246	95-466	246	94-741
Potassium	170	94-270	176	45-298	177	107-291
Phosphate	164	82-294	152	54-262	148	75-230

Table 7.2: Mean nutrient intakes of the subjects.

Protein intakes were adequate in all subjects. Dietary fibre intake was low (<20g/day) in 26 subjects overall, recording as little as 19% of the RDI. There were 13 subjects who had consistently adequate dietary fibre results at each of the 3 collections. At entry, intake was low in 20 subjects (51% of subjects), at year in 14 (37% of subjects) and year two 13 (33% of subjects). Eight of the subjects with low dietary fibre intakes at entry recorded adequate levels at subsequent assessments, whereas 6 were low throughout and another 6 were low at one of the following two time periods. In addition, 6 subjects had an adequate intake initially, only for it to fall at later assessments. The dietary fibre and total energy intakes for all subjects can be viewed in Figure 7.1.

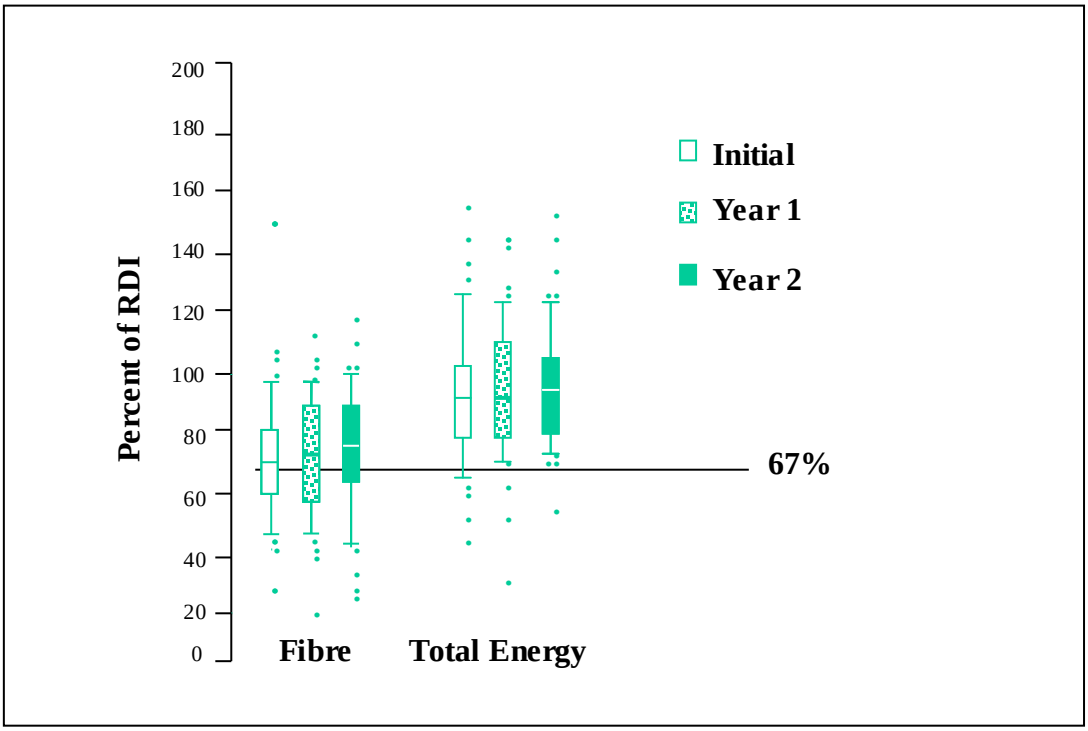


Figure 7.1: Comparison of the dietary fibre and total energy (kJ) intakes, from food only, during the two-year period. The bars show the range from the 10th, 25th, 50th, 75th & 90th percentiles. The dots represent the outliers.

Calcium intake, as calculated from dietary intake alone, was above 67% of RDI in 21 subjects throughout the study, but below 67% of RDI in 14 subjects (33%) at one or more of the time points, with a range of 28-66% RDI. This was the case in 6 at entry (15%), 6 at year one and 6 at year two. Four subjects at year one had a low intake only at that assessment, as did

another four at year two. Iron intake was generally adequate, with only 8 subjects having a low intake (26-66% RDI) [– 3 at entry, another at year one, and four new subjects at year two]. Only two had a low iron intake at more than one time point. There were 27 subjects with iron intakes consistently above 67% RDI. Only two subjects had low magnesium intake, one at entry and another at year one. Nine subjects had low zinc intake (24-66% RDI), but in only three at more than one time point. For most subjects with a low intake it was an isolated finding, with one or two nutrients below 67% of RDI. However, there were three subjects with an inadequate intake of at least four of the above nutrients. One was a 47 year old insulin dependent diabetic woman with a BMI of 26, one a 28 year old female with a BMI of 23 and the third a woman of 27 with a BMI of 19. Selected micronutrient data is shown in Figure 7.2.

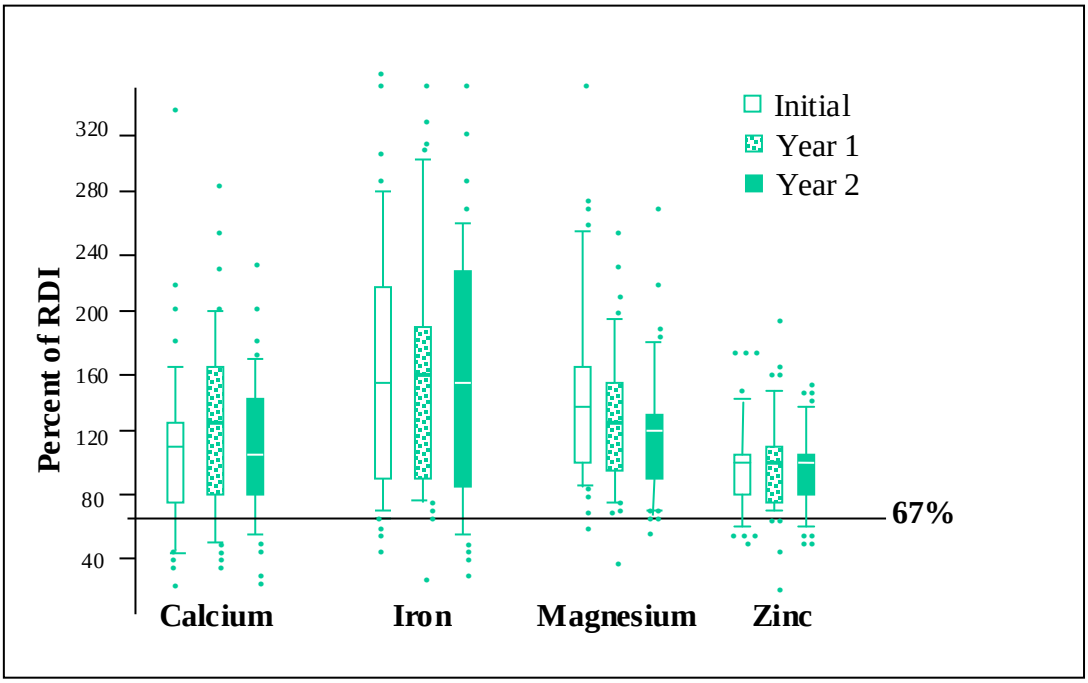


Figure 7.2: Comparison of calcium, iron, magnesium and zinc, from food only, during the two-year period. The bars show the range from the 10th, 25th, 50th, 75th & 90th percentiles. The dots represent the outliers.

Table 7.3 shows the intakes of nutrients when supplements are also considered. In the current study vitamin and mineral supplements were not routinely collected at entry, however ten

supplements were taken during the 2-year period. These were calcium, iron, zinc, magnesium, thiamin, riboflavin, niacin, vitamin C, retinol and potassium.

Supplement taken	Percent of RDI: Food Intake Plus Supplement					
	Year 1 (N=38)			Year 2 (N=39)		
	n	Mean of N	Range of n	n	Mean of N	Range of n
Calcium	14	189	74-314	14	127	114-247
Iron	3	163	66-152	4	268	78-2442
Magnesium	4	162	37-1395	4	129	131-282
Zinc	6	202	65-1946	6	113	92-350
Vitamin C	11	1667	139-10701	10	913	697-4586
Thiamin	9	2057	1080-47542	6	490	383-6387.5
Riboflavin	9	890	820-15681	5	636	976-13809
Niacin Equivalents	9	482	182-6109	4	355	720-1550

N represents the total number of subjects returning weighed food diaries, while n represents the number of subjects taking a tablet supplement that enhances their dietary intakes.
Table 7.3: Mean food and supplement nutrient intakes of the subjects.

The results at year 1 showed that 57% of the subjects (3M, 19F) were taking one or more daily or weekly dietary supplements. At year two, 46% of the subjects (2M, 16F) were taking one or more supplements. The average age of the females taking supplements was 48 years. Most women took more than one nutrient supplement on a daily basis. One of the 3 men taking supplements took calcium and vitamin C, while the other 2 took only daily calcium tablets. Calcium was the most common mineral supplement, used by 18 subjects overall, either as a tablet or part of a multivitamin preparation. Ten subjects were recorded as having taken calcium throughout the study, 4 during year one only and another four only during year two. The dose ranged from 65-1200mg per day. Four of the six of the subjects with a low intake of calcium from food alone took supplements during the first year of the study. In each of these the combined calcium intake was then greater than 67% of RDI (range 74-192%). This was also the case for two of the 6 subjects at year two (114% and 133%).

Only six subjects took iron supplements, with an average dose of 5mg/day in the first year and 55mg/day in the final year. Only two subjects with a low dietary iron intake were also on

supplemental iron. In both, the total iron intake rose to at least 67% of RDI. Zinc supplementation was also uncommon, being taken in 10 subjects overall, but in only two at years one and two. Only one of the subjects with a low dietary intake was also using a zinc supplement, but even with this her combined intake was 66% of RDI. Vitamin C was a popular supplement, being used in 10 subjects at year one and 11 at year two. The average intakes ranged from 500- 900mg per day. Only one subject had a vitamin C intake from food in the first year that was less than 67% of RDI. This was corrected when the vitamin C tablet was included in the calculation of intake.

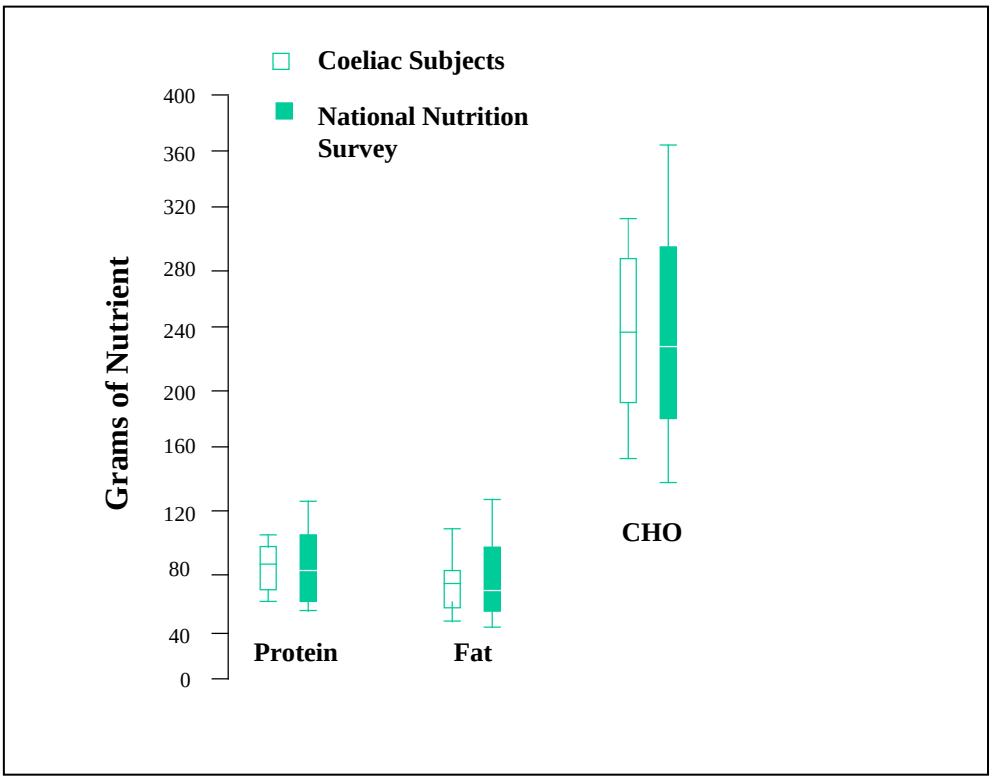


Figure 7.3: Mean daily protein, fat and CHO intakes, for all years of the coeliac study cohort, compared with the 1995 Australian National Average. Bars indicate 10th to 90th percentiles as presented in the NNS.

Figure 7.3 shows the mean intakes of the macronutrients, protein, fat and carbohydrate, compared with the general gluten-consuming population collected in the 1995 Australian National Nutrition Survey *(McLennan & Podger, 1998-b)*.

The total intakes of energy, protein, fat and CHO did not differ from the Australian average, nor did fibre or zinc intake (Table 7.4).

Daily Nutrient Intake	Coeliac Food Diaries n=118		National Nutrition Survey n=13 858		p value (comparison of means)
	mean [10th & 90th percentile]	median	mean [10th & 90th percentile]	median	
Total Energy (kJ)	8552.6 [4080.1 – 14164.6]	8352.5	8768.1 [5817.7 - 13361.5]	8384.3	NS
Protein (g)	87.4 [45 – 135.4]	86.6	86.5 [57 – 131.1]	82.4	NS
Fat (g)	78.4 [46.9 – 170.4]	72.7	77.1 [48.5 - 124.1]	72.8	NS
Carbohydrate (g)	242.4 [102.9 – 379]	237.9	239.5 [157.6 – 371.7]	231.7	NS
Fibre (g)	21.8 [10.3 – 39.8]	21.5	21.4 14.1 – 34]	20.6	NS
Zinc (mg)	11.6 [191.5 – 631.7]	11	11.5 [8 – 16.7]	10.4	NS
Calcium (mg)	978.8 [494.3 – 1927]	892.2	767.3 [449.6 – 1322.6]	712.5	< 0.001
Iron (mg)	12.2 [6.1 – 20.2]	11.9	13.4 [8.8 – 20.3]	12.7	< 0.01
Magnesium (mg)	365.3 [191.5 – 631.7]	340.1	317.2 [214.4 – 464.5]	307.0	< 0.001
Riboflavin (mg)	2.1 [0.6 – 3.5]	2.0	1.9 [1.1 – 3.2]	1.7	< 0.02
Thiamin (mg)	1.2 [0.43 – 2.8]	1.2	1.5 [1 – 2.4]	1.4	< 0.001

Table 7.4. Compares the mean and median nutrient intakes of the group with coeliac disease with the Australian National average. NS means not significant.

Table 7.4 illustrates whether the difference seen in the mean intake of the group with coeliac disease varies significantly from the Australian National average. The mean nutrient intake for the group was determined by initially calculating the mean intake of each nutrient, in 42 subjects, from their Entry, Year 1 and Year 2 weighed food diaries and then finding the mean of these figures. The figures are the result of 118 weighed food diaries because each of the 42

subjects with coeliac disease did not complete all 3 weighed food diaries. A t-test determined that the mean calcium intake was significantly greater in the coeliac subjects than the general population, even when diet alone is considered without supplements. Magnesium and riboflavin intakes were also higher. In comparison, thiamin and iron intake were lower in the study subjects.

Unsupplemented as well as supplemented calcium intakes of the study subjects are plotted in Figure 7.4 for comparison with the data from the National Nutrition Survey *(McLennan & Podger, 1998-b)*, which portrays the intake of nutrients contributed from food alone.

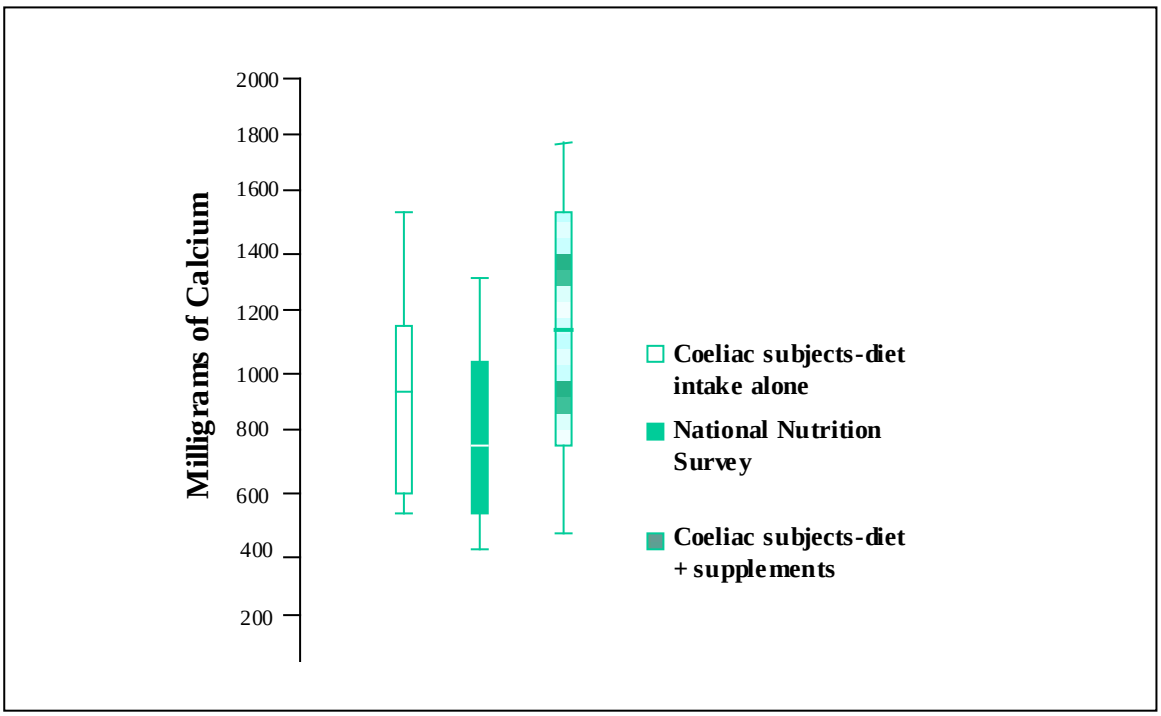


Figure 7.4: Mean daily calcium intake of the coeliac study cohort compared with the 1995 Australian National Average and those who supplemented their diets with calcium tablets. Bars indicate the 10th to 90th percentiles.

The fibre intakes of the subjects with coeliac disease did not differ significantly from those recorded in the National Nutrition Survey. The values have been plotted in Figure 7.5.

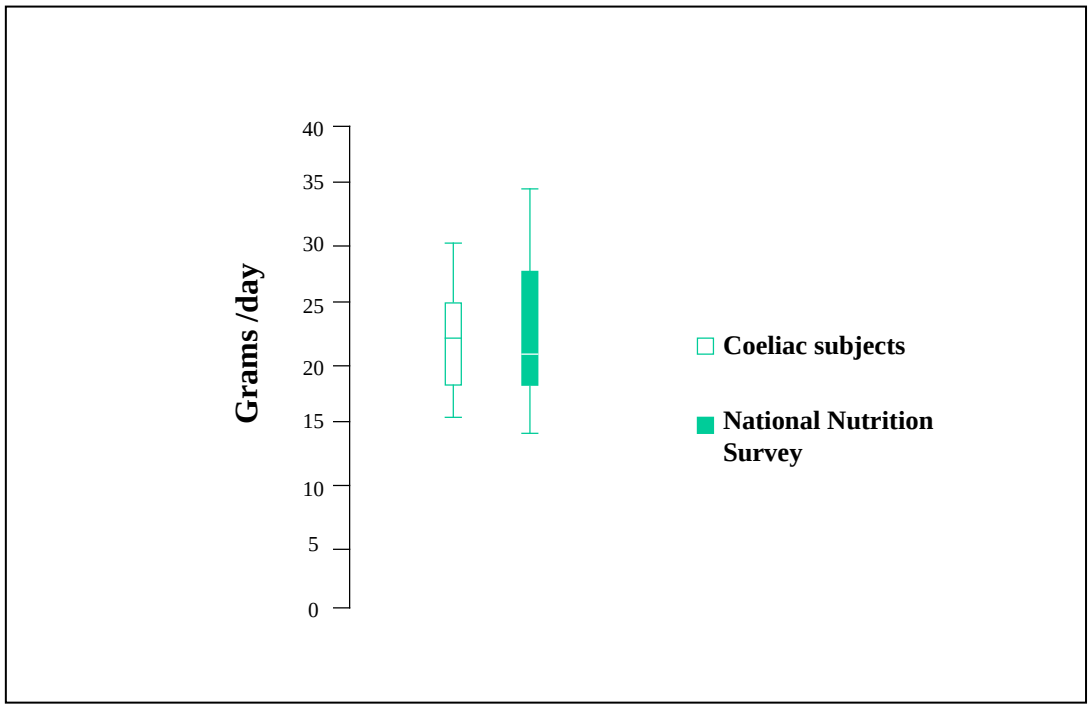


Figure 7.5: Mean daily fibre intake of the coeliac study cohort compared with the 1995 Australian National Average. Bars indicate the 10th to 90th percentiles.

An indication of the fibre content of gluten containing grains and or foods, and their gluten-free equivalent is necessary to appreciate the difficulties of eating sufficient fibre when the diet is changed from gluten-containing to gluten-free. Table 7.5 lists the fibre content of popular gluten-free grains used in commercial products compared with the fibre content of wheat flour sources.

GLUTEN-CONTAINING FOOD	DIETARY FIBRE/100G	GLUTEN-FREE FOOD	DIETARY FIBRE/100G
Wheat flour, white	3.8	Cornflour	0.1
Wheat flour, wholemeal	11.2	Soy flour, low fat	15.5 U
Rye flour, wholemeal	12.2	Soy flour, full fat, soya grits	12.7 U
Barley flour	10.1 U	Rice flour	2.4 U
		Rice flour, brown	4.6 U
		Potato flour	1.8
		Chickpea flour (besan)	10.8 U
		Buckwheat flour (whole groat)	10 U
		Maize flour (wholegrain yellow)	13.4 U
		Maize flour (wholegrain white)	9.6 U
wheat bran	44.7	rice bran	25.5
oat bran	*15.9*	polenta (cornmeal)	2.9
		Amaranth	11 M
		sesame seed	10.1
		sunflower seed	10.8
		poppy seed	10 U
wheat breakfast biscuit (weet-bix)	10.5	gluten-free muesli	8.6 M2
all-bran	28.9		
wheat-based muesli, toasted	8.7		
oat porridge	*1.3*		
white bread	2.7	gluten-free bread, rice	0.9 R4
wholemeal bread	6.5	gluten-free high fibre bread	2.2 R4
multi-grain bread	5.1	rice cake, brown puffed	4.3
		corn cake, puffed	14.2 M
		corn thin, puffed	9 M
		buckwheat cake, puffed	2.1 M
		rice crackers	2

Unless otherwise indicated all content values were taken from the SERVE Nutritional Database. U indicates those taken from the USDA database. M indicates those taken from the Manufacturers food label. M2 indicates that the value is an average of 2 manufactured products. R4 indicates that the value is an average of 4 recipes used by subjects in the study.

Table 7.5. Comparison of dietary fibre content between commonly eaten foods and their gluten-free alternative. *Oat consumption remains controversial and was excluded from the study diet.*

DISSCUSSION

The analysis of the food and supplement consumption of the subjects on a GFD in this study demonstrates that, on average, the diet contains the RDI of each of the macro and micro nutrients studied. In fact, most of the nutrient intakes were above 100% of recommended. None of the average intakes of any nutrient fell below 2/3 of the RDI, a level that is considered inadequate. Although some people were underweight and the mean caloric intake was just below 100% of the RDI, only one subject's weight dropped more than a couple of kilograms during the two years and her energy intakes were above 100% RDI. The mean protein, fat & alcohol intakes were close to the ideal recommended percentage of the total energy, although the mean CHO intake, at 46%, did not quite reach the ideal (50-60%). However, this was mirrored in the findings of the broader Australian population on a normal diet (Figure 7.3). Fibre intake on a GFD was generally low, at 70-75% of the RDI, although again this did not differ from the general population.

Although the mean values on a GFD were reassuring, there were individual subjects whose intakes of various nutrients fell below 67% of RDI. This indicates that attention must be given to each of these nutrients when patients with coeliac disease are undergoing dietary assessment. These are discussed below.

Calcium

Ensuring adequate calcium intake is an important part of the nutritional management of patients with coeliac disease, given its female predominance and the association with osteopenia and osteoporosis. It was therefore reassuring that for the majority of the subjects the amount of calcium in the diet was close to RDI, even without the use of supplements, as assessed at the initial interview. The mean intake was actually higher than that of the general

population, according to the NNS. This would suggest that coeliacs are aware of the need for calcium and the importance of reducing the risk of osteoporosis. However, there were still six subjects whose intake was inadequate at entry to the study. This was still the case later in the study as well, even though the importance of calcium intake was discussed at the initial interview. Follow-up advice on calcium was given individually in the first and second years as dictated by the outcomes of the weighed food diaries. *Collins et al, (1986)* in their study of dietary adequacy, also reported that 2 of 17 subjects had an inadequate dietary intake of calcium. Because of these findings it is essential that attention be paid to maintaining the calcium intake of those with coeliac disease. This is especially important in older subjects, particularly post-menopausal women, and those patients who already have reduced BMD, but should not be overlooked in others. An intake of 1.5g of calcium per day has been recommended for all middle-aged people with coeliac disease *(Heaney et al, 1977)*. As this would be very difficult to obtain by diet alone a supplement of between 500-1000mg of elemental calcium is necessary for most people. As a minimum, all patients with coeliac disease should have careful assessment of their calcium intake and be advised of its importance, even after diagnosis and commencement of a GFD. *Walters (1994)* supports the high calcium intakes suggested by Heaney and states that any calcium salt is suitable if taken for long enough. He suggested over-the-counter products containing calcium citrate and calcium carbonate are acceptable and that these could be taken with or without a vitamin D supplement.

Dietary sources of calcium are not altered considerably by eating gluten-free foods. Much of the calcium in foods is found as inorganic salts that are poorly absorbed by the body (sardines, oysters, dark green leafy vegetables and wheaten bread). However calcium in milk and cheese is more readily absorbed by the gut (*Wahlqvist, 2002, pp-272-273*). Lactose and

other dietary sugars promote the absorption of calcium in the dairy foods. It is difficult to reach the recommended intake for calcium without consuming dairy foods.

Lactose intolerance can be quite common in undiagnosed coeliac disease, due to the inability of the abnormal mucosa to digest lactose. In an effort to decrease symptoms, some of this group chose to follow a dairy free or lactose reduced diet during the many years leading up to the diagnosis of coeliac disease. Even after villous recovery lactase deficiency persists in some with the disease and so the dairy restrictions continue, usually without adequate calcium replacement. Osteopenia, or low bone mineral density, is a recognised complication of coeliac disease, which was discussed in the previous chapter.

It has been reported that wheaten bread makes a significant contribution to the overall calcium intake because it is frequently consumed by the general population *(Wahlqvist, 2002, pp-272-273)*. The NNS reports that most calcium in the Australian diets is obtained from dairy products, but the second highest source was cereals *(McLennan & Podger, 1998-b, p77).* The diets analysed in this thesis indicate that calcium consumption is not greatly affected by changing to a GFD, but calcium requirements increase and are usually met with the help of calcium supplements.

Iron

Iron intake was again, on average, well above RDI, although it was statistically less than the National average. As with calcium intake, there were several subjects who were consuming less than 67% of RDI. *Collins et al, (1986)* also found 5 of their 17 subjects deficient in dietary iron after being gluten-free for one year. Another group found that the daily intake of iron in

women diagnosed with coeliac disease decreased significantly from a mean of 19mg on a normal diet to 15 mg after they commenced their GFD. However, no similar decrease was seen in men (*Björkman et al, 1985)*. The combined average iron intake for both men and women across the entire two-year study period in the present study was 12.0 mg/day, approximately 150% of the RDI.

The above findings indicate that iron intake, like calcium consumption, must not be overlooked in coeliac disease. Iron deficiency is a common presentation of the disorder and is often found at diagnosis in patients presenting with other features *(Farrell & Kelly, 2002; Fasano & Catassi, 2001).* This should correct itself with the institution of a GFD and a short course of iron therapy, in approximately 3 months. After that, iron supplementation may not be needed universally, but may be if intake is considered to be inadequate or if there is persistent iron deficiency. Of course, the latter may require investigation to exclude another cause if this has not been done already.

Meat, containing haem iron, is the best dietary source. This is followed by non-haem sources such as fortified breakfast cereals, wheat bran flakes, whole wheat bread, eggs, legumes and brown rice *(Wahlqvist, 2002, pp-331)*. The NNS found that adults over 19 years obtained most of their iron from cereal and cereal products (males 30.1%; females 29.3%), while only 22.3% and 16.9% respectively, came from meat, poultry and game products and dishes *(McLennan & Podger, 1998-b, p80). Thompson (2000)* also reports that enriched, fortified cereal products contribute a large percentage of the daily intake from iron.

The meat intake of a person with coeliac disease is not greatly influenced by changing to a GFD. Those who ate significant amounts of sausages and processed meats however would

be adversely affected, since many of these types of meats cannot be eaten because gluten products may be used as fillers. A more likely explanation for lower iron intakes may be that the gluten-free range of flours and breakfast cereals are not fortified with iron, as is found in much of the gluten range of products. Thus acquired intakes of iron from fortified grains are absent in those on a GFD *(Thompson T, 2000)*. Today, some fruit juices and milks are fortified with iron and could be consumed on a gluten-free diet. Other iron fortified sources such as Milo® and similar drinks, often contain sources of gluten, so care should be taken with reading food labels.

As with calcium, assessing iron intakes and adding a small iron supplement may be prudent, especially in those with inadequate intakes whose biopsy has not returned to normal.

Magnesium

Magnesium (Mg) absorption also occurs in the duodenum (*Krause & Mahan, 1984, p86).* Whole grain cereals are a major source of magnesium in the diet and therefore exclusion of these in a GFD may adversely affect magnesium intake. Commercial gluten-free products generally do not use wholegrain as their major grain source even though some gluten-free flours contain at least the levels of magnesium as found in wheat flour (e.g. soy flour contains 230mg Mg/100g compared with wholemeal wheat flour 143mg Mg/100g:- *SERVE nutritional database*). Cornflour (6.8mg Mg/100g) and rice flour (23mg Mg/100g) are used most often as the major ingredients in gluten-free products *(SERVE nutritional database)*. The reliance on grains as a source of magnesium is supported by the observations in the NNS. This survey found that the main sources of magnesium (~25%) in the Australian diet were cereals and cereal products *(McLennan & Podger, 1998-b, p79).* Vegetables, dairy, meats and non-alcoholic beverages supplied moderate sources (~10-13%). Despite this, there was no

evidence of inadequate magnesium in the GFDs presented here, with intakes across the 2 years of 120-140% of the RDI. Only one person in each of the first 2 collections recorded intakes of magnesium less than 67% of the RDI. These magnesium intakes recorded were slightly higher than the National average. Magnesium supplementation is therefore not routinely required but should be assessed individually if either intake or serum levels are low.

Zinc

Mean intakes of zinc were between 95 and 99% of the RDI at each time point in the study. Consumption also compared favourably with the National average. However, as with the other nutrients there were individuals with low intake, four at entry, three at year one and four at year two. Zinc is absorbed from the small intestine and may compete with both iron and calcium for the transport mechanism involved and on average only 25-40% of the dietary intake is absorbed *(Davies, 1980; Wahlqvist, 2002, pp-279-280).* Serum zinc levels are decreased in coeliacs who do not maintain a GFD *(Crofton et al, 1983)*. Zinc is present in most tissues, especially liver. It is therefore found in a variety of meat products, some seafood such as shellfish and herring, wheat bran and to lesser extents in legumes, nuts and cooked spinach (*Krause & Mahan, 1984, p146; Stanton R, 1995).* The Australian NNS showed that the zinc requirement of most adults was met by consuming dairy milk, muscle meats and a moderate amount of bread *(McLennan & Podger, 1998-b, p81)*. Dairy and meat sources are usually acceptable when eating gluten-free, so zinc intakes should not be too adversely affected by the changes imposed on a GFD. Zinc in plant foods is bound to phytic acid, oxalates and dietary fibre and is more bioavailable from cereals that are leavened, due to the presence of phytase in the yeast *(Wahlqvist, 2002)*. It can be presumed that less zinc is absorbed in gluten-free breads that do not utilize yeast as a raising agent. As wheaten bran or wheaten breads cannot be eaten, this source of zinc is lost to those on a GFD. However changes in the persons' meat intakes will have the greatest impact on zinc levels and this is

affected little by changing to a GFD. Routine supplementation of zinc in those who adhere to a GFD is not strongly supported by the present findings. However, attention to foods that contain zinc can be given at dietary interview.

Dietary Fibre

The main dietary source of dietary fibre in the NNS was cereals and cereal products followed closely by vegetable products. Fruit supplied only a moderate source of dietary fibre *(McLennan & Podger, 1998-b, p67).* In a normal diet the cereal fibre comes mostly from wheat, particularly wholegrain or bran forms. Although significant amounts are also found in oat bran, this is still excluded from a GFD in Australia. There are some gluten-free grains however which can provide a rich source of fibre (Table 7.5). Examples include soy and chickpea flours, rice bran and also sesame, sunflower and poppy seeds. However, unlike wheat-based breads, gluten-free breads contain substantially less dietary fibre. Commercial gluten-free bread and baking mixes most often use rice flour (2.4g fibre/100g) or maize cornflour (0.1g fibre/100g) as the major component of these mixes. Even the so-called "high-fibre" preparations, which are scarce on the market, contain smaller amounts of dietary fibre compared with their wheat-based equivalents *(Thompson, 2000)*. In the present study, dietary fibre intakes were found to be generally low, approximately 70-75% of the RDI. Nevertheless, they did not differ significantly from the National average, with a mean intake of 22.1g/ day in the subjects on a gluten-free diet compared with 21.4 g/ day for the normal population. Similarly a Swedish study in patients with coeliac disease and dermatitis herpetiformis found that the average dietary fibre intake did not change even though the source of the dietary fibre altered when a GFD was started *(Björkman et al, 1985)*.

Increasing the dietary fibre content of the GFD is challenging due to the dominance of low fibre flours in use in commercial products. In addition to the dietary fibre-rich flours and grains

shown in Table 7.5, non-grain sources include fruits, vegetables, nuts and legumes. Those on a GFD should aim for 4 or more serves/ day of grain-containing foods, such as commercial or homemade muesli's, brown rice, rice and corn cakes, gluten-free varieties of pasta, breads, focchacias, muffins, biscuits etc. This was discussed with the subjects at their entry interview and data collection but, as can be seen in Figure 7.5, this discussion had little effect in raising the overall dietary fibre intake for the rest of the study, although in several subjects it did. It therefore appears that the coeliac subjects in this study, like the Australian population in general, has difficulty consuming the 30g/ day of dietary fibre that is currently recommended.

A recent Swedish study, using a 4-day food record, found that the mean daily intakes of folate and vitamin B12, but not vitamin B6, were significantly lower in coeliac subjects than in normal controls *(Hallert et al, 2002)*. Although both B vitamins were still ingested at levels greater than recommended, the amount of folate ingested was inadequate for many *(Hallert et al, 2002)*. The SERVE nutritional analysis package used in this study was not able to give data on the vitamins B6, B12 and folate levels in the food eaten.

There are several limitations to the interpretation of the data presented here that must be considered. While the 1995 National Nutrition Survey has been used to indicate the normal Australian diet and compare it with the gluten-free diet, there are differences in the 2 groups that should not be overlooked. The subject group in this study is small and contains only 7 males. The NNS is considerably larger (13 858 people) than this and has approximately equal proportions of males and females *(McLennan & Podger, 1998-a)*. To obtain a more accurate comparison of intakes between coeliacs and non-coeliacs would require a very large controlled study, but it is unlikely, given the current results, that any major discrepancies would be found in a GFD.

The Australian nutrient analysis tables (NUTTAB) that have been used to assess the nutritional adequacy of the GFD in this study contained little information about gluten-free grains such as buckwheat, sorghum, almond meal, soy flour and rice flour. Some of these make up the most common ingredients in gluten-free breads, muffins, cakes and other baking goods. Although a database of gluten-free foods was developed, some inaccuracies in the nutritional data exist for the gluten-free foods.

Finally, it should also be considered whether or not the results of this study are representative of coeliacs on a GFD as a whole. It is possible that the subjects who volunteered have a greater awareness of nutrition than average. However, the call for recruitment did not indicate that there would be a nutritional assessment of their diet, only that they should be on a GFD. Moreover, there were still individuals that were well below RDI of several nutrients not just at entry, but throughout the study. It was interesting to note that the second most popular vitamin supplement was vitamin C. Subjects were not advised to add this to their diet but many felt the need to do so. Whether this reflected their thoughts that their diet was inadequate or whether they were aware that it was an anti-oxidant recommended in cancer prevention, was not elicited from the subjects. The use of vitamin and mineral supplements was not universal. The NNS did not publish its results on dietary supplements, however the New South Wales (NSW) Department of Health published vitamin and mineral supplement information in 1994 *(Stickney et al, 1994).* They have concluded that females were more likely to take supplements, than males and that between 23-26% of females and 14-18% of males consumed vitamin or mineral supplements at least once per week *(Stickney et al, 1994, p 179)*. Their compilation of surveys suggests that the use of supplements was greatest between the ages of 60-69 years *(Stickney et al, 1994, p 178)*. The group with coeliac disease, in this study, was predominantly female with only 3 males taking supplements and 50% (45 – 56%) of the group as a whole,

taking regular supplements throughout. This is higher than the figures that reflect usage by the general population as shown above.

Again, only a large, controlled study of unselected coeliac subjects would confirm the generality of these findings. However, the findings still indicate that there are individuals in whom intake of various nutrients may be inadequate and these should be assessed in each case. Even if a larger study shows differences from a normal population it will still mean that each individual with coeliac disease will need a careful nutritional assessment.

In conclusion, the results of this study demonstrate that, for the majority of people with coeliac disease, a GFD is able to provide an adequate nutritional intake. However, for each of the major nutrients examined there were a few subjects whose levels of intake were below 67% of the RDI and thus considered inadequate. The reasons for this have not been addressed in this study but are worthy of further research. The findings also highlight the role of the dietitian in coeliac disease, not just to explain the nature of the gluten free diet to patients, but also to assess their diet generally, with emphasis on calcium, iron, fibre and folate *(Hallert et al, 2002)* in particular. Ways of maintaining the intake of these and other important nutrients can be given. Recommendations can also be made with regard to supplementation if considered appropriate. Routine calcium supplementation is also advisable in at least older patients of both sexes, post-menopausal women and patients with reduced BMD.

CHAPTER 8

8

NUTRITIONAL STATUS

Assessing the nutritional status of adults with treated coeliac disease and persistent abnormal mucosa.

INTRODUCTION

All people with untreated coeliac disease are at risk of malnutrition and decreased nutritional status because their absorbing capacity is impaired. Most of them make a good clinical recovery and achieve a significant improvement in their nutritional status after beginning a GFD *(Radzikowski et al, 1991; Corazza et al, 1994)*. However, some have persistent villous atrophy, persistent mild symptoms and evidence of mild nutritional deficiencies *(Corazza et al, 1994; Kemppainen et al, 1995)*. The continued ingestion of significant amounts of gluten is often blamed for this despite denial by the patient. However, the evidence presented in Chapters 4-6 showed that the subjects in this study were all adherent to a GFD and that the trace amounts of gluten found in their gluten-free diets was not responsible for any persistent villous atrophy or any abnormality in BMD.

There is very little available information on the nutritional status of individuals with coeliac disease. The few papers that have been published have assessed nutritional status before or soon after the gluten-free diet has been instituted *(Schwartz et al, 1968; Gawkrodger et al, 1988; Radzikowski et al, 1991; Corazza et al, 1994; Kemppainen et al, 1995; Capristo et al, 1997)*. This chapter examines the nutritional status, over a period of 2 years, of a group of

subjects with treated coeliac disease who have been on a GFD for between 2 and 59 years (median: 1.8 years). This assessment was performed by integrating the findings of anthropometry, dietary intake, biochemical indices and small bowel histology. The findings in those subjects with persistently abnormal mucosa were compared with those whose mucosa was normal.

METHODS

Subjects

Forty-two subjects, recruited using the criteria outlined in Chapter 3, were studied. There were 33 females and 9 males. The mean age was 48.6 years (range: 20-72 years). The average age at diagnosis of coeliac disease was 39 years (range: 6months-70 years) and the average during following diagnosis was 9.6 years (range: 2-59 years).

Anthropometry and skin-fold measurements

The following measurements were made at entry, year 1 and year 2.

Body Mass Index (BMI)

Body mass index is a measure of the body's energy store corrected for height and compared with a standard range *(Wahlqvist, 2002, pps495-507)*. BMI was calculated from each subject's height and weight using the following formula (*Krause & Mahan, 1984).*

$$BMI = \text{weight (kg)} / [\text{height (m)}]^2$$

Subjects were classified as:

underweight	BMI	< 19.9
normal	BMI	20 - 24.9
overweight	BMI	25 – 29.9
obese	BMI	> 30

Skinfold Measurements

Skin fold measurements were taken from the subscapular and triceps region of the left side of the body. All measurements were taken by the same observer (author) using Harpenden Skinfold Callipers.

Triceps skinfold thickness (TS), measured while the left arm was hanging relaxed, was taken as a measure of body fat mass *(Wahlqvist, 2002, pps495-507).* The triceps reading was measured three times at each visit and then averaged. The results were expressed as a percentage of the standard values: 11.3mm for males and 14.9mm for females (*Jelliffe, 1966)*

Mid-upper arm muscle circumference (MAMC) is a measure of body muscle mass and, indirectly, of total body protein *(Corazza et al, 1994)*. Mid-upper arm circumference (MAC) was measured by tape measure. The mid-upper arm muscle circumference (MAMC) was then calculated using the TS and MAC in the following formula:

$$MAMC\ (cm^2) = MAC\ (cm) - \pi\ [TS\ (cm)] \qquad (Grant\ et\ al,\ 1981)$$

MAMC was then expressed as a percentage of the standard values: 22.8mm for males and 20.9mm for females (*Jelliffe, 1966)*. A value of less than 90% of the standard value was considered to be indicative of mild malnutrition and less than 80% of moderate malnutrition *(Blackburn & Thornton, 1979).*

Dietary data

The trace amounts of gluten in the subjects' GFDs was been quantified and presented in Chapter 4. Assessment of nutrient intake in the GFD was described in Chapter 7. Dietary intakes less than 67%, or two-thirds, of the RDI have been considered to be inadequate *(Briggs & Wahlqvist, 1984,p.13).*

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Blood tests

Blood was taken at the entry, year 1 and year 2 appointments for the following tests:

Vitamins: A, D, E, folate, red cell folate

Minerals: calcium, iron, magnesium, zinc

Haemoglobin, ferritin, percent saturation, albumin and total protein were only done at the one and two-year follow-up visits. The assays were performed in the Central Sydney Area Health Service diagnostic laboratories at either Royal Prince Alfred Hospital or Concord Hospital.

Small bowel biopsy

Duodenal biopsies were obtained endoscopically and assessed as described in Chapter 5. Those findings are used here as well.

RESULTS

Biopsies

At entry the small bowel biopsy was normal in 29 (22F, 7M) subjects and abnormal in 13 (11F, 2M). By the end of the first year the number with an abnormal biopsy had decreased to 6 subjects (5F) and after 2 years, 9 (8F) showed villous damage. Only 5 (4F) subjects had persistently abnormal biopsies throughout the study period.

BMI

The average BMI of the 42 subjects was 23.8 (range 17.1-37; median 23.4). The mean BMI at entry of the five subjects with persistently abnormal mucosa was 22.1 (SD 2.2). Only one, a 53-year-old woman, had a BMI below 20 (underweight) throughout the study. One other female in this group recorded a BMI below 20 at the final data collection only. Eight other subjects had abnormal mucosa at their entry small bowel biopsy. Their mean BMI was 24.4 (SD 2.8) and only one was below 20. The mean BMI of the 29 subjects with normal mucosa at entry was 23.9 (SD 4.4). Six of these had a BMI below 20.

Looked at the other way, four subjects had a BMI consistently less than 20 throughout the study (S-7,18,27,41). Only one of these (female, S-41, aged 53) had persistent villous atrophy despite reporting one of the strictest gluten-free diets recorded in this study (gluten category A at each assessment). Her blood results were consistently within the normal range. Although her entry and year 1 total energy intakes were below 100% of the RDI, none were less than 67% (95% & 97% respectively). Two other female subjects (S-7,27) with a BMI less than 20 had normal mucosa on each biopsy and caloric intakes greater than 100% of the RDI throughout. The last of these four subjects with low BMI began the study with abnormal mucosa, followed by 2 normal biopsies at years 1 and 2 (S-18). She failed to return the year 1 weighed food diary, but the entry and year 2 weighed food diaries indicated total energy

intakes at approximately 90% of recommended and only dietary calcium and zinc less than 67% at the final assessment. This decreased oral intake was not reflected in her serum results.

Skin Fold Measurements

No one at any time in the study was regarded as being malnourished since all measurements of MAMC were above 90% of standard. Four females at entry, four at year 1 and seven at year 2, had MAMC values between 90 and 100%. Duodenal biopsies were normal throughout the study in all but two of these subjects, who had villous atrophy on their final biopsies. Of the subjects with MAMC of 90-100%, two at entry, two at year 1 and three at year 2 had a BMI below 20.

Iron studies

Serum iron was normal at all times in each of the five subjects with persistently abnormal mucosa. One of these female subjects (S-20) had a low percent saturation after one year in the study, but this was not recorded again at the final collection. Another of these 5 (S-15) had a low ferritin level at year 2 only. Ferritin levels and percent saturation were not recorded at entry. Unfortunately, her ferritin was not analysed at year 1 so a low level earlier in the study is also possible.

Of the subjects with normal mucosa, only four (S-27,10,30,45) had low serum iron levels, at entry, years 1, 1 and 2 respectively, despite dietary iron intakes greater than 90% of RDI. Subject 27 did not record low serum iron at any other stage of the study. Low ferritin and low percent saturation was also recorded in subject 30 at year 1. Although serum iron was not low at year 2 the other 2 tests remained so. Subject 45 recorded only a low ferritin at year 1, however at year 2, iron, ferritin and percent saturation were low. The third subject (S-10) had

low iron on entry but normal ferritin, percent saturation throughout. Her dietary iron intake was adequate and her serum iron level rose progressively at the year 1 and 2 measurements.

Subject 36, who recorded an abnormal mucosa only at entry, had a low serum iron at entry only and low serum vitamin A at entry and year 1. Although her BMI was 26 her total energy intake was below 67% RDI throughout with resultant low dietary nutrient intakes.

Three other subjects (S-32,49,50) with normal mucosa recorded low ferritin levels at year 1 which persisted into year 2 in subjects 32 and 50. Subject 32 had concurrent low percent saturation in both years, while this occurred in subject 50 only in year 2.

Two subjects (S-20,37) at year 1 recorded only low percent saturation (one with persistent abnormal mucosa discussed above) and in only (S-37) one this persisted into year 2. Three others in year 2 recorded only a low percent saturation, while 2 others had a low percent saturation with low serum magnesium (S-39) or low serum magnesium and serum calcium (S-44).

There was only one low haemoglobin recorded throughout the study and this was during the final collection for subject 45 who also had low serum iron, percent saturation and ferritin at the final collection, despite having normal mucosa, total protein, albumin and adequate dietary intakes of all nutrients.

Serum Folate

No subject with an abnormal biopsy had a low serum folate at any stage of the study. Serum levels of folate were low at entry in one subject, three in year 1 and one in year 2. One year 1

subject also had a low ferritin (S-32). However, red cell folate levels were within normal limits in every subject throughout the two-year study period.

Serum Total Protein

This was not measured at entry. However, all values at years 1 and 2 were normal.

Serum Albumin

Serum albumin was not measured at entry. It was low in only one subject, at the final data collection. This female had normal mucosa but her diet was regarded as nutritionally inadequate. Although her BMI was 23 throughout, her energy intake was below 67% of the RDI at entry and year 1 and only just above (70% RDI) at year 2. Correspondingly, many of her dietary nutrients were low. Her protein intake however was close to, or above, 100% of recommended throughout. All five subjects with persistently abnormal mucosa had normal albumin levels at years 1 and 2.

Serum Calcium

As with the other parameters, none of the 5 subjects with persistently abnormal mucosa had low serum calcium levels at any stage. Eight other subjects had a low serum calcium level at one point in the study but none had low values at each time point. Two of these subjects had low calcium levels at two of the time points. They had normal mucosa at the time and dietary calcium intakes of at least 100% RDI. One of the other eight had a low calcium level corresponding to an intake below 67% of the RDI, although at other times it was normal.

Serum Magnesium

Only two subjects, both with normal mucosa, had low serum magnesium levels recorded during the study. Their dietary magnesium intakes were normal. Levels were also normal in those subjects with villous atrophy.

Serum Zinc

One of the subjects with persistently abnormal biopsy had low serum zinc at entry, while two others, with normal mucosa, also had low levels at entry. In one of the latter the dietary intake of zinc was inadequate (< 65% RDI). Serum zinc was normal in all subjects at the other time points.

Fat Soluble Vitamins

At entry, 4 subjects had low levels of serum vitamin A. Two of these still had low values at year 1, but not at year 2. Only subject 37 had an inadequate dietary retinol equivalent intake (43.5%) at entry, which remained below 67% of RDI at years 1 and 2 even though her serum vitamin A levels had returned to normal by years 1 and 2. Serum vitamin D levels were low at entry in 3 subjects. None of these were subjects with persistent abnormal mucosa nor did any of them have abnormal BMD at any stage of the study. Serum Vitamin E was not low in any subject at any of the 3 time points when it was measured. Coagulation (a measure of vitamin K levels) was not measured in the study.

DISCUSSION

This study of nutritional status of subjects with long-standing coeliac disease on a gluten free diet has shown that none can be regarded as being undernourished. It was particularly noticeable that this applied also to the 5 subjects who had persistently abnormal mucosa.

There are several methods that used to judge the nutritional status of an individual. Weight and height measurements, and hence BMI, are commonly used as they are simple, quick, non-invasive and require minimal equipment. An overall impression of nutritional status can be obtained by this means *(Wahlqvist, 2002, pps495-507)*. Only four subjects were found to be underweight throughout this study and none were severely compromised. Only one of those with a constantly low BMI had villous atrophy throughout the study and it was clear that low BMI did not reflect inadequate energy intake or the presence of an abnormal mucosa leading to decreased nutrient absorption. Conversely a high BMI did not necessarily imply normal mucosa.

The anthropometric skin-fold measurements are another commonly-performed non-invasive technique to acquiring information about muscle protein status and general nutritional status. *Corazza et al (1994)* used similar anthropometric measurements to show that mild or moderate malnutrition was common in those with a classical presentation of coeliac disease but not in those with subclinical presentations such as anaemia or on screening. Adherence to a GFD corrected any malnutrition. The present study using anthropometric data confirmed that treated coeliacs were well nourished, regardless of what their small bowel biopsy showed.

Haematological and biochemical parameters, including serum levels of important vitamins and minerals, are another way to assess an individual's level of nutrition. Several of the serum measurements appeared to be randomly low throughout except for percent saturation and

ferritin, which is discussed below. The results again show that any low values do not result from villous atrophy and its associated malabsorption. In fact, more abnormal results were found in those with normal mucosa than in those with villous atrophy. *Corraza et al, (1994)* attributed these abnormal serum values to continued gluten intake with resultant mucosal damage. However, there was no association with villous atrophy in the present study or with the trace amounts of gluten being ingested in the subjects' gluten free diets.

Anaemia is common today in adult coeliac disease *(Hoffbrand, 1974).* Serum ferritin is a measure of total body iron stores *(Addison et al, 1972; Cook et al, 1976; Cook et al, 1974; Walters et al, 1973). Souroujon et al, (1982)* studied children with coeliac disease under the age of 18 years and concluded that "serum ferritin levels reflected the iron-deficiency state in coeliac disease better than other laboratory parameters used for the same purpose". They also found that when the diets of these children were changed from gluten-free back to gluten-containing serum ferritin levels declined at an average rate of 4μg /l/month *(Souroujon et al, 1982).* When gluten was again removed from the diet there was an increase of serum ferritin at a rate of 1μg /l/month. They therefore suggested that determination of the serum ferritin level could help decide when it is appropriate to do further biopsies in people with coeliac disease. In our study only one of the six adults with low ferritin had an abnormal biopsy, and this was abnormal throughout. The mucosa in the remaining five was normal at every procedure. In this small group the iron status appeared to be randomly low in some and this was not a reflection of dietary intake. Low ferritin only resulted in anaemia in one subject. The findings also indicate that since iron deficiency appears to be unrelated to mucosal abnormalities in treated coeliac disease, when the patient is on a gluten free diet, a search for other causes is warranted.

Serum total protein was normal in all subjects and serum albumin was normal in all but one. Low measurements of the fat soluble vitamins can be an indicator of fat malabsorption, however low levels of vitamins A and D only occurred in subjects who had normal small bowel mucosa.

Overall, it was found that the subjects in this study were not undernourished. The compilation of measures in this chapter to determine the nutritional status of the group suggest that the small trace amounts of gluten eaten by the study group has had no impact on their overall nutritional status. A study of nutritional adequacy in people with dermatitis herpetiformis, where the mucosal damage is considered to be patchier and less extensive, also found that malnutrition was uncommon *(Gawkrodger et al, 1988).* Perhaps the most important finding of the current study was that the five subjects with persistent villous atrophy had normal nutritional parameters. The reasons for this, compared with untreated symptomatic patients, need to be elucidated. However, it is possible that the changes are limited to the proximal small intestine, reflecting the progressive recovery from distal to proximal once a gluten free diet is instituted. It is also likely that gut function has returned to normal even though histological abnormalities persist. A similar situation may exist in those patients with coeliac disease who have a subclinical or atypical presentation *(Corazza et al, 1994)*. That same group also reported that those whose nutritional status did not improve were noncompliant with their gluten free diet.

In Chapter 5 it was shown that the trace amounts of gluten found in a gluten free diet are not responsible for the persistent villous atrophy found in some patients. The data presented here confirm that people with coeliac disease on long term gluten-free diets achieve acceptable nutritional status. This is true also in those who have ongoing villous atrophy.

CHAPTER 9

9

CONCLUDING DISCUSSION

Persistent Symptoms

It has not been widely appreciated that some patients with coeliac disease have continuing symptoms despite apparent adherence to a gluten-free diet. This has usually been attributed to ingestion of gluten, either intentional or inadvertent. The study described in Chapter 2 indicates that the small amounts of gluten found in a *Codex-GFD* can be responsible for symptoms, but even when this is removed, as in the *NDG-GFD*, symptoms can persist. In many of these subjects, non-gluten food intolerances were shown to be the cause of their continuing symptoms.

Persistent Mucosal Abnormalities

It is also widely believed that complete recovery of villous atrophy should be seen in all patients with coeliac disease provided they are adhering to a gluten-free diet. However, clinical experience and a review of the literature indicate that this is not necessarily the case. In the subjects described in Chapter 5 of this thesis, villous atrophy was found in 32.5% of subjects at entry and 23% at the end of 2 years. This was not related to whether the patient was following a *Codex-GFD* or *NDG-GFD*, or to the quantities of gluten ingested (expressed as estimated annual gluten consumption). It should be emphasised that all patients in this study were adherent to a GFD, as documented by dietary interview and *Food Brand Questionnaire* (performed annually), *One–Week Food* Diary (recorded every second month) and the *Gluten Intake Diary* (kept continuously). The quantities of gluten being consumed

were very small, and certainly less than an amount that would suggest non-compliance. The high level of adherence was confirmed by the fact that endomysial antibodies were negative in all but one subject.

The mechanisms underlying persistent villous atrophy in patients adhering to a GFD are not known but may represent a continuing autoimmune process. Further evaluation of this finding is required, including determination of how far down the small intestine the abnormalities extend and whether they are associated with any of the immunological abnormalities seen in refractory sprue, which these patients did not have clinically. Regardless of the underlying mechanism, it was clearly shown in the small number of subjects with persistent villous atrophy while adhering to a GFD, that this not associated with an increased likelihood of any nutritional or metabolic consequences, including osteopenia or osteoporosis, over a two-year period. Whether such problems might emerge over a longer period of observation remains to be determined, and long-term follow up of these individuals is planned.

It is noteworthy that, though not statistically significant, there was an overall trend towards improvement in symptoms and a reduction in the number of patients with mucosal abnormalities over the two-year study period (Chapter 5), suggesting that avoidance of the small amounts of gluten in *Codex*-permitted 'gluten-free' foods may be of clinical significance for some individuals.

Changes To The GFD

The other aspect of coeliac disease examined in this thesis was the changing composition of the gluten-free diet in Australia. Strictly speaking, until recently the term '*gluten free diet'* was a misnomer, since the *Codex* standards permitted labelling of foods as 'gluten-free' even though

they could contain up to 0.3g/100gm of protein from gluten-containing grains. After introduction of the new food labelling standards in March 1995, foods could only be labelled 'gluten-free' if no gluten could be detected by the best available assay and the product did not contain oats or malt. This excluded foods containing Codex-permitted gluten, principally in the form of wheat starch and malt. The term "*NDG-GFD*" is used in this thesis to define a GFD based on this standard.

Even with this more stringent approach to gluten avoidance, trace amounts of gluten can still be ingested. However, this was found to have no measurable effect on mucosal histology, bone mineral density or other nutritional parameters. The only measure affected by trace gluten and Codex-permitted gluten consumption was the presence or absence of symptoms. This was seen in a subset of patients who may be considered to be at the 'more sensitive' end of the spectrum.

Nutritional Adequacy

Whether this new Australian GFD is nutritionally adequate has not previously been studied. The overall nutritional status of all patients in this study was found to be normal. The mean dietary fibre intake was lower than recommended, but not different from that of the general population on a normal diet. Increasing the dietary fibre content of the diet using gluten-free grains is challenging as the most commonly used gluten-free flours contain less dietary fibre than their gluten containing counterparts. Other nutrients could be adequately supplied by appropriate food substitution in a gluten-free diet. Although mean intake of calcium and iron were adequate in the present study population, they need to be assessed and monitored in routine clinical practice, with particular emphasis on folate *(Hallert et al, 2002).*

Clinical Implications

These findings have important implications for the dietetic management of coeliac disease and also raise important questions. The concept of a single 'gluten-free diet', suitable for all people with coeliac disease may be inappropriate. There are clearly many people with coeliac disease who can consume products containing wheat starch and malt (as permitted on a *Codex-GFD)* without experiencing symptoms and without compromising their villous architecture, BMD or nutritional status. Indeed, this is the GFD that the majority of patients with coeliac disease in Europe are consuming and is also the diet that has been shown to reduce the risk of malignancy to that of the general population.

It must be acknowledged, however, that there is a subgroup of 'more sensitive' coeliac patients who cannot tolerate the small amounts of gluten permitted in a *Codex-GFD.* Even though their symptoms resolve or improve when these Codex-permitted amounts of gluten are removed from the diet, there does not appear to be any further benefit in terms of mucosal histology or bone mineral density.

A few coeliacs claim they are so sensitive to gluten that they avoid any product that has been derived from wheat, including glucose syrup (which is so far down the refining chain that no gluten can be detected by any currently available method). This degree of gluten sensitivity has not been validated scientifically. However the Canadian Food Standard acknowledges this possibility and is the strictest food standard in the world. It does not permit foods to be "…labelled, packaged, sold or advertised as gluten-free if they contain wheat, including spelt and kamut, or oats, barley, rye, triticale or any part thereof."[7web]

[7] www.inspection.gc.ca/english/bureau/labeti/guide/7-0-0ae.shtm#7-15-7 (accessed 20/2/03)

The current Australian food standard indicates that foods can only be labelled ‘gluten-free’ if they contain no detectable gluten (i.e. <0.002%), oats or malt. Doctors and dietitians have adopted these restrictions when giving advice about what ingredients may be purchased when selecting commercial products not labelled as gluten free, while following a GFD. This is more restrictive than the *Codex-GFD*, in which wheat starch, malt, malt extract and thickeners etc, are included. Current recommendations in Australia are to place all newly diagnosed patients with coeliac disease on a *NDG-GFD*. If the small bowel biopsy returns to normal and the patient becomes asymptomatic, it may be reasonable to introduce small quantities of ingredients that contain amounts of gluten acceptable on a *Codex-GFD*. This less restrictive GFD may also be suitable for selected asymptomatic people with coeliac disease picked up on screening (e.g. those with type 1 diabetes or first-degree relatives of patients with coeliac disease). They can be reassured that ingestion of these small amounts of gluten is unlikely to cause any serious harm, at least in the short to medium-term.

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APPENDICIES

Appendix 1:- Informed Consent Form

Consent form signed by participants in the Food intolerance and coeliac disease study.

CONSENT FORM

I agree to participate in a study to investigate the role of food chemical intolerances in people with coeliac Disease whose symptoms persist despite following a strict gluten-free diet.

I have read and understood the information sheet outlining the testing procedure. I have been given the opportunity to discuss both the procedure and the diet with Dr. Loblay and Kim Faulkner-Hogg (Dietitian).

I am aware of the risks and side effects associated with the endoscopy and bowel biopsy. I understand that I may be drowsy after the procedure and will be unable to drive myself home.

I give my consent to allow the researchers access to my previous medical records from my family doctor and/or specialist(s). I understand that confidentiality will be observed at all times. Results of the tests conducted on me in the course of this study will be sent to my doctor(s) with my permission.

I am aware that I may withdraw from this study at any time without prejudice.

Participant's signature _____ Witness _____

Name (block letters) _____ _____

Date: _____

FOOD BRAND QUESTIONNAIRE

NAME:_____

1. How many slices of bread on average do you eat a day?_____

2. Do you buy ready made bread? yes / no
   which brands _____
   _____
   _____

3. Do you buy bread mixes to bake? yes / no
   which brands _____
   _____
   _____

4. Do you make your own breads? yes / no
   which flours do you use? _____
   _____
   _____

5. Which brand/s of cornflour do you use?_____

_____

_____

6. How many days does it take you to consume / use a loaf of bread?
   _____
   _____

7. How often do you eat biscuits or crackers? _____
   _____

8. How often do you buy a packet of biscuits or crackers? _____
   _____
   which brands do you buy? _____
   _____
   _____
   _____
   _____
   _____

9. How often do you eat cakes? _____
   _____

10. Do you buy commercial cakes? yes / no

11. Which cakes/brands do you buy? _____
    _____
    _____
    _____

12. How often do you buy cakes? _____
    _____

13. Do you do your own baking? yes / no
    Which flours do you use? _____
    _____
    _____
    _____
    _____
    _____

14. How often do you bake? _____

15. Which breakfast cereals, (and their brands), do you use?
    _____ _____
    _____ _____
    _____ _____
    _____ _____

16. Which types and brands of pasta do you use?
    _____ _____
    _____ _____
    _____ _____
    _____ _____

17. How often do you eat take-away?_____
    _____

18. Which take-away foods do you have? _____
    _____

19. How often do you eat at restaurants? _____
    _____

Appendix 2:- Food Brand Questionnaire

20. What type of foods do you usually choose at these restaurants?_____
_____

21. How often do you eat at the homes of friends?
_____
_____

22. When eating out: what do you do if you *feel* something *may* contain gluten?
a) at restaurants: _____
_____
_____
_____
b) at friends houses: _____
_____
_____
_____
_____

23. Which alcoholic beverages do you drink?

list brands	how often / how much
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

24. Do you use sauces on your meals? yes / no
which brands _____
_____
_____
_____
_____
_____

25. Which thickeners do you use in home made sauces? _____
_____

26. Do you use sausages, salami or processed meats? yes / no
which brands _____
_____
_____

27. Which brands of chocolate do you consume?
_____
_____
_____

28. Do you take medication? yes /no
If so, please list the medications & their brands
_____
_____
_____
_____
_____

29. Do any of your medications contain gluten? yes / no
please list the ones which contain gluten
_____
_____
_____

30. Do you take vitamin tablets or powder? yes / no
If so, please list them & their brands
_____
_____
_____
_____
_____

31. Do any of these preparations contain gluten? yes / no
please list the ones which contain gluten
_____
_____

32. On what type of occasion would you knowingly eat gluten? _____
_____
_____

33. How frequently does this occur? _____
_____
_____

34. Do you know of any instances where you may have eaten gluten in the past 3 months? _____
_____
_____
_____
_____
_____

FOOD & SYMPTOM DIARY

Please record your USUAL daily diet for one-week leading up to your biopsy appointment.

Please take this with you to your biopsy appointment and leave it there for me to collect.

INSTRUCTIONS

This booklet is to be used to record your food intake and symptoms while you are undergoing dietary testing.

1. In the DIETARY INTAKE column, record
    - *The time of your meals and snacks*
    - Which food and drinks you had
    - Vitamin and mineral supplements

2. In the SYMPTOMS column, record
    - Any symptoms, *regardless of whether you think they're related to your diet*
    - *The approximate time of the onset*
    - Severity (in brackets), graded 1-4
        (1) - mild
        (2) - moderate
        (3) - marked
        (4) - severe

3. In the REMARKS column, record
    - *How long the symptoms lasted*
    - Medications used
    - Any additional factors which may have influenced your symptoms (Smells and fumes, work, periods, stress etc)

Appendix 3:- One Week Food & Symptom Diary

Example page

Day:	Date:	Challenge:		
Time	Dietary Intake	Symptoms	Grade (0-4)	Remarks
7.00 am	Breakfast: Puffed rice Pear syrup Rice bread & sundew marg Pear jam Decaf cof Macro M	Stomach cramps	2	
10.00 am	Morning snack: 2 rice cakes & sundew Pear jam			
1.00 pm	Lunch: Rice bread and sundew Chicken Pear chutney Iceburg lettuce Homemade lemonade 1 fresh pear peeled			
3.30 pm	Afternoon snack: Kettle chips (30g) Decaf coffee			hairdresser very smelly
7.00 pm	Dinner: Lamb (no fat) Home made french fries Choko Brussel sprouts 2 canned pears Meringue Home made lemonade	Headache Diarrhoea	1 2	
9.00 pm	Evening snack: 3 marshmallows (white) Decaf coffee			

Space for subjects to write their information

Day:	Date:	Challenge:		
Time	Dietary Intake	Symptoms	Grade (0-4)	Remark
	Breakfast:			
	Morning Snack:			
	Lunch:			
	Afternoon snack:			
	Dinner:			
	Evening snack:			

Appendix 4:- Simplified Elimination Booklet modified for study.

An updated booklet version of this diet can be viewed by obtaining the

Royal Prince Alfred Hospital
Elimination Diet Handbook
With food and shopping guide

At www.allergydownunder

Appendix 5:- Salicylates, Amines & Glutamate Booklet.

An updated booklet version of this diet can be viewed by obtaining the

Royal Prince Alfred Hospital
Elimination Diet Handbook
With food and shopping guide

At www.allergydownunder

Appendix 6:- Challenge Dosage for Elimination Diet Challenges

Those without asthma or other confounding medical problems or medications received the standard set of challenges to complete at home.

STANDARD CHALLENGE SET (April 1991)	
CHEMICAL	DOSAGE
Salicylate: Aspirin	 500mg
Preservative: Sodium Benzoate + p-Hydroxy benzoic acid + Sorbic acid + Sodium Metabisulphate	 500 mg 200 mg 200 mg 50 0mg
Preservative: Sodium Nitrate + Sodium Nitrite	 25 mg 25 mg
Preservative: Butylated Hydroxy Toluene (BHT) + Butylated Hydroxy Anisole (BHA)	 50 mg 50 mg
Bread Preservative: Sodium Propionate	 500mg
Colour: Tartrazine + Brilliant Blue	 30 mg 10 mg
Amine: Tyramine HCL + Phenylethylamine HCL	 150 mg 4 mg
MSG: Monosodium Glutamate	 5 g
Placebo or active challenge: Lactose	 500mg
Placebo: Potato Starch	 2g
Placebo: Sucrose	 1g

Those with asthma or those taking certain medications were sent a short set to complete at home and other set to complete with the supervision of their General Practitioner.

SHORT CHALLENGE SET (April 1991)	
CHEMICAL	DOSAGE
Preservative: Sodium Benzoate + p-Hydroxy benzoic acid + Sorbic acid	 500 mg 200 mg 200 mg
Preservative: Sodium Nitrate + Sodium Nitrite	 25 mg 25 mg
Preservative: Butylated Hydroxy Toluene (BHT) + Butylated Hydroxy Anisole (BHA)	 50 mg 50 mg
Amine: Tyramine HCL + Phenylethylamine HCL	 150 mg 4 mg
Placebo or active challenge: Lactose	 500mg
Placebo: Sucrose	 1g

THE GP CHALLENGE SET (April 1991)		
CHEMICAL	TOTAL AMOUNT	AMOUNT PER DOSE
Sodium Metabisulphate	500 mg	50 mg 100 mg 150 mg 200 mg
Tartrazine	30 mg	30 mg
Monosodium Glutamate	4.8 g	1.2 g 1.2 g 2.4 g
Aspirin	1.2g	50 mg 100 mg 150 mg 300 mg 600 mg

Appendix 7:- Symptom Record Sheet

SYMPTOM RECORD SHEET

Name:……...

Current date:……..

Date of diagnosis of coeliac disease:…….

Symptom	Symptoms BEFORE diagnosis		CURRENT symptoms	
	severity	frequency	severity	frequency
diarrhoea				
constipation				
nausea				
vomiting				
bloating				
stomach pain				
cramps				
excessive wind or flatulence				
mouth ulcers				
headaches				
fatigue				
Dermatitis Herpetiformis				
other				

Please complete the symptom record table over-page, using the severity and frequency guide below to help you. **Please record the symptoms experienced both BEFORE diagnosis and those you currently have.**

Severity	**Frequency**
0...none	0...never
1...mild	1...less than 1 month
2...moderate	2...monthly
3...severe	3...weekly
	4...daily

Please read the following carefully so that you will be able to classify the severity of a symptom as mild, moderate, or severe.

MILD: You are aware of the symptom, but it is easily tolerated.

MODERATE: This symptom is enough to cause interference with daily life or usual activity.

SEVERE: This is incapacitating with inability to work or to take part in your usual activities.

FOOD INTAKE DIARY

Name: _____

Date: _____

Please record your USUAL daily diet for one week

Please return this, with the questionnaire, in the envelope supplied

INSTRUCTIONS

This booklet is to be used to record your daily food intake

1. In the DIETARY INTAKE column, record:
   - the time of your meals and snacks
   - which foods and drinks you had
   - vitamin and mineral supplements

2. In the REMARKS column, record:
   - medications used
   - any additional factors which may have influenced your diet choice (eg. restaurants, home of friends)

Appendix 8:- One Week Food Intake Diary (no symptoms)

Day: Wednesday		Date: 14:2:96
TIME	REMARKS	DIETARY INTAKE (including vitamin & mineral supplements)
7.00 am		Breakfast: gluten free muesli: rice flakes, rice bran, sunflower seeds, dried fruit & nuts milk gluten free bread, butter & blackberry jam 2 cups of tea/milk/sugar multi-vitamin calcium
10.00 am		Morning snack: coffee/milk/sugar banana small block of Cadbury milk chocolate
12.30 pm	Chinese restaurant	Lunch: spring rolls lemon chicken and cashews with vegetables boiled rice lychees and ice-cream lemonade
3.00 pm		Afternoon snack: rice cakes, margarine & honey coffee/milk/sugar
7.30 pm		Dinner: roast beef gluten free gravy roast potato, pumpkin, onion, choko pavlova, cream and mango glass of white wine
9.00 pm		Evening snack: gluten free chocolate cake tea/milk/sugar

TIME	REMARKS	DIETARY INTAKE (including vitamin & mineral supplements)
		Breakfast:
		Morning snack:
		Lunch:
		Afternoon snack:
		Dinner:
		Evening snack:

Appendix 9:- Information Sheet

The *Symptom Questionnaire* can be found in Appendix 15.

HOW TO FILL OUT THE QUESTIONNAIRES

Please read the instructions below so that you will be able to answer the questions on the accompanying sheets more fully.

Brand Questionnaire:

- Please give as much information about the product as you can where the question asks for a brand name.
    - Example: Some companies market products without using their name in the title so they can be difficult to trace without this information. Please let me know that Pick-Me-Up Worcestershire sauce is marketed by Cornwells (& if possible Cornwells phone number or address which should be written on the food label).

- Similarly:- please state the name of your medication, the dosage, brand name, marketing company (address/phone no.) and whether it is a tablet, capsule or liquid. e.g. Vitamin C, 500 mg tablets by Cenovis. (ph:9567 0371)

Symptom Report:

- The questions about symptoms are to give us an idea what symptoms you are experiencing now, not what your symptoms were like when coeliac disease was diagnosed.
- Please complete the symptom record table over-page, using the severity and frequency guide below to help you. **Please record your current symptoms only in this table.**

Severity	**Frequency**
0...none	0...never
1...mild	1...less than 1 month
2...moderate	2...monthly
3...severe	3...weekly
	4...daily

Please read the following carefully so that you will be able to classify the severity of a symptom as mild, moderate, or severe.

MILD: You are aware of the symptom, but it is easily tolerated.

MODERATE: This symptom is enough to cause interference with daily life or usual activity.

SEVERE: This is incapacitating with inability to work or to take part in your usual activities.

One Week Food Diary:

- Instructions for completing the one-week food diary are outlined on the inside cover of the booklet. If it is not clear, please call Kim Faulkner-Hogg on 9515-8244.

Appendix 10:- Supplement record Sheet

Supplement taken Name of company Contact number/address	Amount: please record the units as written on the bottle eg.					Number of tablets (specific dose of medication) taken
	ug	mcg	mg	g	other	
Example: Didrocal: Proctor and Gamble: 9685 4686						Once a day
Calcium carbonate				1.25		
Vit B6: Natures Own, 93212877			250			Twice a week

GLUTEN INTAKE RECORD

Name: _____

Date: _____

At all times we wish you to be following a no detectable gluten diet, but occasionally you may have not been able to avoid gluten.

Please write down in this diary any time over the next 2 months when you have knowingly eaten gluten-containing foods, or if you think you may have accidentally had some gluten.

DAY & DATE	Incidence where gluten was or may have been eaten
Monday: Jan 1st 1996	**New Years fruit cake, small slice**
Sunday 4.2.96	**Ate at a restaurant & felt a bit ill afterwards so am not sure if there was gluten in the meal**

Appendix 12:- Food Ingredient Check List

INGREDIENT LIST

When buying commercial foods it is expected that you purchase both foods and snacks labelled as gluten-free and those that are not. In the process of reading food labels you therefore make the decision about whether or not you will purchase the product. Please indicate how frequently you may consume some of the following ingredients as written on the product.

Ingredient as listed on the product. *example*	Do you eat this?		How frequently would you eat this?					Which product/s eaten by you may contain this ingredient?
	Yes	No	Daily	Few days a week	Weekly	Monthly	< Monthly	
thickener (1400-1450)	Y		√					yoghurt
						√		mayonnaise
Thickener (1400-1450)								
Vinegar								
Glucose syrup								
Cornflour								
Malto-dextrin								
Malt								
Malt vinegar								
Hydrolysed vegetable protein								

Ingredient as listed on the product.	Do you eat this?		How frequently would you eat this?					Which product/s eaten by you may contain this ingredient?
	Yes	No	Daily	Few days a week	Weekly	Monthly	< Monthly	
Yeast extract								
Starch								
Anti-caking agents								
Caramel colour								
Wheat starch/ cornflour								

FOOD & DRINK INTAKE DIARY

Name: _____

Date: _____

Please record in this booklet the type & amount of food and drink you consume over 4 consecutive days, which include one weekend day.

When completed: Please mail the booklet back in the reply paid envelope provided

Allergy Unit, Suite 210, 100 Carillon Ave, Newtown, 2042

If there are any problems please contact Kim Faulkner-Hogg on
Ph: (02) 9515 8244 OR fax: (02) 9519 8420

This diary has been compiled with the help of the Human Nutrition Unit at the University of Sydney

INSTRUCTIONS

1. Please record your food and drink as you consume it so that nothing is forgotten.
2. Please record food intake for 4 consecutive days. Either Sunday, Monday, Tuesday and Wednesday…. OR ……Wednesday, Thursday, Friday and Saturday.
3. Record the type of food & drink with as much description as possible. Brand names can be useful. For example:
   - Bread gluten free - white - Basco gluten free / wheat free (GF/WF): (not just bread)
   - Margarine - polyunsaturated - Meadow Lea: (not just margarine)
   - Milk - reduced fat - Lite White: (not just milk)
   - Coffee - instant - decaffeinated - Moccona: (not just coffee)
   - Salad - lettuce, tomato, cucumber, beetroot etc: (not just salad)
4. Don't forget to include accompaniments to ordinary foods and drinks. For example:
   - Spreads: Toast with margarine, honey, jam , cheese etc: (not just toast)
   - Drinks: Coffee / Tea with milk, sugar, lemon juice, honey: (not just tea or coffee)
   - Sauces & Dressings: salad with French dressing: (not just salad)
     roast meat with home made GF gravy: (not just meat)
5. Include cooking method. For example.
   - boiled vegetables, roast chicken (ate leg + breast), fried steak - which oil did you use?
6. Measure the amount of food prior to eating it. Use weighing scales (in grams) to calculate the exact weight, or use standard household measures such as cups or spoons. For example:
   - Rice - white - long grain - Calrose - 1 cup
   - Margarine - polyunsaturated - Meadow Lea - 1 tablespoon

   Serving sizes are often listed on packets with the exact weight. For example
   - Kettle chips, plain - 1 medium packet = 75 grams
7. If it is not possible to weigh or measure the amount of food eaten; estimate by describing the dimensions or drawing the food to scale on paper. For example.
   - fillet of bream grilled = 15 x 5 x 0.5 cm (see drawings attached)
8. Remember to record the weight of any leftovers as well as the original weight. For example, core, seed and skin of fruit, chicken bones, fat on meat or any uneaten portion of food etc.
   - Whole pear = 200 grams (g)
   - Pear core and skin = 20 g
   - Therefore the amount of pear eaten = 180 g
9. Include recipes if meals are cooked at home. See back of the diary for recipe record sheets and example. Record in the amount eaten section of the diary how much of the whole recipe you actually ate. For example. The recipe makes 10 scones. But you ate one scone and this scone weighed 20g.
10. If eating at takeaway restaurants, include the name of the restaurant (eg.McDonalds) and the food you ate, trying to include ingredients and estimates of the amount eaten. For example.
    - Cheese burger - GF bread roll (Pavs Allergy Bakery, 25g), margarine (2tsp), meat patty (7cm diameter, 1/2 cm thick), mayonnaise, mustard & tomato sauce (1 tsp each) & large fries (1 &1/2 cups) with extra salt sachet (5g) & large coke (500ml)
11. If eating at a local restaurant, include a detailed description of each of the ingredients (where possible) in the food and drinks you ate, and an estimate of the quantity eaten, in household cup, spoon and ml measures. Draw sizes of odd shaped meat or dessert slices to approximate scale or draw a picture and add the measurements to it. See examples included in the attached sheets.

TIME & REMARKS	Day: Sunday Date: 18.2.96 FOOD & DRINK (plus vitamin/ mineral supplements)	ingredients	brand	cooking method	cups/ spoons/ cms/ mls	amount served (g)	amount left (g)
7 am not a typical breakfast	Breakfast: *instant coffee* *milk* *sugar* *3 gluten free sausages : thick pork* *fried in a tbls of oil* *2 fried eggs* *2 pieces of gluten free toast* *margarine on both slices: monounsaturated*		*Moccona* *lite white* *white, table* *local butcher* *olive oil* *Basco GF/WF* *Farmland*	 *fried* *fried* *toasted*	*1 tsp in 300ml water* *40 ml* *1 tsp* *1 tbls* 	 *3 x 55g* *2 x 40 g* *2 x 15g* *2 x 1tsp*	 *30g*
10.30 am	Morning Tea: *1 scone: see attached recipe* *margarine: as above* *honey* *orange juice*		 *Orchy*	 	 *1 1/2 tsp* *2 tsp* *170 ml*	*20g*	 *30 ml*
12.30 pm	Lunch: *left over fried rice* *instant coffee/ milk/ sugar as above* *peach*	*white, long grain* *soy sauce* *egg* *peas-frozen* *prawns* *oil: peanut* *fresh- no skin*	*Calrose* *Fountain*	*boiled & fried* *fried* *boiled & fried* *fresh, cooked* *to fry*	*2 ¼ cups* *1 1/2 cups* *1 tbls* *1/4 cup* *2 tbls*	 *20g* *30g* *75 g*	 *10g skin, 5g seed*
3 pm	Afternoon Tea: *2 rice cakes* *cream cheese* *black tea, no sugar*	 *tea bag*	 *Pure Harvest* *Philadelphia* *Bushells*	 	 *2 x 1 dsp* *200ml water*	*2 x 10g*	
7 pm Restaurant	Dinner: *steak, rare, with fat on the edge* *potato* *butter* *baked pumpkin* *beans* *pavlova: see drawing* *strawberries* *cream* *red wine*	 *whipped*	*rump* *white skin* *pavlova magic* *fresh*	*grilled* *in jacket* *baked* *boiled*	*13cm x5 x1 (with fat)* *2 tsp* *2x 4x2x2 pces* *1/4 cup* *10 x 12 x 5 cm* *1/2 cup* *1/3 cup* *170 ml*	*90g*	*fat:13x1/2 x1cm* *30 g* *2 x 2 x 5 cm* *1 tbls*
9.30 pm	Evening Snack: *chocolate, plain milk* *coffee* *milk* *sugar*		*Cadbury's* *full cream* *raw*	 *brewed*	*4 squares* *200ml* *30ml* *1 tsp*		

TIME & REMARKS	Day: Date: FOOD & DRINK (+ vitamin/ mineral supplements)	ingredients	brand	cooking method	cups/ spoons/ cms/ mls	amount served (g)	amount left (g)
	Breakfast:						
	Morning Snack:						
	Lunch:						

TIME & REMARKS	Day: Date: FOOD & DRINK (+ vitamin/ mineral supplements)	ingredients	brand	cooking method	cups/ spoons/ cms/ mls	amount served (g)	amount left (g)
	Afternoon Snack:						
	Dinner:						
	Evening Snack:						

RECIPES & DRAWINGS

Name: _____

Date: _____

If you require any help with the diary, please contact Kim Faulkner-Hogg on

PH: 02 95158244.

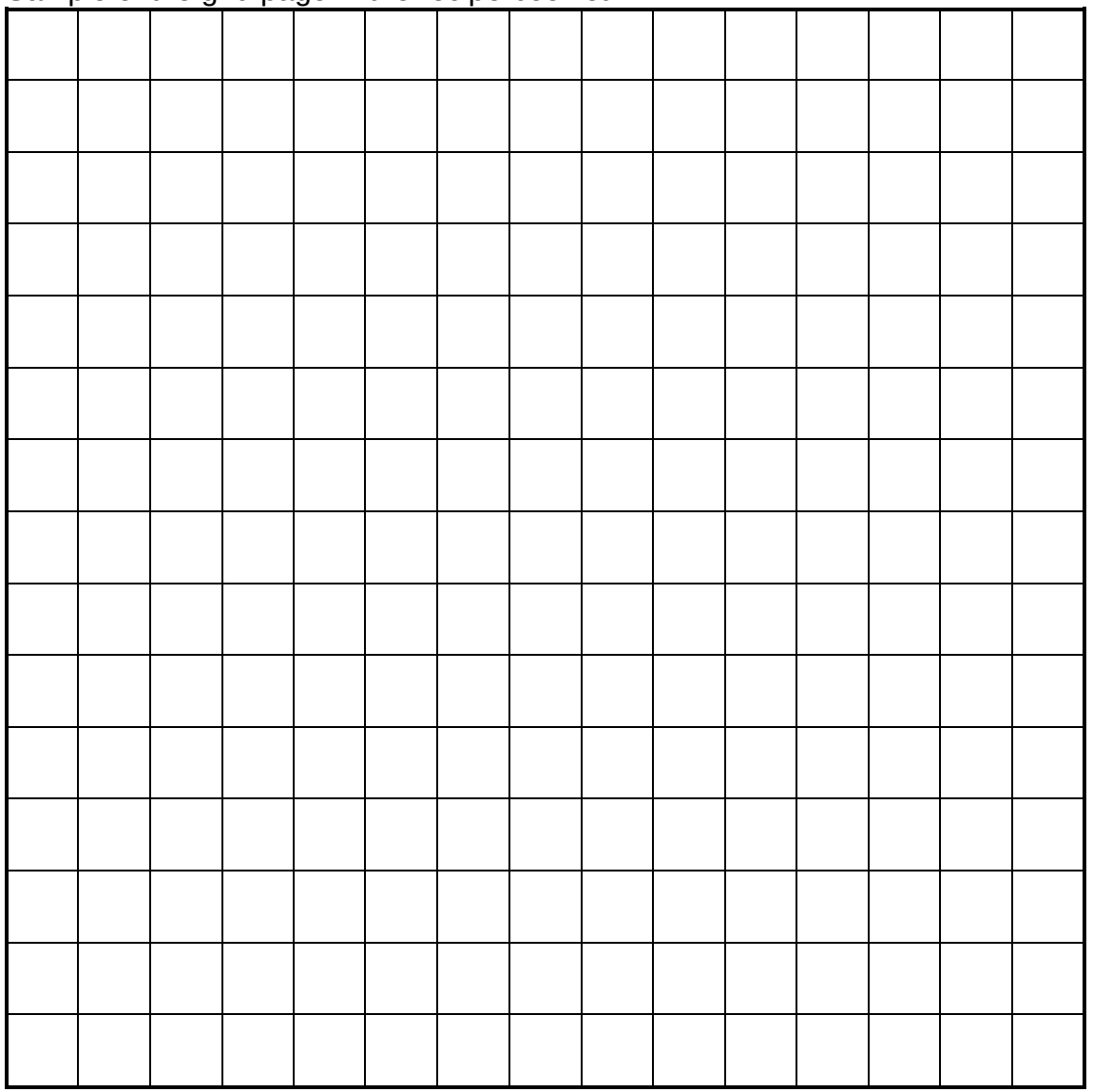
Fax: 02 95501029

RECIPE and INGREDIENTS	brand	cups/ spoons/ cm/ mls/ g	cooking method
Scone: recipe makes 10			*oven*
potato flour		*3/4 cup*	
rice flour		*1 cup*	
baking powder	*Wards*	*2 tsps*	
margarine: monounsaturated	*Farmland*	*4 tbls*	
milk	*lite white*	*1/4 cup*	
1 scone weighs 50 grams			
Tuesdays lunch salad			
Lettuce	*cos*	*2 leaves (10g)*	
Bacon bits		*20g*	*Commercial fried*
Avocado		*40g*	
Mango		*60g*	
Pine nuts		*1 tblsp*	
Tomato	*Cherry*	*15g*	
Salad dressing: home made			
Vinegar	*Red wine*	*2 tblsp*	
Oil	*olive*	*1 tblsp*	
Cracked pepper		*¼ tsp*	
Garlic		*2g*	
½ cup weighs 70 grams			
Thursdays fired rice			
White rice	*Basmati*	*4 cups*	*Boiled*
Shrimps		*150 grams*	*Cooked*
Soy sauce	*Fountain*	*2 tblsp*	
Egg		*2x 60 grams*	*Fried*
Oil	*Sesame*	*2 tblps*	
Peas		*¾ cup*	*Frozen/fried*
All stir fried together			
1 cup weighs 180 grams			

Sample of the blank RECIPE pages left for the subjects to complete.

NGREDIENTS	brand	cups/ spoons/ cm/ mls/ g	cooking method

Sample of the grid page in the recipe booklet



Appendix 15:- Symptom Questionnaire

SYMPTOM REPORT

Name:……..

Current date:……..

Date of diagnosis of coeliac disease:…….

Symptom	which symptoms do you **currently** have?		
	no	yes	
		Severity	Frequency
diarrhoea			
constipation			
nausea			
vomiting			
bloating			
stomach pain			
excessive wind or flatulence			
mouth ulcers			
headaches			
fatigue			
Dermatitis Herpetiformis			
other			

HOW TO FILL OUT THE QUESTIONNAIRES

Please read the instructions below so that you will be able to answer the questions on the accompanying sheets more fully.

Brand Questionnaire:

- Please give as much information about the product as you can where the question asks for a brand name.
    - Example: Some companies market products without using their name in the title so they can be difficult to trace without this information. Please let me know that Pick-Me-Up Worcestershire sauce is marketed by Cornwells (& if possible Cornwells phone number or address which should be written on the food label).

- Similarly:- please state the name of your medication, the dosage, brand name, marketing company (address/phone no.) and whether it is a tablet, capsule or liquid. e.g. Vitamin C, 500 mg tablets by Cenovis. (ph:9567 0371)

Symptom Report:

- The questions about symptoms are to give us an idea what symptoms you are experiencing now, not what your symptoms were like when coeliac disease was diagnosed.
- Please complete the symptom record table over-page, using the severity and frequency guide below to help you. **Please record your current symptoms only in this table.**

Severity	**Frequency**
0...none	0...never
1...mild	1...less than 1 month
2...moderate	2...monthly
3...severe	3...weekly
	4...daily

Please read the following carefully so that you will be able to classify the severity of a symptom as mild, moderate, or severe.

MILD: You are aware of the symptom, but it is easily tolerated.

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One Week Food Diary:

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