The Role of Cow's Milk Protein In Children with Chronic Functional Constipation

Elesa Terese Crowley
BHSc (N&D)

A thesis submitted for the degree of
Masters of Medical Science (Nutrition and Dietetics)

Submitted for examination December 2008

STATEMENT OF ORIGINALITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to provisions of the Copyright Act 1968.

_____

Elesa Crowley

Acknowledgements

I would like to express my sincere thanks to:

My supervisors:

Dr Lauren Williams for her research expertise, patience, guidance and support. I cannot thank her enough.

Professor Peter Jones for his advice and support particularly in the field of paediatrics.

Associate Professor Tim Roberts for his guidance with the big picture of what my research is setting out to do.

Associate Professor Hugh Dunstan for his advice in working out the finer details of the experiments, data collection and laboratory tasks.

My research assistants:

Tony Rothkirch for his sharing his expertise, direction and patience in the lab.

Annette Murphy, Anne Segboer and Loretta Wetherall for assistance with participant recruitment and data entry.

My colleagues:

Dr Libby Ellis for her advice in the development of the research protocol, encouragement and positiveness throughout this research.

Michelle Paul, Paediatric Continence Nurse, Hunter New England Area Health Service, for her assistance with participant recruitment and milk provision to participants.

Dr Trish Davidson and Dr Rajendra Kumar, for assistance with participant recruitment.

Narelle Eddington, Lynn Clarke and Beth Burton from Hunter Area Pathology Services and Marion Mackintosh from New England Pathology for their assistance with the collection, analysis and the transportation of samples.

The staff of the Children's Ward and the Paediatric Clinic at Tamworth Rural Referral Hospital for their assistance with participant recruitment.

The staff of the University Department of Rural Health, Northern NSW, for their support and encouragement that I would see this project completed. In particular, Colette McLaren for her invaluable assistance with Information Technology throughout this research project.

The staff of the Dietetics Department from Tamworth Rural Referral Hospital, particularly Deanne Harris, for approving relief from my clinical duties, for their friendship, encouragement and support throughout this project.

Kim Colyvas and Dr Karin Fisher for assistance with statistical analysis.

My support team:

Friends and family (Maria, Peter, Linda, Shannon, Bev, Tony, Kamilla, Dave, Deanne, Leanne, Jo, Lisa, Paula) for their patience, encouragement and support and assistance with babysitting so that I could complete this thesis. I am also especially grateful to: Dr Kym Rae and Deanne Harris for their encouragement, advice and proofreading of my thesis.

Malcolm, Sophia and Harrison Crowley for their love and support throughout this research and thesis production. I could not have done it without your patience.

Financial and skill development for this project was received from: The University Department of Rural Health, Northern NSW; Primary Health Care Research and Development Program; Sanitarium for the donation of soy milk for Trial 1; and Fairbrae Milk for donation of the A2 milk.

Finally, a special thanks to the children and their families who participated in this research study.

Data collection task assignment

Data collection task	Completed by
Introduction of the study to potential participants	Paediatrician or paediatric continence nurse
Collection of medical history	Paediatrician or paediatric continence nurse
Collection of nutritional assessment data	Dietitian researcher (EC) and research assistant dietitian (3 participants only)
Laboratory analysis of blood samples	HAPS and New England pathology
Laboratory analysis of faecal samples	HAPS and New England Pathology
Entry of data into computer data base	Research assistant
Analysis of nutritional assessments, constipation diaries, blood test result, faecal test reports	Dietitian researcher (EC)

Table of contents

Index of Figures

Index of Tables

List of Appendices

Glossary of abbreviations used in this thesis

AIS	Australian Illawara Shorthorn Cattle
ALT	Alkaline phosphatise
ANOVA	Analysis of Variance
AntiDNase B	Antideoxyribosenuclease B titre
AST	Aspartate Amino Transferase
ASO	Antitreptolysin O
ASOT	Antitreptolysin O titre
β-casein	β-casein
BCM7	Beta-casomorphin-7
CMP	Cow's Milk Protein
CFC	Chronic Functional Constipation
CMPA	Cow's Milk Protein Allergy
CMPI	Cow's Milk Protein Intolerance
Dx	Diagnosis
(EC)	Elesa Crowley
ELISA	Enzyme linked immunosorbent assay
ESR	Estimated Sedimentation Rate
FBC	Full Blood Count
GIT	Gastrointestinal Tract
GGT	Serum Gamma Glutamyl Transpeptidase
hr	hour
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
invent	invention
intol	intolerance

LFT	Liver Function Test
longtitud	Longtitudinal
M	Male
NHMRC	National Health and Medical Research Council
Ob	observation
%	percentage
PCR	Protein C reactive test
+ve	positive
RAST	radioallergosorbent tests
RCC	red cell count
RCT	Randomised Controlled Trial
SIDS	Sudden Infant Death Syndrome
Th1	T helper 1 cells
Th2	T helper 2 cells
TNF-α	Tumor Necrosis Factor [alpha]
WBC	White Blood Cell
wk	week
WCC	white cell count

Synopsis

The idea for this research came from a problem identified in my practices as a clinical dietitian. My interest in cow's milk β casein A2 was sparked after discussion with Professor Tim Roberts, from The University of Newcastle, who described previously cow's milk allergic people who are able to tolerate the consumption of cow's milk β casein A2, without symptoms reoccurring.

The goal of this thesis is to report on research that explored the role of cow's milk protein in children with chronic functional constipation. The research consisted of a systematic review of the literature, two clinical crossover trials, and a qualitative exploration of the lived experience of following a milk-free diet.

Chapter 1 provides the introduction to both allergy and constipation, and the relationship between the two. Causes of constipation can be organic or functional (1). Organic causes of constipation occur in relation to a primary disease classification such as endocrine or metabolic disorders, neurologic disorders, anatomic malformation, collagen vascular disease and some drugs (for example, opiates). Chronic functional constipation is defined as having one bowel motion every three to 15 days (2) and is characterised by painful bowel movements or strain in defecation, hard stools with increased diameter or pellets, and occurs with or without soiling (3). This functional constipation is defined as chronic when it persists for greater than two weeks (4).

Chapter 2 details the methods used in searching the literature for evidence for a role of cow's milk consumption in chronic functional constipation in children from 1980 to 2006. This was published as a systematic review. The literature surrounding cow's milk and constipation was found to be limited. None of the studies previously conducted were population-based or structured to provide evidence-based evaluation or treatment guidelines at either the general practitioner or paediatric specialist level. The strongest evidence found was a double blind

randomised control trial conducted by Iacono and colleagues (3). The research study by Iacono and colleagues (3) provides evidence of an association between cow's milk and constipation. The following research questions were developed from the systematic review:

1. Can the results of the Iacono and colleagues study of children with chronic functional constipation that respond to the replacement of cow's milk protein with soy be replicated in the Australian setting?

2. Does cow's milk β casein A1 cause constipation in children with chronic functional constipation?

3. What are the immunological and biochemical mechanisms underlying chronic functional constipation that respond to the removal of cow's milk protein in children?

4. What factors affect the feasibility of parents administering a cow's milk protein free diet to their children?

The four questions were addressed by two different dietary crossover trials and a qualitative study.

Chapter 3 describes the participants recruited and the methods used for the crossover trials investigating milk protein and paediatric chronic functional constipation including details of the primary outcome measure (number of bowel motions during a two-week trial period) and secondary outcome measures (biochemical, immunological and faecal analysis).

Chapter 4 describes the results of Trial 1, which replicated the Iacono and colleagues study in the Australian setting, investigating the effects of soy and cow's milk β-casein A1 in children with chronic functional constipation. One hundred percent of participants experienced resolution of their constipation during the soy

milk condition compared with 68% experiencing resolution during the soy milk condition in the Iacono and colleagues study (n=65). Thirteen participants were recruited to Trial 1. Nine participants returned constipation diaries for the study period. The mean (SD) number of stools for each of the conditions was: baseline, 5.1 (1.4); cow's milk 9.9 (4.4); washout 13.0 (5.2); and soy milk 15.1 (5.0). The differences between the three dietary conditions were statistically significant, p=0.03. The results confirmed the hypothesis that children in the Australian setting with chronic functional constipation unresponsive to the usual treatments, respond to the removal of cow's milk protein from the diet.

Chapter 5 describes the results of Trial 2, the double blind crossover trial comparing the effects of cow's milk β-casein A1 and cow's milk β-casein A2 in children with chronic functional constipation. Thirty-nine participants were recruited to Trial 2 and 26 participants returned constipation diaries for the trial period. Unlike the soy result, the cow's milk β casein A2 did not give 100% resolution of constipation, in fact, the percentage resolution was almost identical to the cow's milk β casein A1 result. The fact that some children responded during the cow's milk casein A1 condition in both trials could be caused by a threshold effect, given it was likely that participants were consuming less cow's milk protein during the trial (400 mL with elimination of all other sources of cow's milk protein) than on their pre-trial diet. Resolution with both the cow's milk β casein A1 and cow's milk β casein A2 conditions suggests that these children are able to tolerate some cow's milk protein before the symptom of constipation occurs. This could be a food intolerance type reaction or there is some other component in cow's milk that is causing the problem in these children.

Chapter 6 describes a qualitative study of the feasibility for mothers to administer a cow's milk protein free diet to their children. The experiences of mothers following a cow's milk protein free diet to assist in the management of chronic functional constipation in children were reported. A number of themes were identified that are

useful to health professionals educating families. Mothers found the removal of cow's milk protein from the diets of their children challenging but persevered due to the potential benefit to their children. Many mothers planned to continue post study with a modified approach to the cow's milk protein free diet by allowing some cow's milk protein in the diet to make the diet more acceptable to the family but not as much as the pre-trial diet. These experiences provide health professionals with valuable insights and ideas to assist their patients to manage a cow's milk protein free diet.

Chapter 7 discusses all aspects of the research including any limitations. The results of Trial 1 confirmed the hypothesis that children in the Australian settling with chronic functional constipation unresponsive to the usual treatments respond to the removal of cow's milk protein from the diet. Therefore, cow's milk protein is involved in the aetiology of constipation in these children. All the study participants demonstrated an absence or low level of normal gut flora, which may affect bowel regularity. Further research into species present and absent may provide further explanations to the lack of bowel regularity in these children.

The immunological and biochemical mechanisms underlying chronic functional constipation that respond to the removal of cow's milk protein requires further investigation. Although the number of statistically significant variables between the conditions was low, there was a high degree of abnormality. Further investigations are needed, including research into food intolerance reactions that affect the nerve endings in the bowel. The results in Trial 1 and Trial 2 are suggestive of an involvement of blood factors including platelets and monocytes. Other children may have a chronic *Streptococcus A* infection which may be contributing to constipation as well as to liver function abnormalities. Liver function abnormalities were observed for some participants in both trials, independent of milk condition.

The extent to which the research questions have been answered is evaluated in Chapter 7, which includes the conclusions and recommendations of this research. In brief, the findings were:

- Children with chronic functional constipation that is unresponsive to the traditional treatments should trial a cow's milk protein free diet for at least two weeks to determine whether this may resolve the constipation. During this period, the numbers and form of bowel motions should be recorded and results compared to a one week record collected prior to commencing the cow's milk protein free diet.
- Due to the complicated nature of a cow's milk protein free diet, especially the number of processed foods which contain hidden cow's milk protein, consultation with a dietitian is essential for implementation of this diet. The dietitian should consider educating the patient's family, both parents and siblings, to ensure the best outcome in terms of acceptance and compliance of the diet, and provide adequate resources.
- If this dietary modification is successful for the child and alleviates constipation, consultation with a dietitian is recommended to determine the amount tolerated and nutritional adequacy of the diet. Soy milk is recommended as a substitute for cow's milk and a probiotic needs to be prescribed to assist with the normalisation of gut flora.
- Education of health professionals such as general practitioners, paediatricians, and paediatric continence nurses, regarding a cow's milk protein free diet for chronic functional constipation, is essential to support the child and his/her family and integral to the success of this strategy. The findings of this research will be published in the scientific literature and as conference presentations.

  It is hoped that these findings will assist in the management of children with chronic functional constipation unresponsive to the traditional treatments.

Chapter 1: Cow's milk protein allergy and chronic functional constipation

1.0 Introduction

There is an increasing prevalence of food allergy and food intolerances among children. These increases might be related to altered patterns of exposure in early life, where infants were exposed to a much wider range of allergens in infancy (5). Food allergy or food hypersensitivity is defined as, "an IgE-mediated reaction that occurs when the immune system reacts to a normally harmless food protein that the body has erroneously defined identified as harmful" (6) p. 769. These IgE mediated reactions are reproducible through a 'cause and effect' relationship (7). Cow's milk can cause an IgE mediated food allergy response in some people or a food intolerance reaction known as cow's milk intolerance (CMI) in other susceptible individuals (8). Food intolerances are defined as, "an adverse reaction to food caused by idiosyncratic, or non-IgE mediated (IgE) reactions to food or chemical substances in the food," (6) p769. Adverse food reactions are a broad term used by Mahan and colleagues to describe any undesirable food reaction regardless of mechanism, encompassing food allergy or food sensitivity and food intolerances (6), and the term is used in the same context in this thesis. Adverse reactions to food can result in symptoms in various systems throughout the body such as the gastrointestinal tract, skin, respiratory and cardiovascular systems. Gastrointestinal tract symptoms of adverse reactions to food were the primary focus of this thesis and will be discussed in the next chapter.

Adverse reactions to foods have been recognised since the time of Hippocrates (460-370 BCE) and some time later by Galen (131-210 CE). In 1839, Magendie found that repeated injections of the egg albumen in rabbits were lethal. Despite these early investigations, the majority of food-induced diseases were not systematically investigated until the 20th century (8). In 1906, Van Pirquet of

Vienna introduced the term allergy to describe “a deviation from the normal state or normal behaviour of the individual,” (5). In the 1920’s, the antibodies for immediate type hypersensitivity reactions in humans were discovered (8). Over the next thirty years, increasing numbers of cases were described and the range of clinical abnormalities expanded to include reactions that were slower in onset and involved the gastrointestinal tract, skin or respiratory system (8). Before oral challenges and appropriate methods of investigation had been developed, diagnosis was often based on the clinical history and improvement after elimination of the suspected allergen (8). This thesis investigates cow’s milk as a potential stimulator of allergy/sensitivity and aims to investigate whether a causal relationship exists between the dietary intake of cow’s milk protein (CMP) and chronic functional constipation (CFC) in children.

1.1 Adverse reactions to foods

Distinct immunological differences can be observed between food allergies and food intolerances. Food allergies occur when the immune system reacts to an allergen, usually a protein, present in food (9). Food allergies can be classified as either humoral (IgE mediated) or cell mediated response (8) (See Table 1.1). An IgE-mediated or humoral immune response is the most common allergic response usually occurring instantly or within two hours of exposure and is reproducible through a cause and effect relationship (7). Severity of the reaction can vary from mild to life threatening.

Table 1.1: Humoral and Cell Mediated Immunity Components

Type of Immunity	Origin	Function
Humoral Immunity		
B lymphocytes	Bone marrow	Produce: IgA, IgD, IgE, IgG, IgM. These antibodies protect against bacteria and viruses.
Cell Mediated Immunity		
T Lymphocyte Cells	Thymus	Recognise antigens, stimulate T cell growth and produce lymphokines, cytokines.
		Help regulate B cells.
		Cause direct cellular damage to target cells.
Th1 and Th2	Thymus	Resistance of viruses, fungi, tumour cells and other foreign cells.
		Th1-like cells linked to lymphokine profiles stimulate cell mediated immunity and suppress IgE antibody formation.
		Th2 is linked with IgE formation, eosinophils and macrophages resulting in atopic disease.
Macrophages	Monocytes in the blood	Involved in the recognition, clearance and presentation of antigens.
		Engulf and destroy antigens through the process of phagocytosis.

Adapted from: Food Allergy and Intolerance, (8); Allergy and Immunology Secrets (9).

In the cell mediated response, B lymphocytes, known as B cells, produce antigen-specific antibodies in response to the antigen presented, such as viruses and mycobacteria and foreign cells (8). After effective elimination of antigens, the cell mediated response remains primed and able to respond promptly to reoccurrence of the antigen (8). The humoral and cell mediated responses do not occur in isolation and are mutually dependent (8).

Food intolerances are usually triggered by other components in food, usually non-proteins such as food chemicals, and do not involve the immune reactions described for food allergies (8). Food intolerances cause reactions by irritating nerve endings in some parts of the body (10). Examples include cow's milk intolerance, lactose intolerance and idiosyncratic reactions such as sulphite induced asthma (11).

1.2 Increasing prevalence of food allergy: the hygiene hypothesis

In this investigation of CMP as a potential cause of CFC, the increasing prevalence of food sensitivity amongst children needs to be examined. It has been hypothesised that improved hygiene has led to an increase in allergic diseases such as asthma, a proposal known as the 'hygiene hypothesis' (12). This hypothesis was originally proposed by Strachan in 1989. He suggests that cleaner environmental conditions result in the increased prevalence of allergy including asthma in Western countries in comparison to developing countries (13). In Western society it is common practice to 'protect' children from bacteria and microorganisms through indoor isolation and the overuse of antibacterial soaps. A lack of early immune challenge for the post-natal immune system by microbial or parasitic infection may increase the risk for allergy and atopy (14). Determinants such as early exposure to cats (15), growing up in a rural environment (16), larger family size (17) day care attendance (18) and birth order (19); were associated with low levels of allergies and asthma.

The hygiene hypothesis is not without criticism. Changes in prevalence of atopic disorders may have more complex aetiologies than the simplistic theory based on hygiene practices (20-22). Bloomfield and colleagues (21) found that the increase in allergic disorders does not correlate with the decrease in infection with pathogenic organisms nor can it be explained by improved hygiene practices (21). There is evidence that lifestyle changes have led to decreased exposure to microbial or other species that are essential for the development of immunoregulatory mechanisms (21).

1.3 Food allergy and the immune system

The immune system exists to clear the body of foreign substances or potential antigens such as viruses, bacteria, blood cells and tissue cells. During the process of absorbing food, the intestine comes in contact with a wide variety of antigens derived from food, usually food proteins, resident bacteria and invading microorganisms. The intestine usually acts as an effective barrier preventing antigen-entry into the body. However, mechanisms exist that allow antigens to penetrate the mucosal immune system and enter the circulation. Under normal circumstances, these antigens interact with cells of the immune system and are cleared from the body without any adverse reaction (23).

When the immune system reacts to an antigen, this is referred to as an allergic response. This allergic response may be caused by a deficiency of secretory IgA, infection, enzyme deficiency, increased gut permeability or altered gut ecology (9). Immune reactions are classified into four types: types I, II, III (antibody y dependant) and IV (T cell dependant) (23) (See Table 1.2). The most common type of reaction is Type I, immediate sensitivity, which involves IgE. Type II food reactions have not been demonstrated. Type III or Non-IgE-mediated immunologic reactions to food involve circulating food-specific antibodies, IgA, IgG and IgM, and commonly occur (6, 8, 9). Type IV delayed or cell mediated immunity recognises antigens, which then stimulate T cell growth. Type IV reactions are possibly involved in coeliac disease, protein losing enteropathies, eosinophilic gastroenteritis and inflammatory bowel disorders such as ulcerative colitis (6, 23).

1.4 Symptoms

Food allergy can cause a wide range of symptoms in the skin, respiratory system, cardiovascular and gastrointestinal tract (GIT), with skin and GIT the most commonly affected systems (24). Symptoms vary according to the type of immune

Table 1.2: Classification of allergic reactions

Reaction/ Classification	Mechanism	Symptoms	Time	Examples
Type I* Immediate hypersensitivity, anaphylactic IgE mediated, or reaginic reaction	The allergen binds with sensitised IgE antibodies on mast cells (specialized granular cells in the intestines, skin and respiratory tract) or basophils (similar cells in blood). Mediators are released (histamine, eosinophilic chemotactic factor, bradykinin etc.). IgG can also have type of reaction.	Hayfever, anaphylaxis, atopic dermatitis, asthma.	Within seconds or up to 2 h.	Laryngeal oedema, nausea, vomiting, severe abdominal pain, bloating, diarrhoea, angiodema, eczema, erythema, itching, hoarseness, wheezing, cough, chest tightness, hypotension, bronchospasm and shock.
Type II Cytotoxic	IgG antibody reacts with the cell membrane or an antigen associated with cell membrane.			Results from transfusion of incompatible blood types. No food reactions have been demonstrated.
Type III* Antigen-antibody complex	Antigen and Antibodies (IgG and IgM) form a complex called a "precipitating antibody." Complement is also activated in some cases.		6 h or more to appear.	Milk precipitants have been found in the lungs of some children with chronic respiratory infection and GIT of those with gastroenteropathy.
Type IV* Delayed or cell mediated hypersensitivity	T cells interact directly with antigen.			Mechanism of graft rejection. Possibly involved in some food allergies eg coeliac disease and protein losing enteropathies.

Adapted from: Food Allergy and Intolerance (8).

* = Food related reactions

1.5 Foods as Antigens

Any foods that contain residues of protein can trigger an allergic sensitisation (as shown in Figure 1.1). Although over 160 foods have been identified as causing IgE mediated food allergies (26), the majority are caused by cow's milk, wheat, eggs, soyabeans, peanuts, tree nuts (almond, hazel, walnut) fish and shellfish (27). The

Cow's milk allergy has been referred to as the 'most studied and commonest food allergy,' (8) however, the exact immunopathogenesis remains unclear (8, 23). There is some evidence that all four types of allergic reactions can occur in reaction to cow's milk protein. Type I reactions often occur when there is a family history or personal history of atopy (29). Elevated IgE specific for cow's milk protein and positive skin testing occur in these susceptible people (29). Type II reactions are rare but may be responsible for the occasional thrombocytopenia seen in cow's milk intolerance (30). Type III reactions, that is, immune complexes of IgA, IgG and IgM with cow's milk proteins, such as β lactalbumin, have been shown to be present in allergic patients after ingesting milk (10). Type IV reactions have been demonstrated *in vitro* with milk-induced lymphoblast transformation (31) and reduced neutrophil chemotaxis (32).

1.6 Composition of breast milk and breast milk substitutes

Mammals provide milk by lactation for their infants. Milk is composed of carbohydrates, proteins, fat, water, water soluble vitamins (the B complex vitamins), fat soluble vitamins (A, D, E and K) calcium and phosphorus and other minerals (33). The only digestible carbohydrate in milk, lactose, produces intolerance symptoms in people with a lactase deficiency. Lactase, an intestinal brush border enzyme is required to cleave lactose to its constituents, galactose and glucose. The production of lactase declines in all mammals after weaning (23). A comparison between cow's milk and human milk is shown in Table 1.3.

The protein component varies due to the different growth rates and body composition of humans and cows (see Table 1.3). When it is not feasible to provide maternal milk to human babies, cow's milk is usually used as a substitute. Cow's

e proteins in breast and cow's milk are not distinguished by different names (5).

Table 1.3: The composition of mature milk (g/100ml)

	Cow's Milk	Human Milk
Energy (kcal)	62.4	75.0
Total protein	3.5	1.1
Casein	2.8	0.4
Proteins in lactoserum (whey protein)	0.6-0.8	0.7
β-Lactoglobulin	0.37	-
α- Lactoglobulin	0.18	0.35
Immunoglobulins, total	0.05	0.1-0.15
Other proteins	0.13	0.1
Fat	3.4	4.5
Carbohydrate	4.7	7.0
Calcium	121.1	34.0
Phosphorus	94.8	14.0
Sodium	49.9	16.1
Potassium	153.8	57.0
Iron	Trace	0.02

Source: Adapted from Diseases of the Small Intestine in Children (5)

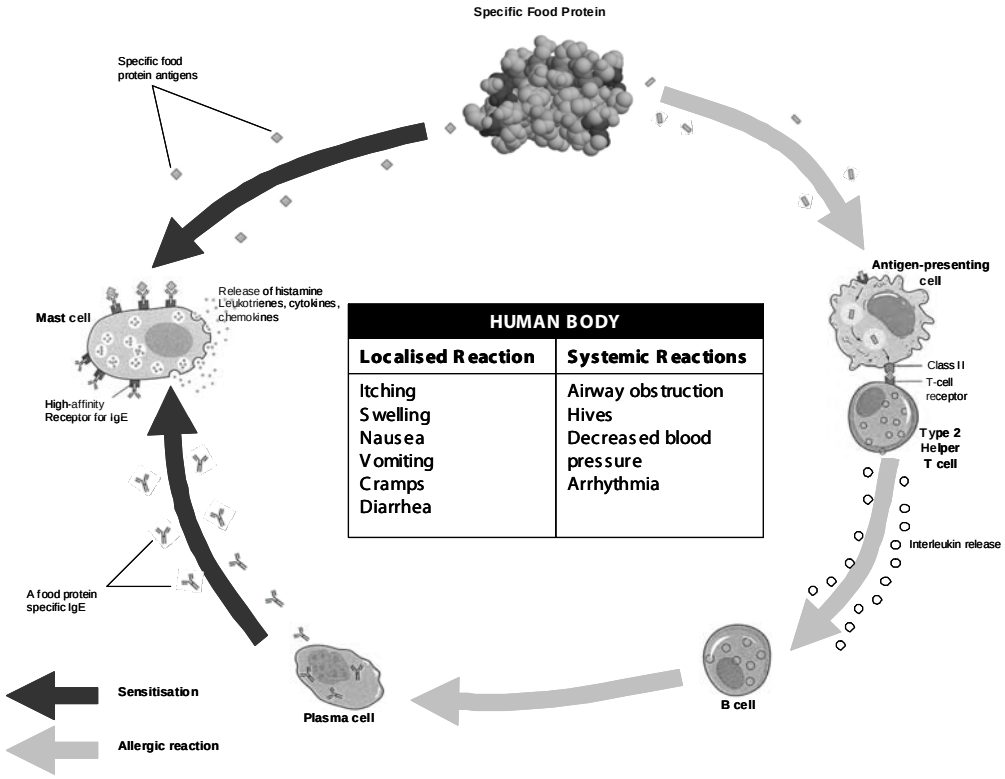
It was once thought that β-lactoglobulin, the predominant whey protein in cow's milk, was responsible for cow's milk allergy especially since it is not present in human milk (34, 35). It is now known that other proteins may act as allergens (5).

Soybean milk has also been used as a substitute for breast milk, from as early as 82BC in eastern countries (36). In the Western world, Ruhrah first described soy as a suitable substitute for cow's milk for infants in 1909 (37). However, it was not until 1929 that Hill and Stuart recommended that soybean be prepared to resemble milk and mass production of soy formula occurred (38) making it a feasible substitute for infants who could not tolerate cow's milk formulae (38). However, 30% of these children are also sensitive to soy and require a specialised hydrolysed formula (39). During the 20th Century, soy formulas progressed through a process

of improvement to ensure an adequate nutritional profile for the growth and development of infants with cow's milk protein allergy and intolerance (36).

1.7 The development of cow's milk protein allergy in children

Shannon (40) provided evidence that breast milk contains traces of the foods consumed by the mother (40). These traces are generally absorbed without harm except to the hypersensitive baby (41, 42). Symptoms of CMP allergy first develop in 10% of hypersensitive babies in the first week of life (8) and in 30% within the first month of life. This early development suggests that the baby may have been sensitised to CMP *in utero* (8, 28). Once sensitisation has occurred, the introduction of formula or cow's milk containing food may trigger an IgE mediated reaction (see Figure 1.1).



Adapted from: Krause's Food, Nutrition and Diet Therapy (6); Food Allergy and Intolerance (8).

Figure 1.1: The sensitisation process and allergic reaction

Cow's milk protein allergy has a remission rate of approximately 45 to 50% at 1 year, 60 to 75% at 2 years and 85 to 90% at three years. Up to 50% of cow's milk

protein allergic infants will develop associated adverse reactions to other foods and 50 to 80% will develop reactions against inhalants before puberty (28).

Intolerance reactions to cow's milk can also occur. While they do not result in an allergic type reaction, they do cause similar symptoms (28).

Prevalence

The prevalence of food allergy in the general Australian population has not been studied and as a result needs to be estimated from selected populations. Hill and colleagues (43) explored the development of food allergy in a cohort of 620 Australian infants at high risk for the development of atopic disease through the antenatal clinic at Mercy Maternity Hospital in Melbourne (43). They estimated the prevalence of cow's milk allergy at age two at 2.0% (43). The prevalence of cow's milk allergy in the Australian population is similar to estimated results from a prospective study in Sweden by Jacobsson, 1.9%, and in Denmark by Host, 2.2% (44) and Host, between 2 to 3% (28).

1.8 Potential causes of cow's milk allergy: A1 and A2 variants

This thesis investigated the possible allergens present in cow's milk. One hypothesis is that the β casein A1 moiety might mediate cow's milk protein allergy.

The ancestors of modern dairy cows originated in the Middle East and Asia some thousands of years ago, eventually domesticated and introduced to Europe (45). The milk producing cows used today are descendants from European breeds, where cross-breeding occurred to suit the various environments (45). The first dairy cattle were bought into Australia by the First Fleet from Britain (46). Between 5000 and 1000 years ago, when cattle were imported into Europe a mutation occurred resulting in a change in β-casein (45). Approximately 80% of total protein in cow's milk, is casein, and 30 to 35% of this is in the form of β-casein (45). β-casein consists of a chain of 209 amino acids (45). There are a number of genetically

determined variants of β-casein, the most common being A1 and A2 (45). The predominant type of β-casein varies between mammals as shown in Figure 1.2, 1.3 and Table 1.4.

Figure 1.2: An example of cows that produce commercially available milk which is predominantly A1

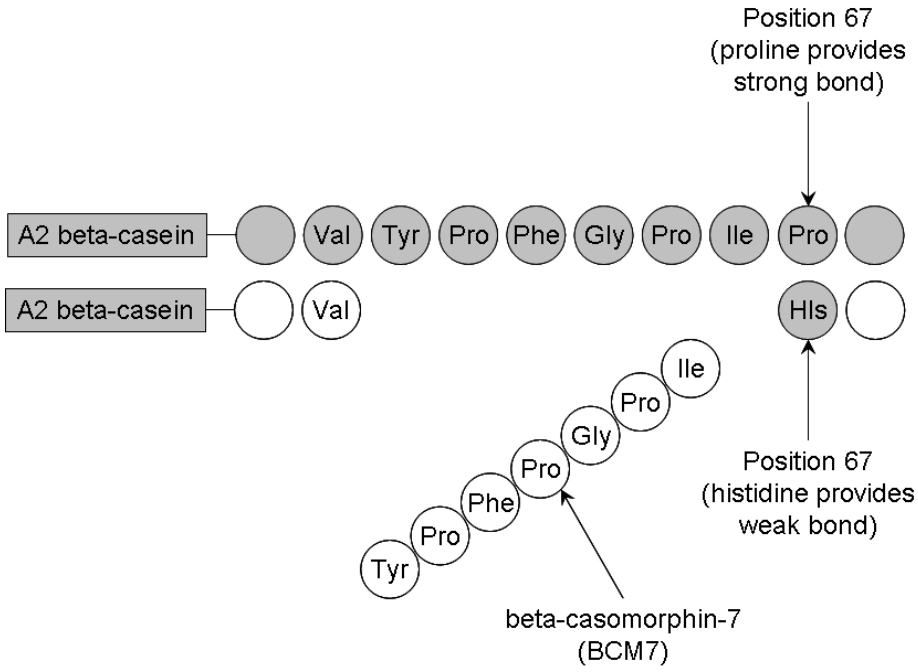
Figure 1.3: An example of the traditional milking cow which produces predominantly A2 milk

Table 1.4: Sources of β casein A1 and β casein A2 milk

Milk predominantly casein A1	Milk predominantly casein A2
Bos taurus cattle	*Bos indicus* cattle milk
AIS	Sheep's milk
Freisian	Yak's milk
Holstein	Goats milk
	African breeds eg Zebu
	Asian breeds
	Guernsey
	Jersey
	Human milk

Source: Adapted from The devil in the milk (45)

β casein A1 and β casein A2 differ in structure (45). It is thought that A2 was the original β casein present in cow's milk and that β casein A1 was formed as a result of an adenine-cystosine substitution mutation, on the sixth chromosome (45). β-casein A2 has a proline at position 67 rather than a histidine (47, 48). The bonds linking proline to adjacent amino acids are cyclic in structure making them very difficult for many human digestive enzymes to use this peptide as a substrate (45). The β casein A1 histidine allows an enzymatic cleavage to occur releasing a peptide of a string of seven amino acids called 'β casomorphin 7' (BCM7) as shown in Figure 1.4 (45, 49).



Source: Reproduced with permission from Craig Potton Publishing, from Devil in the milk (45)

Figure 1.4: Release of β-casomorphin-7 from A1 β casein

Proposed mechanism of absorption of intact peptides

Enhanced permeability of the small intestine can occur in some situations of illness or stress and promotes the absorption of intact peptides. This condition is referred to as a leaky gut or increased intestinal permeability (8). Intact peptides can cross the gastrointestinal barrier by at least three mechanisms, (1) a transcellular route involving diffusion across the brush border membrane, (2) a transcellular route involving carrier mediated transport, or (3) a paracellular route through the 'tight junctions' which are under physiological control (8). It is possible that peptides released during digestion could stimulate the opening of the paracellular pathway across the intestinal epithelium and allow exorphins to pass through the gastrointestinal barrier (8).

Peptides can be exorphins, that is, have opioid and anti-opioid activity (8). This term was first coined by Zioudrou and colleagues in 1979 when they found that digestion of wheat gluten or α-casein with pepsin *in vitro* could result in peptides with opiate-like activities (50). Intact peptides, including exorphins, can be

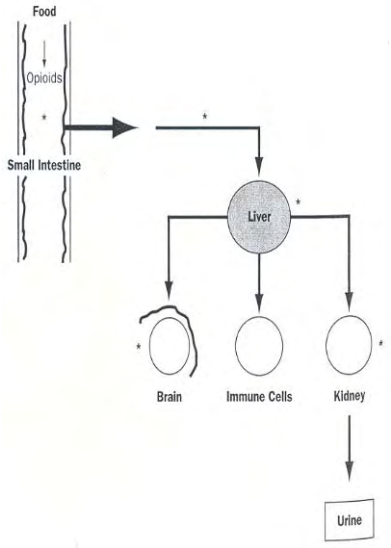


Figure 1.5: A model for the generation of opioid peptides, exorphins, from dietary proteins and their subsequent effects on peripheral tissues, especially the nervous and immune systems.
Reproduced from: Allergy and Intolerance (8) p466.

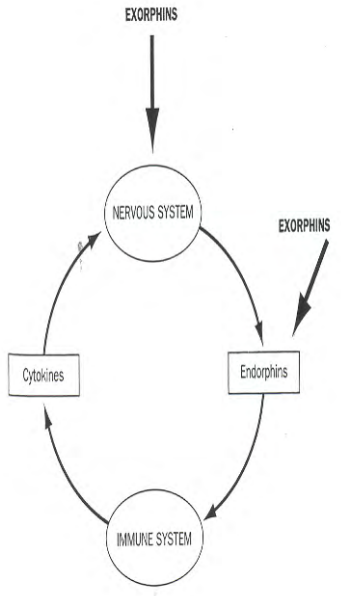


Figure 1.6: Signalling inter-relationships between the nervous and immune systems and how exorphins would be expected to interfere profoundly
Reproduced from: Allergy and Intolerance (8) p473.

In some susceptible people, the exorphins with opioid properties, such as BCM7, can 'leak' from the gastrointestinal tract into the blood stream (45). This is most likely to be by the paracellular mechanism. BCM7 can be identified in the urine of these people (45). BCM7 has also been shown to affect gut transit time without being absorbed into the bloodstream. Becker and colleagues (51) and Delfilipi and colleagues (52) have shown that opioids, including BCM7, can reduce gut transit time. Casein is a known treatment of diarrhoea (45, 51, 52). Codeine, an opioid, has the common side effect of constipation (45). BCM7 has been shown to compromise immune responses (53). These effects are potentially responsible for

symptoms such as constipation in some individuals (45). Woodford (45) suggests that the opioid BCM7 in cow's milk based formula slows down the excretion of waste products from the body. This could give the lactose more time to ferment and cause the intolerance symptoms (45). This may explain why babies fed cow's milk based formula rather than breast milk are susceptible to constipation and, in some severe cases, anal fissures (54).

1.9 Cow's Milk and Constipation

Normal Bowel habits and constipation

Regular bowel motions are considered to be a sign of good health. In the first year of life, parents are particularly observant of the frequency and characteristics of their child's defecation. Motions or habits considered to be unusual result in a visit to the doctor or child and family health nurse (4). Table 1.5 defines the normal frequency of bowel motions for children (4). During the first week of life infants have a mean of four stools per day (4), there is high variability in normal and breastfed infants may not pass stools for several days or pass 2-4 stools per day (55) decreasing to an average of 1.7 stools per day by two years and 1.2 stools per day by four years (55).

Table 1.5: Normal frequency of bowel movements

Age	Bowel movements per week[a]	Bowel movements per day[b]
0-3 months		
Breast fed	5-40	2.9
Formula fed	5-28	2.0
6-12 months	5-28	1.8
1-3 years	4-21	1.4
More than 3 years	3-14	1.0

[a] Approximately mean ± SD
[b] Mean

Source: Fontana M., Bianch C., Cataldo, F. et.al .(55).

The role of the colon is to reabsorb fluid and electrolytes and to store faecal material. Defecation of the stool is then controlled by the pelvic complex, two overlapping muscles sphincters that form a funnel like structure surrounding the

anus (56). The urge to defecate occurs when the stool reaches the external anal sphincter. The Valsalva Manoeuvre, used during defecation, increases intra-abdominal pressure, the external anal sphincter relaxes and the stool is evacuated from the rectum (56). If defecation cannot occur or is painful, the external sphincter and the gluteal muscles can tighten and push the faecal mass back into the rectal vault and the urge to defecate subsides until the rectum is distended again (56). Withholding stool repetitively leads to the stretching of the rectum and lower colon, a reduction of muscle tone and retention of stool, more water reabsorption and a larger, harder stool that can become impacted (56)

Constipation is described as a delay or difficulty in defecation that has been present for more than two weeks (2). Causes of constipation can be organic, non-organic or functional, with the most common cause of constipation after the neonatal period being functional (4). Organic causes of constipation are listed in Table 1.6. Functional constipation usually begins after the neonatal period. Non-organic causes of constipation include: coercive toilet training; school bathroom avoidance; fear of toilet; sexual abuse; and attention deficit disorder (2). Functional constipation, also known as idiopathic constipation or faecal withholding, is diagnosed on the basis of a clinical history and physical examination (2) and is characterised by painful bowel movements or strain in defecation, hard stools with increased diameter or pellets and can occur with or without soiling (3). Some constipated children develop encopresis. Encopresis is described as the involuntary passing of stool which has leaked from a rectum that has been distended by stool (56). A decreased sensitivity to distension can occur and often a child will not be aware of soiling until it is almost complete.

Chronic functional constipation (CFC) is a common problem in children in the Western world. Chronic functional constipation can be defined as having one bowel motion every three to 15 days (2) and is defined as chronic when it persists for greater than two weeks (4). It has been estimated that the frequency is as high as

36% percent of children who attend a consultation with a paediatrician (57), approximately 5% of all outpatient visits by children to general practitioners and 20% to 25% of referrals to paediatric gastroenterologists (56). Encopresis is three to six more times common in boys than in girls and usually presents between 3 and 7 years (56). A number of researchers have described children who do not respond to usual treatments for constipation, that is, medications, a high fibre diet, fluid and behavioural therapy. This suggests that the exact aetiology is unclear in some cases (3). This lack of effective and sustainable treatment poses a problem for health care practitioners including general practitioners, dietitians and early childhood nurses.

Table 1.6: Organic Causes of Constipation

Disease Classification	Disease entities
Endocrine/metabolic	Hypothyroidism
	Hypercalcaemia
	Hypokalemia
	Cystic fibrosis
	Diabetes mellitus
	Gluten enteropathy
Neurologic	Congenital aganglionic megacolon
	(Hirschsprungs disease)
	Myelomeningocele/spinal cord abnormalities
	Botulism
Anatomic malformations	Imperforate anus/anal stenosis
	Anterior ectopic anus
	Pelvic mass
Collagen vascular disease	Scleroderma
	Lupus
	Dermatomyositis
Drugs	Opiates
	Phenobarbital
	Tricyclic antidepressants
	Antacids
	Antihypertensives
	Anticholinergics
Other	Lead poisoning

Reproduced from: Osborn, L. M., DeWitt, T.G., First,L.R., Zenel,J.A. (Ed.)(56)

A medical history, physical examination, radiology and laboratory tests, allow the primary health care provider to differentiate between functional constipation and

organic causes of constipation (2, 56). An algorithm for the management of children older than one year of age has been developed by the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. This algorithm is shown in Appendix 1.

The usual treatment regime is based on the premise that a low intake of dietary fibre and fluid, lack of exercise or behavioural/psychological problems cause CFC (1) and involves disimpaction, maintenance (laxatives, dietary change and behaviour modification) and weaning medications. Dietary recommendations during the maintenance phase include the increased intake of fibre and fluids. Parents should be educated regarding high fibre food sources and naturally occurring sorbitol, found in prunes, pear and apple juices, which result in increased bowel frequency and water content of stools (58, 59). The diet should include wholegrains, fruit and vegetables (4).

The recommendations of the North American Society for Paediatric Gastroenterology, Hepatology and Nutrition (2006) are that when the abovementioned therapies fail, consideration may be given to a time-limited trial of a cow's milk free diet (4).

1.10 Mechanisms of milk allergy causing constipation

There are a number of possible mechanisms that may be causing constipation in children unresponsive to usual treatments. These include: an immune response; a motility disorder; eosinophils; and mucus as outlined below.

An Immune response

The complexity of the immunological reactions surrounding milk allergy and constipation make an explanation of dismotility symptoms, that is, slowing of the bowel movement, difficult (60). Researchers have found that as well as IgE mediated reactions or T-cell mediated reactions, non-IgE mediated or T-cell-

mediated allergic gastrointestinal disorders also exist. Perianal inflammation from milk protein allergy can sometimes initiate the painful stimuli (2). This begins a cycle of events when the child voluntarily prevents the defecation of stools as described above, resulting in a harder, larger stool, causing pain when eventually passed and thus the cycle continues (56).

Neuromuscular motility disorder

Peristalsis is the term used to describe the co-ordinated rhythmic serial contraction of smooth muscles that force food through the gastrointestinal tract (61). There is a close relationship between the nerves supplying the gut and the immune system (62). Neuromuscular secretary function could be altered during an immune response to food protein. Collins and colleagues (62) found that a physical apposition of mast cells to neurons can occur and that immune mediators, such as histamine and serotonin and some interleukins, can function as neurotransmitters (62, 63). They proposed that an immune response to a food protein source such as cow's milk can cause changes in neuromuscular function by affecting nerves in the gut, causing a delay in colonic transit time (62-64). They argued for further research on the immune mechanisms occurring with chronic functional constipation is required.

Other researchers, Shah and colleagues (65) found that anal spasm rather than colonic dismotility was the mechanism of constipation related to cow's milk protein allergy (CMPA). Iacono and colleagues (66) report histological findings (intra-epithelial lymphocytes and lamina propria infiltration by eosinophils) corresponding to rectal inflammation.

Eosinophils

Eosinophils are granulocytes derived from the bone-marrow, involved in both allergic and non-allergic inflammation. In normal conditions, eosinophils reside in

the lamina propria in the stomach and the intestine (25). There is some evidence that eosinophils, recruited by the chemokine eotaxin, are involved in dismotility in allergic diseases and other inflammatory conditions. Their actions are mediated through the release of potent granule constituents, such as histamine that result in inflammation (67). Examination of tissue using an electron microscopy has shown that eosinophils have also been found in the areas surrounding damaged axons. This suggests that eosinophils mediate a pathological response (68).

Scallion and Cadranel (25) hypothesised that similarities occur between eosinophillic oesophagitis associated with eosinophillic infiltration (25) and allergy in the lower large bowel. In motility studies on eosinophillic oesophagitis, spasms have been described. They hypothesised that contractions in the muscle fibres in the muscularis mucosae, resulting in the formation of the typical endoscopic oesophageal rings, may be caused by activation of acetylcholine by histamine (69). Statistically significant differences were found between patients with eosinophillic oesophagitis and controls for mean values of thickness of combined mucosa, submucosa and the muscularis propria (70). Similar reactions in the inflamed rectal walls could affect the biomechanics of the gut causing dismotility (25).

Mucus

The role of mucus is to protect the mucosa against mechanical and chemical antagonism (25). In research on drug induced constipation, using a rat model, the mucus production of crypt epithelial cells was reduced, as was the mucus thickness at the level of the mucosal and faecal surfaces (71). These reactions might change the viscoelasticity of the faeces, resulting in difficulty in defecation (25).

These potential mechanisms could be assessed using biochemical, immunological and microbial markers.

1.11 Biomarkers of constipation caused by cow's milk allergy or intolerance

Blood abnormalities

A number of biochemical abnormalities may occur in children with constipation related to cow's milk allergy or intolerance. Hypothesises for these abnormalities have been developed and are shown in Appendix 2. These possible abnormalities are described in Table 1.7 which has been compiled from several different sources.

Table 1.7: Tests to determine biochemical abnormalities occurring in constipation caused by cow's milk protein allergy or intolerance

Measures	Specific tests	Outcomes in CMP sensitivity	References
FBC	WBC and ESR	Inflammation can occur in constipation and can be determined by testing white cell count and erythrocyte sedimentation rate.	(3)
	Eosinophils	Elevated eosinophils are a common characteristic of cow's milk intolerance.	(3)
LFT's	alkaline phosphatase, serum glutamyl transpeptidase, aspartate amino transferase, alanine amino-transferase, bilirubin total conjugate	Allergens are cleared from the liver. Liver function tests can be used determine whether there are abnormalities in the clearance of antigens.	(8)
Immunoglobulins	IgA, IgG,IgM,IgE	IgA deficiency is associated with the development of GIT food hypersensitivity and with milk hypersensitivity. These is some evidence levels of secretary IgA are low at diagnosis of CMPA but rise when tolerance to CMPA is achieved. Deficiency of IgA enhances intestinal production and secretion of IgM.	(8) (5) (72) (57)
		IgG antibodies may be elevated in patients with food allergy affecting the GIT.	(73) (3)
		Children who are allergic to cow's milk, characteristically, have elevated IgE levels. An IgE mediated response is the most common allergic mechanism in constipation related to CMPA.	
ASOT AntiDnase B		ASOT and AntiDNase B tests confirm a clinical diagnosis of a previous group streptococcal A infection.	(74)
Thyroid Function test		Constipation is a symptom of impaired thyroid function	(4)
B12 and Folate		B12 deficiency causes megoblastic anaemia which then leads to a secondary folate deficiency. Folate is essential for the formation and maturation of red and white bloods. Children with CFC often suffer the symptoms of poor appetite and nausea which can affect their dietary intake of B12 and folate rich foods.	(6) (4)
Zinc and Copper		Zinc is essential for a healthy immune system. Copper has an antagonist relationship with zinc status. If too much copper is being absorbed this will result in poor zinc status. Children with chronic constipation often suffer the symptoms of poor appetite and nausea which can affect their dietary intake of zinc rich foods.	(4, 75)

Faecal abnormalities

The flora of the large bowel contribute to modulation of the immune system (76) through the gut flora species composition and metabolic activities which are primarily determined by diet (8). Numbers and species vary in the specific regions of the gastrointestinal tract (see table 1.8). Gut bacteria are responsible for: digestion of unutilised energy substrates (77); cell growth stimulation; prevention of the growth of harmful organisms; training the immune system to respond only to harmful organisms; and defence against some diseases (78, 79). Long colonic transit times affect the metabolism of macronutrients such as protein and carbohydrates by fermentation and the longer the large bowel transit time, the lower the bacterial mass (80).

Prior to birth the gastrointestinal tract of the foetus is sterile. The initial colonisation of the gut during infancy is important in determining a person's lifelong gut flora composition (78, 79). Colonisation of the gut begins at birth as the foetus passes through the birth canal during a vaginal delivery (81). Bacteria from the surrounding environment also colonise the gut of the new born (82). Infants born by caesarean section are predominantly exposed and colonised by the bacteria of their surroundings with some colonisation from their mother (81). Within the first week of life an anaerobic environment for bacterial communities is created (81).

Zoppi and colleagues (83) studied the composition of the intestinal ecosystem in children with chronic functional constipation, and found that they had an intestinal disturbance which the researchers defined as dysbiosis, that is, a quantitative alteration in the numbers of intestinal bacteria (83). Studies of infants and children with allergies have shown that the gut flora composition differs from children without allergies (22). Those with allergies have higher numbers of the more harmful species, *Clostridia difficile* and *Streptococcus aureaus* and lower numbers of *Bacteroides and Bifidobacteria (21).* It is thought that a lack of the beneficial gut

flora early in life leads to an inadequately trained immune system which overreacts to antigens (22).

Table 1.8: Human intestinal microflora (84)

Section of the Gastrointestinal tract	Number of bacteria/ml	Species present
Stomach	10^3	streptococci lactobacilli Helicobacter pylori
Small intestine	10^8	streptococci lactobacilli bifidobacteria bacteroides fusobacteria Enterobacteriaecae
Colon	10^{10} - 10^{11}	Bacteroides Eubacterium Bifidobacterium Peptostreptococcus
	10^8	Enterobacteriaecae streptococci Lactobacillus

1.12 Summary of Chapter 1

Chapter 1 described allergy and intolerance, its diagnosis and the types of reactions that occur and the symptoms produced. The prevalence of cow's milk allergy in the Australian population has been estimated at 2% of the population. The differences in composition of A1 and A2 cow's milk were also described. There are distinct differences in the digestion of β casein A1 and β casein A2.

Chapter 1 also described the physiology of constipation and the evidence for a relationship between cow's milk and constipation, and the possible mechanisms responsible for constipation. The biochemical abnormalities that can occur with this cow's milk allergic type of constipation were explored. There is biological plausibility to the link between CMP and CFC and the next step was to conduct a systematic

review of the literature investigating a link between cow's milk protein and chronic functional constipation. This review is presented in Chapter 2.

Chapter 2: Systematic review of the literature

2.0 Chapter Outline

This chapter describes the systematic review of the literature to assess the evidence that a causal relationship exists between the dietary intake of CMP and CFC in children. In Chapter 1, the problem of chronic functional constipation in childhood was outlined. There is a biologically plausible link between CMP and CFC. This led to a systematic review of the literature investigating a link between CMP and CFC. Parts of the chapter were published in March 2008 in *Nutrition and Dietetics* (see Publications Arising From This Thesis section of this thesis). The results and discussion in this chapter are more extensive than the published article which was condensed due to the word limit for publication.

2.1 Constipation and cow's milk in the literature

A possible link between CMP and constipation was first referred to in the literature in the 1950's by researchers investigating CMP allergy. In 1954, Clein (85), observed 206 children with cow's milk allergy, six percent of whom had constipation that resolved when cow's milk was withdrawn from the diet (85). In 1978, Buisseret (86) researched an unspecified number of children, aged between 11 months and 17 years, diagnosed with a variety of allergies including CMP allergy (86). For the 79 children diagnosed with allergy to cow's milk, 'constipation was more common than diarrhoea' as an associated symptom of cow's milk protein allergy (86).

This early work provided observational evidence that removal of milk protein from the diet of children who did not respond to usual treatments could be effective in resolving constipation. More recent studies by gut researchers have included immunological and biochemical measures to examine constipation as a possible manifestation of cow's milk sensitivity, expressed as an allergy (IgE mediated

immune response), or intolerance (a non-immune physiological reaction) (3, 87, 88) as outlined in Chapter 1.

2.2 Previous reviews of the literature

In 2001, Motta published a review of the literature from 1954 to 2001 surrounding intolerance of cow's milk and chronic constipation in children (57). The method of review was not defined nor were the number of studies included and excluded. It provides a historical perspective of a possible link between cow's milk and constipation. She found several researchers had demonstrated that constipation unresponsive to the usual treatments was associated with cow's milk allergy or intolerance (57). The most likely cause was stated to be inflammation resulting in painful defecation. She reports evidence that mucosal inflammation caused by immune mechanisms could also affect intestinal motility. Motta went on to provide guidelines to clinicians with respect to clinical assessment, physical examination, laboratory tests food investigations and treatments. She concluded by acknowledging that the results of adverse reaction studies are often inconsistent as children with an allergy or intolerance to cow's milk are not a homogenous group. This lack of homogeneity and the varied results reported needs to be considered before assuming that the findings of these studies are unreliable (57).

Magazzu and Scoglio (89) reviewed the literature from 1991 to 2001 in relation to gastrointestinal manifestations of cow's milk allergy (89). The gastrointestinal manifestations investigated included gastro-oesophageal reflux; constipation, food protein-induced enterocolitis and food induced eosinophilic proctocolitis with respect to diagnostic strategies that might eliminate the need for a double blind, placebo-controlled oral food challenge. A review of Pubmed articles, published between 1992 and 2002 was conducted and only studies that included patients and controls were included. The researchers obtained positive and negative predictive values known as posterior probabilities to calculate the likelihood ratio (89). In terms of

the symptom of constipation, they found only one prospective controlled study by Iacono and colleagues (3). The likelihood ratio and posterior probability of clinical and laboratory variables, histologic abnormalities and signs of hypersensitivity showed that a double blind, placebo controlled oral food challenge of CMP is warranted to diagnose a causal relationship between CMP and constipation (89).

The above mentioned were the only two literature reviews related to CMP and constipation found and both had limitations. Motta's paper lacked specificity in the methodology, yet went on to describe clinical guidelines for the management of constipation that may be occurring as a result of cow's milk protein allergy or intolerance. Magazzu and Scoglio reviewed the literature in terms of gastrointestinal manifestations of cow's milk protein allergy and intolerance not limited to constipation and evaluated diagnostic strategies.

The need for a more thorough approach to a literature review surrounding this clinical problem was identified. The aim of this review was to systematically investigate original research to assess the evidence that a causal relationship exists between the dietary intake of cow's milk protein and chronic functional constipation in children. The methodology is described in detail below. The level of evidence was assessed according to the National Health and Medical Research Council's Hierarchy of Evidence (90) by the researcher.

2.3 Method used for systematic review

A literature search was conducted using the key words: constipation, cow's milk, allergy, intolerance, children and intestinal motility. The date range used was 1980 –2006. The databases searched include: Medline (Ovid and Pubmed), Cochrane, CINAHL and EBESCO. Once articles were identified, reference lists of relevant articles were searched and links to related articles in electronic databases were accessed and reviewed.

Initial searches identified 125 articles as potentially meeting the inclusion criteria. Review of the titles and keywords eliminated 99 articles, leaving 26 full text articles, the abstracts of which were further assessed to see whether they met the inclusion criteria. These search strategies resulted in a total of seven articles being included in this review.

Inclusion and exclusion criteria

Studies using a quantitative methodology were included. This review focused on English language studies investigating dietary interventions for constipation in children (aged from seven days to 15 years). Studies included were required to report immunological and/or biochemical measures. Exclusion criteria included: studies reported in languages other than English; studies investigating adults; studies conducted prior to 1980 because they lacked immunological and biological measures; studies without controls; studies that did not include an intervention and retrospective case studies.

Predictor and outcome measures

All studies included were required to include cow's milk protein allergy or intolerance as a possible predictor. All studies were required to include the removal of cow's milk as the experimental condition or intervention and the resolution of constipation as an outcome measure.

Analysis of published articles: Study methodology

Articles included in the review were analysed according to the following criteria: study design, subject characteristics (age and gender), sample size, method of diagnosing constipation; type and length of intervention; outcome measures (bowel symptoms, immunological and biochemical changes); and statistical analysis methods.

Analysis of published articles: Study results and support for hypothesis

This literature review examined the hypothesis that CMP plays a role in chronic functional constipation in some children. A statement was made on the basis of whether the study findings were consistent with the hypothesis. The studies are summarised in Table 2.1 in terms of subject characteristics, methods of diagnosis and type and length of intervention findings of the study; limitations; conclusions and an indication as to whether the study supported the hypothesis; and the level of evidence as determined by comparison of the studies' design with the NHMRC Hierarchy of Evidence (see Figure 2.1) by the reviewer (90).

Level	Study Design
I	Evidence obtained from a systematic review of all relevant randomised controlled trials.
II	Evidence obtained from at least one properly–designed randomised controlled trial.
III-1	Evidence obtained from well–designed pseudo–randomised controlled trials (alternate allocation or some other method).
III-2	Evidence obtained from comparative studies (including systematic reviews of such studies) with concurrent controls and allocation not randomised, cohort studies, case–control studies, or interrupted time series with a control group.
III-3	Evidence obtained from comparative studies with historical control, two or more single arm studies, or interrupted time series without parallel control group.
IV	Evidence obtained from case series, either post-test or pre-test and post test.

Figure 2.1: NHMRC Hierarchy of Evidence 1999 (90)

2.4 Results

Only seven studies were included in this systematic review, four of which came from the same research team (3, 87, 88, 91). The study designs included randomised control trials, non-equivalent group's time series or pre and post intervention trials. The studies were all conducted between 1995 and 2005 despite the search spanning from 1980 onwards. The researchers used different definitions for a diagnosis of constipation. All studies reported on family and/or personal history of atopy. The type and length of dietary intervention, that is, removal of

cow's milk from the diet, varied between studies. While all studies reported on outcome measures for bowel function these were defined differently. The immunological and biochemical variables measured varied slightly between studies.

Only one of the studies, a double blind, randomised controlled trial, by Iacono and colleagues (3) met level II in the NHMRC hierarchy (90). All other studies were at level III-3 or below. All the studies supported the hypothesis that cow's milk protein plays a role in chronic functional constipation in some children. A summary of the results of the systematic review are shown in Tables 2.1 and 2.2.

2.5 The Iacono and Colleagues Studies

The majority of the higher evidence has been conducted by an Italian researcher, Giuseppe Iacono, and colleagues. In a study published in 1995, Iacono and colleagues (87) monitored 27 children with chronic constipation for seven days. (87). The children were then placed on a CMP free diet for two periods of one month each. The children consumed soy or ass milk instead of cow's milk during this time. Three participants under the age of twelve months received soy formula instead cow's milk based formula. These periods were separated by CMP dietary challenges. After one month, participants whose symptoms abated were challenged with cow's milk for a maximum of 10 days. These participants then consumed a CMP exclusion diet for one month, and then received a second cow's milk challenge. In 21 out of 27 participants, there was resolution of constipation symptoms (87). During the two consecutive challenges, constipation reappeared within 48 to 72 hours of the reintroduction of cow's milk (87). Fifteen out of the 21 participants whose bowel habits normalised with CMP withdrawal, showed a positive immune response (including IgE, IgG anti beta lactoglobulin, circulating eosinophils) at baseline compared with one of the six children whose condition did not improve on a CMP free diet ($p<0.05$) (87). The authors concluded that constipation in infants

Table 2.1: Summary of studies investigating the contribution of CMPA or CMPI to CFC.

Setting, Affiliation, Title, First author and Journal reference	Sample gender and age range (mean ±SD).	Definition of Constipation	Dietary Intervention	Outcome Measures: Bowel +ve response	Outcome Measures: Immunological/Biochemical.	Statistical Analysis
Randomised Control Trial						
Italy, Intolerance of Cow's Milk and Chronic Constipation in Children, Iacono, G N Engl J Med 1998; 339: 1100-1104	N = 65: M: 29 F: 36 11 to 72 (34.6 ±17.1) months	1 bowel movement every 3 to 15 days.	33pts: cow's milk for 2 wks; 32 pts: control: soy milk for 2 wks. 1 wk washout; then alternated milk type for 2 wk period.	≥ 8 bowel motions in 2 wks	• IgE • ESR • Circ. eosinophils • WCC, RCC, PCR test. • Milk specific IgE antibody assay of more than 1 • Skin tests: whole milk, lactalbumin, casein, β-albumin.	*Parametric analysis:* Fisher's exact test: frequency analysis. *Non-parametric analysis:* The Wilcoxan rank-sum test: no. of bowel movements per day and qualitative faecal scores.
Non-equivalent groups time series						
Italy, Persistent cow's milk protein intolerance in infants: the changing faces of the same disease, Iacono G Clinical and Experimental Allergy 1998; 28, 817-823.	Cases: N= 12: M: 6 F: 6 60 -84 months (median = 60 months) Controls N=26: M: 12 F:14 72-108 months (median= 72 months)	1 bowel motion every 3 to 7 days and painful passage of hard stools.	Single challenge: cow's milk or placebo; Challenges started at 5ml and increased to the quantity of a full feed in 3hr. If no reaction occurred, the pt continued the challenge (full feeds) at home for 1 wk.	Resolution of symptoms of constipation	Increase in leucocytes, eosinophils in faecal and nasal mucous, occult blood in stools. Seum levels: total IgE, IgG anti-β-lactoglobulin (by ELISA). RAST: cow's milk, β-lactoglobulin, α-lactoalbumin, casein.	*Parametric analysis:* Fisher's exact test: freq. of acute infectious enteritis prior to CMPI, family hx of allergy; Student t test: gestational age; Chi square test: feeding type, freq. of sympt; *Non-parametric analysis:* Mann-Whitney U-test: analysis of age at onset of CMPI.
Pre and Post Intervention studies						
Finland. Lymphoid nodular hyperplasia & CM hypersensitivity in children with chronic constipation. Turunen, S et al. Journal of Pediatrics 2004; 145;5: 606-611.	N= 50 Cases = 35; M:18 F: 17 3-15 yrs (8.3 ±3.3) yrs Controls = 15; M: 6 F:9 2-15 yrs (11.7± 3.2) yrs	Passing hard stools, with a frequency of fewer than 3 per week.	4 week milk elimination for subjects.	> 3 bowel motions/wk	Serum IgA and IgE. Presence of lymphioid nodules. The no. of eosinophilic granulocytes in lamina propria.	*Parametric analysis:* Chi squared test and student t test: the significance of difference between the subjects and controls. *Non-parametric analysis:* Kruskal-Wallis test.
Italy, Chronic constipation as a symptom of cow's milk allergy, Iacono G et al. J Paed 1995; 126: 34-39.	N= 27: M:15 F:12 5 to 36 months (20.6 ±13.4) Months	1 bowel motion every 3 to 7 days and pain in the passage of hard stools.	7 days usual diet. CMP free: 1 month Challenge with CM: 10 days CMP free: 1 month Rechallenge with cow's milk: 10 days	No. of stools/ day. Quality also scored in terms of hardness on 1-3 scale.	Serum levels: total IgE, IgG, anti-β-lactoglobulin (by ELISA), and peripheral eosinophil counts.	*Parametric analysis:* Chi Square tests: ANOVA; unpaired Student t test to compare means for lab results. *Non-parametric analysis*: The Wilcoxon rank sum test: to compare no.of evacuations/day; & qualitative stool scores during CMF and challenge periods.

Table 2.1 (cont): Summary of studies investigating the contribution of cow's milk protein allergy or intolerance to chronic functional constipation.

Setting, Affiliation, Title, First author and Journal	Sample gender and age range (mean ±SD).	Definition of Constipation	Dietary Intervention	Outcome Measures: Bowel +ve response	Outcome Measures: Immunological/Biochemical	Statistical Analysis
UK, Cow's milk and chronic constipation in children. Shah, N, N Engl J Med 1999; 340:891-2 1999.	N=20: M: 11 F: 9 6 to 79 months (median 37 months)	Intractable constipation (no physical cause) that is unresponsive to treatment.	Six weeks on a cow's milk free diet.	Resolution of constipation (not defined in article).	Intestinal transit study. Mucosal biopsy. Anorectal manometry.	Not defined.
Brazil, Cow's milk protein intolerance and chronic constipation in children, Daher S, Pediatr Allergy Immunol 2001;12: 339-342.	N= 40 (gender not stated) 3mo-122months (Mean not reported)	Hx of painful elimination of hard stools for > 1 month.	CMPF for 4 weeks. Pts with resolved symptoms challenged: cow's milk ad lib without a step wise increase in dose	> 3/wk, & no hard, painful or difficult passage. Response for rechallenge: reappearance of sympt. 48-72hrs	Total IgE, Specific IgE (RAST): cow's milk, α-lactoalbumin, β- lactoglobulin, wheat, peanut, soy, fish, egg white. Skin tests: milk, α-lactoalbumin, β-lactoglobulin, casein.	*Parametric analysis:* Fisher's exact tests. *Non-parametric analysis:* Mann-Whitney test.
Italy, Chronic Constipation and food intolerances: a model of proctitis causing constipation Carroccio, A Scandinavian Journal of Gastroenterology 2005; 40: 33-42.	N=52: M 22 F 30 33 to 69 51.2 ±18 months	1 bowel motion every 3 days or more with painful elimination of hard stools, associated with abdominal pain.	CMPF diet for 4 weeks. If no resolution of bowel symptoms, elimination diet. Double blind food challenge to confirm dx of food intolerance.	At least 5 evacuations per week with soft stools, without painful defacation.	Liver and kidney function tests, metabolic evaluation, ESR, PCR test, no. of eosinophils, WCC, RCC; IgE; RAST: cow's milk, β-lactalbumin, casein, α-lactalbumin and other foods allergens; Skin prick tests: food antigens. Rectal biopsies: all subjects after 2wks ; subjects on elimination diet: at 12 wks	*Parametric analysis:* Fisher's exact test: freq analysis. *Non-parametric analysis:* Wilcoxon rank-sum test: no. of bowel movements/ day and qualitative fecal scores; histologic data with final dx of food intolerance at baseline & >12wks; Mann-Whitney U test: histologic findings; Spearman's rank correlation coefficient: histologic data from rectal mucus gel layer.

Symbols and abbreviations:

α = alpha
β = beta
circ = circulating
CM = cow's milk
CMPA* = cow's milk protein allergy
CMPF = cow's milk protein free
CMPI* = cow's milk protein intolerance
cont. = continued
Dx= diagnosis
ELISA = Enzyme linked immunosorbent assay
ESR = Erythrocyte Sedimentation Rate
f = female
hr = hour
hx = history
IgA= Immunoglobulin A
IgE= Immunoglobulin E
IgG= Immunoglobulin G
intervent = intervention
intol. = intolerance
longtitud. = longtitudinal
M=male
ob = observation
% = percentage
PCR = Protein C reactive test
placebo = hydolysed formula
+ve = positive
RAST = Radioallergosorbent tests
RCC = red cell count
RCT= Randomised Controlled Trial
wks = weeks
WCC = white cell count

* The terms CMPI and CMPA have been used exactly as they have been in the studies. There is no discernible difference between the two.

Table 2.2: Results of intervention studies investigating the hypothesis that CMP has a role in CFC in children

First author & year	Results	Study Limitations	Conclusions	Support for hypothesis	Level of evidence
RCT					
Iacono, G at al. 1998b (3).	44 /65 (68%) had a bowel response to soy milk (ie constipation resolved) (10 motions/2wk) and none had a response to cow's milk. Pts on cow's milk: 4 bowel motions/2 wks. Pts on soy: mean of 10 bowel motions/2 wks. Pts who responded to soy had more sympt. of CMPI and +ve immunological tests.	Subjects selected were patients from paediatric hospital clinic.	CMI (mostly IgE mediated) hypersensitivityb (immunologic, clinical) in ¾ of subjects with CMPI related constipation.	Yes	Level II
Non-equivalent group time series					
Iacono G et al. 1998 (88).	11/12 subjects presented with persistent CMPI and 3/26 controls (P<0.0001) presented with multiple food intolerance. 9/12 had persistent CMPI and 2/26 controls showed atopic dx: asthma, rhinitis, eczema (P<0.0001) Family Hx of atopy in 10/12 cases and in 10/26 controls (p<0.01).	Small sample size.	Persistent CMPI is characterised by: familial atopic disease; possibly a change in CMPI manifestations over time and a delay between CMP consumption and symptoms.	Yes	Level III-3
Pre and post intervention studies.					
Turunen, S et al. 2004 (92).	83% of cases remitted on CMPF diet. Constipation relapsed in 34% during the CM challenge, Hx of atopy 12/35 (34%) subjects versus 3/15 controls (20%)	Small sample size. Mean age varied for case and controls Retrospective selected pts Colonoscopy not repeated during milk elimination.	Cow's milk allergy in children with chronic constipation found in some but not all cases.	Yes	Level IV
Iacono G et al. 1995 (87).	21/27 resolution of sympt; constipation reappeared within 48-72 hr during the 2 CM challenges. 15/21 cured pts, +ve results of 1 or more lab tests (specific IgE, IgG anti β lactoglobulin, circulating eosinophils) at dx compared with 1/6 of uncured pts (p<0.005).	Limited no of subjects, and no control group. No immune mechanism investigation. Method not blinded.	Constipation in infants may be an allergic response. This possibility increases if immunological and biochemical data show abnormalities of the immune system.	Yes	Level IV
Shah et al., 1999 (65).	14/20: hx of atopy. 12/20: family hx of atopy. 8/20: parent with hx of dietary protein intolerance. 8/14 with atopy showed delay in faecal passage as a consequence of retention not motility disorder. 4/8 with atopy showed normal intestinal motility after the CMPF diet.	Small sample size and no control group. Immune mechanisms not investigated. Challenge not blinded	Constipation with atopy is commonly associated with mucosal eosinophilia and increased transit time.	Yes	Level IV
Daher S et. al. 2001 (93).	7/25 (28%) constipation disappeared on diet and reappeared within 48-72 hr of challenge. High serum levels IgE: 5/7 cured (71%), +ve skin test: 2/7(29%); and detectable specific IgE: 2/7(29 %).	Limited immune mechanism investigation. Challenge not blinded, no control group, and high drop out rate.	CMPA or CMPI should be considered as a cause of chronic refractory constipation.	Yes	Level IV
Carroccio, A et. al. 2005 (91).	24/52 dx with CMPI; 6/52 dx with multiple food intolerance. Both had normal stools on elimination diet but constipation reappeared on food challenge (22/52 did not improve on elimination diet). Pts dx with food intol had higher freq. of mucosal erosions, intraepithelial lymphocytes and eosinophils, and eosinophils in the lamina propria than those without food intolerance.	Possible to taste difference between milks provided. No control group	Chronic constipation in children can be due to both cow's milk intolerance and to multiple food allergy in some children.	Yes	Level IV

Symbols and abbreviations:

CM = cow's milk
CMPA* = cow's milk protein allergy
CMPF = cow's milk protein free
CMPI* = cow's milk protein intolerance
cont. = continued
freq. = frequency
hx = history
intol. = intolerance
% = percentage
PCR = Protein C reactive test
+ve = positive
RCT= Randomised Controlled Trial
wks = weeks

* The terms CMPI and CMPA have been used exactly as they have been in the studies. There is no discernible difference between the two.

may have an allergic manifestation particularly in the presence of abnormalities of the immune system (87).

In 1998, Iacono and colleagues published the results of a longitudinal study (88). The aim of this study was to evaluate the clinical and immunological characteristics of a group of infants with persistent cow's milk protein intolerance by following 12 children with this intolerance from birth until age five. The children received a CMP challenge each year and tolerance was evaluated. They found that changes in CMP intolerance occurred over time with a more prolonged delay between consumption and symptoms and that familial atopic disease was associated with CMPA that persists beyond the first few years of age (88). Iacono and colleagues hypothesised that this possible delay is due to a tolerance that is acquired over time that allows a small amount of the antigen to be consumed before clinical symptoms occur. They recommended that a study of the cellular-mediated immune reactions be conducted (88).

The same team investigated very delayed clinical reactions to CMP (94). Eighty-six children with newly diagnosed CMPI were recruited. Tests to demonstrate IgE mediated sensitivity were performed at diagnosis. Participants were placed on a cow's milk free diet for 12 months and then challenged with cow's milk using a double blind, placebo controlled method. Children who continued to be cow's milk sensitive remained on a CMP free diet and were rechallenged with cow's milk on a yearly basis. The number of cow's milk sensitive patients who became insensitive after one, two and three years increased, from 30%, 54.5% and 70%, respectively. At the end of the follow-up period of 40 months, 26 out of 86 subjects showed persistent cow's milk protein sensitivity. These 26 children showed a higher percentage of reactivity to the immunological tests performed, that is, total serum IgE ($P<0.05$), RAST ($P<0.01$) and cutaneous prick tests for cow's milk antigens ($P<0.001$). At diagnosis, all patients had a clinical reaction within seventy-two

hours, however, in later challenges, 10 out of 86 showed very delayed reactions, and an average of 13 days after challenge. The number of late reactors increased with each challenge. The very delayed cow's milk protein intolerance manifestations were: constipation; wheezing, dermatitis plus constipation and dermatitis. The authors concluded that very delayed clinical reactions to reintroduction of cow's milk in the diet, such as constipation, can occur (94).

In a subsequent study, Iacono and colleagues (3) used a double-blind crossover design to compare the response to cow's milk with soy milk in 65 children with chronic constipation. Thirty three children were allocated to cow's milk and 32 to soy milk for two weeks. Parents were asked to withhold foods that contained milk during the study. After one week of an unrestricted diet that could include cow's milk, soy milk and their derivatives, children were given the alternate milk for another two weeks. Constipation resolved for 68% of children consuming soy milk, but not for any of those consuming cow's milk (3). The researchers observed that children whose constipation resolved on the soy milk had a number of symptoms associated with cow's milk protein allergy/intolerance at baseline, suggesting a pre-existing abnormality of the immune system. Iacono and colleagues suggested further research on the immune mechanisms occurring with chronic constipation (3). This was the only study included in this review to reach the NHMRC's Level II of evidence (90, 95).

In a more recent study, 52 consecutive children with a mean age of four years, with CFC unresponsive to the usual treatments, were placed on a CMP free diet for four weeks (96). Those uncured on this diet were placed on an oligoanteric diet, consisting of rice, lamb, carrots, ass milk, olive oil and sugar. A double blind food challenge was performed in hospital to confirm the diagnosis of food intolerance. Children with food intolerance, confirmed by double blind placebo controlled CMP challenge or an open food challenge as well as haematological and immunological assays, rectoscopy and histologic study of the rectal mucosa, showed a higher

frequency of erosions of the mucosa, number of intraepithelial lymphocytes and eosinophils and number of eosinophils in the lamina propria. Study of the rectal mucous gel layer showed food intolerance patients had less thickness than those without food intolerances (91). They concluded that chronic constipation in children could be due to both cow's milk intolerance and to multiple food allergies (91).

2.6 Critiques of the Iacono and Colleagues studies

The Iacono and colleagues studies have not been received without criticism. Eigenmann (97) disagreed with Iacono and colleagues' interpretation of the positive assays for milk specific IgE and positive skin prick tests in the double blind crossover study. He also commented that blinding to the type of milk is difficult but acknowledges that this was recognised by Iacono and colleagues in the publication. Eigenmann (87) suggested that until the immune mechanisms have been clearly established, hypersensitivity to cow's milk should not be identified as a cause of CFC. He expressed concern that diets that do not include milk are detrimental to a child's health (97). In response to Eigenmann's criticisms, Carroccio, a researcher, from the Iacono and colleagues team, described how the cut points for milk specific IgE and positive skin prick tests were derived and quoted other studies with relevant findings that support his teams' results (65).

In the same issue of the New England Journal of Medicine, Daher (98) supported the results published by Iacono and colleagues. These results contribute to existing evidence along with her own research later published (93). Daher investigated CMP allergy in 25 children aged between three months and 11 years. The children were assessed clinically and tested at baseline and after cow's milk was withdrawn from the diet for total serum IgE, radioallergosorbent (RAST) for whole cow's milk, α-lactoalbumin, β-lactoglobulin and other food allergens, that is, wheat, peanut, soy, fish, egg white. Skin prick tests with whole milk, alpha lactalbumin, beta-lactoglobulin and casein were also conducted. The children followed a CMP free diet

for a period of four weeks. During this period, constipation disappeared in seven patients (28%) and reappeared within 48-72 hours of a cow's milk challenge[1]. There were high levels of IgE in five (71%) of the children who showed improvement; a positive skin prick test to cow's milk protein in two (29%) of the children. Daher concluded that cow's milk protein allergy or intolerance should be considered as a cause of CFC in children (98).

In an editorial in the New England Journal of Medicine, Shah and colleagues (65) agreed with the results of Iacono and colleagues' double blind randomised controlled trial (98) and stated that it provided further support to their team's research. Shah and colleagues (65) conducted a prospective study of 20 children, aged between six months and seven years, referred with constipation of no known physical cause and unable to be treated using conventional laxative treatment. At baseline, 14 of the children had a personal history of atopy and 12 children had a family history of atopy. After six weeks on a cow's milk free diet, 11 out of the 14 children improved. In two of the remaining three with the history of atopy, the constipation improved after removal of wheat from the diet. Motility was investigated using intestinal transit studies in the 14 children with atopy before starting the CMP free diet and showed that in eight out of the 14 children, the delay in faecal transit was the result of faecal retention in the rectum and not a motility disorder. This motility study was repeated in four of the eight children after the CMP free diet and all four had a normal transit time. Six children underwent rectal mucosal biopsy and were found to have infiltration of the lamina propria with eosinophils and one had eosinophilic cryptitis. The researchers concluded that CFC in children with atopy is commonly associated with mucosal eosinophilia and increased mouth to anus transit times in association with rectoanal retention, and

[1] Two infants underwent a rectal biopsy which revealed allergic colitis and therefore they did not undergo a cow's milk protein challenge.

removal of CMP should be an integral part of the treatment for constipation in children (65).

Norwicki and Bishop (99) argued that there is insufficient data to support routine screening of children for CMP allergy as a cause of constipation (99). He recommended that further investigation be conducted in unselected populations to determine the prevalence of allergy as a cause of constipation. He did however concur that the trial of a cow's milk free diet with rechallenge may in fact be useful in a child with a history of atopy (99).

Stricker (96) documented that up to thirty percent of infants sensitive to CMP are also sensitive to soy proteins. He questioned whether the children in the and colleagues' double blind randomised controlled trial who did not improve on soy milk were sensitive to both cow and soy protein (39). He agreed that intolerance to cow's milk should be considered in young children with CFC, and recommended further investigation of tools for diagnosis of CMP sensitive constipation (39).

2.7 Other evidence Level III-3 or below

All other studies reviewed for this thesis were at level III-3 or below. Those conducted by Iacono and colleagues (66, 87, 88), Shah and colleagues (65) and Daher and colleagues (93) have been described above. The remaining level III-3 study, by Turunen and colleagues (92), is described here.

In Finland, lymphoid nodular hyperplasia and cow's milk hypersensitivity in children with CFC has been investigated by Turunen and colleagues (92). The aim of the study was to investigate the incidence of cow's milk allergy as evidenced by cow's milk challenge and the findings of endoscopic and immunohistological examinations in children with CFC. Thirty-five subjects, aged between two and 15 years, underwent a colonoscopy at baseline and then commenced a CMP free diet for 4 weeks followed by a CMP challenge (92). After elimination of milk from the diet and

with supportive medication, constipation resolved in 83% of subjects. Constipation and other gastrointestinal or skin symptoms relapsed in 34% of participants during the cow's milk challenge. These subjects had a higher density of intraepithelial $\gamma\delta^+$ T cells in the biopsy samples of the terminal ileum ($P<0.01$) in comparison to control subjects (92).

2.8 Discussion

This systematic review evaluated the quality of the evidence for the hypothesis that CMP has a causal role in chronic functional constipation in some children. A summary of the results of the studies included in this review is presented in Table 2.2 and will be discussed in this section. The current evidence lends some support to this hypothesis. However, the evidence base remains small and needs to be further developed. Despite constipation being a common problem in paediatric practice, the paucity of studies suggests constipation is not a popular area for research. There were no published systematic reviews due to the lack of randomized controlled trials (RCT) studies. Only one of the studies, the double blind randomised controlled trial conducted by Iacono and colleagues (3), provides evidence of an association between cow's milk and constipation. There is a problem with reliance on research from one group given that these results are yet to be attempted to be replicated in other settings.

The limited number of higher level evidence studies could be due to a variety of factors. Conducting studies that withdraw cow's milk presents ethical challenges and difficulties double blinding given the specific flavour profiles of food. Participation in a study with this type of methodology carries a high participant burden particularly biochemical and immunological testing and therefore it would be difficult to enroll large numbers of participants. Despite these considerations, more studies using the double blind, randomised control trial method (3) are required to inform the development of evidence-based guidelines to treat this problem in

practice. However, such studies should consider the limitations of soy milk as a control, since approximately thirty percent of children with cow's milk protein allergy are also sensitive to soy (39). Definitions of constipation should be standarised in further studies published.

A limitation of this review was that studies in languages other than English were not included and it is possible that some evidence was excluded in this way. A further limitation of this review was that the studies included examined children of various ages yet the same conclusions are unlikely for children of different ages. It is important to note that cow's milk protein allergy usually has a remission in 85 to 90% of cases by age four years (28). The strength of the recent evidence is that biochemical and immunological analyses were included showing an association between cow's milk protein and constipation, providing a potential biological explanation for the relationship between CMP and constipation.

The studies reviewed showed that breastfeeding duration, early exposure to cow's milk and familial and personal history of atopy, are all potential factors in the development of cow's milk protein allergy or cow's milk intolerance. Carroccio and colleagues (91) found that constipation can occur as a very delayed clinical reaction to reintroduction of cow's milk in the diet of cow's milk intolerant children (94). Shah and colleagues (65) found that refractory constipation in children with atopy is commonly associated with mucosal eosinophilia and increased mouth to anus transit times in association with rectoanal retention (65).

The existing literature surrounding cow's milk and constipation is limited. None of the studies conducted were population-based nor structured to provide evidence-based evaluation or treatment guidelines at either the primary care or tertiary level (64). The small pool of published studies possesses many limitations: the biased nature of the study population (subjects that have presented clinically to

specialists); study designs unable to prove causality; and the difficulty in blinding subjects and their families to CMP and a suitable milk alternative.

To date, no particular allergy test for milk is currently specific or sensitive enough to recommend routinely (64, 100). Further scientific evidence is required to clarify the physiological, biochemical and immunological mechanisms that occur in susceptible children to establish a process for testing that will contribute to evidence based management of paediatric constipation. A need for evidence based dietary alternatives to cow's milk, for example soy milk, has been identified. The feasibility of families to follow a CMP free diet also needs to be determined.

2.9 Project Aims

This project aims to confirm whether a causal relationship exists between the dietary intake of cow's milk protein and chronic functional constipation in children. The research trials for this thesis were designed to answer four questions:

1. Can the results of the Iacono and colleagues study of children with CFC who respond to the replacement of CMP with soy be replicated in the Australian setting?

2. What effect does the cow's milk β casein A1 and cow's milk β casein A2 have on CFC in children who do not respond to traditional treatments?

3. What are the immunological and biochemical mechanisms underlying CFC that respond to the removal of CMP in children?

4. What factors affect the feasibility of mothers administering a CMP free diet to their children?

The four questions were addressed by two different trials and a qualitative study. Question 1 was addressed by crossover Trial 1, which replicated the Iacono and

colleagues study in the Australian setting (Method in Chapter 3, Results in Chapter 4).

Question 2 was addressed by a double-blind crossover trial, Trial 2, which investigated the effect of cow's milk β casein A1 and cow's milk β casein A2 on CFC in children who do not respond to traditional treatments (Method in Chapter 3, Results in Chapter 5).

Question 3 was addressed through the collection of blood and faeces from children with CFC who do not respond to the traditional treatments, in Trials 1 and 2 (Method in Chapter 3, Results in Chapters 4 and 5).

The answer to question 4 was addressed by the qualitative study described in Chapter 6. Qualitative methodology was used to gain an insight into the experiences of families implementing a CMP-free diet.

The answers to the four research questions and recommendations arising from the conducted trials and qualitative study are outlined in chapter 7.

2.10 Summary of Chapter 2

Chapter 2 described the systematic review of the literature to assess the evidence that a causal relationship exists between the dietary intake of CMP and CFC in children. The evidence showed some support for a causal link between CMP and chronic functional constipation in a group of children, some of whom show increased prevalence of CMP sensitivity in biochemical and immunological tests. This systematic review led to the four research questions presented above and the development of this research project which aims to confirm whether or not a causal relationship exists between the dietary intake of cow's milk protein and chronic functional constipation in children. Chapter 3 describes the participants recruited and the methods used for the two crossover clinical trials investigating cow's milk protein and paediatric chronic functional constipation.

Chapter 3: Methodology

3.0 Chapter outline

This chapter describes the participants recruited and the methods used for the crossover clinical trials investigating milk protein and paediatric chronic functional constipation.

Two separate trials were conducted after the systematic review of the literature reported in Chapter 2. Trial 1 (addressed research Q1) compared cow's milk with soy milk. The second trial (addressed research Q2) compared cow's milk β casein A1 with cow's milk β casein A2. Except for the milk type, the conditions were identical and the study design is described below. The method was designed to measure the hypotheses:

1.1 Hypothesis for Trial 1: *Constipation would resolve for children with CFC whilst consuming soy milk and avoiding cow's milk protein.*

2.1 Hypothesis for Trial 2: *Constipation would resolve for children whilst consuming cow's milk β casein A2 and whilst avoiding all sources of cow's milk β casein A1 protein.*

3.1 Study design

Children aged one to 12 years, with chronic functional constipation, were recruited to the two trials. Both trials used a crossover design trial to compare two milks. Trial 2 was double blind. Each participant started on one milk condition for two weeks. After a two-week washout period, participants were switched to the other type of milk for another two weeks. The order of treatment was randomly assigned according to the participants' date of birth to pathway 1 or pathway 2 by the paediatric continence nurse. This was done as each child and their family consented to participate in the study. The dietitian researcher (EC) was unaware of the order

of treatment. Commercial labels were removed from the cow's milk β casein A1 and cow's milk casein β A2 containers for Trial 2 to allow double blinding. This blinding was possible due to the lack of a discernible taste difference between the two milks. Trial 1 was conducted in Tamworth and Trial 2 in Newcastle. The trials and their pathways are shown below in Figure 3.1 and Figure 3.2.

	Pathway 1	**Pathway 2**
2 weeks	Cow's milk	Soy milk
	↓	↓
2 weeks	CMP and soy protein free	CMP and soy protein free
	↓	↓
2 weeks	Soy milk	Cow's milk

Figure 3.1: Study design for Trial 1, cow's milk β casein A1 versus soy milk

	Pathway 1	**Pathway 2**
2 weeks	Cow's milk β casein A1	Cow's milk β casein A2
	↓	↓
2 weeks	CMP and soy protein free	CMP and soy protein free
	↓	↓
2 weeks	Cow's milk β casein A2	Cow's milk β casein A1

Figure 3.2: Study design for Trial 2, cow's milk β casein A1 versus cow's milk β casein A2

This research project was approved by the Hunter New England Area Health Service Ethics Committee and the University of Newcastle Human Research Ethics Committee (Reference 03/08/13/12/3.23). A variation to extend recruitment by advertising in the Division of General Practitioners newsletters and the paediatrician's rooms was also approved. The approval documentation is shown in Appendix 3a, 3b, 3c and 3d.

Funding was received for this project from: The University of Newcastle's, University Department of Rural Health, Northern New South Wales; a Primary Health Care Research, Education and Development Small Research Grant; soy milk was donated by Sanitarium; and A2 milk was donated by Fairbrae Milk.

3.2 Outcome measures

Primary outcome measures: Resolution of constipation

A Constipation Diary (Appendix 10) was used to record the number of bowel motions per fortnight. A clinical outcome was defined as, eight or more bowel movements during a two week treatment period (3).

Secondary outcome measures: Biomarkers

All biomarkers were monitored at three time intervals throughout the study period: (1) baseline, (2) after the first two week trial and (3) at the end of the study.

Blood

A description of each of the blood factors monitored and a hypothesis statement related to each factor (Hypotheses 3.1) and whether they were expected to change during the study period is described in Appendix 2.

Faeces

Faeces samples were analysed for the presence of normal gut flora and *Streptococcus B*, *Streptococcus agalactiae*. Adequate numbers of gut flora including *Streptococcus B* are required for the maintenance of bowel health and the prevention of constipation (83).

3.2.1 Faeces Hypothesis for Trial 1: *Normal gut flora and Streptococcus B would be low or absent at baseline (time 1), and would return to normal for children during the washout period and whilst consuming soy milk.*

3.2.2 Faeces Hypothesis for Trial 2: *Normal gut flora and Streptococcus B would be low or absent at baseline (time 1), and would return to normal for children during the washout period and whilst consuming cow's milk β casein A2.*

3.3 Dietary intervention

Participants were given a supply of the assigned milk for the two week trial period and advised to consume at least 400 mL of this milk each day and to avoid all other sources of CMP. They were also instructed to continue with the same amount of fibre and fluid per day as per before starting the research protocol. After the nutritional assessment parents and participants were educated by a dietitian to follow a cow's milk protein free diet. Education material was provided consisting of a Milk Free Shopping Guide and a Label Reading Education Tool (Appendix 4a and 4b) and practical, personalised strategies were recommended. Parents were given the name of a suitable calcium supplement that they could give their child if desired. The first 10 participants were provided with fortnightly phone calls by the researcher to answer queries and ensure adherence to the diet. For all other participants, this contact was increased to weekly to improve retention, participation rates and adherence to the diet. Participants were also able to contact the researcher with questions whenever necessary.

The first two-week trial period was followed by a two-week washout period free of all milk and soy protein to remove the physiological and psychological influence of the protein between the trial periods. This washout period acted as another condition in itself given that participants continued on their cow's milk protein free diet with no other milk or soy included. Participants were encouraged to consume an additional 400 mL of other fluid during the washout period in place of the intervention milk to prevent dehydration. This fluid could include rice milk as a substitute for milk. After the washout period, for Trial 1, those consuming soy milk were switched to cow's milk and those on cow's milk were switched to soy milk. After the washout period, for Trial 2, those consuming cow's milk β casein A1 were switched to cow's milk β casein A2 and those on cow's milk β casein A2 were switched to cow's milk β casein A2. Parents obtained the supply of milk for the next

condition from the paediatric continence nurse just before commencing the second two part of the trial.

3.4 Participants

Children aged between one and 12 years with CFC, unresolved by medications, including laxatives or dietary methods were recruited. CFC was defined as chronic faecal retention, that is, less than eight bowel motions per fortnight (3).

All participants

- were consuming dairy products daily
- had previously tried the usual dietary treatment for constipation but had not been successful in the long term.

Children with Hirschsprungs disease, Cerebral Palsy, Coeliac disease and children using medications known to cause constipation were excluded from the study. Children taking laxatives withheld these for the duration of the Trial.

3.5 Study procedure

The aim was to recruit 30 children to each of the two trials. A flowchart of the study procedure is shown in Figure 3.3.

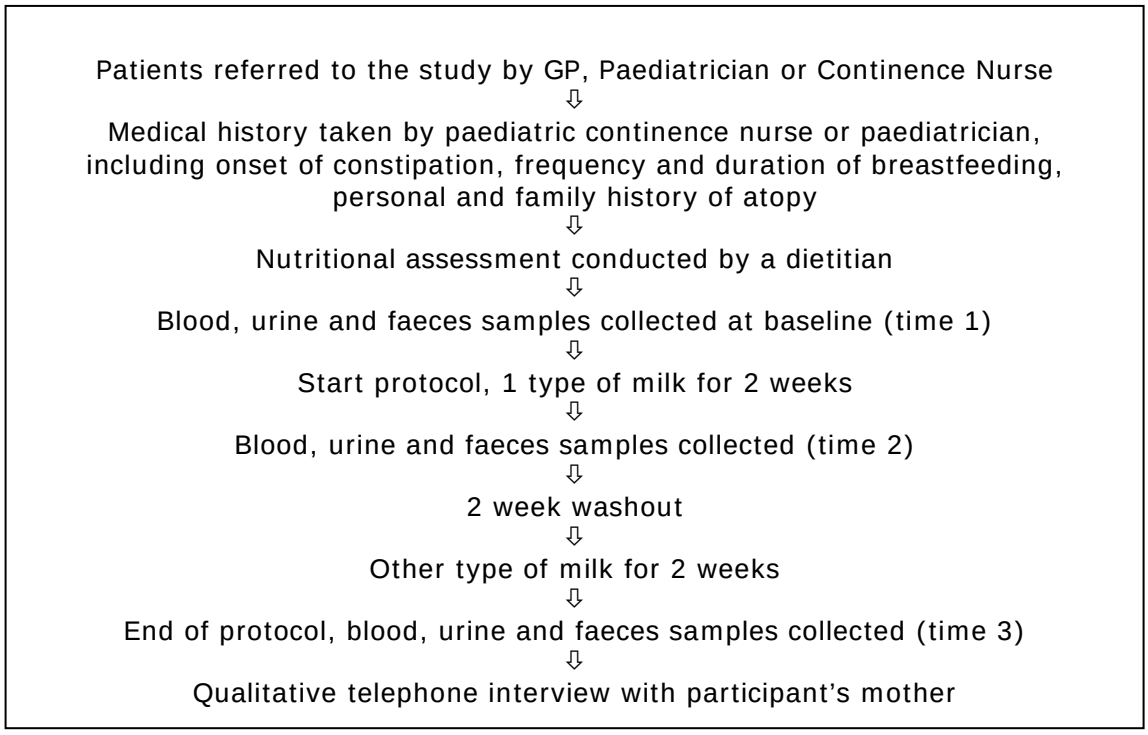


Figure 3.3: Flowchart of the procedure for Trial 1 and Trial 2.

3.5.1 Recruitment Process

All participants were referred to the study by their paediatrician, general practitioner or paediatric continence nurse. Recruitment occurred in Tamworth for Trial 1, from July 2005 to September 2007 and in Newcastle for Trial 2, from July 2005 to May 2008.

Posters (Appendix 5) were displayed in the paediatrician's rooms and the Children's Ward at Tamworth Rural Referral Hospital for Trial 1 and in the waiting room of John Hunter Hospital's Children's Hospital Clinic at Wallsend Community Health Centre for Trial 2. The poster invited parents to approach their doctor, paediatrician or paediatric continence nurse about participating in the study. Advertisements were also placed in the Divisions of General Practice newsletters (Appendix 6) located within the geographical area of the Hunter New England Area Health Service for General Practitioners to refer potential participants to both trials.

The doctor advised the parent of their child's eligibility based on the inclusion criteria. The researcher was then advised of the potential participant and provided the child's parents with an envelope containing an information package about the study (Appendix 7a, Newcastle and 7b, Tamworth) including:

- An information sheet for participants
- An information sheet describing the milk free diet
- An information sheet describing the collection of samples
- A consent form
- A reply-paid envelope

Each potential participant's parents took the envelope away to make a decision about their child's participation in the study in their own time. The researcher remained at arm's length from the parents by recruiting through the paediatrician, general practitioner or paediatric continence nurse. Care was taken to inform parents that the study outcome may not immediately benefit their child. Parents were required to complete the consent form and post it to the researcher in the reply-paid envelope found in the information package. Participation in the study was commenced when the researcher received a signed consent form.

3.5.2 Data collection

After the consent form had been received, the researcher contacted the parent to answer any queries and to arrange a time to meet to conduct the nutritional assessment and for the collection of blood, urine and faeces samples.

After this phone contact, each study participant was mailed a package containing:

- A specimen jar for faeces collection
- Pathology forms to accompany samples to the pathology unit

- A transportation form, to accompany the urine sample from the pathology unit to the Dunstan Roberts Laboratory at the University of Newcastle for analysis by the student researcher.

The Medical History

A detailed medical history was taken by the paediatrician or the paediatric continence nurse using the paediatrician's Encopresis Evaluation Chart (Appendix 8). Data collected included: onset, symptoms and duration of the constipation; medication history; frequency and duration of breast feeding; personal and family history of allergy and intolerance.

The Nutritional Assessment

Each child and his/her parent participated in a consultation with a trained dietitian (either the researcher or the research assistant) who conducted a detailed nutritional assessment (See Appendix 9). Average daily dietary intake including foods and macronutrients of interest such as dairy, fibre and fluid intake was obtained. This information was used to determine: whether 400 mL (or equivalent) of dairy products were being consumed by each participant daily and whether a participant's dietary fibre and fluid intakes were adequate prior to commencing the study. If a participant's dairy intake was inadequate, he/she was required to include 400 mL of dairy products per day in his/her diet for two weeks prior to commencing the Trial. If a participant's fibre and fluid intake was inadequate, they were required to increase their dietary intake of these macronutrients to amounts recommended by the dietitian for two weeks prior to commencing the trial to ensure consistency between participants and to eliminate lack of fibre and fluid as a cause of their CFC.

Each participant's diet was assessed using the Australian Guide to Healthy Eating (101) for recommended number of serves for the relevant age group to determine nutritional adequacy. The consumption of processed foods containing CMP as an

ingredient was also assessed, except for ice-cream which was reported in the Extras category rather than dairy. For this assessment Extras were subcatagorised into the following processed foods containing CMP: convenience foods, for example, pizza, nuggets, hot dogs, fish fingers etc. consumed at least once per week; confectionery (chocolate), at least once per week; biscuits and cakes, at least once per week; snack foods, for example, chips, muesli bars, at least once per day; and ice-cream, at least once per week.

Constipation Study Patient Diary

A 'Constipation Study Patient Diary' (see Appendix 10) using the Bristol Stool Scale (102), a validated scale correlated with gut transit time, (103-105), was used by participants or their parents, to record information about the subjects bowel motions each day. This scale has been found to be a useful tool in clinical research (106), and has been recommended for research by an international working party (107).

Participants and their parents were instructed by the dietitian in how to complete 'Constipation Study Patient Diary' for the duration of the six week study. The participant (over the age of eight) or his/her parent was required to record: the number of bowel movements per day; whether straining was required to pass the motion; stool form and appearance by comparing it to diagrams on the back of the diary; and symptoms of abdominal pain/discomfort and bloating. The constipation diary is shown in Appendix 10.

Blood collection

Children attended either New England Pathology (Trial 1) or Hunter Area Pathology Service (Trial 2) to have blood samples taken at times 1, 2 and 3. Blood was taken by an experienced paediatric blood collector. Children were offered an Emla numbing patch before the procedure. Approximately 22 mL of blood was collected

into a number of specialised vacuette tubes. Samples were analysed by laboratory staff according to standard procedures and results reported to the referring paediatrician or general practitioner, paediatric continence nurse and the researcher.

Faeces

Children provided a faecal sample at times 1, 2 and 3. Participants were advised to collect a sample of faeces into a faeces collection container and to store until in the refrigerator until taken to pathology, ideally on the day of collection. Stool specimens were transported to the pathology laboratory, New England Pathology (Trial 1) or Hunter Area Pathology (Trial 2) in standard plastic stool containers. A transport medium (or preservative medium) was not used in this study as *Streptococcus B* is not considered to be a delicate organism that might be negatively affected by the presence of other organisms or loose viability during the delay of transportation (108).

3.5.3 Qualitative interview at study completion

At conclusion of the Trials, participants' mothers were invited to participate in a qualitative debriefing interview. This interview was used to evaluate the feasibility of following a CMP free diet. Parents were also given the opportunity to debrief over the experience of participating in the research. The method and procedure for this study is described in Chapter 6.

3.6 Analysis of Biomarkers

3.6.1 Biochemistry

Blood samples were analysed for full blood count, liver function tests, total IgA, IgE, IgG, IgM, ASOT, antiDNaseB, thyroid function test, B12, folate, copper, zinc and coeliac antibodies at baseline, after the first two week trial period and at the

conclusion of the final two week trial period. Blood analyses occurred according to standardised commercial laboratory techniques and equipment by Beckman and Coulter (109).

3.6.2 Faeces

The presence and approximate quantity of normal gut flora were assessed using the MacConkey agar while the presence of Enteric Streptococci and *Streptococcus B* were found using the horse blood agar (HBA-CAN agar with added colistin) and nalidixic acid (HBA-CAN), and the Granada or the STRB agar (108). Initially (for the first six samples), the selective plate was Granada agar but this was later replaced by a better chromogenic agar (STRB) which has a slightly better performance than that of the Granada agar. (*Note:* STRB medium was not available when the study commenced).

Specimens were processed on the day of receipt. The media were inoculated with the stool specimen using a swab and streaked out in the usual manner to achieve single colonies. The plates were then incubated for 24 hours at 35°C before being examined (108).

Normal bowel flora appears as mixed red lactose positive and colourless lactose negative colonies on the MacConkey agar, whereas pathogens are identifiable by colour. An approximate amount of growth was recorded as either absent, low, moderate or heavy growth (108).

Suspicious pathogenic colonies were sub-cultured to a new blood agar plate, incubated overnight and examined the next day for characteristics of *Streptococcus B*, including an off-white colony with a narrow zone of β-haemolysis and a negative catalase reaction. The streptococcus grouping test was then performed to confirm the group as Group B. Finally, an antibiotic susceptibility test was then conducted to confirm the presence of normal *Streptococcus B* and to exclude any abnormal

antibiotic resistance pattern that might exist. Any positive *Streptococcus B* were frozen at -70°C for further study if indicated (108). Microscopy was not used as *Streptococcus B* resembles many other bowel organisms in a Gram stain.

3.7 Data management

An *Access*™ data base was purpose built for this study. A research assistant was employed and trained to enter data. Data was collected and entered into the data base using the following process:

Medical history data were sent by the paediatrician's office or the paediatric continence nurse to the postal address of the researcher and entered into the data base by the research assistant.

Nutritional assessment data were analysed by the dietitian researcher (EC) and entered into the data base by the research assistant.

The 'Constipation Study Patient Diary' was posted back to the researcher's postal address by participant's parents in reply-paid envelopes, after each two week condition of the study. Data from the diary was then entered into the data base by the research assistant.

Blood analyses reports and faecal analyses reports were sent to the dietitian researcher (EC) by the pathology laboratory and entered into the data base by the research assistant.

Urine analysis data were forwarded to the dietitian researcher (EC) from the Dunstan Roberts Laboratory and entered into the data base by the research assistant.

Data were entered according to the unique identification number for each participant. They were analysed by the dietitian researcher (EC) after cessation of each of the trials.

3.8 Statistical Analysis

Descriptive statistics were calculated, frequencies, range, mean and standard deviation were reported for each of the outcome variables and correlations between relevant variables. A linear mixed method analysis of the quantitative data was conducted to determine the effect of the treatments for the numeric outcome variables. Repeated measures analysis of variance was also used to compare each of the conditions, intervention milk 1, the washout period and intervention milk 2. Verification of this analysis was conducted using contrasts and pair wise comparisons.

Categorical versions of the numeric variables were also used to statistically compare the number of bowel motions during the three two-week trial periods for each study, that is, intervention milk 1, the washout period and intervention milk 2. The McNemar test for paired observations was used.

For the continuous variables, paired t-tests and non-parametric tests, were used to statistically compare the number of bowel motions per participant per fortnight on each of the milk conditions, intervention milk 1 and intervention milk 2. Continuous variables were checked for normality using measures of skewness and kurtosis. Given the small sample numbers and the concern that the variables may not have been normally distributed, non-parametric tests were performed. The Friedman test and Wilcoxan Ranked Sign were used to verify analysis conducted by the parametric tests.

The analysis techniques were modified for Trial 2 after consultation with a statistician. The mixed model analysis was used in place of the repeated measures analysis of variance to compare the number of bowel motions during the three study conditions, cow's milk β casein A1, the washout and cow's milk β casein A2. (NB it was unnecessary to reanalyse data from Trial 1 because analysis was adequately performed using repeated measures analysis of variance in this

instance). The advantage of this approach was that missing values did not cause a loss of subjects from the analysis. All cases with values are used by this approach.

Estimated marginal means was used to compare the means of uneven sample sizes for the biochemical tests so that each significant mean could be considered in proportion to its sample size. This was necessary since not all participants provided blood samples for analysis.

The statistics package *SPSS*™ version 16.0 was used for analysis.

3.9 Summary of Chapter 3

Chapter 3 described the study groups recruited and the methods used for data collection and analysis for the investigation into the role of CMP in children with chronic functional constipation described in this thesis, that is,

1. Trial 1, cow's milk β casein A1versus soy milk, conducted in Tamworth.

2. Trial 2, cow's milk β casein A1 versus cow's milk β casein A2 study, conducted in Newcastle.

Both Trial 1 and Trial 2 required the collection of data from a medical history, a nutritional assessment, six week constipation diaries, blood, urine and faeces samples and ended with a qualitative interview. The results of these studies are reported in Chapters 4 (Trial 1) and 5 (Trial 2) and 6 (qualitative study).

Chapter 4: Effect of cow's milk versus soy milk on chronic functional constipation: results of Trial 1

4.0 Chapter outline

This chapter describes the results of the first crossover trial comparing the effects of cow's milk β casein A1 with soy milk on chronic functional constipation in children. This trial is referred to as Trial 1 and was conducted in Tamworth, New South Wales. The research questions and hypotheses for Trial 1 were as follows:

Research Question 1: Can the results of the Iacono and colleagues study of children with CFC that responds to the replacement of CMP with soy be replicated in the Australian setting?

1.1 Hypothesis for Trial 1: Constipation would resolve for children with CFC whilst consuming soy milk and avoiding CMP.

Research Question 3: What are the immunological and biochemical mechanisms underlying CFC that respond to the removal of CMP in children?

3.1.1 Blood Hypotheses: A description of each of the blood factors monitored and a hypothesis statement related to each factor and whether they were expected to change during the trial period is described in Appendix 2.

3.1.2 Faeces Hypothesis: Normal gut flora and *Streptococcus B* would be absent or low at baseline (time 1), and would resolve for children during the washout period and whilst consuming soy milk.

The methods for Trial 1 were described in Chapter 3.

4.1 Participant characteristics at baseline

4.1.1 Factors related to allergy

Fourteen children were recruited, with one excluded from the study due to a subsequent diagnosis of coeliac disease leaving 13 participants, six males and seven females. Participants' ages are shown as a categorical variable in Table 4.1. The mean age of participants was 80 months (±38 months) and the range was 16-144 months. The characteristics, family history and clinical history of these participants are also shown in Table 4.1.

Approximately a third of participants had a family history of cow's milk protein allergy or intolerance. Initial clinical assessment of participants, showed that a number of participants had symptoms associated with cow's milk protein allergy or intolerance, including ear infections and grommets (110) but no previous diagnosis of CMPA.

Three participants were delivered by caesarean section. Two out of 13 participants' mothers reported having thrush, *Candida Albicans,* during pregnancy. Approximately half of the participants in this study were breastfed.

Three out of 13 participants were reported by parents to have delayed development in achieving age-related milestones; however, no formal diagnosis of a condition or syndrome had been made. Nearly half of the families had experienced psychosocial disruption due to their child's behaviour, but only two had seen a psychologist.

Several participants had abnormal results for biomarkers at baseline; these data are shown with the post-intervention data.

Table 4.1: Characteristics, age, family history and clinical history of 13 participants recruited to the cow's milk versus soy milk study

Characteristics and history	Participants reporting characteristic of history	
	N	%
Age		
<3 years	2	15.3
4-6 years	4	30.7
7-9 years	5	38.4
10-12 years	2	15.3
Family history of CMPA or CMPI	4	30.7
Personal history of CMPA or CMPI	0	0
Physical symptoms of intolerance to cow's milk:		
Dermatitis, eczema, rhinitis	6	46.1
History of asthma	6	46.1
History of ear infections	9	69.2
Grommets	2	15.3
History of tonsillitis/ throat infections	5	38.4
Tonsils removed	1	7.6
Adenoids removed	3	23.0
Gestation and birth characteristics		
Maternal thrush during pregnancy	2	15.3
Delivered by caesarian section	3	23.0
Breastfed	6	46.1
Development delayed	3	23.0
Behavioural issues		
Consultation with psychologist	2	15.3
Psychosocial disruption of the family	6	46.1
History of recurrent UTI's	3	23.07
Constipation symptoms		
Soiling or encopresis	13	100
Abdominal pain	9	69.2
Anal pain	7	53.8
Anal bleeding	3	23.0
Diagnosis of perianal dermatitis	1	7.6
Current symptoms of		
Poor appetite	5	38.4
Nausea or vomiting	5	38.4

4.1.2 Dietary intake at baseline

Results of the nutritional assessment are reported in Figure 4.1. This assessment identified that some participants were consuming greater than the recommended number of serves of fruits, vegetables and legumes. Seven out of 13 participants were consuming greater than the recommended number of serves of milk, yoghurt and cheese. More than half were consuming red meat less than the recommended 3-4 times per week. All participants were consuming processed foods containing CMP as an ingredient on a daily basis.

Australian Guide to Healthy Eating Food Categories (101)						Extras - Processed Foods containing cow's milk protein as an ingredient					
Participant	Breads and cereals	Vegetables and Legumes	Fruit	Milk, Yoghurt & Cheese	Meat, Fish, Poultry, Nuts & Legumes Red meat no. of times/wk	Fats & Oils	Convenience Foods eg pizza, nuggets hot dogs fish fingers etc At least once per week	Confectionary –Chocolate\ At least once per week	Biscuits and Cakes At least once per day	Snack Foods eg chips pop corn and muesli bars At least once per day	Ice Cream At least once per week
1					3						
2	CMP				<1						
3					2						
4					0						
5	CMP				3						
6					2						
7	CMP				3						
8					2						
9	CMP				3						
10					0						
11	CMP				0						
12	CMP				3						
13					3						

Met recommended no. of serves for age group
Exceeded recommended no. of serves for age group
Lower than recommended no. of serves for age group
Consumed processed foods containing milk as an ingredient as described
Did not consume processed foods containing milk as an ingredient as described
CMP = Breads and cereals consumed contained CMP

Figure 4.1: Nutritional Assessment of the diet history of 13 participants using the Australian Guide to Healthy Eating and showing consumption of processed foods containing cow's milk protein as an ingredient.

4.2 Primary outcome measure: resolution of constipation

Nine participants returned constipation diaries for the study period. The number of cases of constipation resolution, defined by eight or more bowel motions per fortnight and the mean number of bowel motions per fortnight under each condition for participants is shown in Table 4.2. Constipation resolved for all of these nine participants while on soy milk. During the washout period, when no CMP was consumed, eight out of the nine participants had a response. However, five also experienced resolution of constipation on cow's milk.

Table 4.2: Cases of resolution of constipation and mean number of bowel motions during a 2 week condition for 9 participants

Bowel Movements and Descriptions	Prior to study[1] N=9	Cow's milk N=8[3]	Washout[2] N=8[3]	Soy Milk N=9	P
Resolution of Constipation N (%)	0 (0)	5 (62)	8 (100)	9 (100)	0.01*
Bowel motions per fortnight M (SD)	5.1 (1.4)	9.9 (4.4)	13.0 (5.2)	15.1 (5.0)	0.03**

1 All are constipated to be eligible for trial
* Wilcoxan Signed Rank Sum
** Paired T-test
2 The washout period acted as a condition in itself as it was free from all milk and soy protein.
3 The number of participants in each condition is not the same because one participant ceased participation in the study after having a clinical response on the first condition, soy milk, and did not proceed to the washout or CMP condition.

The mean number of stools prior to commencing the trial compared with each of the trial dietary conditions is shown in Table 4.3 for each of the nine participants with constipation diaries. The mean number of bowel motions increased from baseline for all participants. The ninth participant could not be included for statistical analysis because he only participated in the soy milk condition. Using the Greenhouse-Geisser adjustment, the differences between the three conditions was statistically significant, $F(1.88, 13.1) = 4.58$, $p = 0.03$.

Table 4.3: Number of stools per fortnight at baseline and on each of the study conditions and symptoms of straining, abdominal pain/discomfort and bloating reported per week by 9 participants

Participant Number	*Prior to Study*	Stools[1]	*Cow's milk*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]	*Washout*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]	*Soy*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]
														Condition order: Cow's milk/Washout/Soy Milk																			
4		6		15	1	1	-	-	-	-	-	-		10	0	0	-	-	-	-	-	-		21	0	0	-	-	-	-		
6		5		12	0	0	0	0	6	2	0	0		15	1	0	0	0	0	1	0	0		29	0	0	0	0	1	2	0	0
														Condition order: Soy Milk/Washout/Cow's Milk																		
1		2		13	4	5	3	1	2	0	0	0		13	3	1	0	1	2	1	0	0		14	4	3	1	0	0	1	0	0
2		5		5	2	3	6	2*	2	2*	2	2*		9	2	0	1	5	5	4	6	3		9	5	0	7	6	7	7	7	7
3		6		8	0	1	7	7	5	6	7	5		10	0	0	5	7	6	7	7	6		9	2	0	6	6	6	4	3	2
5		6		4*(7)	4	-	-	-	-	-	-	-		11	1	0	5	4	5	3	3	3		20	0	0	2	5	2	5	0	5
7		5		7	2	2	4	6	2	3	0	1		11	3	1	2	7	3	1	2	2		11	2	0	7	2	2	2	1	1
8		2		15	1	-	-	-	-	-	-	-		25	0	0	0	1	0	2	0	3		19	5	0	0	0	0	0	0	0
9		6		**	-	-	-	-	-	-	-	-		**	-	-	-	-	-	-	-	-		27	0	0	-	-	0	0	0	0

[1] = number per fortnight
[2] = number during week 1 of the condition
[3] = number during week 2 of the condition
* = Participant did not complete the full 2 weeks of this condition due to the reoccurrence of constipation
(N) = Number of days on intervention milk before dropping out
** = Dropped out of the trial after a successful outcome on soy milk
- = missing values

Follow up statistical analysis, using contrasts and pair wise comparisons and a Repeated Measures ANOVA identified differences in the number of bowel motions per fortnight between the three conditions, cow's milk, washout and the soy milk condition as shown in Table 4.4. The most significant difference in conditions was between the cow's milk and soy milk conditions, $p=0.02$ (Pairwise comparisons) and $p=0.01$ (Repeated Measures ANOVA). The number of motions in the washout period after the soy milk was higher than that in the washout period after the cow's milk condition, but was not significantly different. Had there been more participants, there may have been a significant result. Non-parametric analysis was also performed to verify the parametric analysis in case lack of normality was important. The Freidman test for 3 related variables was applied to the number of bowel motions in each of the three conditions and showed statistical significance, $x^2(2) = 9.6$, $p=0.01$.

Table 4.4: Differences in the number of bowel motions between the trial conditions, cow's milk, washout and soy milk, N=8

Pair	Contrasts	Pairwise comparison	Repeated Measures ANOVA*	Wilcoxan Signed Ranked Test **
Cow versus washout	0.09	0.09	0.13	0.25
Cow versus soy	-	0.02	0.01	0.17
Washout versus soy	0.31	0.31	0.29	0.588

- \- Missing value due to not all conditions being completed by all participants
- * Wilcoxan Signed Ranked Test
- ** Wilcoxan Signed Ranked Test without participant 5

The number of motions for each subject during the cow's milk versus the soy milk condition for each participant is plotted below in Figure 4.2. The line of no change, that is, the point at which the number of motions during the cow's milk condition equals the number of motions during the soy milk condition is shown. All nine participants had a greater number of motions in the soy condition versus the cow's milk condition. Participant number 5 had an unusually high number of bowel motions per fortnight during the soy condition compared with the cow's milk condition. Participant 5 was removed from the data set and the data was

reanalysed using repeated measures ANOVA as above. Results were now not statistically significant, p = 0.62. This analysis showed that participant 5 had an influential effect on previously conducted analysis reaching statistical significance. Without this participant there would be no difference between the groups, cow's milk, washout and soy milk. However, the tests were repeated using non-parametric analysis with all subjects, p = 0.008, with the Repeated Measures ANOVA, because lack of normality was important with this number of participants, without participant 5. Results were then statistically significant, p = 0.02. As non-parametric methods are not as strongly influenced by very different observations, the change in the p value from the significance of p = 0.008 to 0.2 was not as severe as for the parametric method.

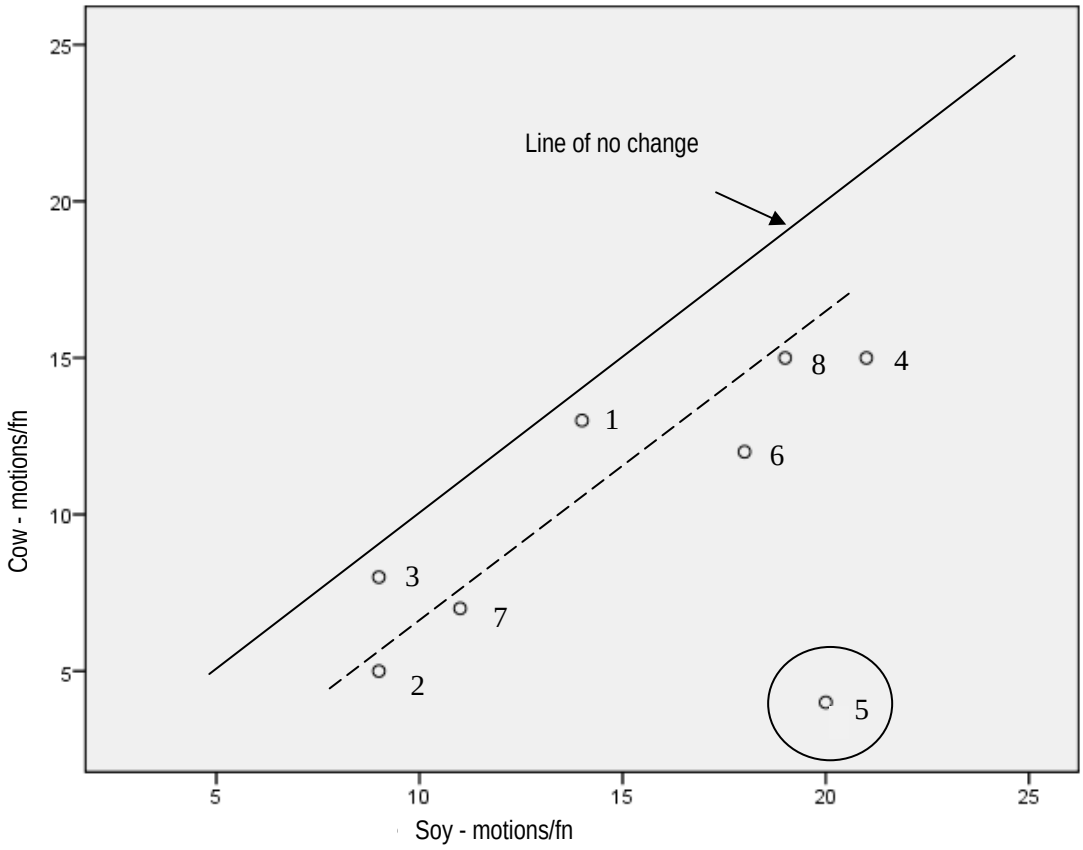


Figure 4.2: Total number of bowel motions per fortnight during the soy condition versus the cow's milk condition

--- shows a 3.7 unit shift towards soy

Follow up analyses using the Wilcoxan signed rank tests (for 2 related samples- paired t test equivalent) showed that the significant difference found with the Friedman test (for 3 or more related samples – Repeated Measures ANOVA equivalent) was due to the difference between soy and cow. This held for both analyses, with and without subject 5. See Table 4.4 for p values.

Stool Form and symptoms of constipation

Participants recorded stool form classified as type 1 through to 7 (see Appendix 10). Types 1 (separate hard lumps like nuts, hard to pass, result of slow transit), 6 (fluffy pieces with ragged edges, a mushy stool) and 7 (watery, no solid pieces, result of fast transit time) were considered abnormal. The number of abnormal stools for each participant during each condition is recorded in Table 4.3. Six out of the nine participants reported abnormal stools in the first week of the study. Five of these six participants commenced on the soy condition and the number of abnormal stools decreased in week 2. The other participant commenced on cow's milk and reported abnormal stools for the duration of the first condition. This suggests that first week of the soy condition acted as a washout period for CMP making the second week of results more reflective of the true effect of soy. Similarly, the most frequent straining and abdominal pain were recorded during the first week of soy as the first condition for seven participants. Six out of nine participants reported an increase in the number of abnormal stools on the cow's milk compared to soy milk.

Only one participant recorded severe pain, grade 3, during the study and this was during the cow's milk condition. Bloating was recorded equally frequently during the cow's milk and washout conditions and slightly less often during the soy period.

Microbiology of stool samples

Seven out of 13 participants (54%) had a low level of normal gut flora independent of milk condition. This result confirms the importance of adequate numbers of gut

flora for regularity of bowel motions (83). No identifiable change was reported in gut flora numbers in these participants within the time period of the trial. *Streptococcus B* was not identified in the faeces of any participants. *Streptococcus B* is usually identifiable in the bowel motions of non-constipated individuals (108).

4.3 Biochemistry

Eleven participants provided blood samples at one or more of the time periods, baseline (pre trial), time 2 (after condition 1) or time 3 (after condition 2). Abnormalities were detected in biochemical and immunological tests and a mixed model analysis of results for all 11 participants are shown in Table 4.5.

There was a high prevalence of abnormal readings at baseline independent of dietary condition. Four participants had liver enzyme abnormalities. High platelet levels were found in seven participants. Low haematocrit levels were identified in two participants. Three participants recorded high copper levels. In five participants ASO titre was detected and in 10 participants AntiDNase B was detected, confirming the presence of a current or chronic *Streptococcus B* infection.

There were statistically significant differences according to milk condition for a number of variables: platelets, monocytes, eosinophils and IgG. According to Estimated Marginal Means, platelets were significantly lower between the pre trial condition and after the cow's milk condition, $p = 0.02$. Monocytes were significantly higher between the soy condition and the cow's milk condition, $p = 0.01$. IgG was significantly lower between time 1 (baseline) and after the cow's milk condition, $p = 0.03$, and between the soy and cow's milk conditions, $p<0.001$. Elevated levels of eosinophils were approaching significance between the pre trial condition and after the cow's milk condition, $p = 0.08$. No other variables differed significantly by the dietary trial.

Table 4.5: Mixed model analysis of blood and serum measures on the trial conditions for N=11 participants

			Mean (N)			N (condition) B= baseline, C = cow's milk condition, S = soy condition			
Laboratory tests and Units	Reference range	Range Min-Max	Pre trial	Soy	Cow	Low	High	F Statistic	P Value (EMM)*
Hemoglobin g/L	(115-135)	116-151	134 (11)	134 (9)	133 (6)	0	0	F(8.3, 6.3) = 0.0	0.9
White cells 10^9/L	(5.5-15.5)	4.0-24.8	9.5 (11)	8.0 (9)	7.5 (6)	1(C)	2 (P)	F(9.4, 8.2) = 0.5	0.6
Platelets 10^9/L	(150-400)	139-489	359 (11)	362 (9)	278 (6)	1 (C)	7(4P, 3S)	F(9.3, 5.7) = 6.7	0.0* (0.0 [a])
Red cell count 10^{12}/L	(3.90-5.3)	3.9-5.4	4.8 (11)	4.8 (9)	4.4 (6)	1(C)	3 (P, C, S)	F(10.4, 6.6) = 0.4	0.7
Haematocrit L/L	(0.340- 0.4)	0.3-0.4	0.4 (11)	0.4 (9)	0.4 (6)	2(B,C)	0	F(8.5, 6.4) = 0.0	0.9
Neutrophils 10^9/L	(1.5-8.5)	1-13	4.7 (11)	3.7 (9)	2.7 (6)	4 (2B, 2C)	0	F(6.9, 8.3) = 1.5	0.3
Eosinophils 10^9/L	(<0.6)	0.1 to 0.8	0.3 (10)	0.3 (9)	0.5 (6)	0	3 (P, 2C)	F (8.8, 7.0) = 5.5	0.1* (0.1[c])
Monocytes 10^9/L	(0.1 -1.1)	0.1-1.2	0.4 (10)	0.6 (9)	0.4 (6)	0	1 (S)	F(7.4, 8.3) = 6.1	0.0* 0.0[b]
B12 pmol/L	(135-600)	151-619	330 (11)	361 (9)	321 (6)	0	2 (P, S)	F(9.1, 6.5) = 0.4	0.7
RC Folate nmol/L	(135-600)	509-1602	1031 (10)	1099 (9)	988 (6)	0	0	F(8.6, 5.8) = 1.01	0.4
Urea mmol/L	(2.5-6.0)	1.3-8.6	5.4 (11)	4.71 (9)	4.3 (6)	1 (C)	6 (3P, 2C, S)	F(11.0, 6.9) = 2.0	0.2
Creatinine umol/L	(40-70)	0-66	43.3 (11)	43.5 (9)	43.1 (6)	6 (3B,2C,S)	0	F(9.0, 6.0) = 0.0	1.0
Total protein g/L	(59-78)	63-83	70.6 (11)	72.2 (9)	70.9 (6)	0	1 (S)	F(7.7, 7.4) = 0.4	0.7
Albumin g/L	(28-41)	37-53	42.9 (11)	43.4 (9)	42.7 (6)	0	25 (11P, 9S, 5C)	F(10.4, 5.9) = 0.1	0.9
Liver enzymes GGT U/L	(<16)	6-28	10.7 (11)	10.7 (9)	11.7 (5)	0	4 (P, 2S, 1C)	F(10.4, 4.9) = 1.6	0.3
Alkaline Phos U/L	(86-315)	100-1818	348 (11)	234 (9)	347 (6)	0	2 (P, S)	F(6.7, 4.27) = 3.6	0.7
ALT U/L	(1-20)	1-236	21.4 (11)	35.7 (9)	29 (5)	0	6 (3P, 2S,1C)	F(9.7, 5.3) = 0.8	0.5
AST U/L	(1-45)	12-90	25.7 (11)	29.3 (9)	26.2 (5)	0	2 (P,S)	F(39.5, 5.7) = 0.4	0.7
Copper umol/L	(11-25)	14-30	19.9 (11)	20.9 (8)	19.9 (6)	0	3 (2P, S)	F(8.1, 6.2) = 0.3	0.7
Zinc (RBC) umol/L	(10-18)	9-15	132 (11)	139 (9)	140 (5)	2 (B,C)	0	-	-
Zinc (Serum) mol/L	(150-260)	88-198	12.4 (11)	12.3 (9)	9.4 (5)	12(3B,6S,3C)	0	F(1.5, 5.6) = 2.6	0.2
ASO titre n/a	(<170)	85-510	153 (11)	91.6 (8)	179 (6)	0	5(2P,3C)	F(6.2, 5.6) = 1.4	0.3
AntiDNaseB n/a	(<60)	60-1360	183 (11)	415 (9)	276 (6)	0	10(4P,3S, 3C)	F(3.48, 3.64) = 2.7	0.2
Serum IgA g/L	(0.3-1.5)	0.5-1.9	1.1 (10)	1.1(6)	1.7 (5)	0	0	F(8.9, 3.2) = 1.4	0.4
Serum IgG g/L	(4.4-11.0)	5.6-14.0	9.4 (10)	10.1 (6)	11.2 (6)	1 (B)	0	F(626 382, 153 506) = 4.30	0.0* (0.0[a]) (0.0[b])
Serum IgM g/L	(0.4-1.7)	0.7-2.8	1.4 (10)	1.5 (6)	1.8 (5)	0	2 (P,C)	F (5.9, 3.8) = 1.5	0.3
Serum IgE IU/mL	(<2-80)	11-852	176 (9)	155 (6)	145 (4)	0	3 (P)	F(31 888, 14.6) = 1.4	0.3

- = A mixed model analysis could not be performed for some variables due to numerous missing values.
c = Approaching significance between pre trial and cow's milk
* = Estimated Marginal Means was performed if p value was significant or approaching significance
a = Significant between pre trial and cow's milk
b = Significant between soy and cow's milk

4.4 Discussion

This study examined children with CFC who at baseline had symptoms associated with CMP allergy or intolerance or a family history of symptoms such as asthma and recurrent ear infections which may have indicated the need for dietary assessment of the constipation (110). All children recruited to the study were passing less than eight bowel motions per fortnight at baseline. There was a high prevalence of abnormal biochemical and immunological readings for participants at baseline. These will be discussed with along with those found for Trial 2 in Chapter 7. Faecal analysis showed a low level of normal gut flora in half of all participants. *Streptococcus B* was not found to be present in any of the participant faecal samples. It can be suggested that lack of *Streptococcus B* may be a simple pathology test of faeces samples to identify children with CFC who may respond to the removal of CMP from the diet. Further investigation with larger numbers is required to confirm this possibility.

Assessment of participants' dietary intakes showed that many participants were consuming greater than the recommended number of serves of milk, yoghurt and cheese. The response of children being drawn to the foods that cause them a problem has been shown in other studies (111). All participants were consuming processed foods containing CMP as an ingredient on a daily basis. This would have contributed to the total amount of dietary CMP consumed.

The results confirm the hypothesis that, children in the Australian setting with CFC that is unresponsive to the usual treatments will respond to the removal of CMP from the diet. Removal of CMP from the diet provided a statistically significant response between the soy and cow's milk condition. One hundred percent of participants experienced resolution of their CFC on soy milk, compared with 68% in the study by Iacono and colleagues (3). However, in 62% of subjects the constipation was resolved while none of the children in Iacono and colleagues study

reported a response during the cow's milk condition (3). However, the Iacono and colleagues study was not completely CMP free for the entire duration of the study. During the one-week washout period, children were allowed to consume an unrestricted diet, including cow's milk and soy milk and their derivatives, and the number of bowel motions during this period was not reported by the researchers. This difference in dietary condition may explain the different results between the two studies.

For this trial, participants were required to consume a CMP free diet for its six week duration. This may have decreased the total amount of CMP consumed in comparison to participant's pre-trial diet (even with the extra 400 mL for the cow's milk condition). This suggests that there may be a threshold to the amount of CMP that can be tolerated, above which constipation occurs. This dose-related response is typical of food intolerances rather than food allergy which elicits an immediate response from a small amount of the food allergen (112).

The most significant difference in mean stool numbers involving dietary conditions was between the cow's milk and soy dietary conditions. Had there been a larger number of participants, the other dietary conditions may have reached statistical significance. Reanalysis of the data using non-parametric analysis confirmed statistical significance even after removal of the outlying participant. One participant in nine may be a typical population response, that is, 10% of the population will respond unusually.

For the majority of participants, stool form which is indicative of transit time (104), normalised or improved during the washout period (free of cow's milk and soy milk). For seven out of nine participants the washout followed the soy milk condition as a result of the randomisation at the beginning of the trial. This could have been avoided with a two week CMP free washout period prior to commencement of the study. It might be that the longer cow's milk has been

removed from the diet, the greater the improvements to stool form and transit time. The number of abnormal stools increased during the cow's milk trial for two thirds of participants. It may not have increased for the other third due to poor reporting of stool form by participants which is very subjective, or these children may have had no adverse effects of CMP.

The strengths of this trial have been identified. The crossover design method with subjects being their own controls is an advantage. The two-week washout period free from cow's milk and soy milk and their derivatives, in comparison with the Iacono and colleagues study, removed any physiological effects of the previous intervention milk. Maintaining a record of bowel outcome measures during the washout period allowed this period to be considered as a dietary condition.

A number of limitations have been identified. The children and their parents were aware of the differences in type of milk during the trial, via both taste and labelling, which may have had a positive psychological effect on some participants' constipation if they believed cow's milk to be a possible cause. Psychological factors have been frequently suggested as a cause of constipation (3, 113, 114). This limitation was overcome in Trial 2 which allowed double blinding. Another limitation is that the immunological and biochemical of variables may have needed more than two weeks to change significantly. The random assignment of participants by the research assistant to intervention milk was not even in the final participant population, 2 participants commenced on cow's milk β casein A1 and 7 commenced on soy. This assignment was done as each participant consented and commenced the study. This would have been more evenly distributed had the trial had more participants. For at least one participant commencing on soy and achieving a resolution of constipation resulted in a withdrawal from the study half way through so the commencing milk affected the research process, if not the results.

4.5 Summary of Chapter 4

The results of Trial 1, cow's milk casein A1 versus soy milk confirm the hypothesis that children in the Australian setting with CFC unresponsive to the usual treatments respond to the removal of CMP from the diet. One hundred percent experienced resolution of their constipation during the soy milk condition compared with 68% experiencing resolution during the soy milk condition in the Iacono and colleagues study. The fact that five out of eight children also reported a response during the cow's milk condition in this study suggests that a threshold is occurring whereby they are able to tolerate some CMP before the symptom of constipation appears.

The results in this trial pose an important question, 'what is it about CMP that is causing the symptom of constipation?' It was hypothesised that cow's milk β casein A1 may be the culprit in chronic functional constipation due to anecdotal reports that people unable to tolerate cow's milk β casein A1 could tolerate cow's milk β casein A2. This hypothesis was investigated in Trial 2, cow's milk casein β A1 versus cow's milk β casein A2. The results of this study are shown in Chapter 5.

Chapter 5: The effect of cow's milk β casein A1 versus cow's milk β casein A2 on chronic functional constipation: results of Trial 2

5.0 Chapter outline

This chapter describes the results of a double blind crossover trial comparing the effects of cow's milk β casein A1 with cow's milk β casein A2 on chronic functional constipation in children. This trial is referred to as Trial 2 and was conducted in Newcastle, New South Wales. The research questions and hypotheses for Trial 2 were as follows:

Research Question 2: What effect does cow's milk β casein A1 and cow's milk β casein A2 have on CFC in children who do not respond to traditional treatments?

2.1 Hypothesis for Trial 2: Constipation would resolve for children whilst consuming cow's milk casein A2 and whilst avoiding all sources of A1 cow's milk protein.

Research Question 3: What are the immunological and biochemical mechanisms underlying CFC that responds to the removal of CMP in children?

3.2.1 Blood Hypotheses: A description of each of the blood factors monitored and a hypothesis statement related to each factor and whether they were expected to change during the study period is described in Appendix 2.

3.2.2 Faeces Hypothesis: Normal gut flora and *Streptococcus B* would be absent or low at baseline (time 1), and would resolve for children during the washout period and whilst consuming cow's milk β casein A2.

The methods for Trial 2 were described in chapter 3.

5.1 Participant characteristics at baseline

5.1.1 Factors related to allergy

Forty participants were recruited, with one excluded from the study due to a subsequent diagnosis of coeliac disease leaving 39 participants, 25 males and 14 females. Participants' ages are shown as a categorical variable in Table 5.1. The mean age of participants was 67 months (±35months) and the range was 21 to 143 months. The characteristics, family history and clinical history of these participants are also shown in Table 5.1.

Some participants reported a family history of cow's milk protein allergy or intolerance. Initial clinical assessment of participants showed that a number had symptoms associated with cow's milk protein allergy or intolerance, including, asthma, ear infections and grommets (110) but only three of these participants had been diagnosed with cow's milk protein allergy or intolerance.

Eleven participants were reported by parents to have delayed development in achieving age related milestones; however, no formal diagnosis of a condition or syndrome had been made. Psychosocial disruption of the family due to their child's behaviour was reported by 17 families.

As for Trial 1, there was a high prevalence of biomarker abnormality in the participants at baseline, which will be discussed together with the intervention results below.

Table 5.1: Characteristics, age, family history and clinical history of 39 participants recruited to the cow's milk casein A1 versus cow's milk casein A2 study

Characteristics and history	Participants reporting characteristic of history	
	n	%
Age		
<3 years	10	25.6
4-6 years	19	48.7
7-9 years	6	15.3
10-12 years	4	10.2
Family history of CMPA or CMPI	7	17.9
Personal history of CMPA or CMPI	3	7.7
Physical symptoms of intolerance to cow's milk:		
Dermatitis, eczema, rhinitis	20	51.3
History of asthma	16	41.0
History of ear infections	9	23.1
Grommets	2	5.1
History of tonsillitis/throat infections	4	10.2
Tonsils removed	2	5.1
Adenoids removed	3	7.7
Gestation and birth characteristics		
Maternal thrush during pregnancy	4	10.3
Delivered by caesarean section	8	20.5
Breastfed	12	30.7
Development delayed	11	28.2
Behavioural issues		
Consultation with psychologist	6	15.4
Psychosocial disruption of the family	17	43.6
History of recurrent UTI's	9	23.1
Constipation symptoms		
Soiling or encopresis	25	64.1
Abdominal pain	28	71.8
Anal pain	27	69.2
Anal bleeding	14	35.9
Diagnosis of perianal dermatitis	8	20.5
Current symptoms of		
Poor appetite	22	56.4
Nausea or vomiting	8	20.5

5.1.2 Dietary intake at baseline

Results of the nutritional assessment are reported in Figure 5.1. This assessment identified that some participants were consuming greater than the recommended number of serves of fruits, vegetables and legumes. Seventeen out of 39 participants were consuming greater than the recommended number of serves of milk, yoghurt and cheese. More than half were consuming red meat less than the recommended 3-4 times per week. All participants were consuming processed foods containing CMP as an ingredient on a daily basis.

Australian Guide to Healthy Eating Food Categories (101)						Extras - Processed Foods containing cow's milk protein as an ingredient					
Participant	Breads and cereals	Vegetables and Legumes	Fruit	Milk, Yoghurt & Cheese	Meat, Fish, Poultry, Nuts & Legumes Red meat no. of times/wk	Fats & Oils	Convenience Foods eg pizza, nuggets hot dogs fish fingers etc At least once per week	Confectionary –Chocolate\ At least once per week	Biscuits and Cakes At least once per day	Snack Foods eg chips pop corn and muesli bars At least once per day	Ice Cream At least once per week
1	CMP				<1						
2	CMP				<1						
3	CMP				1						
4											
5											
6											
7											
8					1						
9					1						
10											
11					0						
12	CMP										
13											
14	CMP				<1						
15	CMP				1						
16	CMP				<1						
17	CMP				1						
18	CMP										
19	CMP										

Met recommended no. of serves for age group
Exceeded recommended no. of serves for age group
Lower than recommended no. of serves for age group
Consumed processed foods containing milk as an ingredient as described
Did not consume processed foods containing milk as an ingredient as described

CMP = Breads and cereals consumed contained CMP

Figure 5.1: Nutritional Assessment of the diet history of 39 participants using the Australian Guide to Healthy Eating and showing consumption of processed foods containing cow's milk protein as an ingredient.

5.2 Primary outcome measure: resolution of constipation

Twenty-six participants returned constipation diaries for the study period, however, one participant did not complete the full two weeks of any of the three conditions due to the reoccurrence of constipation after commencing with the cow's milk β casein A1. This participant was not included in any statistical analysis. The number of cases of constipation resolution, defined by eight or more bowel motions per fortnight and the mean number of bowel motions per fortnight under each condition for participants is shown in Table 5.2. The washout period acted as a condition in itself as it was free from all milk and soy protein.

Table 5.2: Cases of resolution of constipation and mean bowel motions during a 2 week condition for 25 participants

Milk condition	Pre trial[1]	Cow's milk β casein A1	Mean (SD) Washout[2]	Cow's milk β casein A2
	N = 26	N = 22 [3]	N = 23[3]	N = 25[3]
Resolution of Constipation N (%)	0 (0)	14 (64)	18 (78)	16 (64)
Bowel motions per fortnight Mean (SD)	4.42 (1.55)	10.05 (5.75)	10.43 (5.05)	10.56 (5.24)

1 All are constipated to be eligible for trial
2 The washout period acted as a condition in itself as it was free from all milk and soy protein.
3 The number of participants in each condition is not the same because some participants ceased participation in the study after the various conditions

The highest observed resolutions, 18 (78%) participants, occurred during the washout period, when no cow's milk protein was being consumed. There was no difference in the mean number of bowel motions per fortnight, although there was a tendency to a higher number for the cow's milk β casein A2 condition.

Mean number of stools prior to commencing the trial compared with each of the trial dietary conditions is shown in Table 5.2 for each of the twenty-five participants with constipation diaries. The mean number of bowel motions increased from baseline for all participants.

Table 5.3: Number of stools per fortnight at baseline compared with each of the trial conditions and symptoms of straining, abdominal pain/discomfort and bloating reported per week by participants with condition order A1, washout, A2, N=11

Participant Number	*Prior to Study*	Stools[1]	*Cow's milk casein A1*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]	*Washout*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]	*Cow's milk casein A2*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]
1		6		10	0	0	1	0	0	0	5	2		12	1	0	0	0	0	1	-			6	1	0	0	0	0	0	2	0
4		4		6	0	0	1	0	1	1	1	0		9	0	0	0	0	0	0	0	0		7	1	0	0	0	0	0	1	0
5		6		13	2	3	1	6	3	6	3	5		13	0	0	4	4	2	3	4	3		16	2	1	3	2	5	1	3	3
8		4		3	0	0	0	0	3	1	4	0		4	0	0	0	0	0	1	0	0		3	0	2	0	0	2	0	0	0
10		2		11	0	0	3	1	6	2	0	0		6	0	0	2	0	5	2	0	0		8	0	1	0	3	0	0	0	0
11		4		9*	2	0	1	3	1	0	4	0		-	0	0	0*(1)	-	0*(1)	-	0*(1)	-		-	0	0	0*(1)	-	0*	0*	0*(1)	-
13		2		7	0	0	0	2	2	3	4	3		8	0	0	1	0	2	0	2	0		8	2	0	1	1	1	0	1	2
17		4		8	0	0	0	1	1	1	0	0		11	4	2	0	0	0	0	0	0		9	2	1	0	0	1	0	0	0
18		6		23	0	0	5	3	1	0	0	1		14	0	0	3	5	0	0	0	0		20	1	0	5	2	0	0	0	0
19		6		18	1	0	0	0	0	0	1	1		8	0	0	0	0	0	0	0	0		7	0	0	0	0	0	0	0	0
20		6		8	7	5	0	0	0	0	0	0		10	7	7	0	0	0	0	0	0		10	0	1	0	0	0	0	0	0

[1] = number per fortnight
[2] = number during week 1 of the condition
[3] = number during week 2 of the condition
* = Participant did not complete the full 2 weeks of this condition due to the reoccurrence of constipation
(N) = Number of days on intervention milk before dropping out
- = missing values

Table 5.4: Number of stools per fortnight at baseline compared with each of the study conditions and symptoms of straining, abdominal pain / discomfort and bloating reported by participants with condition order A2, washout, A1, N=15

Participant Number	*Prior to Study*	Stools[1]	*Cow's milk casein A1*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]	*Washout*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]	*Cow's milk casein A2*	Stools[1]	Abnormal[2]	Abnormal[3]	Straining[2]	Straining[3]	Pain[2]	Pain[3]	Bloating[2]	Bloating[3]
2		6		9	0	0	4	4	0	0	0	0		13	1	0	3	3	1	0	0	0		12	0	0	5	5	3	2	0	0
3		7		*	0	0	-	-	-	-	-	-		*	0	0	-	-	-	-	-	-		6	3	1	0	0	0	2	2	1
6		6		4(5)**	2	0	4*(4)	-	3*(4)	-	0*(4)	-		17	4	2	2	3	2	3	0	0		23	2	2	5	4	3	0	3	0
7		6		8	0	0	0	0	0	-	0	-		17	0	0	0	0	0	0	0	0		19	2	2	0	0	0	0	0	0
9		4		14	1	0	4	2	4	0	5	1		18	1	1	1	2	2	2	0	0		10	2	0	4	2	3	2	4	2
12		4		-	0	0	0**	-	0**	-	0**	-		-	0	0	1*(2)	-	0**	0**	0**	0**		6	0	0	0	2	2	1	4	1
14		2		-	0	0	0*(0)	-	0*(0)	-	0*(0)	-		3(7)*	0	0	0*(1)	-	0*(1)	-	0*(1)	-		6	0	0	2	0	2	6	4	6
15		4		24	0	1	4	4	5	4	6	5		22	1	2	7	1	7	0	7	4		6	1	3	4	7	4	7	6	7
16		2		15	1	1	3	2	2	0	2	0		11	3	1	0	3	1	1	0	2		13	2	1	5	5	5	3	3	3
21		6		10	2	0	0	0	0	0	0	0		9	1	1			0	0	0	0		12	3	0	0	0	0	0	0	0
22		4		-	0	0	0**	0**	0**	0**	0**	0**		-	0	0	0**	0**	0**	0**	0**	0**		18	7	4	0	3	5	5	3	6
23		4		5	0	0	0	1	0	0	0	1		6	1	0	1	1	1	0	2	1		6	1	1	2	2	2	1	0	0
24		4		5	0	0	1	1	1	1	0	1		5	0	0	1	0	0	2	1	2		7	0	0	0	1	3	0	0	0
25		2		5	2	3	0	0	0	0	0	0		10	0	1	0	0	0	0	0	0		13	1	1	2	0	0	0	0	0
26		4		6	0	0	5	7	5	6	0	0		12	1	0	2	0	2	2	0	0		13	2	0	5	4	3	0	3	0

[1] = number per fortnight
[2] = number during week 1 of the condition
[3] = number during week 2 of the condition

* = Participant did not complete the full 2 weeks of this condition due to the reoccurrence of constipation
(N) = Number of days on intervention milk before dropping out
\- = missing values

A comparison of the number of resolutions during the cow's milk β casein A1 and cow's milk β casein A2 conditions is shown in Table 5.5.

Table 5.5: Clinical outcomes for participants who completed all 3 conditions: cow's milk β casein A1 condition compared with the cow's milk β casein A2 condition, N=21.

		Cow's milk casein A2		
		Less than 8 motions	More than 8 motions	Row Total
Cow's milk β casein A1	Less than 8 motions	3	6	9
	More than 8 bowel motions	3	9	12
	Column Total	6	15	21

From the total column in Table 5.5, twelve participants (57%) had an improved number of bowel motions on cow's milk β casein A1. From the row total, fifteen participants (71%) improved on cow's milk β casein A2. However, because each participant participated in both conditions in the study there is more to be understood about Table 5.5. Examining the results jointly, that is, for the A1 and A2 conditions together, three participants (10%) did not resolve during either the cow's milk β casein A1 or cow's milk β casein A2 conditions. Nine participants (43%) resolved under both conditions. Further, six (29%) participants resolved on A2 but did not resolve on cow's milk β casein A1 (four out of these six commenced on A2). Three (14%) did not resolve on cow's milk β casein A2, but did resolve on cow's milk β casein A1 (two out of the three commenced on A1). It is these last two cells that provide the information as to whether A2 differs from A1 and is assessed using the McNemar test for paired categorical data. Based on this analysis, the percentages that resolved on cow's milk β casein A2, 15 participants (71%) and cow's milk casein A1, 12 participants (57%), were not significantly different, p= 0.51.

The resolution rate was highest during the washout condition at 81%, when no CMP was consumed at 81%. The results for all three conditions are summarised in Table

5.6. The testing of the differences is by the McNemar test as described in the Table 5.5, but the details of the paired tables have not been provided. The difference between washout and the two milk conditions was not significant, for cow's milk β casein A1 p=0.12 and for cow's milk casein A2, p=0.62.

Table 5.6: Percentage of participants with constipation resolved for the three conditions, cow's milk casein A1, washout condition and cow's milk casein A1, N= 21

	Percent resolved	Mean (SD)
Cow's milk casein A1	57	0.57 (0.507)
Cow's milk casein A2	71	0.71 (0.463)
Washout	81	0.81 (0.402)

Table 5.6 shows a higher percentage resolution on cow's milk β casein A2 than cow's milk β casein A1, but the results were not statistically significant.

The data for the 21 participants who completed all three dietary conditions were also analysed using the number of motions per fortnight. A mixed model analysis was conducted using a grouping variable comprising the three experimental conditions (A1, A2 and washout) as the explanatory factor and an unstructured covariance structure within subject residuals. There was no statistical significance between the three conditions, cow's milk β casein A1, washout and cow's milk casein A2, $F(2, 39.2) = 0.90$, $p = 0.42$.

In summary, there was no statistical significance found between the three conditions. As neither of the two conditions was demonstrably different, nor different to washout, it appears participating in the trial was the most important effect, raising the percent resolved from 0% prior to the trial to an average level of (57+71+82)/3 = 70% over the 6 weeks of the study.

Stool form and symptoms of constipation

The number of abnormal stools for each participant during each condition is recorded in Table 5.4. Twenty-two out of 26 participants recorded abnormal stool forms during the six week trial. Those consuming cow's milk β casein A2 in the first

two weeks reported more abnormal stool forms and there were more abnormal stool forms recorded in first week of the dietary condition than the second week of the condition. Participants consuming cow's milk β casein A1 in the first two weeks reported more occasions of abnormal stool forms than in the washout condition and the cow's milk β casein A2 condition. Symptoms lessened as the trial progressed. Thirteen out of 21 participant's symptoms of straining, abdominal pain and bloating decreased as the six week trial progressed from week 1 to week 6, independent of the condition. Symptoms remained equal for three out of 21 participants independent of trial milk.

Microbiology of stool samples

Analysis showed that eight out of 39 participants (20.5%) had a low level of normal gut flora. *Streptococcus B* was not identified in the faeces of any of the 39 participants.

Blood results

Thirty-three participants provided blood samples at one or more of the time periods, baseline (time 1), time 2 or time 3. Abnormalities were detected in biochemical and immunological tests for all participants. The mean (SD) values and results of a mixed model analysis are shown in Table 5.7.

There was a high prevalence of abnormality at baseline. Seventeen participants had liver enzyme abnormalities. High platelet levels were found in 14 participants, low haematocrit levels in 12 participants and high albumin levels in the thirty participants who had it tested. Twenty-one participants recorded low red blood cell zinc levels. Zinc levels were shown to improve for participants during the trial. In seven participants ASO titre was detected and in nine participants AntiDNase B was detected, confirming the presence of a current or chronic *Streptococcus A* infection.

There were statistically significant differences for urea, creatinine, total protein, ALT and AST. Estimated Marginal Means was used to test statistical significance. Urea was significantly different due to an increase between the pre trial condition and after the cow's milk casein A2 condition, $p<0.001$. Creatinine was significantly lower between the pre trial condition and after the cow's milk casein A2 condition, $p<0.001$. The liver function enzymes, ALT and AST were significantly increased between the pre trial tests and after the cow's milk β casein A1 condition, $p<0.001$. No other variables differed significantly by the dietary trial.

Table 5.7: Results of mixed model analysis for the trial conditions and abnormal blood and serum for N= 33 participants

			Mean (N)			N (condition) B= baseline, A1= A1 condition, A2= A2 condition			
Laboratory tests and units	Reference range	Range Min-Max	Pre trial	A1	A2	Low	High	F Statistic	P Value (EMM)[x]
Hemoglobin g/L	(115-135)	97-144	121.6(31)	122.5(13)	120.4(16)	9(5B,A1,3A2)	0	F(11.6,10.5)=0.7	0.5
White cells 10^9/L	(5.5-15.5)	3.8-13.3	8.6(31)	8.2(13)	7.4(16)	4(B,A1,A2)	0	F(19.4,13.8)=1.9	0.2
Platelets 10^9/L	(150-400)	175-560	355.3(31)	346.9(13)	304.3(16)	0	18(10B,5A1,3A2)	F(14.9,12.9)=1.6	0.2
Red cell count 10^{12}/L	(3.90-5.30)	3.2-5.5	4.4(31)	4.4(13)	4.3(16)	4(2B,2A2)	1(B)	F(9.8,9.4)=2.4	0.1
Haematocrit L/L	(0.340-0.40)	0.3-0.4	0.4(31)	0.4(13)	0.4(16)	16(8B,A1,4A2)	0	F(12.4,12.8)=1.2	0.3
Neutrophils 10^9/L	(1.5-8.5)	0.5-7.4	3.7(31)	3.6(13)	3.1(16)	3(3B)	0	F(16.9,12.3)=1.9	0.2
Eosinophils 10^9/L	(<0.6)	0.0-1.0	0.3(31)	0.3(13)	0.2(16)	0	6(4B,A1,A2)	F(20.4,15.8)=1.6	0.2
Monocytes 10^9/L	(0.1-1.1)	0.0-1.2	0.5(31)	0.5(13)	0.5(16)	0	3(B,A1,A2)	F(17.4,13.2)=0.3	0.8
B12 pmol/L	(135-600)	0-850	333.0(31)	352.5(13)	357.4(16)	1(B)	4(2B,A1,1A2)	F(19.5,13.5)=0.4	0.7
RC Folate nmol/L	(135-600)	180-2011	1011.4(31)	982.5(13)	1002(16)	1(B)	6(5B,1A2)	F(16.9,15.7)=0.1	0.9
Urea mmol/L	(2.5-6.0)	2.9-8.4	5.5(33)	4.8(12)	4.8(16)	0	11(7B,2A1,2A2)	F(21.6,16.7)=5.0	p<0.001* p<0.001[a]
Creatinine umol/L	(40-70)	14-53	38.2(33)	34.9(12)	34.6(16)	38(22B,8A1,82)	0	F(17.3,14.9)=3.4	0.1* 0.1[a]
Total protein g/L	(59-78)	62-80	70.4(33)	72.7(12)	71.7(16)	0	0	F(19.5,15.4)=2.7	0.1
Albumin g/L	(28-41)	35-50	42.0(33)	41.7(12)	42.2(16)	0	56(32B,12A1,12A2	F(12.9,12.0)=0.3	0.7
Liver enzymes								F(19.5,14.5)=0.5	0.6
GGT U/L	(<16)	1-18	10.5(33)	9.7(12)	10.5(16)	0	8(5B,3A2)		
Alkaline Phos U/L	(86-315)	116-524	239.9(33)	235.6(14)	241.7(14)	0	4(2B,A1,A2)	F(21.4,13.8)=0.4	0.6
ALT U/L	(1-20)	10-52	21.6(33)	18.0(14)	19.6(14)	0	8(7B,A2)	F(13.9,14.4)=4.9	p<0.001* p<0.001[b]
AST U/L	(1-45)	16-58	31.0(33)	28.5(14)	29.4(14)	0	1(A2)	F(20.6,15.4)=2.5	0.1* p<0.001[b]
Copper umol/L	(11-25)	0-27	17.8(33)	16.5(14)	18.3(14)	1(B)	0	F(12.1,10.6)=0.9	0.4
Zinc (RBC) umol/L	(10-18)	0-192	127.3(33)	129.5	120.6(14)	14(10B,2A1,2xA2)	0	F(18.4,17.1)=0.2	0.9
Zinc (Serum) mol/L	(150-260)	8-18	11.4(33)	12.1(14)	12.0(14)	41(20B,11A1,10A2	0	F(17.6,16.5)=1.6	0.2
ASO titre n/a	(<170)	80-510	92.8(33)	107.6(14)	129.8(14)	0	10(5B,A1,4A2)	F(16.8,14.9)=1.4	0.3
AntiDNaseB n/a	(<60)	60-2720	322.9(33)	243.6(14)	272.3(14)	0	16(8B,3A1,5A2)	F(20.9,14.8)=0.6	0.6
Serum IgA g/L	(0.25-1.52)	0.24-170	1.0(33)	26.8(14)	1.0(14)	8(6B,2A2)	3(3A1)	F(14.1,14.1)=1.7	0.2
Serum IgG g/L	(4.44-11.90)	0.69-12.7	8.8(33)	8.2(12)	9.1(16)	2(2B)	0	F(19.4,14.6)=1.4	0.3
Serum IgM g/L	(0.41-1.86)	0.4-9.6	1.1(33)	1.6(12)	1.1(16)	3(1B,A1,1A2)	2(A1,A2)	F(16.0,14.2)=0.5	0.6
Serum IgE IU/mL	(<2-80)	1-2153	122.9(33)	96.3(12)	107.2(16)	0	11(6P,2A1,3A2)	F(21.1,14.7)=1.2	0.3

a = Significant between pre trial and cow's milk β casein A2

b = Significant between pre trial and cow's milk β casein A1

* = Estimated Marginal Means was performed if p value was significant or approaching significance

5.3 Discussion

This study examined children with CFC many of whom at baseline reported symptoms associated with or had a family history of cow's milk protein allergy with cow's milk protein allergy or intolerance, including, asthma, ear infections and grommets (110) Only three of these participants had been diagnosed with cow's milk protein allergy or intolerance. All children recruited to the study were passing less than eight bowel motions per fortnight. There was a high prevalence of abnormal biochemical and immunological findings for participants at baseline. These will be discussed in conjunction with those found for Trial 1 in Chapter 7 as there were similarities in the findings between the two studies. There were no distinguishable trends in more than a few participants who showed improvement during the trial period. This may have been because many participants provided only the baseline blood sample. Faecal analysis showed a low level of normal gut flora in some participants and lack of *Streptococcus B* in all participants. The presence of normal gut flora is essential for normal gut transit time and the prevention of constipation (83). *Streptococcus B* is usually present in the bowels of healthy individuals (108). It is suggested that the lack of *Streptococcus B* may be a simple pathology test of faeces samples to identify children with CFC who may respond to the removal of CMP from the diet. Further investigation with larger numbers of participants is required to confirm this possibility.

Many participants were consuming greater than the recommended number of serves of milk, yoghurt and cheese at baseline. The response of children being drawn to the foods that cause them a problem has been shown in other studies (111). All participants were consuming processed foods containing CMP as an ingredient on a daily basis. This would have contributed to the total amount of dietary CMP consumed.

The results disproved the hypothesis that constipation would resolve for children whilst consuming cow's milk β casein A2 and whilst avoiding all sources of cow's milk protein. However, the number of bowel motions increased in each of the conditions, in comparison to the number of motions reported prior to the study for all participants. Removal of CMP from the background diet increased the number of bowel motions for participants: cow's milk β casein A1 (64%), cow's milk β casein A2 (64%) and the washout condition (78%) in comparison to the number of bowel motions prior to the study. Although there was an increase in the number of bowel motions, results were not statistically significant unlike Trial 1 that was statistically significant despite its small number of participants. This lack of statistical significance is due to the closeness in number of participants who reported a response in the three conditions.

The fact that for many of the children the number of bowel motions increased during the three dietary conditions, cow's milk β casein A1 condition, the washout, and the cow's milk β casein A1 condition in comparison to their background diet prior to the trial suggests that a threshold is occurring. Children were consuming much less CMP during the trial than their previous diets. Children are able to tolerate some CMP before the symptom of constipation appears. As occurred in Trial 1, this suggests that it could be a food intolerance reaction occurring or there is some other component in cow's milk that is causing the problem in these children.

All participants reported more occasions of abnormal stool in the first two weeks of the trial despite whether they started on cow's milk β casein A1 or cow's milk β casein A2 condition. Participants' symptoms of straining, abdominal pain and bloating also decreased as the trial progressed. Symptoms all lessened as the trial progressed. This may have been due to a carryover effect from the pre trial diet since it takes seven to eight days to clear cow's milk protein and its influence from the body (39). The longer that cow's milk has been decreased in the diet, the greater the improvements to stool form and other abdominal symptoms.

A strength of this trial is the crossover design with subjects being their own controls. Another strength is the two week washout in between the two intervention milks to clear any physiological or psychological effects of the first intervention milk before commencing the second intervention milk. The double blind study design is also a strength of the study. This prevented any psychological bias participants may have toward one intervention milk or the other. Psychological factors have been frequently suggested as a cause of constipation (4, 113, 114). It also prevents any bias by the researcher towards the trial results from participants on one type of milk intervention.

A number of limitations have been identified. A washout period prior to commencement of the trial would have been beneficial for more accurate results and remove any effects of the background diet. Longer periods on the intervention milk may have shown more changes in immunological and biochemical variables. However, this would have increased participant burden further. The random assignment of participants by the research assistant to intervention milk was not even, 11 commenced on cow's milk β casein A1 and 15 commenced on cow's milk β casein A2. This may have been more evenly distributed had the diet continued for a longer period of time however, it was necessary to close of the trial due to time constraints.

5.4 Summary of Chapter 5

In this Chapter the results of Trial 2, cow's milk β casein A1 versus cow's milk β casein A2 were presented. The results of this trial disproved the hypothesis that constipation would resolve for children during the washout period and whilst consuming cow's milk β casein A2. Even though the number of stools reported by participants increased in comparison to prior to commencement of the trial, there was no statistical significance between the conditions cow's milk β casein A1, washout and cow's milk β casein A2. The fact that the number of bowel motions

improved for participants in comparison to the pre-trial diet suggests that it is not an allergic reaction occurring but an intolerance. These children are able to tolerate some CMP before the symptom of constipation appears.

Chapter 6 describes the qualitative study that was conducted with participants at the conclusion of Trial 1 and Trial 2. This study examines the experiences of families whilst being on a cow's milk free diet and being involved in a dietary trial.

Chapter 6: Maternal experiences of applying a CMP free diet: a qualitative exploration

6.0 Chapter outline

In this chapter, the qualitative study that formed the last part of the study protocol for Trial 1 and Trial 2 is described. A qualitative phone debriefing at the conclusion of each of the trials was used to evaluate the ease or difficulty of following the study protocol, in particular, following a milk free diet. Parents were also given the opportunity to debrief over the experience of participating in the research. The qualitative study was specifically undertaken for this master's research and aimed to investigate the experiences of families following a CMP free diet. For qualitative reports, most of the discussion occurs in the results section. The implications of these findings for practice are described.

6.1 Introduction and rationale

The results of Trial 1 showed that children who avoid CMP had resolution of their constipation. The results of Trial 2 showed that cow's milk β casein A2 was not the answer to this problem. There is some other component in cow's milk causing the problem. These children unresponsive to the usual treatments for constipation will need to avoid CMP. Therefore, it is important to determine whether the CMP free diet is feasible for families.

Few studies have focused on the lived experience of following a specialised diet for allergy or intolerance. Two studies on autism were identified that found dietary modification is often difficult and more expensive to implement than a family's usual self chosen diet. A special diet raises difficulties such as social isolation, changes required to shopping and cooking practices and lack of support from medical practitioners (115). Parents reported being faced with deciding whether the benefits of the diet outweigh the difficulties of its implementation (116). A review of

the literature revealed a lack of studies of this type in relation to a CMP free diet and CFC, therefore exploratory research was required.

6.2 Method

Qualitative research methods are used to explore an area where little is known or not previously understood. The methods can be used to understand lived experiences (117). The research aim for this study was to find out how feasible it was to implement a CMP free diet for a child under 12 with CFC.

6.2.1 Development of questions for the qualitative debriefing interview

A series of six open ended questions were developed to gather information about the lived experience of following a milk free diet. These questions are shown in Appendix 11. The questions focused on what it was like to follow a milk free diet and whether participants felt they received enough information and support to follow the diet adequately. The interview schedule was administered in a semi-structured fashion, with all questions covered, but with some variations in the order and wording to allow for participant responses and the conversational style of the interview. Some prompts were added after the first three interviews to encourage participant to verbalise ideas.

A variation to extend this research in order to include this qualitative study was approved by Hunter New England Area Health Service Ethics Committee and the University of Newcastle Human Research Ethics Committee (Reference 03/08/13/12/3.23). The approval documentation is shown in Appendix 12. The existing consent form was modified to include the qualitative debriefing (see Appendix 13) and a letter was developed to invite previous participants to participate (see Appendix 14).

6.2.2 Procedure

Mothers of the children who had completed one of the six week dietary trials, Trial 1, cow's milk β casein A1 versus soy milk or Trial 2, cow's milk β casein A1 versus cow's milk β casein A2, were invited to participate in the qualitative interview.

Participants' parents were contacted as close as possible to completing the final dietary intervention and an appointment made for a telephone interview. Because the qualitative interview was added to the protocol after its initial design, some parents had already completed the dietary interventions protocol up to 24 months previously. A letter was sent to previous participants offering them the opportunity to participate (see Appendix 14). The mothers were given the opportunity to verbally consent or decline participation. The researcher documented this on the consent form (see Appendix 15).

6.2.3 Telephone interviews

The phone interviews were conducted by the researcher between September 2007 to June 2008 using the protocol (see Appendix 11). The interview was generally able to be conducted in 10 to 20 minutes. Participants' responses were voice-recorded with a digital recorder by the researcher with either the verbal or written consent of the participant (Appendix 15) and then transcribed into a Word document. Transcription was performed by an administrative assistant from an independent company, Tamworth Secretarial. The tapes were erased after transcription. Transcribed data were stored using the participant's unique identification code. All names and other identifiable data were changed for the purposes of reporting. Participants were assured of anonymity.

6.2.4 Data analysis

Thematic analysis of the individual interviews was performed using NVIVO 7 (QSR International Pty Ltd) to manage the data. Data was formatted with suitable

headings and exported from Word files into the NVIVO program where auto coding was used to determine descriptive categories relevant to each question. Data was then searched for themes arising from these categories. The transcripts were also independently analysed by another researcher experienced in qualitative methods and some categories were changed to reach agreement.

6.3 Results

The key themes and issues arising from the interviews are presented below. Narrative explanation of the themes is supported by direct quotes by participants, which are indented from the text and identified using the participant's age and the trial which they participated.

6.3.1 Feasibility of the milk free diet

Mothers as providers of food for the family

Concern to please their child and the entire family with food was of strong importance to mothers. This was challenged by the milk free diet where mothers had to balance the clinical needs of the child with the taste preferences of the rest of the family. Some mothers felt that family members were not receptive to the avoidance of dairy products and the consumption of CMP free foods.

> *It was more his brothers and his father that found it hard because I just stopped buying cheese and that sort of thing to reduce the temptation for him (Mother of child aged 4, A1 versus A2 trial).*

Some mothers reported that the milk free diet was difficult due to their child being a 'fussy eater' and unwilling to try new foods,

> *It was quite hard at first because he's very fussy and he doesn't like the taste of new things, he just likes what he likes and is used to what he's always had (Mother of child aged 4, A1 versus A2 trial).*

It was hard because she likes her dairy; she likes her yoghurts and her cheese (Mother of child aged 1, Cow versus Soy trial).

Some mothers reported that they had pre-conceptions; they believed that the CMP free food would be unpalatable and unacceptable to their child.

I thought he wouldn't want to try the new foods because they wouldn't have a lot of flavour in them. I thought it would turn him off and he would not want them, but he actually took it on pretty well, he didn't complain, he didn't give me a hard time about the change in food (Mother of child aged 4, A1 versus A2 trial).

I was just lucky that he didn't eat a lot of the packaged stuff that was excluded anyway (Mother of child aged 6, A1 versus A2 trial).

These quotes show that the mother retains the responsibility for pleasing the child with food, even in the context of a special diet.

Overcoming difficulties and developing new habits

Some mothers expressed that the milk free diet was difficult initially but became easier as the trial progressed as they became more familiar with label reading and shopping. Others saw it as a challenge and found innovative ways to cope. Some reported that the instructions and the milk free shopping guide assisted in this process,

At first I thought oh, there's going to be so much restriction but with the list that you gave me of products, if I just stuck to those, then it was heaps, heaps easier than I was expecting it to be (Mother of child aged 2, A1 versus A2 trial).

Some mothers reported the development of coping mechanisms for challenging social situations,

If we went to a birthday party, I just made my own fairy bread and took some dairy-free chips and things like that with me so that he then had his own party food; he didn't have what the other kids were having. I made a little dairy free cake for him, so that he still felt like he was he was included in the party, but he wasn't actually eating their food (Mother of child aged 3, A1 versus A2 trial).

Some mothers reported that a family approach made it easier for the child,

We just adjusted our whole diets around it so that it wasn't difficult. We tried to make it as easy as possible for him so that he didn't have to see us eating dairy (Mother of child aged 4, A1 versus A2 trial).

Sharing responsibility with the child

While mothers where clearly the gatekeepers of food provision, some mothers chose to involve the child in the special diet. Those who did reported it assisted with the child's acceptance and compliance during the dietary trial,

The diet was a lot easier than I thought it was going to be. Once we explained to him what was happening he didn't have a problem eliminating some things from his diet and having a little more of other things, yeah, he didn't mind it at all (Mother of child aged 6, A1 versus A2 trial).

Johnny was great with it too, you know he'd go to a friend's place and he'd be offered something and he'd say "does that have dairy in it?" (Mother of child aged 6, Cow versus Soy trial).

Completing the constipation diary also provided responsibility for some children,

She became interested and I think she was quite proud of herself that she disciplined herself enough to remember every day to fill out a form. It gave

her a little bit of responsibility too (Mother of child aged 10, A1 versus A2 trial).

This strategy is likely to have had a beneficial effect in improving compliance.

Using tools to assist dietary change

All participants reported that they used the milk free shopping guide. Many mothers reported that they took the list to the supermarket each time that they shopped for groceries,

I just carried it in my handbag the whole time and if I went to buy something I'd look on the list or I'd look at the ingredients of the food so yeah, I had it with me all the time (Mother of child aged 2, A1 versus A2 trial).

Others took it the first few times, only until they developed their skills in label reading and shopping for CMP free products,

I took it the first time and then I referred to it a couple of times at home. After the first time I knew what she could and couldn't have so it was fine, I didn't have to take it every time (Mother of child aged 4, A1 versus A2 trial).

While the guide was seen as a useful tool, one mother suggested an adaptation,

If you actually had a label or samples of the products wrapping, packaging or just the trademark, it makes it easily identifiable, like ah, I think it was Sweet William chocolate, which I'd never heard of, never bought, found it very hard to find (Mother of child aged 10, A1 versus A2 trial).

Other mothers took initiative and asked shopkeepers for help,

> *I actually found that shopkeepers were very good if you asked specifically if things had certain ingredients. They would go and get me the original packaging and let me stand there and read it if they didn't have time to do it for me, so yes, there's a lot more awareness and acceptance of it out there (Mother of child aged 6, A1 versus A2 trial).*

Other ideas suggested by participants included: a milk free product aisle in the supermarket, more milk free products in the supermarket and improved labelling to identify products containing milk.

Other ideas reported by mothers to make the milk free diet easier included recipes and meal ideas,

> *Maybe some recipes and a few meal ideas there might have helped (Mother of child aged 2, A1 versus A2 trial).*

6.3.2 Change as a result of following the diet

Some mothers expressed that the trial and the shopping list led them to an increased interest in label reading and greater awareness of what they were consuming in general,

> *I'm quite used to reading labels on products, but it just made us look a bit further and think about what's in our food that can affect us, dairy just being one of them (Mother of child aged 10, A1 versus A2 trial).*

Some mothers were very surprised at the foods containing hidden dairy,

> *It was actually quite an eye opener really, I found a lot of products that I didn't think, even think, would have dairy in them actually do (Mother of child aged 4, Cow versus Soy trial).*

Some mothers went beyond the original brief of using the milk free guide and learned how to adapt to manufacturing changes that resulted in the new version of the product containing milk,

> *When I first started I could go down to the supermarket with a list in hand and sort of scoot around and pick things out, but what I realised pretty quickly was that you have to be vigilant and read labels constantly (Mother of child aged 6, Cow versus Soy trial)*

6.3.3 Intention to continue with dietary changes post study

When asked if they planned to continue with dietary modification after the trial, three response categories emerged: (1) continuing with an exclusive CMP free diet (2) continuing with a modified approach, that is, a small amount of dairy allowed in the diet (3) not continuing with a CMP free diet. Key issues arising in each category are presented below.

Continuing with an exclusive CMP free diet

Some mothers were motivated to continue with a CMP free diet by the difference in their child's symptom of constipation,

> *From what we've seen we think it has improved the constipation an awful lot, he doesn't mind it, he seems to be healthy enough so we're going to keep him on it for the time being (Mother of child aged 6, A1 versus A2 trial).*

Some were keen to continue despite the acknowledged difficulties,

> *The milk free diet made a difference to Jack. We'll definitely be sticking to it. The results are obvious, it helps his constipation. The hardest thing is eating out at friends houses. You feel out of place taking your own food for Jack. I always try to make sure we've offered to bring something. I usually choose*

dessert because they're always full of dairy and at least Jack can eat what I've made (Mother of child aged 4, A1 versus A2 trial).

Some mothers commented that the development of a support group for parents of sufferers of CFC may be useful,

I don't know how we'd do it though, to get the contacts of other mothers with children with the same, you know, the same sort of thing so you can sort of, you know, be able to have something in common with another person and be able to help each other out in that regard (Mother of child aged 6, A1 versus A2 trial).

Continuing with a modified approach

Other mothers believed that there was benefit to their child in following a milk free diet, but that, due to the difficulties with social situations and family food preferences they would modify the level of restriction,

The trial has certainly made Jane's stools become normal. I think we'll do a modified diet. If her symptoms worsen I'll be stricter with the diet (Mother of child aged 8, Cow versus Soy trial).

Some mothers had included dairy in the diet due to their strong beliefs about its importance as a source of calcium,

We're mainly trying to stick to the diet, but because of the requirement for calcium, allowing some milk (Mother of child aged 4, A1 versus A2 trial).

Not continuing with a CMP free diet

Some mothers expressed that they did not feel that the CMP free diet helped with their child's constipation and had discontinued the diet.

For at least two mothers, this decision was influenced by the opinion of their paediatrician,

> *Because I spoke to the paediatrician about it and he said that, well, it won't work because he's constipated and it's not from any allergies (Mother of child aged 6, A1 versus A2 trial).*

> *I think she's going to be that way inclined forever, and that is what the paediatrician said, that she will always have to have wholemeal everything and just be careful (Mother of child aged 2, A1 versus A2 trial).*

Some were awaiting the results of the constipation diaries, blood, urine and faeces tests before they made a final decision,

> *I don't think so at this stage, we've got to wait for the results and then find out what it is causing the constipation (Mother of child aged 10, A1 versus A2 trial).*

6.3.4 Experiences of the dietary trial

Some mothers commented on the positives of the trial despite the need to do more home cooking,

> *It was a useful trial and helped us to find an answer to the problem. I had to do more cooking which was a little more time consuming for me as a working mum (Mother of child aged 4, A1 versus A2 trial).*

Some mothers welcomed the opportunity to try an option other than medication,

> *It was good to take part in it and try something new. It was more than the doctors could offer me (Mother of child aged 2, A1 versus A2 trial).*

Many mothers expressed that they were satisfied with the trial and grateful of the opportunity to participate,

> *I know I had a Paediatrician tell me it was all in my head and you get very disheartened and you think, you know, have you as a parent done something wrong. I believe that if parents are offered the opportunity to do something like this it's definitely really a good angle to try to see if it helps out (Mother of child aged 8, Cow versus Soy trial).*

One mother recognised the study as belonging to a specific approach,

> *My mother-in-law's brother who is a naturopath said, you've got to look at it this way, doctors have their procedure, and that's the way they run through it, we've got ours and dietitian's have theirs. Which is fair enough, but I guess the hardest thing is being a parent, I'm the one that sees her everyday and see's what she goes through and how much of a drama it is for her to go to the toilet (Mother of child aged 1, Cow versus Soy trial).*

Some mothers commented on the negative aspects of the blood testing for their child and its effects on their child. Some mothers reported that blood tests were too numerous or the amount of blood collected too much. Some participants reported previous negative experiences of blood tests,

> *The blood tests were difficult. Had I not been a nurse we would have probably dropped out (Mother of child aged 6, Cow versus Soy trial).*

Another factor that assisted families to follow a CMP free diet was the ongoing support from the researcher. The importance of ongoing support has been reported by other researchers. Researcher contact with subjects has been shown to improve participation and retention (118).

> *If I had a question I knew that I could contact you for help. If you weren't available, I'd leave a message and you'd always get back to me with the answer (Mother of child aged 2, Cow versus Soy trial).*

6.4 Discussion

The qualitative phone interview at the conclusion of the trials revealed that a CMP free diet provided a variety of experiences for participants and their families. A number of key themes and issues were identified. Concern to please their child and entire family was of strong importance to mothers and is referred to by other researchers (119, 120). However, Verplanken and Aarts (121) found that father's food preferences have the strongest influence on changes to the family eating patterns and whether changes will be adopted by the family. Many mothers found the milk free diet difficult initially, becoming easier as the trial progressed. New concepts and behaviours often require time to be accepted and become habits (121). Birch and Marlin (122) found that eight to 15 repeated exposures are required to enhance children's food acceptance (122). Parental pre-conceptions of the taste of milk free foods and expectations of how their child would cope with the milk free diet often did not match the experiences of their child. Mothers expressed surprise at how well their child adapted and accepted the milk free diet shows that the mothers not only need to be convinced of the need for dietary intervention but also reassured that their children will not suffer in following the diet.

A family approach, with all members of the family following the milk free diet, was expressed as assisting in the management of a milk free diet. A family approach provided a supportive environment for the child, decreased temptation and increased compliance with the CMP free diet. Giving responsibility to the child also seemed to assist with acceptance of the milk free diet. Children over the age of six could be involved in their own treatment.

Mothers needed tools and support to implement the diet. The milk free shopping guide needs to be updated regularly to keep abreast of manufacturing changes to products and identify new products. Labels and packaging were identified as a useful tool to be used by dietitians in the education of families in regards to

specialised diets. The establishment of a support group for parents of children with CFC and education for other health practitioners were identified by mothers as other supportive strategies to assist with the implementation of the milk free diet, and could be established by dietitians.

While many found the diet feasible, a number of difficulties following a milk free diet were identified. Socialising made compliance with the CMP free diet difficult. Mothers were required to be well organised and develop coping strategies to overcome social situations, such as, offering to contribute to the meal provided and bring a dish that their child could consume. Some mothers reported that their child's strong preference for dairy products made the CMP free diet difficult. It is possible that these children were having a withdrawal response from CMP. This response has been reported by other researchers in relation to casein free and gluten free diets followed for Autism Spectrum Disorders (111).

The intention to continue on the milk free diet post dietary trial varied. If there was a strong observed effect on constipation the mothers planned to continue the CMP free diet even if they planned to modify the approach to make it less restrictive. This suggests that many mothers believed that there was a threshold occurring, their child was able to tolerate some CMP before the symptom of constipation reoccurred. This supports the findings from Trial 1 and Trial 2, that some participants reported symptom resolution of constipation during the cow's milk casein A1 condition. Others were not going to continue with the CMP free diet because no obvious benefit had been found for their child or they did not have the support of their paediatrician. Doctor's opinions strongly affect the beliefs of their patients (123). Practitioners having a better understanding of the benefits of the CMP free diet would assist with a multidisciplinary approach to the management of a CMP free diet for children with CFC unresponsive to traditional treatments.

Limitations of the qualitative method have been identified. The structured interview questions may have limited the responses provided by participants. Some interviews occurred some time after the dietary trial which could have limited the memories of some of the experiences. Researcher bias may also have occurred since it was the researcher conducting the interviews rather than a research assistant. There is the possibility that some mothers were giving socially desirable responses to the questions. As the researcher, it was difficult to be totally objective because there is a subjective element to analysis of qualitative data. All efforts were made to control for researcher bias by having data analysed independently by another researcher experienced in qualitative methods.

6.5 Summary

Qualitative data on the experiences of mothers following a CMP free diet were reported in this chapter. Thematic analysis identified several key themes in terms of challenges to the food-related care-giving role of mothers and increased nutrition awareness. Intention to continue was explored. These experiences provide health professionals with valuable insights and ideas to assist their patients to manage a cow's milk protein free diet for chronic functional constipation and other conditions requiring a cow's milk protein free diet. Chapter 7 draws together and discusses all the components of this research into cow's milk protein in children with chronic functional constipation (CFC).

Chapter 7: Discussion and limitations

7.0 Chapter outline

This chapter discusses the way in which the research reported in this thesis has contributed to the understanding of the role of cow's milk protein in children with chronic functional constipation. Findings from the results of the trials and the qualitative study will be discussed under the headings of the research questions. Strengths and limitations of the study are discussed. Recommendations are made for children with CFC unresponsive to the usual treatments of medication, behavioural/psychological therapy, exercise and traditional diet therapies of a high fibre, high fluid diet.

7.1 Introduction

This research into the role of cow's milk protein in chronic functional constipation (CFC) consisted of four key studies: a systematic review of the literature on the role of CMP in CFC; Trial 1, a crossover trial comparing the effects of cow's milk β casein A1 with soy milk on CFC in children; Trial 2, a double blind crossover trial comparing the effects of cow's milk β casein A1 with cow's milk β casein A2 on CFC in children; and a qualitative study to obtain insights into mothers' lived experiences of administering a CMP free diet to children.

The systematic review of the literature showed there was some evidence for the hypothesis that CMP has a causal role in CFC in children, some of whom showed increased prevalence of CMP sensitivity in biochemical and immunological tests. The strongest evidence (Level 2) came from a double blind randomised controlled trial conducted by Iacono and colleagues in Italy and showed an association between CMP and constipation when cow's milk was consumed in comparison to a resolution of constipation in 68% of children when soy milk was consumed. However, this study had not been replicated. The systematic review showed that despite

constipation being a common problem in paediatric practice the evidence base remained small. Further scientific evidence was required to clarify the physiological, biochemical and immunological mechanisms that occur in children with CFC. The review led to the development of four research questions, investigated through two crossover trials and a qualitative study. Those questions and the way in which they were answered, are discussed below.

7.2 Research Question 1: Can the results of the Iacono study of children with CFC who respond to the replacement of CMP with soy be replicated in the Australian setting?

1.1 Hypothesis for Trial 1: Constipation would resolve for children with CFC whilst consuming soy milk and avoiding cows milk protein.

Trial 1 compared the effects of cow's milk β casein A1 with soy milk in nine children with CFC. Some of these children had signs of CMPI, but no diagnosis of allergy or intolerance. It is possible that constipation may be the only symptom of CMP allergy or intolerance for some children (124, 125). There were some biochemical abnormalities at baseline and faeces showed low levels of normal gut flora and a lack of *Streptococcus B.* During the soy condition of the crossover trial, all nine participants experienced resolution of their CFC. This confirms the hypothesis that constipation would resolve for children whilst consuming soy milk and avoiding all sources of CMP. Similarly, all nine participants had resolution of their constipation in the washout condition. However, five children also experienced resolution of constipation in the cow's milk condition, while none of the children in Iacono and colleagues' study (3) reported a response during the cow's milk condition. The reasons for this were discussed in Chapter 4. The most likely explanation is that the effect of CMP on constipation is an intolerance reaction, that involves a threshold, and with other sources of CMP removed, the 400 mL of the cow's milk condition may have been below the threshold for these five children (3, 87, 88).

The hypothesis that constipation would resolve for children with CFC whilst consuming soy and avoiding cows milk protein was proven. The research question was answered with some improvements in study design on the Iacono and colleagues research, such as, the two week washout between the two intervention milks to remove the physiological and psychological effects of the previous intervention milk, the reporting of the bowel outcome measure during the washout in addition to the intervention milk trials which allowed this period to be considered as a dietary condition.

The results of the first crossover trial showed that the removal of CMP had a positive effect on alleviating constipation and conversely that CMP caused constipation in at least four out of nine children. The next study went on to test whether the β casein moiety is the part of the CMP causing the constipation.

7.3 Research Question 2: What effect does the cow's milk β casein A1 and cow's milk β casein A2 have on CFC in children who do not respond to traditional treatments?

Hypothesis: Constipation would resolve for children whilst consuming cow's milk casein A2 and whilst avoiding all sources of cow's milk β casein A1 protein.

If β casein were the protein fraction causing the problem then perhaps the A1 variant of β casein (a result of a mutation some 5000 years ago in certain dairy cattle) was the cause. If so, the A2 variant of β casein may be free of the effect. This led to Trial 2 which used a double blind crossover methodology. This was the first experiment of its kind to compare the effects of β casein A1 and β casein A2 moiety on children with CFC.

While the mean number of bowel motions increased and some participants reached resolution, the two conditions, cow's milk β casein A1 and cow's milk β casein A2,

had identical effects, and reproduced the results of the cow's milk β casein A1 condition in Trial 1. It seems that it is not the β casein moiety, a chain of 209 amino acids, in cow's milk that is causing constipation or if it is, it is not the section that differs in the A1 and A2 variant so the hypothesis was disproved. There is some other component in cow's milk causing a problem in these susceptible children that is common to both A1 and A2 milk but not soy milk.

7.4 Research Question 3: What are the immunological and biochemical mechanisms underlying CFC that responds to the removal of CMP in children?

Although extensive testing was conducted, this question was not completely answered by the research, but some key observations were made.

Firstly, the children participating in the study showed abnormalities at baseline. The absence or low levels of normal gut flora identified in seven out of 13 participants in Trial 1 and 8 out of 39 participants in Trial 2, may have affected gut transit time since the number of species of gut flora has been shown to be important for bowel regularity (83). Several participants reported conditions that are suggested to affect initial colonisation of the gut including being delivered by caesarean section (81), mothers having thrush (*Candida albicans)* during pregnancy (82) and being formula fed (126). *Streptococcus B* was absent from the faecal samples of participants at baseline, but is usually identifiable in the bowel motions of non-constipated individuals (108). The absence of *Streptococcus B* might be useful as an indicator of CFC. A small number of participants, three in Trial 1 and five in Trial 2, were shown to have elevated IgE levels at baseline. Elevated IgE is the most easily identifiable immune response in investigations of cow's milk protein allergy (110). In IgE mediated allergy, the tolerance for the offending food is low and quickly results in symptoms (127). These particular participants reported a history of recurrent urinary tract infections, ear infections and eczema. Their immunoglobulin response

suggests that these particular participants actually have a cow's milk protein allergy as opposed to an intolerance. One child in Trial 1 and two in Trial 2 of participants showed low IgG at baseline, indicative of an immunodeficiency that usually manifests as a difficulty in clearing viral infections in children (5). The presence of *Streptococcus A* identified in 6 participants in Trial 1 and 11 participants in Trial 2, by testing for ASO titre and AntiDNase B serology, suggests the presence of an undiagnosed condition that may confound results of this study.

Secondly, although biochemical variables changed between baseline and after the dietary conditions, only platelets, monocytes, and eosinophils were statistically significant in difference. An increase in mean eosinophils between baseline and after the cow's milk condition in Trial 1 was significant. An increase in eosinophils in cow's milk allergic children has been previously documented (3). There is some evidence that eosinophils have been involved in dismotility in allergic diseases and other inflammatory conditions (67). Platelet levels were found to be significantly higher after the cow's milk condition compared with baseline, echoing the results of other studies investigating CMPA (112). Monocytes were significantly higher after the soy condition compared with after the cow's milk condition. This was not an expected result and may have occurred in relation to inflammation and immune mediated disease (128).

Mean serum urea was significantly higher and after the cow's milk casein A2 condition in and creatinine was significantly lower after the cow's milk casein A2 condition in Trial 2 compared with baseline. Urea is a by-product of amino acid metabolism and creatinine normally reflects muscle mass and increases with catabolism of skeletal muscle beyond normal levels (129). Reasons for these significant differences are unknown and require further investigation. The liver function enzymes, ALT and AST were significantly higher after the cow's milk casein A1 condition compared with baseline. Liver function abnormalities were observed

for some participants in Trial 1 and Trial 2, independent of milk condition. Liver function abnormalities occur in response to inflammation and infections (130).

Low serum zinc levels were identified at baseline and independent of dietary condition in seven children in Trial 1 and 22 children in Trial 2. Nutritional assessment of participants showed that these children had a poor or minimum intake of red meat which is a rich source of dietary zinc. Zinc is essential for the functioning of the immune system and zinc deficient subjects may experience increased susceptibility to a variety of pathogens (131). Poor appetite and nausea are also symptoms associated with constipation (4), which may exacerbate poor dietary intake of this nutrient. Bioavailability of zinc can be reduced by high fibre, high phytate foods and some participants were consuming greater than the recommended number of serves of fruits, vegetables and legumes.

The biochemical and immunological data show that some of the children who responded to CMP being withdrawn from the diet were likely to have a true food allergy. In terms of the mechanism for those other children, the results are suggestive of CMPI but further research is needed.

7.5 Research question four: What factors affect the feasibility of mothers administering a CMP free diet to their children?

The qualitative study aimed to obtain insights into the lived experiences of mothers administering a CMP free diet to children in order to assess the feasibility of this as a method of treating CFC.

Many mothers found the milk free diet difficult initially, becoming easier as the trial progressed. The mothers expressed surprise at how well their child adapted and accepted the milk free diet and that involving the child in the process made it easier. This was a positive outcome in this assessment of the feasibility of a CMP

dietary intervention since children's food preferences and food intakes have been shown to be related to those of their mothers (132). The concern of the mother in pleasing the family through food provision was an important theme and a potential barrier to the successful implementation of a CMP free diet and is referred to in the literature (119, 120).

A number of factors limiting the feasibility of the CMP free diet were identified including: socialising; 'fussy eaters' reluctant to accept and consume new CMP free foods and a child's strong preference for dairy products. Factors that increased the feasibility the CMP free diet including: giving the child some responsibility led to acceptance and compliance; taking a family approach included provision of resources such as the milk free resources and ongoing support from the researcher. Researcher contact with subjects has been shown to improve participation and retention (118). This suggests that regular contact from dietitians will assist with acceptance and compliance with the diet.

Overall feasibility was evaluated by assessing whether the mothers of children who improved on the CMP free diet intended to continue the milk free diet. Some mothers were going to continue due to the benefits of the trial to their child's condition despite a noticeable increase in cooking and shopping time. Others planned to continue with a modified CMP approach, allowing some CMP but not as much as the pre-trial diet. This was due to the degree of restriction not being perceived as being sustainable, and their deduction that partial removal of CMP would bring some relief from constipation. Other mothers were not going to continue with the CMP free diet because no obvious benefit had been found or they did not have the support of their paediatrician. So the answer to question 4 is that some mothers, when motivated by clinical improvement in their children found it feasible, while others found it necessary to ease the level of restriction to some extent.

7.6 Study strengths and limitations

This research into the role of cow's milk protein in children with chronic functional constipation had a number of strengths including: the four components of the study, the qualitative study conducted to evaluate the feasibility of mothers administering a cow's milk protein free diet to their children; the two crossover design trials, the immunological and biochemical investigations, the two-week washout period in between the intervention milks to remove any physiological and psychological effects of the previous intervention milk; and the recording of bowel outcome measures by participants during the washout period as well as during the dietary intervention periods.

As with any research, these studies had limitations.

Participants in the crossover trials

There was some bias in the participant groups. While Trial 1 had equal gender representation, Trial 2 was 64% male, reflective of dominance of CFC in boys (56). However, this male bias did not cause a problem for statistical analysis since these trials were of crossover design and subjects were compared against themselves and not controls. The age range and mean age of participants of each trial were similar although Trial 1 participants were slightly older (mean 80 months, range 16-144 months) than Trial 2 (mean 67 months, range 21-143 months). There may have been a bias in Trial 1 in that those volunteering for a dietary trial could have considered cow's milk a cause for constipation and psychological factors contribute to CFC (4). The exclusion of medication for the trial period also caused reluctance to participate on the part of some families.

The rigorous design of the crossover trials carried a high participant burden, which proved a challenge to retention. Thirteen participants were recruited to Trial 1 with only eight completing the bowel diary for all three conditions. Thirty-nine

participants were recruited to Trial 2 with only 21 completing the bowel diary for all three conditions. Nutrition intervention studies are notorious for difficulty in retaining participants because of the burden of dietary change (133). Unfortunately, the families who ceased participation were not formally interviewed but some reported reasons for dropout to the dietitian researcher (EC) including: death in the family, six weeks being too long and some participants reported that they had participated only for results of the baseline blood test, in order to identify any abnormalities. Of more concern in terms of biasing the sample were two cases both in Trial 1: one where a positive result for one participant during the soy condition resulted in the family ceasing the study because the child's constipation was cured. Another participant developed an allergic response, a rash, to the soy milk and ceased participation.

Design

While the crossover design is strong, Trial 1 was potentially limited by small numbers. During Trial 1, the children and their mothers were aware of each respective milk condition given appearance, smell and taste profiles of cow's milk and soy milk are easily distinguishable. This may have had a psychological effect on the symptom of constipation in some participants. This limitation was removed for Trial 2, where the children and their mothers were unaware of the differences in type of milk. Labelling was removed and there are no discernible differences in the appearance or taste of A1 and A2 milk, therefore preventing any psychological effect on participants' constipation.

As mentioned in Chapter 4 and 5, each Trial should have commenced with at least a one week washout period to eliminate the effects of the pre-trial CMP from participants. Participants would ideally have kept a food diary for the six week trial to monitor compliance to each trial condition, but this would have further increased the already heavy participant burden.

Another potential limitation is the specified minimum amount of 400 mL of intervention milk per day that was used to standardise intake amongst participants of various ages. This possibly should have been greater to clearly show the effect of CMP on these children with CFC.

Measures

There were limitations in the measures. The number of bowel motions prior to the commencement of the study was verbally reported by parents rather than measured using the constipation diary and therefore may not be an exact representation of pre trial bowel motions. Some children did not provide samples for all trial tests due to an aversion, or a parental aversion to particular types of testing. The time between measures may not have been extensive enough to detect changes in immunological and biological parameters, but an increase in time following each condition would have further increased participant burden.

7.7 Conclusions: an enhanced understanding of CFC

As a result of this research, we understand more about the effect of CMP on CFC. The systematic review showed that there was some evidence for a role of CMP in CFC. Trial 1 confirmed the hypothesis that constipation would resolve for children whilst consuming soy milk and avoiding all sources of CMP.

While all the children in Trial 1 responded to soy milk, approximately two thirds of the children on one of the forms of cow's milk in both trials had a response on cow's milk, while the other third remained constipated. Either the exact same number of children was not sensitive to CMP or these children exhibited a dose-related response typical of food intolerance reactions. Some CMP, in this case 400 mL per day, can be tolerated by these children before constipation occurs and the less CMP the higher the number of mean bowel motions and percentage of participants with resolution. As described in Chapter 1, food intolerances are not

cell mediated reactions but result in similar symptoms to food allergies. They can have an effect on the nerve endings in various parts of the body which in this case would be the nerve endings in the GIT involved in bowel regularity (63). In comparison, an allergic reaction with an identifiable immunoglobulin response may have been the mechanism for the other third of children on cows milk (7). An immune response to even small amounts of food protein such as cow's milk can cause changes in neuromuscular function by affecting the nerves in the gut, causing a delay in colonic transit time (63).

Thus, rather than one definitive reaction that results in constipation, the findings of this study suggest a mixed model. The children in these trials, who all presented with CFC unresolved by usual treatments, are likely to have had different causes for their constipation. While some turned out to have a chronic *Streptococcus A* infection as well as liver function abnormalities which may have contributed to constipation, some had a CMP allergy, while the majority are likely to have had CMPI. None of the children who completed Trial 1 were sensitive to soy but one who withdrew had an allergic reaction. Children with CMPA also have a high likelihood of soy allergy (39).

Trial 2 showed no difference in mean number of bowel motions between A1 and A2 milk. This suggests that it is not the A1 variant of the β casein moiety that causes constipation in susceptible children but perhaps some other protein component. This trial ruled out the A1 moiety as the culprit and further research is needed to identify the fraction responsible for CFC in susceptible children.

Those children who are allergic or sensitive to CMP need to follow a CMP free or modified diet, respectively and the qualitative study enhanced our understanding of whether this might be feasible. Many mothers planned to continue some dietary modification after the study. A number of themes were identified that will be useful

to health professionals educating families in terms of the compliance and acceptance of a CMP free diet.

7.8 Recommendations for practice

The findings of this study have implications for children with CFC unresponsive to the traditional treatments of medication and a high fibre, high fluid diet.

- Children with CFC that is unresponsive to the traditional treatments should be checked for CMPA by measuring IgE levels. If allergic, they need to follow a CMP free diet, and be reassessed at annual intervals. If not, check for other biochemical abnormalities and coeliac disease as a possible cause.
- If these results are negative, trial a CMP-free diet for at least two weeks to determine whether this may resolve the CFC. During this period, children should drink at least 400 mL per day of soy milk and record the numbers and form of bowel motions. Results should be compared to a one week record collected prior to commencing the CMP free diet.
- If this dietary modification is successful for the child and alleviates constipation, a modified approach to a CMP-free diet may be able to be adopted. To determine the amount tolerated and nutritional adequacy of the diet consultation with a dietitian is recommended.
- Due to the complicated nature of a CMP-free or modified diet, especially the number of processed foods which contain hidden CMP, consultation with a dietitian is essential for implementation of this diet. The dietitian should educate the whole family, both parents and siblings, to ensure the best outcome in terms of acceptance and compliance of the diet. The results of the qualitative study, especially the concern to please their child and entire family with food need to be taken into account in the education.
- Adequate resources need to be provided to children and their families to support the nutrition education consultations, including: a list of milk free products and

visual aids such as samples of CMP product packaging to show families; a supermarket tour to assist with the identification of products and label reading; ongoing support from a dietitian to answer queries; CMP free recipes and meal ideas for children and their families; and the establishment of a support group for parents of children with CFC to share ideas and decrease isolation.

- A probiotic, suitable for the child's age, is recommended to assist with the normalisation of gut flora and may also have immune enhancing effects.
- The qualitative results showed that mothers were confused and frustrated by the differing opinions of health professionals. Education of health professionals such as general practitioners, paediatricians, and paediatric continence nurses, regarding a CMP-free diet strategy for CFC, is essential to support the child and their family and integral to the success of the strategy. This education would need to occur via a variety of media such as scientific literature, conference presentations, articles in profession specific magazines or newsletters and materials for patient education.

7.9 Further research

While there are some clear practice implications from this research, several areas of further research are required.

- In any further investigations, chronic functional constipation should be clearly and commonly defined in research as well as in practice. Replications of this study should commence with a two-week washout period. Bowel motions should be recorded and reported during this time.
- Higher volumes of cow's milk need to be consumed to determine the threshold that causes constipation in CMPI.
- Further investigations into the immunological or biochemical mechanism that is occurring in CFC that appears to have characteristics of a food intolerance is

required. These should include investigations of the intolerance reactions and how they affect nerves in the gastrointestinal tract.

- Exact faecal analysis of the gut flora present in these children is recommended to determine probiotic requirements for normalising this gut flora.
- A longitudinal study of children aged one to 12 years is recommended to better understand this type of CFC affected by CMP over time.
- Baseline blood and serum tests are recommended for assisting in the determination of biochemical and chemical abnormalities that may be occurring. There appears to be no advantage in testing at the end of each condition as two weeks is not a sufficient time frame to detect changes in many of these blood and serum variables.

7.10 Summary of Chapter 7

In Chapter 7, the questions in this master's research have been addressed and the way in which knowledge on CFC has been enhanced was discussed. The findings were used to make a series of recommendations for practice and for further research.

The idea for this research came from a problem identified in clinical dietetic practice. It is hoped that these findings will assist in the management of children with CFC that is unresponsive to the traditional treatments. It is recommended that further research with greater numbers of children will be undertaken to further clarify the immunological and biochemical reactions that are occurring in this potentially food intolerance related CFC.

References

1. Coughlin EC. Assessment and management of pediatric constipation in primary care. Pediatr Nurs. 2003 Jul-Aug;29(4):296-301.

2. Behrman RE, Kilegman RM, Jenson HB. Nelson's Text Book of Paediatrics. Philadelphia: Saunders; 2004.

3. Iacono G, Cavataio F, Montalto G, Florena A, Tumminello M, Soresi M, et al. Intolerance of cow's milk and chronic constipation in children. N Engl J Med. 1998 Oct 15;339(16):1100-4.

4. North American Society for Pediatric Gastroenterology HaN. Evaluation and treatment of constipation in infants and children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Journal of pediatric gastroenterology and nutrition. 2006;43(3):1-13.

5. Walker-Smith J, Murch S. Diseases of the Small Intestine in Childhood. Fourth Edith ed. Oxford; 1999.

6. Mahan K, Escott-Stump S, editors. Krause's Food, Nutrition and Diet Therapy 11th Edith ed. Philadelphia: Saunders; 2004.

7. Taylor SL. Food allergies and avoidance diets. Nutrition Today. 1999;34(1):15.

8. Brostoff J, Challacombe SJ, editors. Food Allergy and Intolerance. 2nd Edition ed. London: Saunders; 2002.

9. Gershwin ME, Naguwa M, editors. Allergy and Immunology secrets. Philadelphia: Elservier-Mosby; 2005.

10. Brostoff J, Carini C, Wraith DG, Johns P. Production of IgE complexes by allergen challenge in atopic patients and the effect of sodium cromoglycate. Lancet. 1979 Jun 16;1(8129):1268-70.

11. Allen DH, Van Nunen S, Loblay R, Clarke L, Swain A. Adverse reactions to foods. The Medical journal of Australia. 1984 Sep 1;141(5 Suppl):S37-42.

12. Thompson-Chagoyan OC, Maldonado J, Gil A. Digestive Diseases and Sciences. 2007;52(9):2069-77.

13. Strachan DP. Hay fever, hygiene, and household size. BMJ (Clinical research ed. 1989 Nov 18;299(6710):1259-60.

14. von Hertzen L, Haahtela T. Disconnection of man and the soil: reason for the asthma and atopy epidemic? J Allergy Clin Immunol. 2006 Feb;117(2):334-44.

15. Fasce L, Tosca M, Silverestri M, Olcese R, Pistorio A, Rossi GA. "Early" cat ownership and risk of sensitization and allergic rhinitis in ligurian children with respiratory symptoms. Ann Allergy Asthma Immunol. 2005;561:561-5.

16. Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. Clin Exp Allergy. 2000;20(201-8).

17. Karamus W, Botezan C. Does a higher number of siblins protect against the development of allergy and asthma? A review. J Epidemiol Community Health. 2002;56:209-17.

18. Celedon JC, Wright RJ, Litonjua A, Sredl D, Ryan L, Weiss S. Day care attendance early in life, maternal history of asthma, and asthma at age 6 years. Am J Respir Crit Care Med. 2003(167):1239-43.

19. Matricardi PM, Franzinelli F, Franco A, Caprio G, Marru F, Cioffi D, et al. Sibship size, birth order, and atopy in 11,371 Italian young men. J Allergy Clin Immunol. 1998;101:439-44.

20. Flohr C, Pascoe D, Williams HC. Atopic dermatitis and the hygiene hypothesis: too clean to be true. Br J Dermatol. 2005;152:202-16.

21. Bloomfield SF, Stanwell-Smith R, Crevel RW, Pickup J. Too clean or not too clean: the hygiene hypothesis and home hygiene. Clin Exp Allergy. 2006;36:402-25.

22. Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol. 2001 Oct;108(4):516-20.

23. Metcalfe D, Sa H. Food allergy: adverse reactions to foods and food additives. Oxford: Blackwell; 2003.

24. Sampson HA. Food allergy. Part 2: diagnosis and management. J Allergy Clin Immunol. 1999 Jun;103(6):981-9.

25. Scaillon M, Cadranel S. Food allergy and constipation in childhood: how functional is it? European journal of gastroenterology & hepatology. 2006 Feb;18(2):125-8.

26. Hefle S, Nordlee J, Taylor S. Allergenic foods. Crit Rev Food Sci Nutr. 1996;36:S69-S89.

27. Food and Agriculture Organization of the United Nations. Report of the FAO Technical Consultation on Food Allergies. Rome; 1995 13-14 November Contract No.: Document Number|.

28. Host A. Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol. 2002 Dec;89(6 Suppl 1):33-7.

29. Dannaeus A, Johansson S. A follow-up study of infantswith adverse reactions to cow's milk. I. Serum test, skin test reactions and RAST in relation to clinical course. Acta paediatrica Scandinavica. 1979;68:377-82.

30. Jones R. Milk induced thrombocytopenia. Arch Dis Child. 1979;52:744-5.

31. Scheinmann P, Gendrel D, Charlas J, Paupe J. Value of lymphoblast transformation test in cow's milk protein intestinal intolerance. Clinical allergy. 1976 Sep;6(5):515-21.

32. Butler HL, Byrne WJ, Marmer DJ, Euler AR, Steele RW. Depressed neutrophil chemotaxis in infants with cow's milk and/or soy protein intolerance. Pediatrics. 1981 Feb;67(2):264-8.

33. Gruskay FL. Comparison of breast, cow, and soy feedings in the prevention of onset of allergic disease: a 15-year prospective study. Clin Pediatr. 1982;21:486-91.

34. Visakorpi JK. Intolerance to food proteins in malabsorption syndrome. Acta paediatrica Scandinavica. 1967:Suppl 177:22-3.

35. Freier S, Kletter B, Gery I, Lebenthal E, Geifman M. Intolerance to milk protein. J Pediatr. 1969 Oct;75(4):623-31.

36. Cantani A, Lucenti P. Natural history of soy allergy and/or intolerance in children, and clinical use of soy-protein formulas. Pediatr Allergy Immunol. 1997 May;8(2):59-74.

37. Ruhrah J. The soybean in infant feeding. Preliminary report. Arch Pediatr. 1909;26:496-501.

38. Hill L, Stuart H. Soybean food preparation for feeding infants with milk ideosyncrasey. JAMA. 1929;93:985-7.

39. Odze RD, Wershil BK, Leichtner AM, Antonioli DA. Allergic colitis in infants. J Pediatr. 1995 Feb;126(2):163-70.

40. Shannon W. Demonstration of food proteins in human breast milk by anaphylactic experiments in guinea pigs. Am J Dis Child. 1921;22:223.

41. O'Keefe E. The relation of food to infantile eczema. Boston Med Surg J. 1920;183:569.

42. Talbot F. Eczema in childhood. Med Clin North AM. 1918:986-96.

43. Hill D, J., Hosking C, S.,, Zhie CY, Leung R, Baratwidjaja K, Iikura Y, et al. The frequency of food allergy in Australia and Asia. Environmental Toxicology and Pharmacology. 1997;4:101-10.

44. Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. Allergy. 1990 Nov;45(8):587-96.

45. Woodford K. Devil in the milk. Nelson, New Zealand: Craig Potton Publishing; 2007.

46. Rogers J, editor. What food is that? and How Healthy Is It? Sydney: Weldon; 1990.

47. Hartwig A, Teschemacher H, Lehmann W, Gauly M, Erhadt G. Influence of genetic polymorphisms in bovine milk on the occurence of bioactive peptides. Milk Protein Polymorphism, International DairyFederation Special Publication 9702. 1997:459-60.

48. Jinsmaa Y, Yoshikawa M. Enzymatic release of neocasomorphin and beta-casomorphin from bovine beta-casein. Peptides. 1999;20(8):957-62.

49. Elliott RB, Harris DP, Hill JP, Bibby NJ, Wasmuth HE. Type I (insulin-dependent) diabetes mellitus and cow milk: casein variant consumption. Diabetologia. 1999 Mar;42(3):292-6.

50. Zioudrou C, Streaty RA, Klee WA. Opioid peptides derived from food proteins. The exorphins. The Journal of biological chemistry. 1979 Apr 10;254(7):2446-9.

51. Becker A, Hempel G, Grecksch G, Matthies H. Effects of beta-casomorphin derivatives on gastrointestinal transit in mice. Biomedica biochimica acta. 1990;49(11):1203-7.

52. Defilippi C, Gomez E, Charlin V, Silva C. Inhibition of small intestinal motility by casein: a role of beta casomorphins? Nutrition (Burbank, Los Angeles County, Calif. 1995 Nov-Dec;11(6):751-4.

53. McLachlan CN. beta-casein A1, ischaemic heart disease mortality, and other illnesses. Medical hypotheses. 2001 Feb;56(2):262-72.

54. Andiran F, Dayi S, Mete E. Cows milk consumption in constipation and anal fissure in infants and young children. Journal of paediatrics and child health. 2003 Jul;39(5):329-31.

55. Fontana M, Bianchi C, Cataldo F, Conti Nibali S, Cucchiara S, Gobio Casali L, et al. Bowel frequency in healthy children. Acta paediatrica Scandinavica. 1989 Sep;78(5):682-4.

56. Osborn LM, DeWitt, T.G., First,L.R., Zenel,J.A., editor. Pediatrics. Philadelphia: Elsevier Mosby; 2005.

57. Motta M. Intolerance of Cow's Milk and Chronic Constipation in Children. International Paediatrics. 2001;16(2):66-71.

58. Kneepkens C. What happens to fructose in the gut? Scand J Gastroenterol. 1989;24(Supplement: 1-8).

59. Gryboski J. Diarrhea fro dietetic candy. N Engl J Med. 1966;266:818-9.

60. Murch S. Allergy and intestinal dysmotility-causal or coincidental links? Journal of pediatric gastroenterology and nutrition. 2005 Sep;41 Suppl 1:S14-6.

61. Glanze W, editor. Mosby's Medical, Nursing and Allied Health Dictionary. St Louis: The CV Mosby Company; 1990.

62. Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology. 1996 Dec;111(6):1683-99.

63. Mayer EA, Collins SM. Evolving pathophysiologic models of functional gastrointestinal disorders. Gastroenterology. 2002 Jun;122(7):2032-48.

64. Putnam PE. The milk of human constipation. J Pediatr. 2004 Nov;145(5):578-80.

65. Shah N, Lindley K, Milla P. Cow's milk and chronic constipation in children. N Engl J Med. 1999 Mar 18;340(11):891-2.

66. Iacono G, Bonventre S, Scalici C, Maresi E, Di Prima L, Soresi M, et al. Food intolerance and chronic constipation: manometry and histology study. European journal of gastroenterology & hepatology. 2006 Feb;18(2):143-50.

67. Bengtsson U, Knutson TW, Knutson L, Dannaeus A, Hallgren R, Ahlstedt S. Eosinophil cationic protein and histamine after intestinal challenge in patients with cow's milk intolerance. J Allergy Clin Immunol. 1997 Aug;100(2):216-21.

68. Hogan SP, Mishra A, Brandt EB, Royalty MP, Pope SM, Zimmermann N, et al. A pathological function for eotaxin and eosinophils in eosinophilic gastrointestinal inflammation. Nat Immunol. 2001 Apr;2(4):353-60.

69. Mann NS, Leung JW. Pathogenesis of esophageal rings in eosinophilic esophagitis. Medical hypotheses. 2005;64(3):520-3.

70. Fox VL, Nurko S, Teitelbaum JE, Badizadegan K, Furuta GT. High-resolution EUS in children with eosinophilic "allergic" esophagitis. Gastrointest Endosc. 2003 Jan;57(1):30-6.

71. Shimotoyodome A, Meguro S, Hase T, Tokimitsu I, Sakata T. Decreased colonic mucus in rats with loperamide-induced constipation. Comp Biochem Physiol A Mol Integr Physiol. 2000 Jun;126(2):203-12.

72. Isolauri E, Suomalainen H, Kaila M, Jalonen T, Soppi E, Virtanen E, et al. Local immune response in patients with cow milk allergy: follow-up of patients retaining allergy or becoming tolerant. J Pediatr. 1992 Jan;120(1):9-15.

73. Cunningham-Rundles C, Brandeis W, Pudifin D, Day N, Good R. Autoimmunity in selective IgA deficiency: relationship to anti-bovine protein antibodies, circulating immune complexes and clinical disease. Clin Exp Immunol. 1981;45:299-304.

74. Kaplan E, Rothermel C, Johnson D. Antistreptolysin O and Anti-Deoxyribonuclease B Titers: Normal Values for Children Ages 2 to 12 in the United States. Pediatrics. 1997;101(1):86-8.

75. Shils ME, Olson JA, Shike M, editors. Modern Nutrition in health and Disease. Philadelphia: Lea and Febriger; 1994.

76. Hentges D, editor. Human Intestinal Microflora in Health and Disease. London: Academic Press; 1983.

77. Wynne AG, McCartney AL, Brostoff J, Hudspith BN, Gibson GR. An in vitro assessment of the effects of broad-spectrum antibiotics on the human gut microflora and concomitant isolation of a Lactobacillus plantarum with anti-Candida activities. Anaerobe. 2004 Jun;10(3):165-9.

78. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet. 2003 Feb 8;361(9356):512-9.

79. Sears CL. A dynamic partnership: celebrating our gut flora. Anaerobe. 2005 Oct;11(5):247-51.

80. Stephen AM, Wiggins HS, Cummings JH. Effect of changing transit time on colonic microbial metabolism in man. Gut. 1987 May;28(5):601-9.

81. Schwiertz A, Gruhl B, Lobnitz M, Michel P, Radke M, Blaut M. Development of the intestinal bacterial composition in hospitalized preterm infants in comparison with breast-fed, full-term infants. Pediatr Res. 2003 Sep;54(3):393-9.

82. Bettelheim KA, Breadon A, Faiers MC, O'Farrell SM, Shooter RA. The origin of O serotypes of Escherichia coli in babies after normal delivery. J Hyg (Lond). 1974 Feb;72(1):67-70.

83. Zoppi G, Cinquetti M, Luciano A, Benini A, Muner A, Bertazzoni Minelli E. The intestinal ecosystem in chronic functional constipation. Acta Paediatr. 1998 Aug;87(8):836-41.

84. Dunne C. Adaptation of bacteria to the intestinal niche: probiotics and gut disorder. Inflamm Bowel Dis. 2001 May;7(2):136-45.

85. Clein N. Cow's milk allergy in infants. Pediatr Clin North Am. 1954;4:949-62.

86. Buisseret PD. Common manifestations of cow's milk allergy in children. Lancet. 1978 Feb 11;1(8059):304-5.

87. Iacono G, Carroccio A, Cavataio F, Montalto G, Cantarero MD, Notarbartolo A. Chronic constipation as a symptom of cow milk allergy. J Pediatr. 1995 Jan;126(1):34-9.

88. Iacono G, Cavataio F, Montalto G, Soresi M, Notarbartolo A, Carroccio A. Persistent cow's milk protein intolerance in infants: the changing faces of the same disease. Clin Exp Allergy. 1998 Jul;28(7):817-23.

89. Magazzu G, Scoglio R. Gastrointestinal manifestations of cow's milk allergy. Annals of Allergy, Asthma, and Immunology. 2002;89(6):65-8.

90. National Health and Medical Research Council. Evidence Based Medicine. 2006.

91. Carroccio A, Scalici C, Maresi E, Di Prima L, Cavataio F, Noto D, et al. Chronic constipation and food intolerance: a model of proctitis causing constipation. Scand J Gastroenterol. 2005 Jan;40(1):33-42.

92. Turunen S, Karttunen TJ, Kokkonen J. Lymphoid nodular hyperplasia and cow's milk hypersensitivity in children with chronic constipation. J Pediatr. 2004 Nov;145(5):606-11.

93. Daher S, Tahan S, Sole D, Naspitz CK, Da Silva Patricio FR, Neto UF, et al. Cow's milk protein intolerance and chronic constipation in children. Pediatr Allergy Immunol. 2001 Dec;12(6):339-42.

94. Carroccio A, Montalto G, Custro N, Notarbartolo A, Cavataio F, D'Amico D, et al. Evidence of very delayed clinical reactions to cow's milk in cow's milk-intolerant patients. Allergy. 2000 Jun;55(6):574-9.

95. Council NHaMR. A guide to the development, implementation and evaluation of clinical practice guidelines. Canberra: Commonwealth of Australia; 1999 Contract No.: Document Number|.

96. Stricker T. Constipation and Intolerance to Cow's Milk. Journal of Pediatric Gastroenterology and Nutrition. 2000;30(2):224.

97. Eigenmann PA, Zamora SA, Belli DC. Cow's milk and chronic constipation in children. N Engl J Med. 1999 Mar 18;340(11):891; author reply 2.

98. Daher S, Sole D, de Morais MB. Cow's milk and chronic constipation in children. N Engl J Med. 1999 Mar 18;340(11):891; author reply 2.

99. Nowicki MJ, Bishop PR. Organic causes of constipation in infants and children. Pediatr Ann. 1999 May;28(5):293-300.

100. Norwicki MJ, Bishop PR. Organic causes of constipation in infants and children. Pediatr Ann. 1999;28(5):293-300.

101. Children's Development Foundation, Women's and Children's Hospital (SA), and Faculty of Health and Behavioural Aciences DUV. Australian Guide to Healthy Eating. In: Aging DoHa, editor. Canberra: Australian Governement; 1998.

102. Heaton K, Thompson W, editors. Irritable Bowel Syndrome. Oxford: Health Press; 1999.

103. O'Donnell L, Heaton K. Pseudo-diarrhoea in the irritable bowel syndrome: patients records of stool form reflect transit time while stool frequency does not. Gut. 1988;29:A1455.

104. Heaton K, Ghosh S, Braddon F. How bad are the symptoms and bowel disfunction of patients with the irritable bowel syndrome? A prospective, controlled study with emphasis on stool form. Gut. 1991;32:73-9.

105. Degen L, Phillips S. How well does stool form reflect colonic transit? Gut. 1996;39:109-13.

106. Reigler G, Esposito I. Bristol scale stool form. A still valid help in medical and clinical reseach. Tech Coloproctol. 2001;5:163-4.

107. Talley N, Nyren O, Drossman D, Heaton K, Veldhuyzen van Zanten S, Koch M. The irritable bowel syndrome: towards optimal design of controlled treatment trials. Gastroenterol Int 6. 1999:189-211.

108. Isenberg H, editor. Diagnostic microbiology - Laboratory manuals. Second edition ed. Washington: ASM Press; 2004.

109. Beckman Coulter I. Assay manual. Fullerton: Beckman Coulter, Inc; 2002 [cited.

110. Hill DJ, Duke AM, Hosking CS, Hudson IL. Clinical manifestations of cows' milk allergy in childhood. II. The diagnostic value of skin tests and RAST. Clinical allergy. 1988 Sep;18(5):481-90.

111. Schreck KA, Williams K, Smith AF. A comparison of eating behaviors between children with and without autism. J Autism Dev Disord. 2004 Aug;34(4):433-8.

112. Host A, Halken S. The role of allergy in childhood asthma. Allergy. 2000 Jul;55(7):600-8.

113. Olatawura MO. Encopresis. A review of thirty-two cases. Acta paediatrica Scandinavica. 1973 Jul;62(4):358-64.

114. Pinkerton P. Psychogenic megacolon in children: the implications of bowel negativism. Arch Dis Child. 1957;24:371-80.

115. Cornish E. Gluten and casein free diets in autism: a study of the effects on food choice and nutrition. J Hum Nutr Diet. 2002 Aug;15(4):261-9.

116. Alano A. A Whole New World: Diet Modification in Children with ASD An in-depth qualitative study; 2006 Contract No.: Document Number|.

117. Richards L. Handling Qualitative Data. A practical guide. London: Sage Publications; 2006.

118. Connell CM, Shaw BA, Holmes SB, Foster NL. Caregivers' attitudes toward their family members' participation in Alzheimer disease research: implications for recruitment and retention. Alzheimer Dis Assoc Disord. 2001 Jul-Sep;15(3):137-45.

119. Skinner JD, Carruth BR, Bounds W, Ziegler PJ. Children's Food Preferences: A Longitudinal Analysis. Journal of the American Dietetic Association. 2002;102(11):1638-47.

120. Brown JL, Miller D. Couples' gender role preferences and management of family food preferences. J Nutr Educ Behav. 2002 Jul-Aug;34(4):215-23.

121. Verplanken B, Aarts H. Habit, attitude and planned behaviour: Is habit an empty construct or an interesting case of goal-directed automaticity. European Review of Social Psychology. 1999;10.

122. Birch LL, Marlin DW. I don't like it; I never tried it: effects of exposure on two-year-old children's food preferences. Appetite. 1982 Dec;3(4):353-60.

123. Armstrong K, Stopfler J, Calzone K, Fitzgerald G, Coyne J, Weber B. What does my Doctor think? Preferences for knowing Doctor's Opinions amoung women considering testing for BRCA 1/2 Mutations. Genetic Testing. 2002;6(2):115-8.

124. Chin KC, Tarlow MJ, Allfree AJ. Allergy to cows' milk presenting as chronic constipation. Br Med J (Clin Res Ed). 1983 Nov 26;287(6405):1593.

125. McGrath J. Allergy to cow's milk presenting as chronic constipation. Br Med J (Clin Res Ed). 1984 Jan 21;288(6412):236.

126. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: composition and development. Acta Paediatr Suppl. 2003 Sep;91(441):48-55.

127. Mekori YA. Introduction to allergic diseases. Crit Rev Food Sci Nutr. 1996;36 Suppl:S1-18.

128. Zeigler-Heitbrock L. The CD14+ CD16+ Blood Monocytes: their role in infection and inflammation. Review. J Leukocyte Biology. 2007;81:584.

129. Nicoll D, editor. Pocket book of diagnostic tests. 5th Edition ed. New York: McGraw-Hill; 2008.

130. Schiff LS, ER, editor. Diseases of the Liver. 7th Edition ed. Philadelphia: Lippincott; 1993.

131. Prasad AS. Zinc in Human Health. J Trace Elem Exp Med. 1998;11:63-87.

132. Skinner J, Carruth B, Moran J. Toddlers' food preferences: concordance with family members' preferences. J Nutr Educ. 1998;30:17-22.

133. Keller CS, Gonzales A, Fleuriet KJ. Retention of Minority Participants in Clinical Research Studies. West J Nurs Res. 2005 April 1, 2005;27(3):292-306.

Publications Arising From This Thesis

This is a copy of the literature review, 'Evidence for a role of cow's milk consumption in chronic functional constipation in children: Systematic review of the literature from 1980 to 2006,' published in *Nutrition and Dietetics* 2008. The published literature review is a condensed version of Chapter 2 due to the word limit allowed for publication.

Evidence for a role of cow's milk consumption in chronic functional constipation in children: Systematic review of the literature from 1980 to 2006

Elesa CROWLEY, [1,2] Lauren WILLIAMS, [3] Tim ROBERTS, [4] Peter JONES [1] and Richard DUNSTAN [5]

[1] University Department of Rural Health Northern New South Wales, The University of Newcastle, Newcastle, [2] Tamworth Rural Referral Hospital, Tamworth, [3] School of Health Sciences, The University of Newcastle, Newcastle, New South Wales, Australia, [4] The University of Newcastle, Singapore, and [5] School of Environmental and Life Sciences, The University of Newcastle, Newcastle, New South Wales, Australia

Nutrition & Dietetics 2008, 65: 29-35

doi: 10.1111/j.1747-0080.2007.00225.x

ABSTRACT

Aim: This article examines the evidence for a role of cow's milk protein in chronic functional constipation in children.

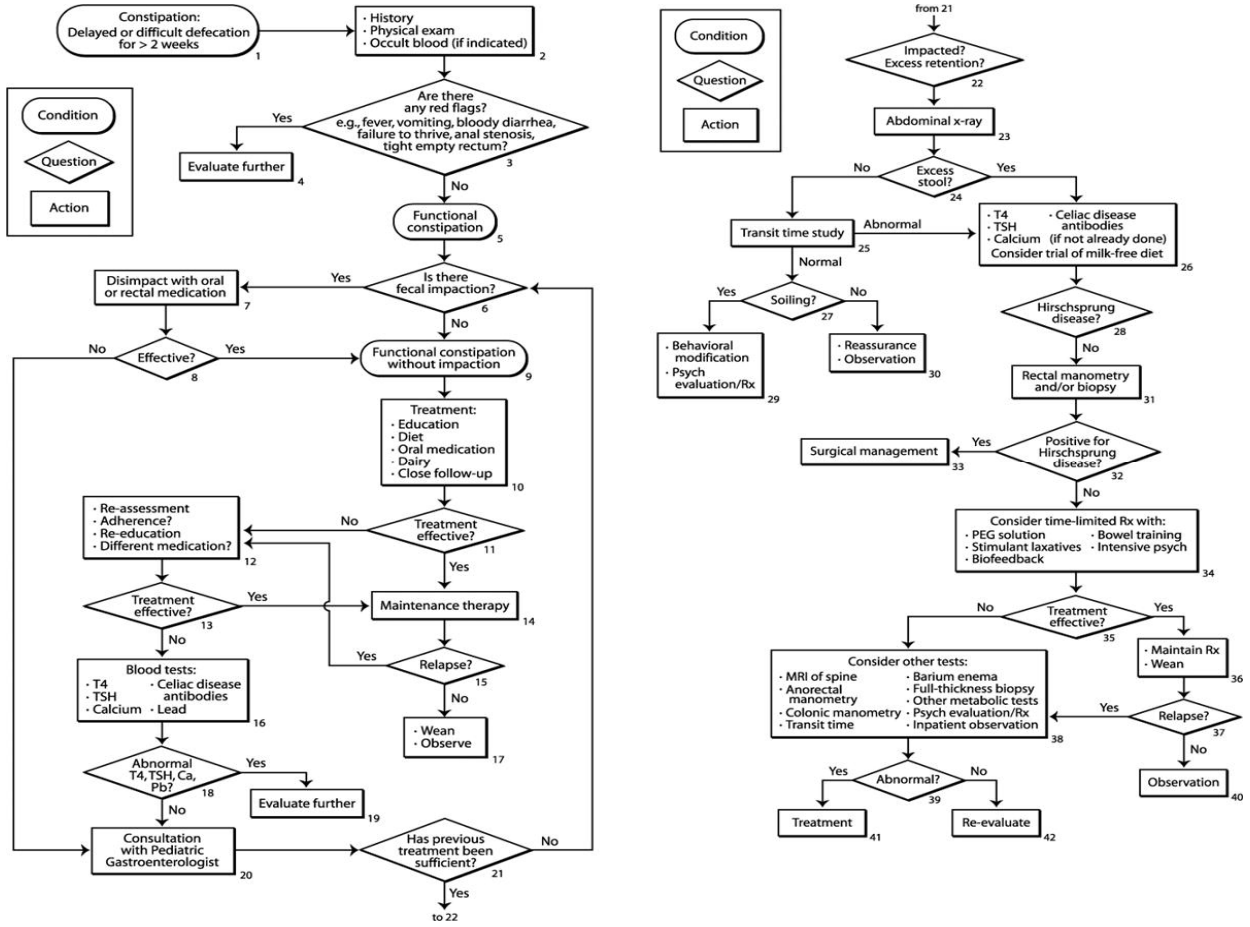
Methods: A literature search was conducted using Ovid and Pubmed, the Cochrane data bases, CINHAHL and EBSCO. Keywords searched included: constipation, cow's milk, intolerance, allergy, children and intestinal motility. This systematic review focused on dietary intervention studies in children (aged from 7 days to 15 years) with chronic functional constipation. All articles were required to include measures of cow's milk protein allergy or intolerance and include resolution of constipation as an outcome measure.

Results: The keyword search identified 125 articles. Seven of these articles met the criteria for inclusion, including one double-blind, randomised controlled trial. The results of this review provide support for the hypothesis that a proportion of children with chronic functional constipation respond well to the removal of cow's milk protein from the diet, particularly if serum analysis shows abnormalities of immune mechanisms.

Conclusion: The evidence surrounding cow's milk constipation was limited with only one of the assessed studies being at level II of evidence according to the NHMRC. In order to develop evidence-based guidelines, further high-level evidence is required to clarify the physiological, immunological and biochemical changes that occur in some constipated children who respond to the removal of cow's milk protein from the diet.

Appendices

Appendix 1: Algorithm for the Management of Paediatric Constipation



Appendix 2: Hypothesis statements for blood factors analysed

- Haemoglobin an indicator of nutritional status, more specifically, an adequate presence of iron in the blood (Sacher, 2000). It was hypothesised that haemoglobin be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- White cell count is an indicator of how the immune system reacts to infection (Dunstan, 2000). It was hypothesised that white cell count be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- Platelets are also an indicator of how the immune system reacts to infection (Dunstan, 2000). It was hypothesised that platelets be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- Red blood cell count is an indicator of nutritional status, more specifically one of the measures of anaemia (Dunstan, 2000). Its principal action is to transport oxygen in the blood. It was hypothesised that red blood cell count be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- Hematocrit is an indicator of nutritional status, more specifically one of the measures of anaemia (Dunstan, 2000). It was hypothesised that hematocrit be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- Neutrophils are the circulating white blood cells essential for phagocytosis and proteolysis in which bacteria and other foreign particles are removed and destroyed (Collan, 1972). A mixed model analysis was undertaken to determine whether the dietary trial affected the neutrophil numbers of participants, that is, were they more susceptible to infection or other foreign bodies. It was

hypothesised that neutrophils be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.

- Eosinophils, white cells normally found in the gut mucosa, increase dramatically with inflammation such as allergy or infection (Collan, 1972). Eosinophil numbers have been reported to increase in the intestinal mucosa of children with cow's milk allergy (Withrington and Challacombe, 1979). It was hypothesised that eosinophil numbers be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.

- Monocytes, the white cells produced by cells in the bone marrow, enter the blood stream for transport and differentiate into macrophages in the connective tissues (Cohn et al., 1966). A mixed model analysis was undertaken to determine whether the dietary trial affected the monocyte numbers of participants, that is, were they more susceptible to infection or other foreign bodies. It was hypothesised that monocytes be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.

- A decreased absorption of B12 is associated with pernicious anaemia. B12 was ensured the children in this study did not have pernicious anaemia. Changes in bowel habits such as constipation or diarrhea can be a symptom of pernicious anaemia (Sleisinger and Fordtran, 1983). It was hypothesised that B12 be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.

- Red Cell Folate may be low in persons with macrocytic anaemia. Folate, found in plant and animal foods, can be absorbed from the entire length of the small bowel. Red cell folate was measured to ensure children in this study were not anaemia. It was hypothesised that red cell folate be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.

- Urea is a by-product of protein metabolism (Dunstan, 2000). It was hypothesised that urea be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- Creatinine is also a by-product of creatine metabolism and an indicator of renal function. High levels also occur in the blood when there is a higher breakdown of skeletal muscle (Dunstan, 2000). It was hypothesised that creatinine be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- Total protein is a measure of the total proteins, albumin and globulins present in the blood serum (Sacher, 2000). It was hypothesised that total protein be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- Albumin is an indicator of nutritional status (Sacher, 2000). It was hypothesised that albumin be normal at baseline and it was not expected to change throughout the dietary trial periods of the study.
- GGT, Alkaline Phosphatase, ALT and AST are liver function enzymes. It was hypothesised that these liver function enzymes be normal at baseline and not expected to change throughout the dietary trial periods of the study.
- Copper is absorbed from the intestinal tract and used as an enzyme in many important reactions in the body (Shils et al., 1994). It was hypothesised that copper be normal at baseline and not expected to change throughout the dietary trial periods of the study.
- Serum zinc and red cell zinc are absorbed from the small intestine tract and is used as a co-factor for making energy, plays an important role in growth and development, the immune response and reproduction (Shils et al., 1994). Zinc deficiency causes changes in taste acuity and can be related to fussy eating. It

was hypothesised that zinc be normal at baseline and not expected to change throughout the dietary trial periods of the study.

- ASO titre and AntiDnaseB titre are indicators of the presence of an acute or chronic streptococcous A infection in the blood (Kaplan et al., 1997). It was hypothesised that ASO titre and AntiDNaseB titre be normal at baseline and not expected to change throughout the dietary trial periods of the study.
- IgA, IgG, IgM, IgE are biochemical markers indicating an immune response (Brostoff and Challacombe, 2002). It was hypothesised that these markers may have been abnormal at baseline and may have changed throughout the dietary trial periods of the study.

Appendix 3a: Ethics approval certificate 2003

The UNIVERSITY of NEWCASTLE
AUSTRALIA

Form HREC/2/98

HUMAN RESEARCH ETHICS COMMITTEE

Certificate of Approval
for a research project involving humans

Applicant

Chief Investigator/Project Supervisor: (First named in application)	*Associate Professor Tim Roberts*
Other Investigators:	*Ms Elesa Towers* *Associate Professor Trish Davidson* *Associate Professor Peter Jones* *Ms Elizabeth Ellis* *Associate Professor Hugh Dunstan*
Project Title:	*An investigation into constipation in children*

In approving this project, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the *National Statement on Ethical Conduct in Research Involving Humans, 1999*, and the requirements within this University relating to human research.

Details of Approval

HREC Approval No: *H-682-1003*	**Date of Approval:** *15 October 2003*
Approval valid for: *3 years*	**Progress reports due:** *Annually*

Comments or conditions:

Considered in consultation with the Hunter Area Research Ethics Committee (HAREC).

Approved subject to a satisfactory response to issues identified by the Committee and the HAREC.

22 December 2003
Approval confirmed.

Signed: A J O'Connor

Ms Susan O'Connor
Secretary to the Committee

Appendix 3b: Ethics approval certificate New England Health

New England Health

23 December 2003

Ms Elesa Towers
Nutrition & Dietetics
Tamworth Base Hospital
PO Box 83
TAMWORTH NSW 2340

Dear Ms Towers

Re: Research Project
DB158: "An investigation of the relationship between the dietary ingestion of cow's milk proteins and constipation in children"

The New England Area Health Service Research Ethics Committee at its meeting of 9th December 2003 discussed your submission on the above proposal.

It was resolved that: No ethical concerns. Approval given with the inclusion of alternative counselling being offered (section 2.13 of the ethics form)

Ethical approval is conditional upon adherence to the conditions outlined below.

1. The research project will be carried out as described in the application and in accordance with ALL subsequent correspondence.
2. The Chief Investigator will advise the NEAHS Research Ethics Committee of any changes in the research protocol or conduct, if any unforeseen events that might affect continued ethical acceptability of the project or adverse events take place, or should the project be abandoned for any reason. New ethical approval must be sought for substantially altered or revised research protocols.
3. In order to fulfil monitoring requirements of the Committee, a report is required at annually and at the completion of the study. The committee should receive your first annual report by December 2004 for its consideration. *Ethical approval will lapse unless the report is received.*

Should you require any additional information please contact Virginia Braack (02) 6766 2288.

Please quote Project No. DB158 in all correspondence.

Yours sincerely

Karin Fisher
A/Director
NEH Research and Development Institute

New England Public Health Unit
PO Box 597
Tamworth NSW 2340
Tel 02 6766 2288
Fax 02 6766 3003

Appendix 3c: Ethics approval certificate Hunter Area Research Ethics Committee

HUNTER HEALTH
Improving Health in the Hunter

Hunter Area Research Ethics Committee
Ph: (02) 2421 4950
Ph: (02) 4921 4943
Fax: (02) 4921 4818
Email: nicole.gerrand@hunter.health.nsw.gov.au
michelle.lane@hunter.health.nsw.gov.au
http://hal.hunter.health.nsw.gov.au/harec/index.asp
www.hunter.health.nsw.gov.au/index.php?=2006

3 November 2004

Associate Professor T Roberts
Environmental & Life Sciences
University of Newcastle

Dear Associate Professor Roberts,

Re: An Investigation into Constipation in Children (03/08/13/3.12)

The above protocol was approved by the Hunter Area Research Ethics Committee on **3 December 2002.** *The National Statement on Ethical Conduct in Research Involving Humans* requires that an annual report is required for all research protocols that received approval from an Human Research Ethics Committee on the anniversary of that approval. Could you please complete the attached form and return it and any additional documentation (for example a half to one page summary of the progress of the protocol so far) by **31 December 2004.**

Yours sincerely,

Ms Michelle Lane
Administrative Officer
Hunter Area Research Ethics Committee

Appendix 3d: Ethics approval certificate for variation

Hunter Area Research Ethics Committee
Locked Bag No. 1
New Lambton NSW 2305
Telephone (02) 4921 4950 on [02] 4921 4943
Facsimile (02) [4921 4818]
Email nicole.gerrand@hnehealth.nsw.gov.au
michelle.lane@hnehealth.nsw.gov.au
http://hal.hunter.health.nsw.gov.au/harec/index.htm
http://www.hunter.health.nsw.gov.au/harec/

Hunter New England
Area Health Service

18 July 2005

Dr L Williams
School of Health Sciences
University of Newcastle

Dear Dr Williams,

RE: An Investigation into Constipation in Children(03/08/13/3.12)

Thank you for the submission of the following variations:

1. Change project supervisor from A/Prof Tim Roberts to Dr Lauren Williams;
2. Amend the research design/method;
3. Amend the timetable for the research data collection;
4. Amend the proposed participant group; and
5. the revised study recruitment documentation

for the above protocol, *the Hunter Area Research Ethics Committee* has resolved that:

The above variations for the protocol **An Investigation into Constipation in Children,** the information sheet for the dietary intervention group – Newcastle (version 5 dated 27 April 2005), the information sheet for the dietary intervention group – Tamworth (version 5 dated 27 April 2005), the dietary intervention information sheet (version 5 dated 27 April 2005), the consent form dietary intervention group – Newcastle (version 5 dated 27 April 2005), the consent form dietary intervention group – Tamworth (version 5 dated 27 April 2005), the collection instructions for parents (version 5 dated 27 April 2005) and the form for participant samples (version 5 dated 27 April 2005) have been approved and may now be implemented.

This amendment has also been reviewed by *the University of Newcastle Human Research Ethics Committee* and you will receive separate notification of their approval.

Approval from the Hunter Area Research Ethics Committee for the above protocol is given for a maximum of 3 years from the date of the approval letter of your initial application, after which a renewal application will be required if the protocol has not been completed. The above protocol is approved until **December 2006.**

- The *National Statement on Ethical Conduct in Research Involving Humans, (1999),* which the Committee is obliged to adhere to, include the requirement that the committee monitors the research protocols it has approved. In order for the Committee to fulfil this function, it requires:

- a report of the progress of the above protocol be submitted at 12 monthly intervals. Your review date is **December 2005**. A proforma for the final report will be sent two weeks prior to the due date.

- A final report be submitted at the completion of the above protocol, that is after data analysis has been completed and a final report compiled. A proforma for the final report will be sent two weeks prior to the due date.

- All variations or amendments to this protocol, including amendments to the Information Sheet and Consent Form, must be forwarded to and approved by the Hunter Area Research Ethics Committee prior to their implementation.

- Adverse events, however minor, must be recorded as observed by the Investigator or as volunteered by a participant in this protocol. Full details will be documented, whether or not the Investigator or his deputies considers the event to be related to the trial substance or procedure.

- Serious adverse events that occur during the study or within six months of completion of the trial at your site should be reported to the Professional Officer of the Hunter Area Research Ethics Committee as soon as possible and at the latest within 72 hours.

- Copies of serious adverse event reports from other sites should be sent to the Hunter Area Research Ethics Committee for review as soon as possible after being received.

- Serious adverse events are defined as:

  Causing death, life threatening or serious disability.
  Cause or prolong hospitalisation.
  Overdoses, cancers, congenital abnormalities whether judged to be caused by the investigational agent or new procedure or not.

- It is the responsibility of the investigator to inform the Hunter Area Research Ethics Committee of serious adverse events.

If for some reason the above protocol does not commence (for example it does not receive funding); is suspended or discontinued, please inform Dr Nicole Gerrand, the Professional Officer of the Hunter Area Research Ethics Committee as soon as possible.

If you have any enquires please contact Dr Gerrand, as per her contact details at the top of the previous page. I wish you well with your research.

Yours Sincerely,

For Ms J. MacDonald
Chair

CC: Ms S O'Connor
University of Newcastle's Human Research Ethics Committee

Hunter New England Area Health Service
(Locked Bag No 1)
(New Lambton NSW 2305)
Telephone (02) 49214 960 Facsimile (02) 49214 696
www.hnehealth.nsw.gov.au

Appendix 4a: Milk Free Shopping Guide

Milk Protein Free	Contains Milk Protein
Fruit and Vegetables ➢ All fresh fruit and vegetables ➢ Tinned fruit in natural juice ➢ Juice 100% No added Calcium	***Fruit and Vegetables*** ➢ Mushrooms in Butter sauce ➢ Juice with added calcium
Margarine ➢ Milk Free Margarine ➢ Becel ➢ Nuttlelex ➢ Meadow Lea Free from Lactose, Salt and Cholesterol ➢ Naytura Soya margarine ➢ Home made garlic butter made on dairy free margarine	***Margarine*** ➢ All other margarine ➢ All other garlic butter
Dairy ➢ Allowed milk provided by research team	***Dairy*** ➢ Milk ➢ Flavoured Milk ➢ Yoghurt ➢ Fruche ➢ All dairy desserts eg Yogo ➢ Cream ➢ Soy Yoghurt ➢ Cheese ➢ Cottage Cheese ➢ Ricotta Cheese ➢ Philadelphia Cream Cheese ➢ Spreadable Cheese ➢ Cheddar Cheese ➢ All Soft Cheese
Ice Cream and Alternatives ➢ Vitari ➢ Icy Poles ➢ Frosty Fruits ➢ Weiss Sorbet ➢ Gelativo passionfruit or lemon lime	***Ice Cream and Alternatives*** ➢ Splice ➢ Paddle Pops ➢ Milk Pops ➢ Weiss Fruitia
Pasta and Rice ➢ Pasta ➢ Rice ➢ Rice Noodles ➢ Cous Cous	***Pasta and Rice*** ➢ Continental pasta and sauce ➢ Tinned Spaghetti with Cheese Sauce
Bread ➢ All types of bread except those with added milk	***Bread*** ➢ Wonder White, Milk Bread

Milk Protein Free	Contains Milk Protein
Meat and Meat Alternatives ➢ Tuna ➢ Salmon ➢ Fresh fish ➢ Beef ➢ Devon (check ingredients) ➢ Frankfurts (check ingredients) ➢ Ham, Bacon ➢ Chicken ➢ Turkey ➢ Roast Beef ➢ Tofu ➢ Sanitarium Not Bacon ➢ Soy sausages ➢ Vegie hotdogs ➢ Rice noodles ➢ Baked Beans (No Cheese Sauce)	***Meat and Meat Alternatives*** ➢ Salami ➢ Pepperoni ➢ Strasburg ➢ Sandwich slice ➢ Sandwich Ham
Cereals and Cereal Bars *Uncle Toby's* ➢ Crisp and Crunchy Plus ➢ Sports Plus ➢ Muesli Flakes Plus ➢ Fibre Flakes Plus ➢ Lite Start Plus ➢ Vita Brits ➢ OT's ➢ Crispy O's ➢ Crunchy Squares ➢ Rolled Oats ➢ Roll- Ups ➢ Be Natural - Trail Bars ➢ Nice & Natural Muesli Bars ➢ Sunbeam Fruit & Nut ➢ Carmans Classic Fruit Muesli Bars	***Cereals and Cereal Bars*** *Uncle Toby's* ➢ Oat Temptations ➢ Chocolate Coated Muesli Bars ➢ Yoghurt Topped Muesli Bars ➢ Fruit Twists ➢ Chewy Muesli Bars ➢ Fruit Filled Bars

Milk Protein Free	Contains Milk Protein
Kellogg's ➢ Sustain ➢ Sultana Bran ➢ Nutragrain ➢ Just Right ➢ Special K ➢ Cornflakes ➢ Crunchy Nut Cornflakes ➢ Mini Wheats ➢ Crispix ➢ Bart Simpson Eat My Shorts ➢ Frosties ➢ Fruit Loops ➢ Rice Bubbles ➢ K-time Honey Nut Crunch Bar ➢ K-Time Just Right Bars ➢ K-Time Mixed Berries ➢ Special K Bar ➢ Oat Brits ➢ Shredded Wheat ➢ Weeties ➢ Fruity Bites ➢ Oat Flakes ➢ Natural Style Muesli ➢ Crunch Nut Clusters ➢ Guardian ➢ Mini Wheats	*Kellogg's* ➢ Milk and cereal bars ➢ Plus Bars ➢ Coco Pops ➢ K-Time Muffin Bars ➢ K-Time Twists ➢ Komplete
Sanitarium ➢ Light and Tasty ➢ Weet-Bix ➢ Weet-Bix Honey Crunch ➢ Good Start ➢ Fruity Bix ➢ Honey Wheats ➢ Hi-Bran ➢ Puffed Wheat	*Sanitarium* ➢ Up and Go ➢ Fruity Bix Bars ➢ Light & Tasty
Lowan ➢ Multiflakes ➢ Honey O's ➢ ***Dick Smiths*** Bush Foods Breakfast ➢ ***IXL*** Fruit Snacks	

Milk Protein Free	Contains Milk Protein
Biscuits *Arnott's* ➢ The Original Teddy Bear (Large) ➢ Honey Jumble ➢ Nice ➢ Salada ➢ Vita Wheat ➢ Ryvita ➢ Salada ➢ Watercracker - wholegrain ➢ Granita *Coles* ➢ Smart Buy - Gingernut biscuits ➢ Smart Buy - Nice biscuits ➢ Watercracker ➢ Paradise - Marie *Freedom Foods* ➢ Lactose Fee biscuits ➢ Choc chip ➢ Choc blitz ➢ Crunchy coconut ➢ Blueberry cookie ➢ Spicy apple *Woolworths FreeFrom Biscuits* ➢ Double Choc ➢ Coconut Crunch	***Biscuits*** *Arnott's* ➢ All Cream Filled Biscuits ➢ All Chocolate Biscuits ➢ Tiny Teddies ➢ Milk Arrowroot ➢ Tina Wafers ➢ Scallywags ➢ Hundreds and Thousands ➢ Cruskits ➢ Sao's ➢ Ginger Nut ➢ Scotchfinger ➢ Jatz ➢ Shapes
Paradise Lites ➢ Wholewheat and Sesame Lites ➢ Sesame and Poppy Crackers ➢ Soy and Linseed Crackers ➢ Malt ➢ Jam Fancies ➢ Strawberry Mallows ➢ Marie ➢ ***Captain Table*** Water crackers ➢ ***Dick Smith's*** Water Crackers	**Flavoured Rice Crackers** **All Shortbread** *Western's* ➢ Shortbread ➢ Highland Oatmeal ➢ Rich Tea

Milk Protein Free	Contains Milk Protein
Nabisco ➢ Premiums ➢ Ritz (plain) ***Snack Foods*** ➢ Orgran fruit Bars ➢ Classic Choc biscotti ➢ Smith's Original Chips (plain) ➢ Red Rock Deli salt Sea salt ➢ CC's Original Chips ➢ Parkers Pretzels ➢ Mother Earth Popcorn Sea Salt Flavour ➢ All Natural Waffle cones ➢ Beta- Natural Ice Cream Wafers ***Convenience Foods*** ➢ Campbell's Tomato Soup ➢ Heinz tomato Soup ➢ Kan Tong Sweet and Sour ➢ Chinese Sweet and Sour ➢ Honey Teriyaki ➢ Tomato Based Pasta Sauce without cheese ➢ Oven Fries ➢ Wedges ➢ Birds Eye Potato Gems ➢ Oven Roast Vegetables ➢ Frozen vegetables (without sauces) ➢ I&J Fish Fingers ➢ I&J Light & Crispy Crumbed Fish ➢ Birds Eye Fish Fingers ➢ Home Brand Fish Fingers ➢ Baiada Chicken Tenders ➢ Maggi 2 minute noodles (not tomato) ***Coles*** ➢ Patties Milk Free Party Pies ➢ Patties Milk Free Sausage Rolls ➢ Patties Milk Free Pasties ➢ Jelly ➢ Easy Bake Bread Mix	*Nabisco* ➢ Chicken in a biscuit ***Snack Foods*** ➢ All other flavoured chips ➢ All other flavoured CC's ➢ All other flavours of the Mother Earth popcorn ***Convenience Foods*** ➢ All Crème Soups ➢ Chicken Tonight Creamy Sauces ➢ Baked Beans with Cheese Sauce ➢ Potato Smiles ➢ Animals ➢ Birds Eye Lil Fishies ➢ Maggi Pommes ➢ Fish in Parsley Sauce ➢ Fish in Cheese Sauce ➢ Steggles Chicken nuggets ➢ Maggi 2 minute Noodles (tomato) ➢ Packet Cake and Muffin Mixes ➢ Creamed Rice ➢ Yoghurt Mixes

Milk Protein Free	Contains Milk Protein
Condiments and Sauces	***Condiments and Sauces***
➢ Corn Relish ➢ Mustard ➢ Salad dressing ➢ Praise Traditional Mayonnaise ➢ Tomato Sauce ➢ BBQ Sauce	➢ Home Brand Coleslaw Dressing ➢ Home Brand Mayonnaise ➢ Kraft Mayonnaise
➢ Massel Supreme Gravy	➢ Gravox ➢ Green's Gravy
➢ Jam ➢ Honey ➢ Peanut Butter ➢ Vegemite	➢ Choc Chip Peanut Squeeze ➢ Nutella ➢ Hazelnut Chocolate Spread ➢ Nuts about Chocolate
Chocolate and Lollies	***Chocolate and Lollies***
➢ Lindt Excellence Dark Chocolate 85% Cocoa ➢ Lindt Excellence Dark Chocolate 70% Cocoa ➢ Sweet William(health food section of supermarket) ➢ Whittman's Dark Chocolate	➢ All other chocolate
➢ Allen's Fruit Pastilles ➢ Banana's ➢ Natural Confectionary Co. ➢ Starburst jubes ➢ Starburst gummy Bears ➢ Jelly Beans ➢ Boiled Lollies ➢ Minties ➢ Shakes ➢ Marshmallows	➢ Werther's Butter Scotch ➢ Butter menthols ➢ Allen's Chico's ➢ Home Brand Choc Mint Crunch ➢ Jersey Caramels ➢ Chocolate Peanuts ➢ Chocolate Bullets ➢ Freckles ➢ Chocolate Éclairs ➢ Fantales ➢ Columbines

Appendix 4b: Ingredients to look for on packaging

Cows milk protein free diet

Following a dairy free diet is not simply eliminating dairy products such as milk, yoghurt and cheese. These foods are commonly included as ingredients in other foods.

Look for the following words or items in ingredients lists (ingredients to avoid)

- Dairy
- Milk, milk powder, milk protein
- Milk solids, non-fat milk solids
- Skim milk solids, skim milk powder, skim milk protein
- Casein or sodium caseinate
- whey protein, whey powder
- Cheese, cheese powder
- Yoghurt
- Cream
- Sour cream
- Ice-cream
- Butter, margarine
- Chocolate
- Lactose (milk sugar)
- Beverage whitener

Appendix 5: Poster advertising study

The UNIVERSITY of NEWCASTLE
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 6984

DOES YOUR CHILD HAVE CONSTIPATION?

Poster for Newcastle. An investigation into constipation in children. Version 1 dated 13/09/2005

IS HE/SHE AGED BETWEEN 1 AND 12 YEARS?

WOULD YOU BE WILLING TO GIVE PERMISSION FOR THEM TO BE INVOLVED IN A RESEARCH TRIAL BEING CONDUCTED AT THE UNIVERSITY OF NEWCASTLE?

You would be asked to:

- arrange a time convenient to you to discuss your child's diet.
- have your child follow a dairy free diet for 6 weeks.

What would you have to do?

Give permission for your child to:

- provide a urine specimen and stool sample at the beginning of the study, after 2 weeks and then after 6 weeks on a dairy free diet.
- have additional blood taken when the routine samples are obtained.

How do you become involved?

- speak with your Doctor about your interest in the study during your consultation OR
- contact the researchers on the details listed below.

Contact: Elesa Crowley
Telephone: (02) 49215649
Mobile: 0428 612511
Email: elesa.crowley@hnehealth.nsw.gov.au
School of Health Sciences, The University of Newcastle

Appendix 6: Notices for Division of GP Newsletter

The University of Newcastle
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 7902
Email: lauren.williams@newcastle.edu.au

DOES YOUR PAEDIATRIC PATIENT HAVE CONSTIPATION?

Are they aged between 1 and 12 years?
Are they willing to be involved in a dietary research trial being conducted by researchers from the University of Newcastle?

Chronic functional constipation (CFC) is a common problem in children in the western world. It has been estimated that the frequency is as high as thirty-six percent of children that attend a consultation with a paediatrician. Causes of constipation can be organic or functional. The usual treatment regime is based on the premise that low intake of dietary fibre and fluid, lack of exercise or behavioural/psychological problems cause CFC (Coughlin, 2003). However, a number of researchers have described children who have not responded well to these usual treatments for constipation, suggesting that the exact aetiology is unclear in some cases (Iacono et al., 1998).

Several researchers have examined CFC as a possible manifestation of cow's milk protein allergy or intolerance. There is evidence (NHMRC Level II) that cow's milk protein removal from the diet results in symptom resolution for some children who are unresponsive to the usual treatments for constipation. This double blind RCT aims to further investigate this possibility in the Australian setting.

The child and or their parent would be asked to:

- Discuss their child's diet at a time convenient to them.
- Have their child follow a dairy free diet for 6 weeks (alternative sources of calcium will be discussed).
- Include the milk provided for the trial periods. This milk will be either A1 milk, A2 milk or soy milk. A1 and A2 refers to the type of casein protein in the milk. A1 milk is the cow's milk currently commonly available to consumers. A2 milk is cow's milk from the traditional milking cow's, Jerseys and Guernseys, and has recently become commercially available in New South Wales. All alternative milk will be provided at no cost to participants.
- Provide a blood, urine specimen and stool sample at the beginning of the study, after 2 weeks and then after 6 weeks on a dairy free diet.

How do they become involved or to find out more?

- Contact the researcher on the details listed below.

Contact: Elesa Crowley

Telephone: 0428 612511 Email: elesa.crowley @hnehealth.nsw.gov.au
School of Health Sciences, The University of Newcastle

Appendix 7a: Information package for participants, Newcastle

The University of Newcastle
AUSTRALIA

School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 6984

CONSENT FORM

Consent form, dietary intervention group, Newcastle. An investigation into constipation in children. Version 10 dated 16/05/05

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

I understand that the project will be conducted as described in the Information Sheet, a copy of which I have been given.

I understand that I am free to withdraw my child from this study at any time and do not have to give any reason for withdrawing. I also understand that withdrawing my child from the study will not incur disadvantages or penalties to my child or my self regarding his/her medical treatment in any way.

I consent to:

- A standard nutrition consultation with Elesa Crowley, Dietitian,
- Placing my child on a prescribed, normal cow's milk-free diet for 6 weeks with 2 milk trials, as decided by chance assignment for six weeks.
- Having three additional 6ml samples of blood collected while blood required for routine tests is taken from my child.
- Providing three samples of my child's urine and faeces.

I understand that my child's personal information will remain confidential to researchers.

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

I give permission for the dietitian conducting the research to contact me on the details I have provided below, to arrange a consultation at a time convenient to my child and myself.

Print Name:.....

Signature: ..... Date:.

Preferred method of contact - Telephone/Fax/Email:

.....

The University of Newcastle
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 6984

INFORMATION SHEET

Information Sheet for dietary intervention group, Newcastle. An investigation into constipation in children. Version 10 dated 16/05/07

Your child is invited to take part in the research project identified above which is being conducted by Elesa Crowley from the School of Health Sciences at The University of Newcastle. Elesa Crowley is a dietitian currently undertaking research for her Masters Degree at the University of Newcastle. Please ensure you discuss this study with your child. Your child's refusal to participate will mean that he/she will not be included in the study.

What is the purpose of this research?

The purpose of this research is to investigate a possible relationship between the dietary intake of milk and constipation. Constipation may have a number of causes. There are therefore a number of treatment options, one of which may be a cow's milk protein free diet. Cow's milk is a popular drink and food base containing many nutrients. This study is not seeking to alter this perception. Instead, it is thought that some children, a small percentage of the population, are sensitive to cow's milk although they may not realise it. Elesa is attempting to refine our understanding of this possible adverse reaction to milk by measuring changes in blood, urine and faeces.

Who can participate in this research?

We are seeking 60 children, aged between 18months and 12 years, with constipation to go on a cow's milk protein free diet for 6 weeks. Your child has been identified by your paediatrician as a suitable participant for this study.

What choice do you have?

Participation in this research is entirely your choice and the choice of your child. Only those people who give their informed consent will be included in the project.

Whether or not you decide to allow your child to participate, your decision will not disadvantage your child in any way and will not affect the care provided by your child's paediatrician. If you and your child decide to participate, you may withdraw from the project at any time without giving a reason. The researchers may withdraw a participant if it is considered in the participant's best interest to do so or for another reason. If the researchers withdraw your child from the study they will explain why and advise you about any follow-up procedures or alternative arrangements for your child as appropriate.

What would you be asked to do?

If you agree to participate, a standard nutrition consultation with a qualified dietitian Elesa Crowley, will be conducted at a time convenient to you. The aim of this consultation is to discuss your child's usual diet. This will take no more than 45 minutes.

After this consultation, the research assistant will provide you with milk labelled A or B. This will be the milk your child will be asked to use for the first 2 weeks of the study. This could be soy milk, or A2 milk or the commercially available cows milk,(predominantly A1). The middle 2 weeks will be totally milk free. You will receive another alternative milk for the final 2 weeks of the study. Please note that both A1 and *A2* are cow's milk. A1 and A2 refers to the type of casein protein in the milk. A1 milk is the cow's milk currently commonly available to consumers. Some reports have suggested that A1 milk may be linked to specific health conditions however, further research is needed before the questions can be answered with any certainty. It is predicted that it is also linked to gastrointestinal disturbances such as constipation. A2 milk is a modified type of cow's milk and has recently become commercially available in Northern New South Wales. All alternative milk will be provided for you at no cost.

Three samples of blood, urine and faeces would be required, one before and one at the end of the first 2 week milk trial and one at the end of the 6 weeks in addition to usual tests requested by your child's paediatrician. If your child is already having a blood sample collected, we would like permission to collect an additional 6ml (just over 1 teaspoon) of blood from the arm of your child. A nurse qualified in the collection of blood collection nurse will use a needle and syringe to extract the blood.

The blood will be couriered to the laboratory at the University of Newcastle. The results of the tests will be kept strictly confidential and upon your request, will be made available through your doctor. We do not expect that the individual results obtained in the study will be of clinical significance, however, any abnormalities found will be indicated on the result sheet and your child's paediatrician will be notified.

The tests on your child's samples would examine if there are specific milk related molecules present and if the composition of faecal bacteria and urine are altered with constipation and then with milk exclusion. Specifically, these tests are:

- Full blood count, milk specific molecules in blood called TABMs, anti-milk antibodies;
- faecal analysis;
- urinary amino acids; and
- urinary breakdown products of milk.
- Depending on the results of the study, that is, if constipation is resolved for participants, participants will be offered the opportunity to rechallenge with cows milk. This means your child will be offered the opportunity to consume cow's milk again. If constipation then occurs after consuming the cow's milk, this will assist in the confirmation that it is in fact the cause of the constipation.

What are the risks and benefits of participating?

It is important to understand that the project is speculative research and there may be no immediate benefits to participants. It is not expected that there will be any risk to participants.

How will your privacy be protected?

Confidentiality will be assured by assigning a number to the specimens and data will be recorded against that number. Information will be stored in the computer databases and locked cabinets within the laboratory, access to which is limited to authorised personnel only.

How will the information collected be used?

The data collected will be reported by Elesa Crowley in a thesis to be submitted for her Masters Degree and published in scientific journals.

What do you need to do to participate?

Please read this Information Statement and be sure you understand its contents before you agree for your child to consent to participate. If there is anything you do not understand, or you have any questions, please contact the researchers.

If you and your child would like to participate, please complete the enclosed consent form and return it to the University in the enclosed reply paid envelope. Elesa will then contact you to arrange a time at your convenience to conduct the nutrition assessment, discuss the diet, blood, urine and faeces collection and any additional nutrition issues. A calcium supplement will also be offered at this time.

Further Information

Dr Lauren Williams of the School of Health Sciences at the University of Newcastle will supervise the research. Elesa Crowley can be reached by telephoning (02) 49215630 during business hours for further information about the research project. You may ask any questions you wish and expect clear, comprehensible answers.

It is entirely your choice whether your child participates in this study and your decision will in no way disadvantage your child or alter their usual medical treatment. If your child does participate, you may withdraw them (or they may withdraw) at any time and ask for all samples and data to be destroyed.

Thank you for considering this invitation.

Yours sincerely

Dr Lauren Williams
Senior Lecturer
School of Health Sciences
The University of Newcastle
University of Newcastle
Telephone: (02) 4921 5649

Elesa Crowley
Student Researcher
School of Health Sciences
The University of Newcastle
University of Newcastle
Telephone: (02) 67678467

This research was approved by the former Hunter Ares Research Ethics Committee (whose functions are now performed by the Hunter New England Human Research Ethics Committee) ref 03/08/13/12/3.23).Should you have any concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308. Phone 02 49 216333 email *Human-Ethics@newcastle.edu.au* or to Dr Nicole Gerrand, Professional Officer (Research Ethics), Hunter New England Area Health Research Ethics Committee, Hunter Health, Locked Bag 1, New Lambton NSW 2305. Phone: (02) 49214950, email Nicole.Gerrand@hunter.health.nsw.gov.au

The University of Newcastle
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 0249216984

What would a dairy free diet involve?

Dietary intervention information sheet. An investigation into constipation in children. Version 10 dated 16/05/07

If you consent to your child taking part in this study, it will be necessary for them to follow a diet free of commonly available cows milk protein for a six-week period. This would involve reading all food labels and avoiding all foods containing the following ingredients:

Ingredients to AVOID

Milk, milk solids, non-fat milk solids, skim milk solids, casein, sodium caseinate, whey, whey powder, cheese, cheese powder, yoghurt including soy yoghurt, cream, butter and lactose.

Instead, your child may be assigned an alternative milk group. These will be labelled A or B. You will assigned one type for the first 2 and another for the last 2 weeks of the study.

Cows Milk Group This will be the ordinary commercially available .

Soy Milk Group This will be Sanitarium So Good.

A2 Milk Group This will be Fairbrae A2 Milk. A2 milk is already commercially available in Northern New South Wales. This milk will be provided for you at no cost.

Whichever group your child is assigned to, the appropriate milk substitute will be provided for you at no cost.

Instead of your usual butter or margarine, your child will need to use Nuttelex, Becel or Milk-Free Canola margarine.

Additional information:

- Most breads are naturally milk free.
- Some breakfast cereals contain milk products so you would need to check the label for the above ingredients.
- Many commercially prepared cakes and biscuits contain milk products. Specifically labelled milk free biscuits and cakes are available.
- You could also alter your existing recipes and simply replace butter *or margarine* with one of the suggested margarines and milk with the type of milk alternative you are assigned.

Other suitable food and beverage replacements will be discussed with you and your child during the dietary consultation should you choose to take part in this study. Calcium intake would also be addressed as would any other dietary issues for you and your child.

Appendix 7b: Information package for participants, Tamworth

The University of Newcastle
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 6984

CONSENT FORM

Consent form, dietary intervention group, Newcastle. An investigation into constipation in children.
Version 10 dated 16/05/05

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

I understand that the project will be conducted as described in the Information Sheet, a copy of which I have been given.

I understand that I am free to withdraw my child from this study at any time and do not have to give any reason for withdrawing. I also understand that withdrawing my child from the study will not incur disadvantages or penalties to my child or my self regarding his/her medical treatment in any way.

I consent to:

- A standard nutrition consultation with Elesa Crowley, Dietitian,
- Placing my child on a prescribed, normal cow's milk-free diet for 6 weeks with 2 milk trials, as decided by chance assignment for six weeks.
- Having three additional 6ml samples of blood collected while blood required for routine tests is taken from my child.
- Providing three samples of my child's urine and faeces.

I understand that my child's personal information will remain confidential to researchers.

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

I give permission for the dietitian conducting the research to contact me on the details I have provided below, to arrange a consultation at a time convenient to my child and myself.

Print Name:.....

Signature: ..... Date:.

Preferred method of contact - Telephone/Fax/Email:

.....

The UNIVERSITY of NEWCASTLE
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 6984

INFORMATION SHEET

Information Sheet for dietary intervention group, Tamworth. An investigation into constipation in children. Version 10 dated 16/05/07

Your child is invited to take part in the research project identified above which is being conducted by Elesa Crowley from the School of Health Sciences at The University of Newcastle. Elesa Crowley is a dietitian currently undertaking research for her Masters Degree at the University of Newcastle. Please ensure you discuss this study with your child. Your child's refusal to participate will mean that he/she will not be included in the study.

What is the purpose of this research?

The purpose of this research is to investigate a possible relationship between the dietary intake of milk and constipation. Constipation may have a number of causes. There are therefore a number of treatment options, one of which may be a cow's milk protein free diet. Cow's milk is a popular drink and food base containing many nutrients. This study is not seeking to alter this perception. Instead, it is thought that some children, a small percentage of the population, are sensitive to cow's milk although they may not realise it. Elesa is attempting to refine our understanding of this possible adverse reaction to milk by measuring changes in blood, urine and faeces.

Who can participate in this research?

We are seeking 60 children, aged between 18months and 12 years, with constipation to go on a cow's milk protein free diet for 6 weeks. Your child has been identified by your paediatrician as a suitable participant for this study.

What choice do you have?

Participation in this research is entirely your choice and the choice of your child. Only those people who give their informed consent will be included in the project. Whether or not you decide to allow your child to participate, your decision will not

disadvantage your child in any way and will not affect the care provided by your child's paediatrician. If you and your child decide to participate, you may withdraw from the project at any time without giving a reason. The researchers may withdraw a participant if it is considered in the participant's best interest to do so or for another reason. If the researchers withdraw your child from the study they will explain why and advise you about any follow-up procedures or alternative arrangements for your child as appropriate.

What would you be asked to do?

If you agree to participate, a standard nutrition consultation with a qualified dietitian Elesa Crowley, will be conducted at a time convenient to you. The aim of this consultation is to discuss your child's usual diet. This will take no more than 45 minutes.

After this consultation, the research assistant will provide you with milk labelled A or B. This will be the milk your child will be asked to use for the first 2 weeks of the study. This could be soy milk, or A2 milk or the commercially available cows milk,(predominantly A1). The middle 2 weeks will be totally milk free. You will receive another alternative milk for the final 2 weeks of the study. Please note that both A1 and *A2* are cow's milk. A1 and A2 refers to the type of casein protein in the milk. A1 milk is the cow's milk currently commonly available to consumers. Some reports have suggested that A1 milk may be linked to specific health conditions however, further research is needed before the questions can be answered with any certainty. It is predicted that it is also linked to gastrointestinal disturbances such as constipation. A2 milk is a modified type of cow's milk and has recently become commercially available in Northern New South Wales. All alternative milk will be provided for you at no cost.

Three samples of blood, urine and faeces would be required, one before and one at the end of the first 2 week milk trial and one at the end of the 6 weeks in addition to usual tests requested by your child's paediatrician. If your child is already having a blood sample collected, we would like permission to collect an additional 6ml (just over 1 teaspoon) of blood from the arm of your child. A nurse qualified in the collection of blood collection nurse will use a needle and syringe to extract the blood.

The blood will be couriered to the laboratory at the University of Newcastle. The results of the tests will be kept strictly confidential and upon your request, will be made available through your doctor. We do not expect that the individual results obtained in the study will be of clinical significance, however, any abnormalities found will be indicated on the result sheet and your child's paediatrician will be notified.

The tests on your child's samples would examine if there are specific milk related molecules present and if the composition of faecal bacteria and urine are altered with constipation and then with milk exclusion. Specifically, these tests are:

- Full blood count, milk specific molecules in blood called TABMs, anti-milk antibodies;
- faecal analysis;
- urinary amino acids; and
- urinary breakdown products of milk.
- Depending on the results of the study, that is, if constipation is resolved for participants, participants will be offered the opportunity to rechallenge with cows milk. This means your child will be offered the opportunity to consume cow's milk again. If constipation then occurs after consuming the cow's milk, this will assist in the confirmation that it is in fact the cause of the constipation.

What are the risks and benefits of participating?

It is important to understand that the project is speculative research and there may be no immediate benefits to participants. It is not expected that there will be any risk to participants.

How will your privacy be protected?

Confidentiality will be assured by assigning a number to the specimens and data will be recorded against that number. Information will be stored in the computer databases and locked cabinets within the laboratory, access to which is limited to authorised personnel only.

How will the information collected be used?

The data collected will be reported by Elesa Crowley in a thesis to be submitted for her Masters Degree and published in scientific journals.

What do you need to do to participate?

Please read this Information Statement and be sure you understand its contents before you agree for your child to consent to participate. If there is anything you do not understand, or you have any questions, please contact the researchers.

If you and your child would like to participate, please complete the enclosed consent form and return it to the University in the enclosed reply paid envelope. Elesa will then contact you to arrange a time at your convenience to conduct the nutrition assessment, discuss the diet, blood, urine and faeces collection and any additional nutrition issues. A calcium supplement will also be offered at this time.

Further Information

Dr Lauren Williams of the School of Health Sciences at the University of Newcastle will supervise the research. Elesa Crowley can be reached by telephoning (02) 49215630 during business hours for further information about the research project. You may ask any questions you wish and expect clear, comprehensible answers.

It is entirely your choice whether your child participates in this study and your decision will in no way disadvantage your child or alter their usual medical treatment. If your child does participate, you may withdraw them (or they may withdraw) at any time and ask for all samples and data to be destroyed.

Thank you for considering this invitation.

Yours sincerely

Dr Lauren Williams
Senior Lecturer
School of Health Sciences
The University of Newcastle
University of Newcastle
Telephone: (02) 4921 5649

Elesa Crowley
Student Researcher
School of Health Sciences
The University of Newcastle
University of Newcastle
Telephone: (02) 49215649

This research was approved by the former Hunter Ares Research Ethics Committee (whose functions are now performed by the Hunter New England Human Research Ethics Committee) ref 03/08/13/12/3.23).Should you have any concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308. Phone 02 49 216333 email *Human-Ethics@newcastle.edu.au* or to Dr Nicole Gerrand, Professional Officer (Research Ethics), Hunter New England Area Health Research Ethics Committee, Hunter Health, Locked Bag 1, New Lambton NSW 2305. Phone: (02) 49214950, email Nicole.Gerrand@hunter.health.nsw.gov.au

The University of Newcastle
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 0249216984

What would a dairy free diet involve?

Dietary intervention information sheet. An investigation into constipation in children. Version 10 dated 16/05/07

If you consent to your child taking part in this study, it will be necessary for them to follow a diet free of commonly available cows milk protein for a six-week period. This would involve reading all food labels and avoiding all foods containing the following ingredients:

Ingredients to AVOID

Milk, milk solids, non-fat milk solids, skim milk solids, casein, sodium caseinate, whey, whey powder, cheese, cheese powder, yoghurt including soy yoghurt, cream, butter and lactose.

Instead, your child may be assigned an alternative milk group. These will be labelled A or B. You will assigned one type for the first 2 and another for the last 2 weeks of the study.

Cows Milk Group This will be the ordinary commercially available .

Soy Milk Group This will be Sanitarium So Good.

A2 Milk Group This will be Fairbrae A2 Milk. A2 milk is already commercially available in Northern New South Wales. This milk will be provided for you at no cost.

Whichever group your child is assigned to, the appropriate milk substitute will be provided for you at no cost.

Instead of your usual butter or margarine, your child will need to use Nuttelex, Becel or Milk-Free Canola margarine.

Additional information:

- Most breads are naturally milk free.
- Some breakfast cereals contain milk products so you would need to check the label for the above ingredients.
- Many commercially prepared cakes and biscuits contain milk products. Specifically labelled milk free biscuits and cakes are available.
- You could also alter your existing recipes and simply replace butter *or margarine* with one of the suggested margarines and milk with the type of milk alternative you are assigned.

Other suitable food and beverage replacements will be discussed with you and your child during the dietary consultation should you choose to take part in this study. Calcium intake would also be addressed as would any other dietary issues for you and your child.

Appendix 8: Encopresis Evaluation Chart

Encopresis Clinic – Evaluation and Management Chart

NAME:
ADDRESS:
MRN:
DOB:
PHONE:

Height: **Weight:** **Head Circumference:**

MAIN PRESENTING PROBLEM:

SYMPTOM	INITIAL	CURRENT
Frequency of stools	□ daily □ 2nd daily □1/wk □2/wk	□ daily □ 2nd daily □1/wk □2/wk
Consistency	□ soft □ hard □ formed □ loose □ sticky	□ soft □ hard □ formed □ loose □ sticky
Abdominal pain	□ No □ Yes	□ No □ Yes
Waxing and waning of symptoms	□ No □ Yes	□ No □ Yes
Anal Pain	□ No □ Yes	□ No □ Yes
Anal bleeding	□ No □ Yes	□ No □ Yes
Age of onset of constipation	□ 1□ 2 □ 3 □ 4 □ 5 □ 6 □7 □ 8 □ 9 □ >9	□ 1□ 2 □ 3 □ 4 □ 5 □ 6 □7 □ 8 □ 9 □ >9
Toilet Training Urine	□ <2 □2 □ 3 □ 4 □ 5 □>5 □ never	□ <2 □2 □ 3 □ 4 □ 5 □>5 □ never
Toilet Training Faeces	□ 1□ 2 □ 3 □ 4 □ 5 □>5 □ never	□ 1□ 2 □ 3 □ 4 □ 5 □>5 □ never
Faecal soiling	□ No □ Yes	□ No □ Yes
Withholding behaviour	□ No □ Yes	□ No □ Yes
Change in appetite	□ No □ Yes	□ No □ Yes
Nausea or vomiting	□ No □ Yes	□ No □ Yes
Weight loss	□ No □ Yes	□ No □ Yes
Perianal fissures, dermatitis abscess or fistula	□ No □ Yes If yes, details	□ No □ Yes If yes, details
Dietary intake: Fibre Fluid Dairy	 □ Adequate □ Inadequate □ Adequate □ Inadequate □ Adequate □ Excessive	 □ Adequate □ Inadequate □ Adequate □ Inadequate □ Adequate □ Excessive (>3 cups)

Previous medications: Oral □ Lubricant □ Stimulant □ Softener □Bulking agent □ Nil			
Current Medications: Oral □ Lubricant □ Stimulant □ Softener □Bulking agent □ Nil			
Enema/week	□ No	□ Yes	If yes, frequency
Suppository	□ No	□ Yes	If yes, frequency /week
Herbal	□ No	□ Yes	If yes, frequency /week
Other medications/vitamins □ No		□ Yes	If yes, details
Family History			
Significant Illnesses □ Thyroid □ Parathyroid □ Cystic fibrosis □ Coeliac disease □ Asthma □ Diabetes Type I □ Diabetes Type II □ Other (please detail)			
Gastrointestinal Hx (constipation, Hirschsprung's disease)(please detail)			

Medical History			
Gestational age, born at:	□ 24 wks	□ 24-36 wks	□ 36-38 □ 38-40 □ 40+
Maternal thrush during pregnancy	□ No	□ Yes	
Maternal Strep B during pregnancy	□ No	□ Yes	
Delivery (tick if yes)	□ Induced	□ Vaginal delivery	□ Caesarian
Time of passage of meconium	□ in the first 48 hours	□ after the first 48 hours	□ unsure
Condition at birth	□ Satisfactory	□ NICU	□ other please detail
Breast fed	□ No	□ Yes	Duration /months
Bottle fed	□ No	□ Yes	Name of formula:
Acute injury or disease	□ No	□ Yes If yes, details	
Hospital admissions	□ No	□ Yes If yes, details	
Immunisations	□ All completed	□ Not completed	
Allergies	□ No	□ Yes If yes, details	
Surgery	□ No	□ Yes If yes, details	
Sensitivity to cold	□ No	□ Yes	
Coarse hair	□ No	□ Yes	
Dry Skin	□ No	□ Yes	
Eczema	□ No	□ Yes	
Recurrent Urinary tract infections	□ No	□ Yes	
Other:			

Developmental History	□ Normal	□ Delayed development	
Diagnosis (tick if yes)	□ ADHD	□ ODD □ ADD	□ Other,detail
School performance	□ Average	□ Below average	□ Above average
Has your child seen a psychiatrist	□ No	□ Yes	
Has your child seen a psychologist	□ No	□ Yes	

Psychosocial History		
Psychosocial disruption of child or family	□ No	□ Yes
Interaction with peers	□ good	□ poor

Temperament	☐ Normal	☐ Abnormal	
Toilet habits at school	☐ Always	☐ Occasionally	
	☐ Never	☐ Urine only	☐ Soiling

Physical Examinations		
General Appearance	☐ normal	☐ abnormal, please detail:
Head, ears, eyes, nose and throat	☐ normal	☐ abnormal, please detail:
Neck	☐ normal	☐ abnormal, please detail:
Cardiovascular	☐ normal	☐ abnormal, please detail:
Lungs and chest	☐ normal	☐ abnormal, please detail:
Abdomen		
Distension	☐ No	☐ Yes
Palpable liver and Spleen	☐ No	☐ Yes
Faecal mass	☐ No	☐ Yes
Anal Inspection		
Position	☐ normal	☐ abnormal, please detail:
Stool present around anus or on clothes	☐ No	☐ Yes
Perianal erythema	☐ No	☐ Yes
Skin tags	☐ No	☐ Yes
Anal fissures	☐ No	☐ Yes
Rectal Examination		
Anal tone	☐ normal	☐ abnormal, please detail:
Faecal mass/presence of stool	☐ No	☐ Yes
Consistancy of stool	☐ Soft	☐ Hard
Explosive stool on	☐ No	☐ Yes
withdrawral of finger	☐ No	☐ Yes
Occult blood in stool	☐ No	☐ Yes, please detail:
Other masses		
Back and Spine		
Dimple/Tuft of hair	☐ No	☐ Yes
Investigations Blood		
T3, T4, TSH	☐ normal	☐ abnormal, please detail:
Coeliac antibodies	☐ normal	☐ abnormal, please detail:
Calcium	☐ normal	☐ abnormal, please detail:
Rectal Biopsy		
Plain X-ray abdominal	☐ normal	☐ abnormal, please detail:
Colonic transit time	☐ normal	☐ abnormal, please detail
Barium enema	☐ normal	☐ abnormal, please detail
Other tests:		
MRI of spine	☐ normal	☐ abnormal, please detail:
Manometry-anorectal	☐ normal	☐ abnormal, please detail:
Colonic	☐ normal	☐ abnormal, please detail:

Diagnosis		
Functional constipation with or without impaction	□ No	□ Yes
Functional faecal retention	□ No	□ Yes
Hirchsprung's disease	□ No	□ Yes
NID	□ No	□ Yes
Others		

Management (Use guidelines)			
Education:			
Disimpaction:	□ No	□ Yes	
Osmotic:	□ No	□ Yes	
Dietary Education:	□ Fibre	□ Fluid	□ Dairy Free
Willingness to participate in study	□ No	□ Yes	
Would you Like to try without medication initially:	□ No	□ Yes	

Further questions for study participants

Asthma	□ No	□ Yes
Recurrent UTI's	□ No	□ Yes
Ear Infections	□ No	□ Yes
Grommets	□ No	□ Yes
Tonsilitis/Throat infection	□ No	□ Yes
Tonsils removed	□ No	□ Yes
Adenoids removed	□ No	□ Yes

Investigations Blood T3, T4, TSH	□ Normal □ Abnormal Exact measures	**Investigations Blood** LFT	□ Normal □ Abnormal Exact measures
Coeliac disease Abs	□ Normal □ Abnormal Exact measures	Total IgA, IgE, IgG, IgM	□ Normal □ Abnormal Exact measures
FBC	□ Normal □ Abnormal Exact measures	ASOT & antiDNaseB	□ Normal □ Abnormal Exact measures
EUC	□ Normal □ Abnormal Exact measures	B12 & Folate (blood +serum)	□ Normal □ Abnormal Exact measures
Copper	□ Normal	Zinc	□ Normal

	□ Abnormal Exact measures		□ Abnormal Exact measures

Investigations **Faeces** **Aerobic** NormaL Gut Flora	□ Absence □ Presence □ Scanty □ Moderate □ Profuse
Strep B	□ Absence □ Presence □ Scanty □ Moderate □ Profuse

Investigations **Urine** **HPLC** **Exophins**	□ Absence □ Presence

Investigations **Serum** **TABM**	□ Absence □ Presence Exact Measure

Investigations **Serum** **ELISA**	□ Absence □ Presence Exact Measure

DIETARY ASSESSMENT DIETITIAN TO COMPLETE

Name:	DOB:
Address:	Phone No:

Date: Referred by:

NO. OF FAMILY MEMBERS AT HOME: YEAR AT SCHOOL:

COOKING: SHOPPING:

DIET HISTORY:

Milk/d:	Cereal type /d:	Fish:	Soft drink:	KFC:
Cheese/d:	Rice:	Spag Bol:	Cordial:	Pies:
Yoghurt/d:	Pasta:	Lasagna:	Juice:	Sausage rolls:
Ice-cream/d:	Croissants:	Pizza:	Water:	Eat Out:
Custard/d:	Cakes:	Beef Strog:	Prunes:	Other Takeaway:
Cream/d:	Biscuits:	Fish fingers:	Bran:	Before/after school care:
Butter/Marg/d:	Fruit/d:	Chips:	Psyllium:	Day care:
Soy milk/d:	Vegetables/d	Choc/lollies:	Canteen:	Sleepovers:
Rice milk/d:	Meat:	Eggs:	McD:	Dip:
Bread/type/d:	Chicken:	Legumes:	Hungry Jacks:	Any Other foods:

ASSESSMENT/PLAN:

H/OUTS: □ Std Milk Free □ Milk free Food List □ Constipation Diaries
□ Path form □ Recipe book □ Alt Milk supply organized

Appendix 9: Dietitian assessment form

DIETARY ASSESSMENT DIETITIAN TO COMPLETE

Name:	DOB:
Address:	Phone No:

Date: Referred by:

NO. OF FAMILY MEMBERS AT HOME: YEAR AT SCHOOL:

COOKING: SHOPPING:

DIET HISTORY:

Milk/d:	Cereal type /d:	Fish:	Soft drink:	KFC:
Cheese/d:	Rice:	Spag Bol:	Cordial:	Pies:
Yoghurt/d:	Pasta:	Lasagna:	Juice:	Sausage rolls:
Ice-cream/d:	Croissants:	Pizza:	Water:	Eat Out:
Custard/d:	Cakes:	Beef Strog:	Prunes:	Other Takeaway:
Cream/d:	Biscuits:	Fish fingers:	Bran:	Before/after school care:
Butter/Marg/d:	Fruit/d:	Chips:	Psyllium:	Day care:
Soy milk/d:	Vegetables/d	Choc/lollies:	Canteen:	Sleepovers:
Rice milk/d:	Meat:	Eggs:	McD:	Dip:
Bread/type/d:	Chicken:	Legumes:	Hungry Jacks:	Any Other foods:

ASSESSMENT/PLAN:

H/OUTS: □ Std Milk Free □ Milk free Food List □ Constipation Diaries
□ Path form □ Recipe book □ Alt Milk supply organized

Appendix 10: Constipation diary

Constipation Study Patient Diary

Date of the first day of the week:
Please complete one row at the end of each day.

day month year

Day of the week	Bowel Movements: Number of bowel movements (0 = None, 1 = One, 2 = Two, >3 = More than Three)	Bowel Movements: Straining?	Stool form (refer to diagram on back)	Abdominal Pain/Discomfort (0 = None, 1 = Present but tolerable, 2 = Present and interfering with but not preventing normal daily activities, 3 = Preventing normal daily activities)	Bloating (0 = None, 1 = Present but tolerable, 2 = Present and interfering with but not preventing normal daily activities, 3 = Preventing normal daily activities)
1	0 1 2 3 >3	yes no	1 2 3 4 5 6 7	0 1 2 3	0 1 2 3
2	0 1 2 3 >3	yes no	1 2 3 4 5 6 7	0 1 2 3	0 1 2 3
3	0 1 2 3 >3	yes no	1 2 3 4 5 6 7	0 1 2 3	0 1 2 3
4	0 1 2 3 >3	yes no	1 2 3 4 5 6 7	0 1 2 3	0 1 2 3
5	0 1 2 3 >3	yes no	1 2 3 4 5 6 7	0 1 2 3	0 1 2 3
6	0 1 2 3 >3	yes no	1 2 3 4 5 6 7	0 1 2 3	0 1 2 3
7	0 1 2 3 >3	yes no	1 2 3 4 5 6 7	0 1 2 3	0 1 2 3

Stool Form	Appearance	Type
Separate hard lumps like nuts (hard to pass) Result of slow transit.		1
Sausage - shaped but lumpy.		2
Like a sausage but with cracks on its surface.		3
Like a sausage or snake - smooth and soft.		4
Soft blobs with clear cut edges (easy to pass).		5
Fluffy pieces with ragged edges, a mushy stool.		6
Watery, no solid pieces. Result of fast transit.		7

Appendix 11: Questions for debriefing interview

QUESTIONS FOR DEBRIEFING INTERVIEW

Hello, it's Elesa Crowley, the researcher from the investigation into constipation study speaking. I'm calling today to conduct a debriefing interview. I'm going to ask you a few questions about how you as a family found the study. You will remember that your child was required to follow a milk free diet for six weeks and the first two weeks and the last two weeks you included the trial milk provided to you in his/her diet.

1. What was it like putting your child on the milk free diet?
2. Did you use the milk free shopping guide?
    a. If no, why not?
    b. If yes, how did you use it?
3. Can you think of anything that would have made the milk free diet easier?
4. Is there any other information that you think would have made the milk free diet easier to follow? (Prompt – a supermarket tour).
5. Now that you've finished the study, what do you think you'll do in terms of the diet? Why?
6. Is there anything else you'd like to comment on about having your child participate in the study?

Appendix 12: Ethics approval for qualitative study

21 May 2007

HUNTER NEW ENGLAND
NSW HEALTH

Dr L Williams
School of Health Sciences
University of Newcastle

Dear Dr Williams,

RE: An Investigation into Constipation in Children (03/08/13/3.12)

Thank you for submitting a request for an amendment to the above project. This amendment was reviewed by the Deputy Chair of the Hunter New England Human Research Ethics Committee under the provisions of expedited review. This Human Research Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research (2007)* (National Statement) and the *CPMP/ICH Note for Guidance on Good Clinical Practice.*

I am pleased to advise that the Hunter New England Human Research Ethics Committee has granted ethical approval for the following amendment request:

- to extend recruitment via the Division of General Practice in the Hunter New England Area and from the private practices of Dr Ranjendra Kumar and the Newcastle Private Hospital Paediatric Clinic;
- The inclusion of a qualitative telephone interview;
- The Information Package for Participants (attachment 5 which includes the information sheet and consent form for the dietary intervention group (version 10 dated 16 May 2007), the collection instructions for parents (version 10 dated 16 May 2007), the form for participant samples (version 10 dated 16 May 2007);
- The letter to previous participants (version 1 dated 16 May 2007);
- The questions for debriefing interview (attachment 1); and
- The consent form for the debriefing interview (Attachment 4).

For the protocol **An Investigation into Constipation in Children.**

Approval from the Hunter New England Human Research Ethics Committee for the above protocol is given for a maximum of 3 years from the date of the approval letter of your initial application, after which a renewal application will be required if the protocol has not been completed. The above protocol is approved until **December 2009.**

The *National Statement on Ethical Conduct in Human Research (2007)* which the Committee is obliged to adhere to, include the requirement that the committee monitors the research protocols it has approved. In order for the Committee to fulfil this function, it requires:

- a report of the progress of the above protocol be submitted at 12 monthly intervals. Your review date is **December 2007.** A proforma for the annual report will be sent two weeks prior to the due date.

- A final report be submitted at the completion of the above protocol, that is after data analysis has been completed and a final report compiled. A proforma for the final report will be sent two weeks prior to the due date.

Hunter New England Human Research Ethics Committee

(Locked Bag No 1)
(New Lambton NSW 2305)
Telephone (02) 49214 950 Facsimile (02) 49214 818
Email: Nicole.gerrand@hnehealth.nsw.gov.au
Michelle.lane@hnehealth.nsw.gov.au
http://intranet.hne.health.nsw.gov.au/human_research_ethics
http://www.hnehealth.nsw.gov.au/ human_research_ethics

- All variations or amendments to this protocol, including amendments to the Information Sheet and Consent Form, must be forwarded to and approved by the Hunter New England Human Research Ethics Committee prior to their implementation.

- The Principal Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including:

    - any serious or unexpected adverse events:

        - Adverse events, however minor, must be recorded as observed by the Investigator or as volunteered by a participant in this protocol. Full details will be documented, whether or not the Investigator or his deputies considers the event to be related to the trial substance or procedure.

        - Serious adverse events that occur during the study or within six months of completion of the trial at your site should be reported to the Professional Officer of the Hunter New England Human Research Ethics Committee as soon as possible and at the latest within 72 hours.

        - Copies of serious adverse event reports from other sites should be sent to the Hunter New England Human Research Ethics Committee for review as soon as possible after being received.

        - Serious adverse events are defined as:

            - Causing death, life threatening or serious disability.
            - Cause or prolong hospitalisation.
            - Overdoses, cancers, congenital abnormalities whether judged to be caused by the investigational agent or new procedure or not.

    - unforeseen events that might affect continued ethical acceptability of the project.

- If for some reason the above protocol does not commence (for example it does not receive funding); is suspended or discontinued, please inform Dr Nicole Gerrand, the Professional Officer of the Hunter New England Human Research Ethics Committee as soon as possible.

The Hunter New England Human Research Ethics Committee also has delegated authority to approve the commencement of this research on behalf of the Hunter New England Area Health Service. This research may therefore commence.

Should you have any queries about your project please contact Dr Nicole Gerrand as per her contact details at the top of the previous page. The Hunter New England Human Research Ethics Committee Terms of Reference, Standard Operating Procedures, membership and standard forms are available from the Hunter New England Area Health Service website:

Should you have any queries about your project please contact Dr Nicole Gerrand as per her contact details at the bottom of the page. The Hunter New England Human Research Ethics Committee Terms of Reference, Standard Operating Procedures, membership and standard forms are available from the Hunter New England Area Health Service website:

Hunter New England Human Research Ethics Committee

(Locked Bag No 1)
(New Lambton NSW 2305)
Telephone (02) 49214 950 Facsimile (02) 49214 818
Email: Nicole.gerrand@hnehealth.nsw.gov.au
Michelle.lane@hnehealth.nsw.gov.au
http://intranet.hne.health.nsw.gov.au/human_research_ethics
http://www.hnehealth.nsw.gov.au/ human_research_ethics

Intranet address:
http://intranet.hne.health.nsw.gov.au/human_research_ethics

Internet address:
http://www.hnehealth.nsw.gov.au/ human_research_ethics

Please quote **03/08/13/3.02** in all correspondence.

The Hunter New England Human Research Ethics Committee wishes you every success in your research.

Yours faithfully

For: Dr M Parsons
Chair
Hunter New England Human Research Ethics Committee

Hunter New England Human Research Ethics Committe

(Locked Bag No
(New Lambton NSW 230
Telephone (02) 49214 950 Facsimile (02) 49214 8
Email: Nicole.gerrand@hnehealth.nsw.gov.
Michelle.lane@hnehealth.nsw.gov.
http://intranet.hne.health.nsw.gov.au/human_research_ethi
http://www.hnehealth.nsw.gov.au/ human_research_ethi

Appendix 13: Revised consent form for qualitative interviews

The UNIVERSITY of NEWCASTLE
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 6984

CONSENT FORM

Consent form, dietary intervention group, Newcastle. An investigation into constipation in children. Version 7 dated 16/5/2007

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

I understand that the project will be conducted as described in the Information Sheet, a copy of which I have been given.

I understand that I am free to withdraw my child from this study at any time and do not have to give any reason for withdrawing. I also understand that withdrawing my child from the study will not incur disadvantages or penalties to my child or my self regarding his/her medical treatment in any way.

I consent to:

- A standard nutrition consultation with Elesa Crowley, Dietitian,
- Placing my child on a prescribed, normal cow's milk-free diet for 6 weeks with 2 milk trials, as decided by chance assignment for six weeks.
- Having three additional 6ml samples of blood collected while blood required for routine tests is taken from my child.
- Providing three samples of my child's urine and faeces.
- A follow up debriefing interview after the conclusion of the dietary trials

I understand that my child's personal information will remain confidential to researchers. I have had the opportunity to have questions answered to my satisfaction.I give permission for the dietitian conducting the research to contact me on the details I have provided below, to arrange a consultation at a time convenient to my child and myself.

Print Name:.....

Signature: ..... Date:.

Preferred Method of contact: Telephone/Fax/Email.....

Appendix 14: Letter to previous participants for qualitative study

The University of Newcastle
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 7902
Email: lauren.williams@newcastle.edu.au

(Name)
(Address)

(Date)

Dear (Name)

Re: An investigation into constipation

Previously, you and your child participated in a research study, 'An investigation into constipation.' We appreciate the time and effort you put into completing the study. In order to evaluate the ease or difficulty of following a milk free diet we would like to contact you for a short telephone interview.

You will be contacted by telephone by Elesa Crowley, and given the opportunity to participate. If you choose to participate it is envisaged that the interview could be conducted in ten minutes. This information will be used for the development of practical guidelines for the management of constipation in some children who are unresponsive to the usual treatments.

The phone interview will be conducted by Elesa. Your verbal answers will be recorded onto a tape recorder. Data will be stored using a unique identification code. All names will be removed. You will receive feedback about the study after its conclusion.

If you do not wish to participate in this component it will not affect your participation in the remainder of the study. You are free to withdraw from the study at any time.

Thank you for your consideration.

Yours sincerely

Dr Lauren Williams
Senior Lecturer
School of Health Sciences
The University of Newcastle
University of Newcastle
Telephone: (02) 4921 5649

Elesa Crowley
Student Researcher
School of Health Sciences
The University of Newcastle
University of Newcastle
Telephone: (02) 49215630

This research has been reviewed and approved by the Hunter Area Research Ethics Committee Reference No. 03/08/13/3.23 and the University of Newcastle Human Research Ethics Committee No. H-682-1003.
Should you have any concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308. Phone 02 49 216333 email *Human-Ethics@newcastle.edu.au* or to Dr Nicole Gerrand, Professional Officer, Hunter Area Research Ethics Committee, Hunter Health, Locked Bag 1, New Lambton NSW 2305. Phone: (02) 49214950, email Nicole.Gerrand@hunter.health.nsw.gov.au

Appendix 15: Consent for debriefing interview

The UNIVERSITY of NEWCASTLE
AUSTRALIA

Dr Lauren Williams
School of Health Sciences
The University of Newcastle
CALLAGHAN NSW 2308
Telephone: 024921 5649
Facsimile: 024921 6984

CONSENT FORM

Consent form for debriefing interview. An investigation into constipation in children.

Consent form for debriefing, dietary intervention group, Newcastle. An investigation into constipation in children. Version 1 dated 16/5/2007

I agree ..... to participate in the above research project debriefing interview and have the interview recorded, I give my consent freely. I understand that I am free to end the interview whenever I choose, without penalty.

Participants Name:.....

Telephone: .....