LACTOSE SENSITIVITY

AND

INFLAMMATORY BOWEL DISEASE

A Dissertation for the Degree of Doctor of Medicine

School of Medicine

Cardiff University

April 2013

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SUMMARY OF THESIS

LACTOSE SENSTIVITY AND INFLAMMATORY BOWEL DISEASE

Controversy still exists as to the incidence, role and impact of lactose sensitivity in inflammatory bowel disease. The thesis shows that there is a higher than previously reported incidence of lactose sensitivity determined by a combination of genotype, breath test and symptoms after a lactose challenge. Lactose sensitivity in patients with inflammatory bowel disease who are in remission is 70%. There was no difference compared to healthy volunteers in terms of lactase genotyping; however there was a significantly greater prevalence of positive breath test and symptoms after lactose challenge. This suggests that lactose sensitivity in inflammatory bowel disease is related to the disease itself or a consequence of it and not due to a genetic predisposition. A significant proportion of inflammatory bowel disease patients [16%] are methane producers which warrants further investigation. A pilot study of reduced lactose intake in patients with Crohn's disease and lactose sensitivity, who were in remission, showed a promising improvement in symptoms reported and quality of life scores.

The Real-Time Polymerase Chain Reaction is simple and quick compared to Restrictive Fragment Length Polymorphism for assessing the lactase genotype. The *Quintron MicroLyzer* to assess breath samples after lactose challenge is preferred to the hand held *Micro* H_2 meter. This detects methane in addition to hydrogen and without this a number of cases of lactose sensitivity would be

missed. It may be possible to predict a negative breath test with the absence of any GI symptoms after a breath test and vice-versa a positive breath test is very likely if multiple GI symptoms are reported. The ‘hidden’ lactose in drugs used to treat inflammatory bowel disease and co-existing conditions should be considered as it is present in many drugs and can make a significant contribution to the amount of lactose ingested; lactose free alternatives are widely available.

DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed:……Date: ……

Dr Praveen Eadala

STATEMENT 1

This thesis is being submitted in partial fulfillment of the requirements for the degree of Doctor of Medicine [MD].

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STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references.

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STATEMENT OF ATTRIBUTION OF WORK

The work described in this dissertation was performed in the following institutions: Department of Gastroenterology & Department of Medical Biochemistry, Llandough Hospital, Cardiff, Department of Gastroenterology and Department of Medical Genetics and Department of Medical Biochemistry, University Hospital of Wales, Cardiff and Department of Infection, Immunity & Biochemistry, Cardiff University, Cardiff.

The Milk Allergy testing and Coeliac Serology was carried out by the Department of Medical Biochemistry, University Hospital of Wales, Cardiff.

ACKNOWLEDGEMENTS

I am chiefly indebted to Dr John Green, Department of Gastroenterology, Llandough Hospital, Cardiff, for his constant support, guidance and advice during this work and in the preparation of this dissertation.

I am indebted to Prof Anthony Campbell, Welsh School of Pharmacy, Cardiff University, Cardiff, for his supervision, guidance and advice during this work.

I am also grateful to Dr Stephanie Matthews, Department of Medical Biochemistry, Llandough hospital, Cardiff, for her advice on many aspects relating to this work.

Dr Jon Jenkins-Waud, Department of Medical Biochemistry, Llandough hospital, Cardiff, who taught me the techniques described in chapters 2, 4 & 6 and gave me the much needed guidance and supervision in performing these. I thank him for all of his support.

Ms Rachel Butler, Department of Medical Genetics, University Hospital of Wales, Cardiff, provided me laboratory space in performing genetic analysis in chapter 2 and 4.

I would also like to thanks Ms Jan Newmann, Department of Medical Biochemistry, Llandough Hospital and her staff who provided guidance and allowed me free access to the facility for the work presented in Chapter 3 & 4; for this I am grateful.

Dr Robert Hills, Senior Lecturer in Translation Statistics, Cardiff University, I am grateful for his advise on statistical methods used in this thesis.

I would also like to thank Mr. Adrian Davies and Ms. Awen Jones, Pharmacists, Llandough Hospital Cardiff and Vale NHS Trust, Cardiff, for sourcing and supplying lactose used in chapter 3 and 4 and for providing the samples of drugs used in chapter 6.

I also thank Clinical Research Facility, Cardiff and Vale NHS Trust, Cardiff in providing the facility to for conducting the tests.

I would also like to thanks all the patients who took part in this study without their support and time this would not be possible.

LIST OF ORIGNAL PUBLICATIONS

Some of the work in this dissertation has been published in peer reviewed journals.

Eadala P, Matthews SB, Waud JP, Green JT, Campbell AK. Association of lactose sensitivity with inflammatory bowel disease - demonstrated by analysis of genetic polymorphism, breath gases and symptoms. Aliment Pharmacol Ther. 2011 Oct; 34(7):735-46.

Eadala P, Waud JP, Matthews SB, Green JT, Campbell AK. Quantifying the 'hidden' lactose in drugs used for the treatment of gastrointestinal conditions. Aliment Pharmacol Ther. 2009 Mar 15; 29(6):677-87.

Eadala P, Green JT, Lactose intolerance. CME Gastroenterology, Hepatology & Nutrition. 2006 7(2):48-51.

ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
BNF	British National Formulary
CARD15	Caspase Recruitment Domain 15
CD	Crohn's Disease
CLD	Congenital lactase deficiency
CMA	Cow's milk allergy
CMSE	Cow's Milk Protein-Sensitive Enteropathy
CO2	Carbon Dioxide
CH4	Methane
DNA	Deoxyribonucleic Acid
EC	Enzyme Commission
ECM	Electronic Medicines Compendium
EDTA	Ethylene Diamine Tetra Acetic Acid
FODMAP	Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols
GI	Gastro-intestinal
H2	Hydrogen
HBT	Hydrogen Breath Test
HLA	Human Leukocyte Antigen
HPLC	High Performance Liquid Chromatography
HRQOL	Health Related Quality Of Life
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IFX	Infliximab
IPAA	Ileal Pouch-Anal Anastomosis
LCT	Lactase – Official Nomenclature
LI	Lactose intolerance
LM	Lactose Malabsorption/Maldigestion
LPH	Lactase-Phlorizin Hydrolase
LR	Likelihood Ratio

LTT	Lactose Tolerance Test
LTTE	Lactose Tolerance Test with Ethanol
MAP	*Mycobacterium avium paratuberculosis*
MC	Medicines Compendium
MCM6	Mini Chromosome Maintenance 6
MMX	Multi-Matrix System
NCBI	National Centre for Biotechnology Information
NF-κB	Nuclear Factor Kappa-Light Chain Enhancer of Activated B Cells
NOD2	Nucleotide Binding Oligomerization Domain 2
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PPM	Parts Per Million
PPV	Positive Predictive Value
RT-PCR	Real-Time Polymerase Chain Reaction
RFLP	Restrictive Fragment Length Polymorphism
RNA	Ribonucleic Acid
SCFA	Short Chain Fatty Acid
SD	Standard Deviation
SIBDQ	Short Inflammatory Bowel Disease Questionnaire
SNP	Single Nucleotide Polymorphism
TNF	Tumour Necrosis Factor
UC	Ulcerative Colitis
UCSC	University of California, Santa Cruz

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CHAPTER 1

INFLAMMATORY BOWEL DISEASE AND LACTOSE SENSITIVITY – REVIEW OF LITERATURE

The literature review will focus on three areas - inflammatory bowel disease [its epidemiology, pathogenesis, clinical features and treatment], lactose sensitivity [its definition, epidemiology, clinical features, diagnostic tests and treatment] and finally the clinical significance of lactose sensitivity in inflammatory bowel disease.

1.1: INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease [IBD] is a chronic relapsing and remitting inflammatory condition of the gastro-intestinal [GI] tract. The exact cause is not known but it is likely to arise from a combination of genetic and environmental factors. It is a common cause of morbidity and currently there is no cure. Crohn's Disease [CD] and Ulcerative Colitis [UC] are the two most common forms of IBD that are seen in clinical practice. They are diagnosed using clinical, endoscopic, radiological and histological criteria; no single finding from these is absolutely diagnostic of UC or CD.

UC was first described by the British physician Sir Samuel Wilks in 1859 (1) and CD is named after the American physician Burril B. Crohn who described the disease in 1932 (2). UC is characterised by a continuous inflammation of the colonic mucosa without granulomas on biopsy. It affects the rectum and then extends proximally along the colon to a variable extent. On the other hand CD is characterised by patchy, transmural inflammation, which may affect any part of the GI tract from mouth to anus (3, 4). The presentation of colitis [UC or CD] is characterised by frequency of bowel motions which are usually loose and can be bloody or associated with mucus. The CD affecting other areas of the bowel can often present insidiously with features such as abdominal pain and weight loss

with or without diarrhoea (5). CD may also present with fistulae and/or peri-anal disease.

1.1.1: EPIDEMIOLOGY OF IBD

IBD is primarily a disease of the western world and has a particularly high incidence in North America and Europe. In the UK the reported prevalence of IBD is 400 per 100,000 people [UC: 243/100,000 & CD: 145/100,000] (6). The last published data from the city of Cardiff, UK where my research is based showed the incidence of CD (7) was 6.6 and UC (8) 6.4 per 100,000 people for the decade up to 2005. Gender distribution in IBD is dependent on the disease subtype. In CD, there is a greater prevalence of females ranging from a published ratio of 1.1 to 1.6 (7, 9, 10), whilst in UC population-based studies have shown no significant differences between the sexes (9).

1.1.2: PATHOGENESIS OF IBD

The pathogenesis of IBD is unknown but is believed to arise as a result of the interplay between genetic and environmental factors.

1.1.2.1: FAMILY GENETICS

Epidemiological studies have shown a familial tendency for both types of IBD. Patients with CD have an affected first degree relative in 2.2-16.2% of cases and with either form of IBD in 5.2-22.5% of cases (11, 12). Patients with UC have an affected first degree relative in 5.7-15.5% of cases and with either type of IBD in 6.6-15.8% of cases (12-15). Twin studies (16-19) also demonstrate a greater genetic influence for CD compared with UC. The combined results from studies

show a concordance rate for monozygotic twins of 36% for CD and 16% for UC; for dizygotic twins it is 4% for both diseases (16-19).

1.1.2.2: IBD GENETICS

The complementary strategies of genome-wide linkage scanning and candidate gene analysis have uncovered a number of genetic loci associated with IBD susceptibility. A recent publication cited 99 IBD susceptibility loci/genes: 71 associated with CD, 47 with UC, and 28 with both CD and UC (20). These loci are depicted by lead gene name attaining genome-wide significance and are shown in Table 1.1 (20). CD is associated with nucleotide binding oligomerisation domain 2 [NOD2]/caspase recruitment domain 15 [CARD15] gene mutations on chromosome 16 (21, 22). The protein encoded by this gene is an intracellular receptor involved in the immune response against pathogens. It detects the N acetyl muramic acid moiety of muramyl dipeptide in bacterial cell walls (23). This recognition event triggers an inflammatory response by activation of Nuclear Factor Kappa-Light Chain Enhancer of activated B cells [NF-kB]. The relative risk of developing CD is 2.4 in a simple heterozygote and 17.1 in homozygotes (24). The presence of this mutation is also a risk factor for the development of ileal disease, increased disease severity, increased risk of surgery, diagnosis at a younger age and it also confers a greater risk for development of stricturing and penetrating disease (24-26). In contrast, the allele frequency of NOD2/CARD 15 variants has been similar in UC patients and healthy controls (27) except for one study that has shown that it may interact with the IBD 5 locus to increase the risk of UC (28).

Table 1.1: Inflammatory bowel disease susceptibility loci [adapted from Lees CW et al] (20). The loci depicted by lead gene name attaining genome-wide significance are shown for CD, UC and both UC & CD.

	CD	Both	UC
Th17		IL23R*, AK2*,STAT3*J, TYK2, IL12B*	
HLA		DRB*103	
Epithelial barrier			ECM1, HNF4A, CDH1, LAMB1, GNA12
Cellular innate immunity	NOD2, ATG16, IRGM, LRRK2		
Immune mediated	PTPN22, CCR6, IL2RA, IL18RAP, IL27, ERAP2, ITLN1, CCL2/CCL7,TNFSF11, BACH2, TAGAP, VAMP3	MST1*, IL10*, CARD9*, REL*, PRDM1*,TFSF15*, ICOSLG*, IL1R2*, YDJC, SMAD3, PTPN2	IL26/IFNγ, IL8RA/IL8RB, IL2/IL21, IL7R, TNFRSF9, TNFRSF14, IRF5, LSP1, FCGR2A
Others	DENNDIB, DNMT3A, GCKR, THADA, SP140, PRDX5, ZPF36L1, ZMIZI, MUCI/SCAMP3, CPEB4, FADS1, 5q31	NKX2-3*, CREM*, C11Orf30*, ORMDL3*, RTEL1*, PTGER4*, KIF21B*, CDKAL1, ZNF365	OTUD3/PLAG2G2E, DAP, PIM3, CAPN10

The human leukocyte antigen [HLA] complex on chromosome 6 encompasses genes which have an immuno-regulatory role. The HLA-DRB1*0103 allele (29) has been implicated in the pathogenesis of UC. The association is particularly

strong in patients with extensive disease and in those fail to respond to medical therapy needing surgery (30). In addition, the HLA-DRB1*1502 allele is also positively associated with the presence of UC whilst there is a negative association with HLA-DRB1*04 (29, 30). The most consistently reported association of CD is with the HLA-DRB1*07 (29). This is associated with ileal disease and is only present in the absence of NOD2/CARD15 variant (31, 32). HLA-DRB3*0301 and HLA-DRB1*04 [if possessing NOD2/CARD15 variants] are positively associated with CD (31, 32). The HLA-DRB1*0103 is strongly associated with CD of the colon (31, 32). The HLA-DRB1*1501 allele is negatively associated with CD and appears to confer protection against the condition (29).

1.1.2.3: ENVIRONMENTAL FACTORS

Epidemiological studies have strongly suggested environmental factors in the pathogenesis of IBD.

1.1.2.3.1: SMOKING

The prevalence of UC in smokers is reduced when compared with lifelong non-smokers and ex-smokers (33). The pooled odds ratio has been shown to be 0.41 for current smokers compared with lifelong non-smokers, 1.64 for ex-smokers compared to non-smokers and 2.9 for lifelong non-smokers compared to those who had ever smoked (33). There is a particularly high incidence in the onset of UC in people who have recently quit smoking (34). The relationship between smoking and CD is the opposite to that observed in UC (35) - smokers run a higher risk of developing the disease than ex-smokers (36). Smoking adversely

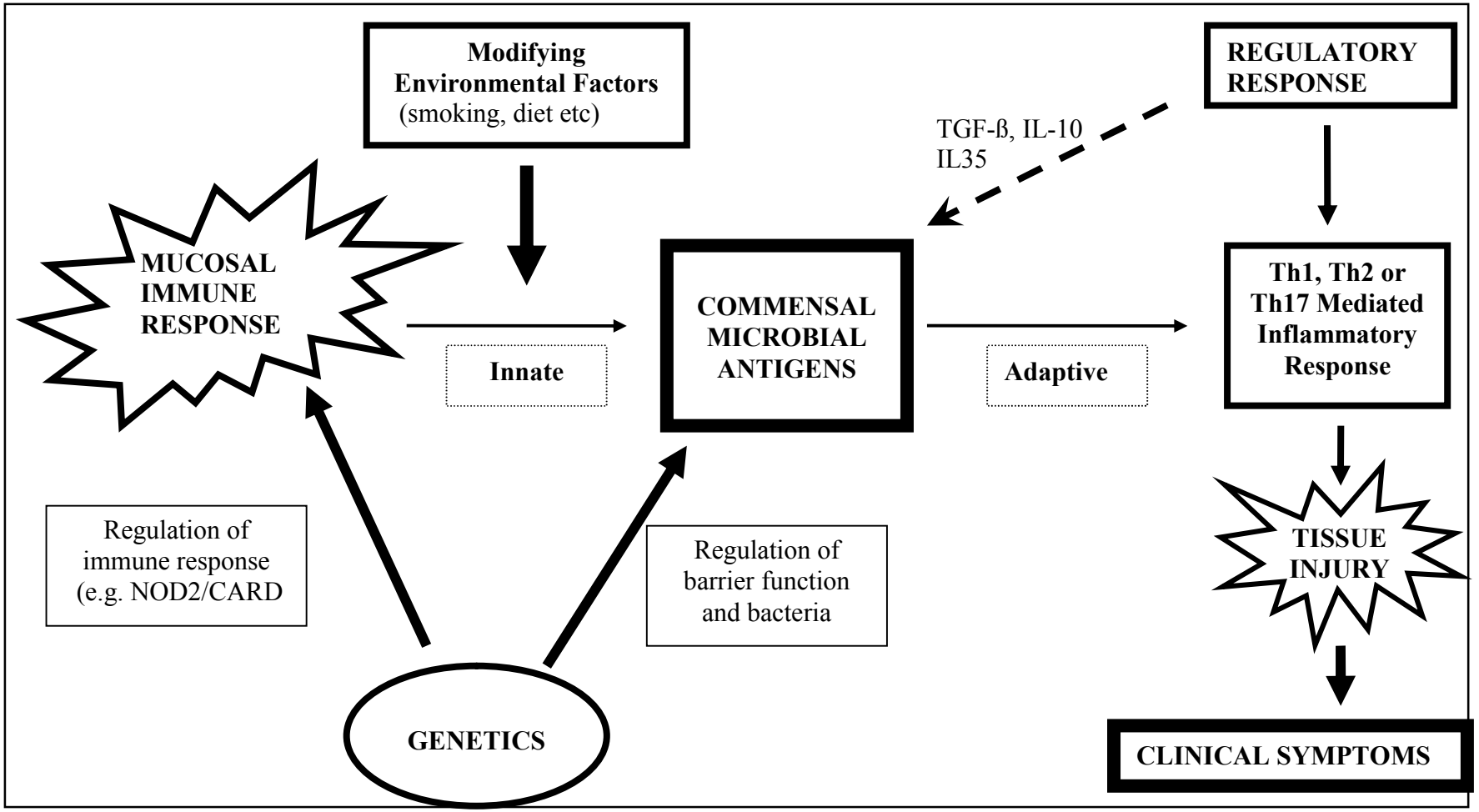
affects the clinical course of CD (37), it increases disease activity, promotes formation of fistulas, accelerating the need for surgery (36) and increasing the risk of recurrence after operations (38).

1.1.2.3.2: GUT FLORA

Humans maintain homeostasis with luminal bacteria until extrinsic factors such as diet, infection etc alter this balance. It is postulated that this alteration may lead to the development of IBD in susceptible individuals. A variety of microbial agents have been thought to have a role in the pathogenesis of IBD, but there is no convincing evidence to support any specific one. It has been proposed that the exposure to infectious diseases during the early years of life could decrease the incidence of IBD (39). Increased levels of antibodies directed against intestinal bacteria have been observed in IBD. In addition patients with CD may show clinical response to antibiotics and also faecal diversion has been shown to benefit some patients with peri-anal disease (40). The disease activity increases rapidly following reconnection of bowel loops after resection or after reperfusion with faecal material (41). Animal models of IBD require the presence of intestinal flora for the condition to manifest (42, 43) and inflammation is attenuated in germ free mice (44). It has also been possible to transfer colitis between animals via T cells that are reactive to enteric bacteria (45). In summary, the current consensus of opinion is that IBD appears to occur when there is a loss of regulation in the immune response to the normal commensal microbial antigens in a genetically susceptible host (46). The genetic basis of this disease is centred on factors that regulate the components of innate and adaptive immune responses. The composition of the bacteria in the bowel and the barrier function of the mucosal

epithelial cells are regulated by these immune responses. A variety of environmental factors affect genetic susceptibility and they act by regulating the immune response of the host, epithelial barrier activity and the type and function of the commensal bacteria. A model proposed by Blumberg for pathogenesis of IBD is summarised in the figure 1.2 (47). In this model IBD is apparently determined by the genetically defined, innate immune responsiveness of the intestinal tissue to components of the commensal bacteria. When this response is initiated, it is characterised by an exaggerated adaptive immune response by the production of immunoglobulin G antibodies that drive inflammation by T and B lymphocyte cells as shown in Figure 1.2 (47). Under the influence of factors derived from innate immune cells, the T cells which include T-helper 1 [TH1], TH2 or TH17 cells secrete cytokines such as interferon, tumour necrosis factor, interleukin [IL] IL 4, 5, 6, 13, 17 & 22 which cause the inflammation (47). These inflammatory factors result in tissue injury and the clinical symptoms of IBD. The T-regulatory cells secrete immunosuppressive cytokines such IL10, IL 35 and tumour growth factor- ß (47) which help to overcome the inflammatory response to achieve remission in IBD (47).

Figure 1.2: A Proposed Model for the Pathogenesis of Inflammatory Bowel Disease. Adapted from Blumberg 2009 (47).



1.1.2.3.3: DIET

Increased incidences of IBD in countries like Korea and Japan during the 1990's and the evidence from migration studies, may implicate westernised diet as an underlying cause (48-50). As a result a variety of different food groups have been studied. A positive association has been demonstrated for consumption of refined sugar, protein and fat consumption with both forms of IBD (48, 51-53), in addition fast foods, margarine and cola drinks have also been implicated as risk factors (53, 54). Also, an inverse association has been shown for the ingestion of both fruit and vegetables with IBD indicating that these could have protective effect in its development (51, 55). Antibodies to bakers yeast have been reported as being common in patients with CD but not in UC (56, 57). Studies have shown conflicting results for any association between IBD and the ingestion of coffee (54, 58), alcohol (58, 59) and cornflakes (60, 61). Curry contains curcumin which has antioxidant and anti-inflammatory properties which could potentially be one of the factors for the lower incidence of IBD in South Asians (62). IBD has also been proposed to be a secondary phenomenon to an allergic inflammatory response to food antigens. Patients with CD have a stronger response to food antigens than healthy controls (63). It has also been shown that food additives can mediate immune reactions (64) and so some authors have proposed that they are a cause of IBD, one study showed a diet lacking these reduced disease activity (65).

1.1.2.3.4: OTHER POTENTIAL ENVIRONMENTAL TRIGGERS

The current consensus is that environmental factors such as oral contraceptives (66), early weaning and childhood vaccination (67, 68) do not play any significant role in IBD pathogenesis. The development of IBD has been reported to occur

more frequently after gastrointestinal infections caused by contaminated food or water (69, 70). Appendicectomy may protect against the subsequent development of UC, whilst it may increase the risk of CD (71).

1.1.3: CLINICAL FEATURES

IBD primarily presents in late adolescence and early adulthood, although the diagnosis may be made at any age (72). The cardinal symptom of ulcerative colitis is bloody diarrhoea but patients may also complain of colicky abdominal pain, urgency, tenesmus and nocturnal defecation. The onset of UC is usually insidious and symptoms are often present for weeks. The clinical course is marked by exacerbation and remission with about 50% of patients experiencing a relapse in any year. Although the disease usually presents with intermittent episodes, it can present as a severe attack in about 15% with systemic symptoms which include weight loss, fever, tachycardia, nausea and vomiting (73). Crohn's disease patients have a wide range of symptoms and presentations. They include chronic diarrhoea, abdominal pain and/or weight loss, malaise, anorexia, and fever. Sometimes non-specific symptoms mimicking irritable bowel syndrome [IBS] and unexplained anaemia can be the only presentation (74). CD may also cause intestinal obstruction due to strictures, and peri-anal fistulas or abscesses. In 10% of patients these are present at the time of diagnosis or may be the only presenting complaint (75). 10% of patients with IBD present with extra-intestinal manifestations, which include an axial or peripheral arthropathy, episcleritis and erythema nodosum - these can precede intestinal symptoms (76).The clinical course of both these diseases is characterised by exacerbations and remission. Crohn's disease tends to cause greater disability than ulcerative colitis with only

75% of patients fully capable of work in the year after diagnosis and 15% of patients unable to work after 5-10 years of disease (77).

1.1.4: INVESTIGATIONS

The current national and international guidelines (4, 78 & 79) recommend that a full history should include enquiry about the onset and presentation of symptoms, together with travel, food intolerances, medications, and a prior history of appendicectomy. In addition information about smoking, family history, and recent infectious gastroenteritis (78, 79) should also be obtained. Clinical examination should include pulse rate, blood pressure, temperature, abdominal examination and digital rectal examination. The perineum and oral cavity should also be inspected together with the measurement of weight (78, 79). Laboratory tests should include full blood count, urea and electrolytes, liver function tests and erythrocyte sedimentation rate or C reactive protein, ferritin, vitamin B12 and folate. In both new presentations and in those who have a 'flare up' stool cultures should be sent – apart from bacteria that commonly cause gastroenteritis, the presence of clostridium difficile and cytomegalovirus should be assessed.

Abdominal radiography is often very helpful. It can exclude small bowel or colonic dilatation, show proximal constipation, calcified calculi, sacro-ilitis or an impression of mass (4). It can be particularly helpful in suggesting disease extent in active UC. In suspected UC or CD, the preferred procedure to establish the diagnosis and extent of disease is ileo-colonoscopy with segmental biopsies including the rectum. CT/MRI/Ultrasound has no role in the diagnosis of UC but they may aid in the assessment/ management of complications in CD where they can detect intestinal/extra-intestinal involvement and penetrating lesions (79).

1.1.5: DIAGNOSIS

A gold standard for the diagnosis of UC or CD is not available. It should be established by a combination of medical history, clinical evaluation and typical endoscopic and histological findings. Where there is doubt about the diagnosis, repeat endoscopic and histological confirmation is necessary after an interval (79).

1.1.6: PATHOLOGICAL FEATURES

A histological diagnosis of established UC is based upon the combination of: basal plasmacytosis, heavy and diffuse increase of inflammatory cells in the lamina propria and widespread mucosal or crypt architectural distortion. Mucosal atrophy and a villous or irregular mucosal surface appear in the later stages of the disease. General or widespread crypt epithelial neutrophilia [cryptitis and crypt abscesses] favours ulcerative colitis (79). In CD there is a focal [discontinuous] chronic [lymphocytes and plasma cells] pattern of inflammation, focal crypt irregularity [discontinuous crypt distortion] and granulomas [not related to crypt injury] (78).

1.1.7: TREATMENT

Currently there is no cure for inflammatory bowel disease. Medical and surgical treatments are used to reduce symptoms, maintain remission, decrease disease-related complications and improve the quality of life. Guidelines have been recently published by the British Society of Gastroenterology and European Crohn's and Colitis Organisation on the management of these conditions (4, 80). Drug treatment is normally the primary treatment option before surgery is considered. Medical treatments aim to induce remission when the condition

becomes more active and also maintain the patient in remission thereby preventing relapses. Commonly used medical therapy includes anti-inflammatory therapy [e.g., 5-Amino-Salicylates or Corticosteroids] and immuno-modulatory treatment [e.g., Azathioprine, 6 Mercapto-purine, Methotrexate or anti-TNF drugs like Infliximab or Adalumimab]. These can cause significant side-effects and are tolerated to a variable degree.

Surgery is performed when medical therapy fails or is not tolerated and when complications develop. Indications for surgery in UC include a poor response to intensive medical therapy, presence of colonic dysplasia or carcinoma and poorly controlled disease (4, 81). Indications for surgery in CD again include a sub-optimal response to medical therapy, obstructive symptoms, abdominal abscesses, strictures and the presence of dysplasia or carcinoma (4, 80). If surgery is necessary for ileo-colonic disease or localised small or large bowel disease then resecting only the affected part is preferable (4, 80). For strictures in CD, stricturoplasty is a safe and more effective alternative to resection.

It is therefore apparent from this review that IBD is a common problem that causes significant morbidity. We are not aware of its exact pathogenesis but it appears to arise, be affected by a combination of genetic predisposition and environmental triggers such as intestinal microbes, diet and smoking. Our current treatments are suboptimal as they cannot provide a cure or indeed reliably control the symptoms of all the patients who suffer from IBD.

1.2: LACTOSE SENSITIVITY

Lactose sensitivity describes the symptoms and/or the abnormal investigations that some people have after the ingestion of the sugar lactose. It is due to hypolactasia i.e. reduced levels of the enzyme lactase in the intestinal wall. Specific focus will be given to how it is diagnosed, its clinical significance and relationship with gastro-intestinal disorders.

1.2.1: TERMINOLOGY

HYPOLACTASIA, LACTASE NON-PERSISTENCE OR LACTASE INSUFFICIENCY

These refer to a very low activity of lactase in the small bowel mucosa. It can be primary [genetic] or secondary. The 'primary' form is an autosomal recessive condition resulting from the physiological decline in activity of the lactase enzyme in intestinal cells after weaning (82). The exact mechanism and the reasons behind this change are not understood yet. It is thought that the lactose content of food falls rapidly after weaning, and so the activity of lactase becomes less important to life. The 'secondary' form is the most frequent cause of insufficient lactose hydrolysis in individuals with genetic lactase persistence and occurs as a result of GI conditions such as coeliac disease (83), radiation enteritis (84), chemotherapy (85), tropical sprue (86) or Crohn's disease (87-89).

CONGENITAL ABSENCE OF LACTASE ENZYME SYNTHESIS

Congenital lactase deficiency [CLD] is a very rare autosomal recessive gastrointestinal disorder. It affects approximately 1:60000 newborns in Finland

(90) whilst worldwide less than 50 cases have been reported (91). Affected babies present with watery diarrhoea soon after starting drinking milk containing lactose. This causes weight loss, dehydration and acidosis (90). There is an almost total lack of lactase activity in jejunal biopsies of these individuals (91) which is due to distinct mutations in the coding region of the lactase gene (82, 92). These children grow and develop normally when placed on a lactose free diet (90).

LACTOSE MALDIGESTION/MALABSORPTION

This indicates that a significant proportion of the ingested lactose is not absorbed in the small bowel which is then delivered to the colon. This is objectively demonstrated via measurements of hydrogen/methane in breath or blood glucose concentrations following ingestion of a lactose load.

LACTOSE INTOLERANCE

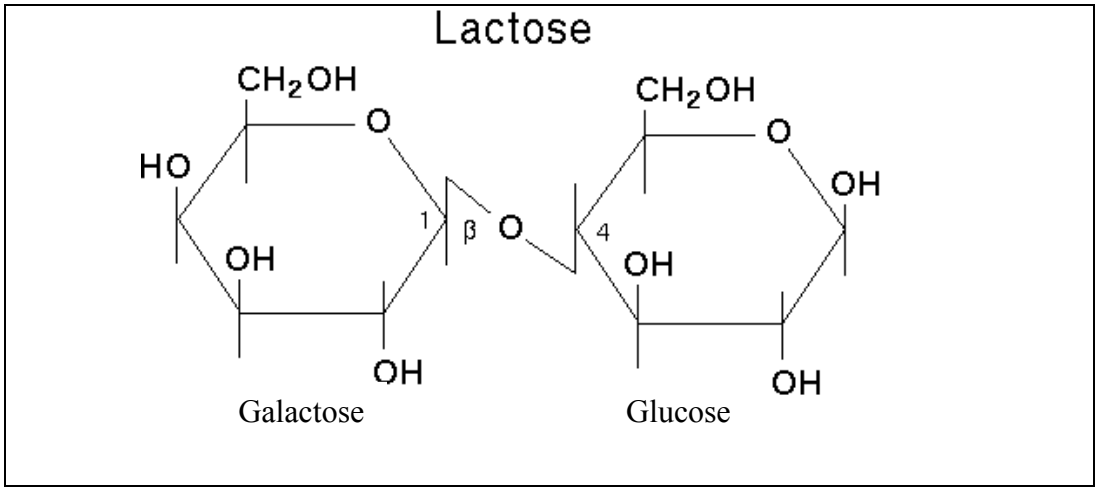
Lactose intolerance [LI] refers to the gastrointestinal and systemic symptoms associated with the incomplete digestion of lactose. The prevalence of lactase non-persistence or LM could exceed the prevalence of LI as the latter is a symptomatic response that is linked to the quantity of lactose malabsorbed. In the literature the term "lactose intolerance" is sometimes incorrectly used to mean lactose maldigestion (93).

1.2.2: LACTOSE

Lactose is a disaccharide that consists of galactose and glucose in equimolar quantities bonded through a β1-4 glycosidic linkage as shown in figure 1.5. Lactose is made by lactose synthase in the mammary gland during late

pregnancy and lactation before it is excreted in milk. Lactose makes up around 2-8% of the solids in mammalian milk (89, 94-95). Cow's milk, which is the most commonly consumed milk by humans contains 5% lactose i.e. one ml of cow's milk contains 47.2mg of lactose (89, 94-95). Lactose is an important energy source for newborns as it is solely the carbohydrate present in the breast milk which accounts for 45% of total caloric content of the breast milk.

Figure 1.5: Structure of Lactose showing glucose and galactose joined by β1-4 glycosidic linkage.



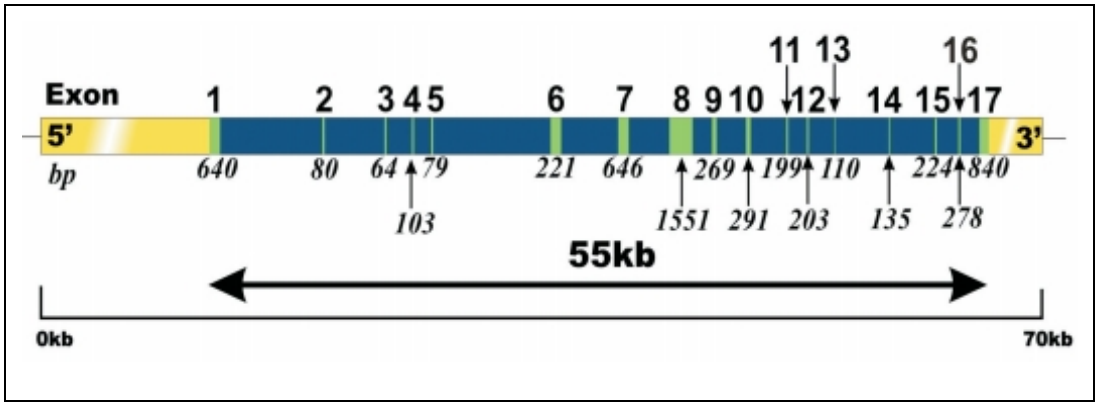
1.2.3: LACTASE - THE ENZYME

Lactose cannot be absorbed by the small intestinal mucosa and therefore has to be broken down into its constituent monosaccharides glucose and galactose by the enzyme lactase. Hydrolysis of lactose occurs at approximately half the rate of sucrose (96). After hydrolysis, glucose and galactose are absorbed from the intestine by the sodium dependent glucose transporter 1. Galactose is metabolized mainly in the liver into glucose, most of which then enters the body's glucose

pool (97). Lactase is located on the apical surface of the brush border of the intestinal epithelium. It is anchored into the membrane by its C-terminal end, with the bulk of the molecule projecting into the lumen. Lactase is encoded by a single gene [LCT - is the official nomenclature for Lactase] of 55 kb located on the long arm of chromosome 2 (98, 99). and the DNA sequence contains 17 exons, and lies within a 70 kb sequence containing regulatory response elements (82) [Figure 1.6]. It is on the reverse strand. In humans regulation involves both transcriptional and post-transcriptional mechanisms, transcriptional regulation controlling appearance of lactase in the foetus just before birth, and its loss on weaning. In spite of extensive searching, no mechanism causing hypolactasia after weaning has been identified.

Figure1.6: The Lactase gene taken from Campbell 2005 (89).

Lactase gene showing the position of the 17 exons and number of base pairs in each of these



Lactase is a large glycopeptide with two active sites within one polypeptide chain. One hydrolyses lactose, while the other was identified originally by its ability to

hydrolyse glycoside phlorizin to glucose and phloretin. Phlorizin is a competitive inhibitor of the lactose site but lactose does not appear to inhibit the phlorizin site (89). Lactase-phlorizin hydrolase is the full name of lactase with two enzyme commission [EC] numbers – EC 3.2.1.62 for its phlorizin hydrolase (100) activity and EC 3.2.1.108 for its β-galactosidase activity. The natural substrates for the phlorizin site are cerebrosides, hydrolysis of which provides sphingosine, found in the cell membranes of the brain. This explains why we need to keep some lactase after weaning (89).

Lactase activity is present in a characteristic gradient from the proximal to the distal small intestine mucosa with maximal activity in the proximal to mid jejunum and lower activity in the duodenum & ileum (101). The expression of enzyme activity is high from the late third trimester until the weaning period. Lactase enzyme activity then decreases after weaning to the lower levels as found in adults (102, 103). In certain groups of the world population, Caucasians and nomadic tribes of Africa, the capacity to digest lactose after weaning is maintained by a genetically determined process (104). This is probably caused by the selection of a spontaneous mutation in the lactase-phlorizin hydrolase gene (105).

1.2.4: PREVALENCE OF ADULT-TYPE HYPOLACTASIA

The prevalence of adult-type hypolactasia has a marked variation between races and populations. The prevalence of adult hypolactasia in different populations is affected by the use of different indirect diagnostic methods of varying sensitivity and specificity, differing diagnostic criteria and the numbers of participants (106, 107). The worldwide prevalence of adult-type hypolactasia is lowest in

populations of Northern Europe with a higher prevalence in the south of the continent. In Denmark and Sweden, its frequency is said to be only around 1-5% (107) however, based on the molecular diagnosis (108) the prevalence in one Swedish cohort was found to be about 10% (109). The prevalence in the UK has been found to be 10-15% (89). In general, adult-type hypolactasia is more common in populations outside Europe. However, in Caucasians and their descendents in North America and Australia the prevalence of adult-type hypolactasia is still low. In American whites, the prevalence is in the order of 15%, in African Americans about 80% and in Mexican American approximately 53% (106, 107). The prevalence in Latin America is generally high, around 70% in Mexico and 65% in Uruguay. In Asia, the prevalence is lower in the western parts: in Northern India around 30% and in Southern India 60-70%. The world's highest prevalence is in the populations of the Far East: in Thailand it is as high as 97-100%, and in Indonesia 91%. In China, a prevalence of adult-type hypolactasia of approximately 90% is observed (106, 107). The prevalence of adult-type hypolactasia in the black African populations ranges from 70 to 95%. Prevalence figures greater than 90% are observed among populations with low milk consumption such as those in Nigeria and Zaire in contrast to the populations with a tradition of milk consumption, nomads and the people who raise cattle, amongst whom the prevalence of hypolactasia is around 10-20% (107).

1.2.5: GENETICS OF ADULT-TYPE HYPOLACTASIA

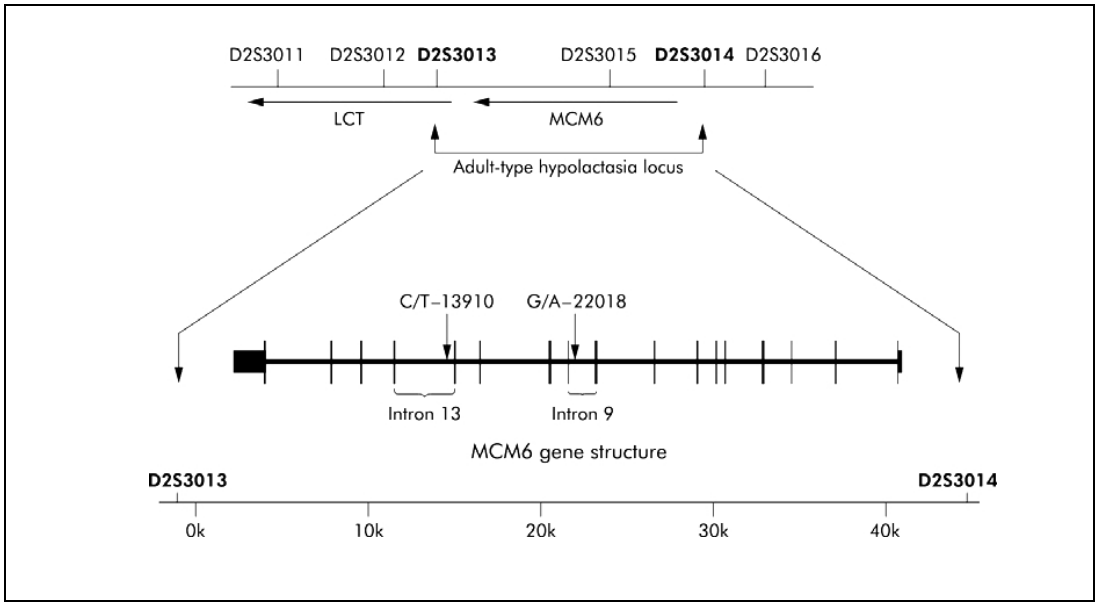
Lactase non-persistence is controlled by a single gene defect, inherited in an autosomal recessive manner (102). This finding was originally based on an analysis of segregation for adult-type hypolactasia in Finnish families (102). The

lactase phenotype had a complete concordance in monozygous twins whereas in dizygous twins the adult-type hypolactasia prevalence was compatible with the prevalence in the background population (110). A trimodal distribution in lactase activity, representing the homozygous recessive, heterozygous and homozygous dominant subjects has been reported in several studies (103, 105 & 111). This meant that the lactase persistence/non-persistence trait is most likely due to polymorphisms within or near the lactase gene on chromosome 2. Despite the cloning and the sequencing of the complete cDNA and 1 kb of the promoter region of the lactase gene, no sequence differences segregating with the lactase persistence trait were identified until recently (112). Several polymorphisms have been found in the introns and exons of the lactase gene and its promoter, but none consistently correlate with lactase persistence/non-persistence (82). There are four common haplotypes world wide, designated A, B, C and U. Only A, B and C are found in Europe, A being found in well over 80% of northern Europeans. The four haplotypes, A, B, C, and U are not related and have different distributions. The haplotypes appear to be in a large region of linkage disequilibrium, where there is evidence of genetic drift in evolution, but they do not help in identifying the true basis of lactase persistence. Haplotype A is associated significantly with high lactase expression (104) and is the most frequent one found in northern Europeans, consistent with the high frequency of lactase persistence in these populations (113).

Enattah and his colleagues (108) analysed the region flanking the LCT gene for microsatellite polymorphisms in nine well-characterised Finnish families which showed a 200 kb region of linkage disequilibrium. The locus for lactase persistence was restricted to a 47-kb interval, covering only one gene, MCM6

[mini chromosome maintenance 6]. They felt that this region was identical by descent and this region was not disrupted by ancestral re-combinations. Full sequence analysis of the 47 kb region upstream of the LCT gene resulted in the identification of a total of 52 non-coding variants. Two of the variants, C to T-13910 and G to A-22018, showed complete co-segregation with lactase persistence. The C/T variant is located 13,910 base pairs from the initiation codon of the LCT gene, in intron 13 of the MCM6 gene, and the G/A variant 22,018 base pairs upstream of LCT, in intron 9 of MCM6 [Figure 1.7].

Figure 1.7: Location of the single nucleotide polymorphisms [SNPs] suggested of being associated with adult-type hypolactasia taken from Enattah 2002 (108).



This was found in both Finnish and non Finnish subjects and provided support for the complete association of the C/T-13910 polymorphism with the lactase persistence/non-persistence trait however, the G/A-22018 did not

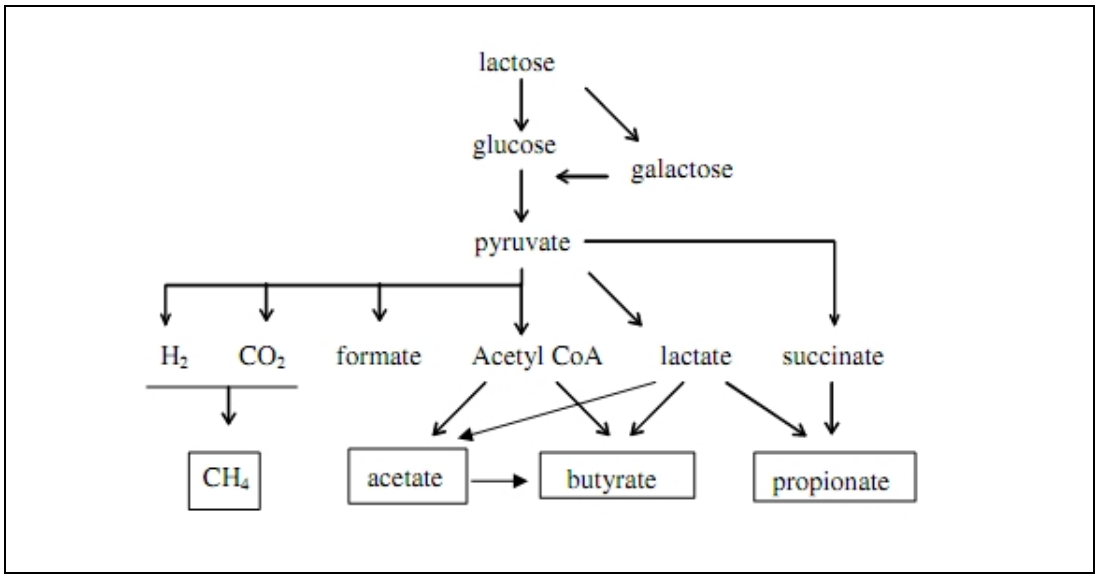
segregate with lactase activity in all cases. The C/C-13910 genotype, based on these findings, was concluded to associate with adult-type hypolactasia, whereas subjects with the genotypes C/T-13910 and T/T-13910 were shown to be associated with lactase persistence (108). CC and GG homozygotes had the lowest level of lactase. Homozygote TT/AA had full levels of lactase, with heterozygotes being in the middle. The genetics of lactose intolerance have been studied extensively in white European populations and non-white populations throughout the world (82, 114). People who are homozygous for lactase persistence retain high levels of lactase into adulthood whilst those who are homozygous for lactase non-persistence have low levels of lactase in adult life. Adults who are heterozygous have intermediate lactase levels. However, lactase persistence behaves as a dominant trait because half levels of lactase activity are sufficient to allow significant digestion of lactose. There is a huge variation between individual phenotypes, both in hypolactasia and the threshold for lactose intolerance. Quantification of relative expression of the LPH mRNA transcripts from the C-13910 and T-13910 alleles by allele - specific reverse transcription polymerase chain reaction in Finnish adults, showed several times higher expression of LPH mRNA from the T-13910 allele (115). Although there have been reports that these lactase polymorphisms can regulate lactase expression in vitro, it is not clear whether either of the two polymorphisms are mechanistically the cause of hypolactasia. There appears to be no correlation between the expression of mRNA for MCM6 and lactase in the gut cells of individuals with hypolactasia or lactase persistence (89). The possible explanation suggested for this are the C/T and G/A polymorphisms are simply a closely linked marker to lactase persistence/non-persistence and there is genetic heterogeneity (89). Two molecular mechanisms

can be responsible for a low level of lactase - a down-regulation of the lactase gene or a reduction in the number of villi cells expressing lactase. The patchy occurrence of lactase in the mature small intestine of humans and animals, suggests that the latter developmental mechanism is most likely to be responsible for hypolactasia in adults (116-120).

1.2.6: METABOLISM OF LACTOSE IN HUMANS

The β1, 4-glycosidic linkage between glucose and galactose is hydrolysed by lactase. Glucose enters directly into the body's glucose pool, but galactose is first metabolised to glucose, mainly in the liver. Galactose which is not metabolised in the liver is excreted in urine or metabolised by red blood cells (121). If the lactose in not absorbed in the small bowel it reaches the colon. The colonic cells do not absorb lactose but it can be metabolised by colonic bacteria. It is first hydrolyzed by bacterial ß-galactosidase into glucose and galactose. Galactose is converted into glucose via the 'Leloir' pathway, and is subsequently fermented. Short-chain fatty acids [SCFA] such as acetate, propionate and butyrate as well as the gases CO_2, H_2 and CH_4 are the end-metabolites from the bacterial fermentation of lactose [Figure 1.8]. Some intermediates, for instance, lactate, ethanol and succinate, are produced and then further metabolized to SCFA. Gases and SCFA are thought to be readily absorbed from the colon (97, 122). Acetate is the principal SCFA produced [~50%] and it passes through the liver before being finally metabolised in the peripheral tissues (123). Absorbed propionate and butyrate are metabolised in the liver (123). Gases are partially absorbed from the intestine into the blood and partially excreted through the lung whilst some is passed as flatus or used for the synthesis of other bacterial metabolites (124).

Figure 1.8: Fermentation of lactose by the colonic bacteria taken from Rombeau 1990 (122).



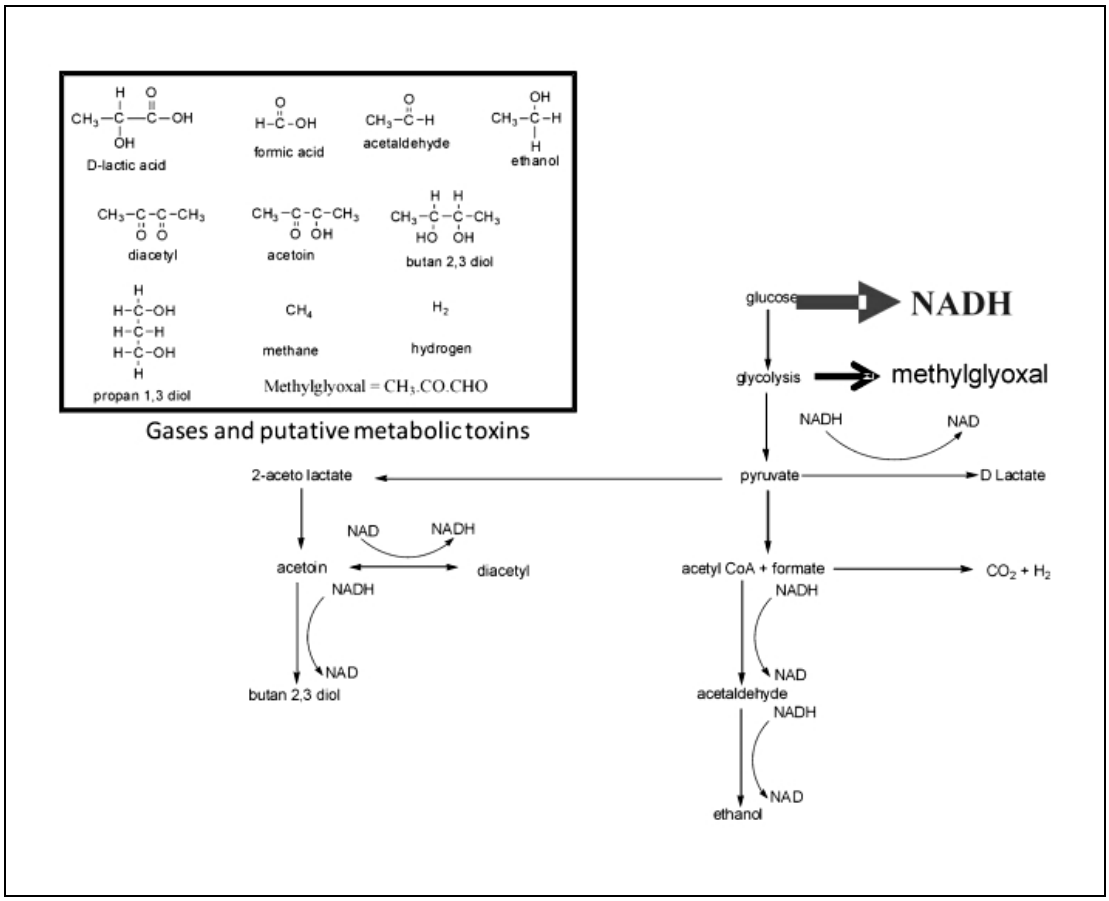
1.2.7: CLINICAL FEATURES OF LACTOSE SENSITIVITY

The peak age of presentation for lactose sensitivity is around 20-40 years. Males and females are affected equally. The classical symptoms are abdominal pain, bloating, flatulence, diarrhoea, and borborygmi (125, 126). These symptoms usually begin about 30 minutes to 2 hours after eating or drinking foods containing lactose. Recently, lactose sensitivity has been associated with systemic symptoms like headaches, fatigue, muscle and joint pains, palpitations, cognitive dysfunction, increased micturition, eczema and mouth ulcers (89, 127-131). Osteopenia has also been reported as a complication of this disorder (132). The severity of the symptoms correlates with not only the amount of lactose consumed, but also with the diet in which lactose is consumed, the rate of gastric emptying, the small-intestinal transit time, individual sensitivity to the stretching of the intestinal wall and also the degree of adaptation to lactose (133, 134).

1.2.7.1: MECHANISM FOR THE SYMPTOMS OF LACTOSE SENSITIVITY

The mechanisms by which lactose malabsorption causes symptoms of lactose sensitivity are not fully understood yet. Generally it is felt that they are related to the osmotic load of maldigested lactose which increases the secretion of fluid and electrolytes into the lumen. This causes intestinal dilatation which increases small intestinal transit and thereby further aggravates maldigestion of lactose (133). The symptoms are also due to the distension caused by the additional water and the gaseous products of fermentation together with the possible effects of SCFA's on colonic motility (96). However, these symptoms can persist for some time after lactose is ingested, suggesting other mechanisms beyond this osmotic effect may be responsible. These systemic and GI symptoms experienced by people with lactose sensitivity can be explained by the **'bacterial toxin'** hypothesis (95, 135) which is shown in figure 1.9. This proposes that the carbohydrate metabolites influence the balance of microflora in the large intestine, through effects on gene expression and growth. This affects host cells, by acting on cell signalling mechanisms and ion channels in gut bacteria which in turn influence many different host cells including neurons, skeletal, smooth and cardiac myocytes, mast cells and cells of the immune system. This would explain the range of GI and systemic symptoms that have been described in lactose sensitivity (95, 135 & 136). Bacteria anaerobically metabolise carbohydrates to produce hydrogen, and toxic metabolites such as alcohols, diols such as butan 2, 3 diol, ketones, acids, and aldehydes e.g. methylglyoxal (89, 95, 128, 135, 137-139). This is because in order to generate ATP, under the anaerobic conditions of the gut, bacteria have to get rid of the hydrogen equivalents if glycolysis is to continue.

Figure 1.9: Hypoxic metabolism of lactose and production of metabolic toxins taken from Campbell 2009 (95).



These 'toxins' induce calcium signals in bacteria and affect their growth, thereby acting to modify the balance of microflora in the gut (131, 137-139). These bacterial 'toxins' also affect signalling mechanisms in cells around the body, modifying molecules, such as hormones and neurotransmitters, thereby affecting their biological activity (95, 135).

Disordered peristalsis and water absorption in the colon caused by products of lactose fermentation leads to the development of loose stool (140). However, as it is generally believed that SCFA's are rapidly absorbed from the

colon. Colonic fermentation is suggested to help to reduce the amount of lactose present in the lumen thereby reducing the osmotic load in the colon (96, 141). When the threshold to do this is exceeded, water is retained which retards the absorption of SCFA's. When lactose is converted to SCFA by fermentation, an 8 fold increase in osmotic load occurs, which makes the efficiency of the colon to absorb these fermentation metabolites an important determinant as to whether symptoms are produced (142). There are differences in the absorption of individual SCFA's (141).

The osmolar load of malabsorbed lactose in the colon is determined by the balance between the ability of the faecal bacteria to ferment lactose and the efficiency with which the colonic mucosa absorbs these fermentation products (142). Colonic fermentation of 50 g of lactose is thought to produce 17 L of hydrogen. Most of the gas produced is consumed by intestinal bacteria. Intestinal gas tolerance is normally high as expeditious gas transit and evacuation prevent its accumulation. When gas transit and/or evacuation is impaired, gas retention occurs, which causes abdominal symptoms and distension.

Colonic fermentation of lactose may also be involved in the development of symptoms in patients with lactose sensitivity. Colonic bacteria are involved in the metabolism of lactose (97) individuals with similar oro-caecal transit and degree of lactose digestion in the small intestine develop symptoms of different severity (143). Also adaptation of long-term lactose ingestion may be related to changes in the microflora and function of the large intestine. Continuous lactose consumption reduces breath hydrogen excretion, increases the bacterial ß-galactosidase activity and improves lactose intolerant symptoms (144, 145). Adaptive changes in colonic functions [motility, transit, and pH] and the colonic

bacterial population lead to reduced bacterial hydrogen production and a decreased perception of symptoms by subjects, however placebo effects have been suggested as explanations for these observations (146, 147). The composition and metabolic activities of the colonic bacteria, the ability of the colon to remove fermentation metabolites and visceral sensitivity also determine the nature of symptoms experienced by lactose intolerant people (133, 142).

1.2.8: TESTS USED TO DETERMINE LACTOSE DIGESTION

There are many methods to measure lactose digestion in humans, these tests are based on different principles ["direct & indirect"] and have variable accuracy as well as diagnostic reliability. In clinical practice, judging the symptomatic response after the withdrawal of lactose from the diet is often suggested to patients who describe symptoms after ingesting dairy products. However, the diagnosis of the adult type hypolactasia based on symptoms alone is inaccurate (126). There are a number of different tests that have been used to try and determine hypolactasia and lactose sensitivity.

1.2.8.1: MEASUREMENT OF LACTASE ENZYME ACTIVITY IN SMALL BOWEL BIOPSY

Biochemical methods can be used to measure lactase activity from the small bowel mucosa. Specimens are obtained distal to the ligament of Treitz (126) either by endoscopy or capsule. It has been shown that both the biopsy techniques have no effect on the enzyme measurement (148). The tissue is subsequently homogenised and incubated with lactose. Lactase hydrolyses the substrate into glucose and galactose and the glucose concentration of the supernatant is

measured. Dahlqvist first described this method in 1968 (149). To correct for confounding influences of the variable water content in the specimen, results are expressed as activity units per gram of protein. This test has been said to be the gold standard for the diagnosis of hypolactasia which is diagnosed when lactase activity is < 10U/g protein (126). Similarly sucrase levels can be measured in the biopsy sample using sucrose instead of lactose and hypolactasia is diagnosed if lactase to sucrase ratio is < 0.3 (150). Recently Kuokkanen (151) described a Quick Lactase Test [Biohit PLC, Helsinki, Finland] for diagnosing adult-type hypolactasia. The endoscopic biopsy from the post-bulbar duodenum is incubated with lactose on a test plate, and a colour reaction develops within 20 minutes as a result of hydrolysed lactose [a positive result] in patients with normolactasia. No reaction [a negative result] develops in patients with severe hypolactasia. When compared with biochemical lactase assays, the sensitivity and specificity of this test was 95 % and 100 %, respectively.

Since only a very small part of the mucosa can be tested by biopsy techniques, the relation between the measured lactase activity with the overall physiological lactase activity of the whole small intestine has not been well established.

1.2.8.2: STOOL ACIDITY TEST

This measures the amount of acid in the stool and has been traditionally used in paediatric practice. Undigested lactose fermented by bacteria in the colon creates lactic acid and other short-chain fatty acids that can be detected in a stool sample- they produce a pH of less than 5.5. In addition, if reducing substances are found in the stool it indicates that carbohydrates are not being absorbed.

1.2.8.3: LACTOSE TOLERANCE TEST [LTT]

The lactose tolerance test is based on the principle that, after consumption of lactose, the substrate will be hydrolysed into glucose and galactose. These monosaccharides are then absorbed with much of the galactose being converted into glucose by the liver. The lactose tolerance test is based on determining the increase in blood glucose from samples taken at intervals after 15 to 30 min and then up to two hours after an oral load of 50 g of lactose. A rise in blood glucose > 1.7 mmol/l is indicative of normolactasia and that of < 1.1 mmol/l suggests hypolactasia. Symptoms after the test also need to be recorded – if these occur in conjunction with a marginal rise in blood glucose then this is said to suggest hypolactasia (126). It has been estimated, using assays of lactase from mucosal biopsies as reference methods, that the specificity of the LTT is 77-96% and the sensitivity is 76-94% (126). It should be noted that the test is not reliable in diabetics (152) and false positive results can occur with delayed gastric emptying (153). This test is not routinely used in clinical practice due to the above problems and the need for repeated blood sampling.

There is a modification of the LTT where ethanol is given in addition [50-150mg/kg orally depending on laboratory protocol]. Ethanol inhibits the hepatic conversion of galactose into glucose. Galactose is subsequently released by the liver into the blood stream and cleared by renal excretion. The concentration of galactose in the urine or blood therefore represents the hydrolysis of lactose in the intestine. Blood galactose concentration is determined in a single blood sample taken 40 minutes after an oral lactose load. Blood galactose concentration of < 0.3 mmol/l at 40 min after the ingestion of lactose and ethanol indicates hypolactasia. The measurement of galactose instead of glucose increases the specificity (126)

between 96% to 100% and sensitivity of 81% to 100% (126). Galactose can also be measured in a urine sample taken 40 minutes after the lactose-ethanol load and hypolactasia is diagnosed if the urinary galactose is < 2.0mmol/l. The sensitivity and specificity of this method are 94 and 98% respectively. The test is suitable for diabetics as well, and is less vulnerable to changes in gastric emptying rates (126). This test is not recommended in infants, children and pregnant women due to the unwanted effects of ethanol in these vulnerable groups.

1.2.8.4: RADIO-LABELLED LACTOSE BREATH TEST

After the consumption and hydrolysis of lactose labelled with radio carbon, the exhaled labelled CO_2 can be measured. The cumulative amount of exhaled CO_2 is related to the hydrolysis of the substrate. It has been shown that there is a good correlation [r=0.77- 0.87] of radio labelled carbon with jejunal biopsies in the diagnosis of lactose malabsorption (154, 155). A study by Heile (156) using naturally enriched ^{13}C-lactose as a substrate showed a 14.5% $^{13}CO_2$ excretion was the best cut-off point for discrimination between patients with low and normal lactase activity. The $^{13}CO_2$ breath test was found to be more sensitive [0.84 versus 0.68] and more specific [0.96 versus 0.89] than the H_2 breath test. The radioactivity of the substrate limits its applicability in medical practice, especially in infants, children and pregnant women.

1.2.8.5: OTHER BREATH TESTS

The colonic bacteria ferment lactose and produce gasses including Hydrogen [H_2] and methane [CH_4] as well as lactate and SCFA's. These gases are absorbed by the colonocytes and transported via the blood and exhaled in breath air. Biological

processes in the human body do not produce H_2 or CH_4, the concentration of these gases in exhaled breath represents the fermentation of carbohydrate in the colon. After a standard oral load of lactose, breath samples are collected at intervals of 15 to 60 min for two to six hours, and the change in hydrogen concentration in the expired air determined. The result of the test is positive [lactose malabsorption] if the breath H_2 concentration rise by 20 ppm or rise in methane by 5 ppm above the base line or nadir. The specificity of the breath hydrogen test varies between 89-100% and sensitivity ranges from 69-100% (126).

There are several factors that could influence the results include exercise before the test, the ingestion of antibiotics and cigarette smoking all of which will increase the rise in hydrogen concentration (157-160). In addition bacterial fermentation of other carbohydrates can also produce gaseous products in breath similar to lactose. As a result subjects undergoing the test should not eat or drink anything other than water for the period of the test.

This technique introduced in the 1970's (161-163) is the most widely used test that is employed to try and diagnose lactose digestion in clinical practice. The advantages are the simplicity of the test and its low cost. The major drawback is that there is no standardised protocol and it is also time consuming, taking 2-6 hrs depending on the protocol. There are different ways in which the test is carried out i.e. the substrate used [lactose or milk], the quantity of lactose administered [25g or 50g], sampling time intervals [15-60mintes], the duration of the test [from 1hr to 6hrs], the measurement of gases [i.e. hydrogen, methane or both] and the equipment used to measure the gases [handheld devices or gas chromatography]. There is also a lack of consensus regarding whether symptoms should be recorded after a lactose challenge and if so which ones are to be noted and for how long.

There is limited information on the sensitivity and specificity of symptoms which developed after a lactose challenge.

1.2.8.6: GENETIC TEST

The test is used to detect adult-type hypolactasia. DNA is extracted from buccal swabs or blood samples and analysed to detect either of the two single nucleotide polymorphisms [SNPs] located about 14 kb and 22 kb upstream of the lactase gene [LCT-13910 C/T and LCT-22018 G/A] (108). The CC or GG genotype is associated with genetic adult type lactase non-persistence and genotypes CT, TT, GA & AA with lactase persistence.

In a sample of 236 individuals, C/T−13910, was found to be completely associated with lactase non-persistence when compared to their biochemically verified lactase persistence/non-persistence status by disaccharide assay (108). The C/C genotype was associated with very low lactase activity [<10 U/g protein] in the majority of children tested at 8 years of age and in every child older than 12 years of age giving a specificity of 100% and sensitivity of 93% for this genetic test (164). In a study by Kuokkanen (115), all individuals with genotype CC−13910 and GG−22018 had both low lactase activity and a low lactase:sucrase ratio whereas all those either heterozygous for CT−13910 and GA−22018 or homozygous for T−13910 and A−22018 had lactase activities >13 U/g and lactase:sucrase ratios >0.35 U/g.

Recent studies have demonstrated an excellent correlation between the CC genotype and a positive H_2-breath test with 97%-100% of people with the CC genotype having positive lactose HBT (117, 136 & 165). In those with a CT or a TT genotype suggestive of lactase persistence, 72%-86% tested negative, whilst

the remainder had a positive HBT. In another recent study in patients who had a positive HBT, the sensitivity, specificity, positive and negative predictive values for the CC genotype variant were 91.4, 96.0, 98.1 and 82.8%, respectively (166). In a study with Austrian subjects, the 24% frequency of the C/C was concordant with the frequency of lactose intolerance diagnosed by the HBT (167). In another Austrian study (165), a 97% correlation was observed between the C/C genotype and a positive test result in HBT. Of those with C/T and T/T genotypes 14%, however, had a positive HBT. In a German cohort, the frequency of the C/C genotype was 21.4%; somewhat higher than that diagnosed by HBT [5%] (168). In a Swedish study, the results from the LTT correlated perfectly with the genotyping results in subjects with C/C and T/T genotypes, whereas in three subjects with a C/T genotype, LTT results were suggestive of lactose malabsorption (169). In a study from the UK, a perfect association between the C/T-13910 genotypes with lactase persistence/non-persistence phenotypes was seen in northern European samples studied but not in 2 out of 40 samples from southern European (82, 170).

Unlike the HBT, results of LCT genotyping are not influenced by factors such as incomplete fasting, antibiotic therapy or disease states. There are several limitations to this test. However, genotyping could only be used as a rule out test since it does not tell anything about the age at which a child with the CC genotype begins to express less lactase. Secondly, in patients where secondary hypolactasia is suspected e.g. coeliac disease or IBD, genotyping should be done together with other tests in order to establish hypolactasia is present. Also the same genotype abnormalities may not be responsible for lactose sensitivity in different populations. A recent study reports the C/T-13910 variant frequencies in 20

distinct African cultural groups (171). In the sub-Saharan groups, the T-13910 allele was found too seldom in order to underlie the lactase persistence phenotype, thus it was suggested that C/T-13910 might not be the causing variant in these groups (171).

The results of the various tests used in the diagnosis of adult type hypolactasia are summarised in table 1.10.

Table 1.10: The specificity and sensitivity of the methods used in the diagnosis of adult-type hypolactasia. [LTT: Lactose tolerance test, LTTE: LTT with ethanol, HBT: Hydrogen Breath Test]

Test	Sensitivity	Specificity
LTT	76-94%	77-96%
LTTE	81-100%	96-100%
HBT	69-100%	89-100%
$^{13}CO_2$ Breath Test	84%	96%
Genetic test [C/T-13910]	93%	100%
Jejunal Biopsies	100%	100%

The current best practice to diagnose lactose sensitivity is to initially ascertain the lactase genotype which avoids invasive testing or provoking significant symptoms in susceptible individuals after a lactose challenge. There appears to be an excellent correlation between C/T-13910 polymorphism and lactase persistence/non-persistence phenotype in all populations- except for the sub-

Saharan Africans- studied so far. In the UK population, if the CC status is detected then this is in keeping with lactase non-persistence and the patient will have a positive breath test therefore no further evaluation is necessary. If they are CT or TT this is associated with genetic lactase persistence, then a breath test and analysis of symptoms after lactose challenge should be carried out where there is suspicion of lactose sensitivity.

In addition to the problems associated with the individual tests described earlier there are other issues which cause difficulties in measuring products of lactose metabolism. Factors which affect the amount of the H_2 in the breath sample include smoking, diet and antibiotic treatment (157, 172). Colonic metabolism of carbohydrates by bacteria is a pH dependent and fall in pH can lead to decreased metabolism of the carbohydrates as seen by decreased breath H_2 excretion, (146) this can occur with chronic lactose ingestion in maldigesters. Factors which affect colonic bacterial production of H_2 include the ingested amount of disaccharides, the functional status of the small bowel and the type of colonic bacteria present (173, 174). Regional or ethnic differences are partly a result of different dietary habits. These factors may explain some of the difficulties in the measurement and the results seen in several studies. Brummer et al recommended that the test should be carried on for up to 4 hours to improve the accuracy. Sampling time duration negatively influences the accuracy of the HBT, with its sensitivity lower as the time is shortened (175). The bacterial flora adapt to chronic lactose exposure, producing less hydrogen and causing fewer symptoms with repeated ingestion which may also account for decreased symptoms and lower response to lactose challenge seen in chronic lactose intolerance (144, 176).

There are a number of issues relating to the use of symptoms in the diagnosis of lactose intolerance. Firstly, there are no studies that report symptoms following a blinded lactose challenge i.e. GI or systemic symptoms occurring after ingestion of lactose that are not observed when compared to ingestion of a placebo. All published studies that have looked at symptoms of LI following a lactose challenge are un-blinded. If subjects without prior GI symptoms are given a lactose load, 4-23% develop them thereafter (177, 178), in those who had baseline symptoms prior to challenge it was 32 to 71% (179, 180) and in those studies which had subjects with or without symptoms prior to challenge it was 38-72% (181-183). The problems in the studies that are published report symptoms in subgroups of their populations [participants with positive tests or only in people who previously had symptoms]. Self reported history of LI related symptoms without empirical evidence of symptoms following a lactose challenge is very difficult to interpret. The prevalence of LI is 12% to 16% in three such studies (184-186), based on self reported symptoms of LI in Caucasians. The results from the above studies show that the prevalence of LI from self-reported symptoms is lower than those that report symptoms after lactose challenge. Studies that reported results in people both with and without LM, reported significantly greater frequency of symptoms in those with positive breath hydrogen tests compared to those with negative tests (178, 183 & 187).

Some of the difficulties in assessment of symptoms are related to the age of the participants [paediatric Vs adult subjects], the ethnic origin of the study population and to individual variations in the daily dose of lactose that is tolerable to subjects with LS. Hypolactasia generally manifests at an earlier age in populations who have a high prevalence than in those with a low prevalence. In

high prevalence populations, symptoms can start at the age of 2 to 7 years whilst in lower prevalence areas they begin 4 to 5 years later (107). A study by Suarez, however, has shown no difference in the metabolism and tolerance of lactose between young adults and the elderly (188). Men and women have the same prevalence of hypolactasia (189, 190). However, women seem to report higher symptom scores than men after a standard lactose challenge - the reason for this is not known (190).

A lower rate of symptoms was observed with lower doses of lactose challenge [50g Vs 12 g] (178, 187). Symptom perception is susceptible to psychological factors. It is generally thought that a small amount of lactose [6-7 g] does not induce symptoms of lactose intolerance (191) and the amount of lactose [12 g] in one cup of milk [240 ml] can be tolerated by most people with hypolactasia (192). This is not absolutely correct as symptoms have been reported in sensitive people ingesting smaller quantities than this (89). Ingestion of 50 g of lactose causes symptoms in 80% to 100% of those with hypolactasia (193, 194) and this is the quantity that is most commonly used in the diagnostic tests. It should be noted, however that a small percentage of people with hypolactasia remain symptom-free even after the ingestion of this amount of lactose (106), the reason for this is not known. Some individuals, whether they had the ability to digest lactose or not, report symptoms suggestive of intolerance after taking various placebos used in double-blinded studies (195-197). Familiarisation with the test procedure also influences symptom recording (144). These observations suggest the possible involvement of psychological factors and that some GI complaints may therefore be mistakenly attributed to the consumption of lactose or milk. People with hypersensitivity of the GI tract such as IBS are likely to

report more symptoms from lactose ingestion when compared to others. Tests have used milk and lactose to record symptoms, lactose should only be used to avoid confusion from symptoms that can occur from the non-lactose fraction of milk. When using self reported symptoms for analysis, there may be variations if lactose is ingested throughout the day which may result in fewer symptoms than if a similar quantity of lactose is taken as a single dose. It is also thought that tolerance can develop if lactose is ingested chronically and this can therefore cause under reporting by patients.

Gastric emptying of lactose is reduced if it is consumed with other foods. Delayed gastric emptying and slowing intestinal transit enable a longer contact between lactase and lactose; this may improve lactose digestion and reduce any symptoms. It may be the mechanism behind the observed differences in many studies where full fat milk or ingestion of milk with meals, both of which prolong gastric emptying, was compared with skimmed milk or milk drunk on its own (198, 199). Studies have shown that a prolonged oro-caecal transit time contributes to fewer symptoms (143, 200 & 201), but this may not be a consistent finding as no difference was seen in another study (202).

Drugs which alter GI motility could also have an influence on the digestion and absorption of lactose. Loperamide is shown to prolong oro-caecal transit time has shown to reduce breath hydrogen concentration and reduced gastrointestinal symptoms (201), whilst propantheline improves symptoms by reducing the rate of gastric emptying and metoclopramide which causes the opposite effect has shown to worsen the symptoms of LI (203, 204). Finally, biases in attributing abdominal symptoms following un-blinded challenges can

make it difficult to accurately identify the prevalence of symptoms truly attributable to lactose.

1.2.9: MANAGEMENT, TREATMENT AND NUTRITIONAL CONSEQUENCES OF LACTOSE INTOLERANCE

The basis of the treatment of lactose sensitivity is to reduce the amount of lactose in the diet; the degree of the restriction depends on the individual's tolerance (205). In primary hypolactasia, a diet which completely excludes milk and dairy products is needed to obtain symptom remission. In the secondary form, a temporary lactose-free diet may only be necessary until a complete recovery of the causative pathological condition. Primary hypolactasia is a lifelong phenomenon, and so long term reduction in lactose intake is necessary to control symptoms (205). Lactose content varies between different dairy products, and for example cheese which has low lactose content is well tolerated, meaning that not all milk products need to be restricted in the diet. Some of the common foods which contain lactose and the quantity of lactose contained in them are shown in table 1.11. After exclusion, a gradual reintroduction of dairy products can then be made to a level determined by the threshold at which symptoms appear. Some of the strategies which will help in this are consumption of fermented dairy products, eating lactose containing products with other foods or dividing the total quantity consumed between several meals (206). Yogurt delays gastric emptying and intestinal transit leading to slower delivery of lactose to the intestine, thus optimising the action of residual lactase in the small bowel (200).

Table 1.11: Lactose content of some common foods (207).

Food	Type	Percent by weight
Milk	Whole Milk	4.6
	Semi-skimmed	4.7
	Skimmed	4.8
	Evaporated	8.5
	Condensed	12.3
	Goat	4.4
Cream	Single	2.2
	Double	1.7
	Sour	2.7
Cheese	Feta	1.4
	Cheddar	0.1
	Cottage cheese	3.1
	Stilton	0.1
	Mozzarella	Trace
Yoghurt	Plain	4.7
	Fruit	4.0
	Fromage frais	4.0
Puddings	Milk Shake	4.5
	Ice cream	5.2
	Custard	5.2

It has also been demonstrated that commercially available plain yogurt is as effective in reducing H_2 and symptoms as pre-hydrolysed milk (208, 209).The tolerance to fermented milk is based on the presence of endogenous lactase activity of yogurt microorganisms. The bacterial ß-galactosidase is structurally different to lactase found in small bowel mucosa. The ingestion of fat improves

carbohydrate absorption by slowing down gastric emptying and intestinal transit time which as a consequence increases the contact time between the enzyme and the substrate which may one of the reasons why full-fat milk tolerated when better compared to skimmed milk and aqueous lactose solution (210). The addition of exogenous β- galactosidase in the form of tablets or capsules with meals or drinks has shown to be effective, practical and with no side effects (211, 212). Furthermore, the absorption of lactose can be facilitated by exogenous lactase preparations (213). In some countries, low-lactose products in which lactose has been pre-hydrolysed, as well as lactose-free milk in which lactose is removed from the milk with chromatographic separation, are available.

The main nutritional consequences of avoiding lactose containing products may be a reduced calcium intake in the diet which may impair bone health (214, 215). Moreover, several studies have shown an increased incidence of adult-type hypolactasia, among subjects with osteoporosis or bone fractures (167, 216). The effect of lactose on calcium absorption is dependent on intestinal lactase activity (217). Calcium absorption is increased in subjects with normal lactase activity, when accompanied with lactose, whereas in subjects with adult-type hypolactasia lactose decreases calcium absorption (217). On the other hand, it has been observed that lactose does not have a beneficial effect on calcium bioavailability in lactose tolerant subjects (218). The mean calcium absorption from both lactose-hydrolysed and un-hydrolysed milk is significantly greater in subjects with adult-type hypolactasia in comparison to subjects who have lactase persistence (219). Calcium supplementation may be required in patients on a restricted lactose diet and the recommendation of calcium fortified foods should also be considered.

1.3: LACTOSE SENSITIVITY IN GI DISORDERS

Although primary lactose sensitivity has been increasingly recognised as a clinically significant entity, the importance of secondary lactose intolerance in patients with established GI diseases is less clear. It has been described in several disorders of the GI tract including Inflammatory Bowel Disease which will be discussed later on.

1.3.1: LACTOSE SENSITIVITY IN IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome [IBS] is a very common diagnosis, with a prevalence of up to 25% in the western world (220). Patients present with at least one of the following symptoms: abdominal pain, bloating, constipation and/or diarrhoea. IBS symptoms are often indistinguishable from those of lactose sensitivity, but it has been shown that lactose sensitivity does not lead to IBS per se (182). However, LM is reported as being very common in those with IBS symptoms with reported rates of 24–27% (220, 221). In an Italian study (222) LM was diagnosed in as many as 68.2% of participants with IBS. In this study, 43.6% of the patients who complied with the lactose free diet reported that their symptoms subsided, in 39% they were reduced and in 17% they remained unchanged. In another study by Alpers, 45% of IBS patients had lactose sensitivity, but only 30% related their symptoms to milk and dairy products; dietary exclusion improved symptoms in 52% of the cohort (223). On the other hand, some IBS patients without objective evidence of lactose malabsorption, describe symptoms of lactose intolerance (223). Lactose sensitivity should be excluded before the diagnosis of IBS is made (221, 224) as it is apparent that a large number have clinically unrecognised

lactose malabsorption, which cannot always be discriminated by symptoms and dietary history alone but may be effectively treated with a lactose-restricted diet.

1.3.2: LACTOSE SENSITIVITY IN COELIAC DISEASE

Coeliac disease is a life-long inflammatory condition of the gastrointestinal tract that affects the small intestine in genetically susceptible individuals. It improves morphologically when gluten, the causative agent, is removed from the diet. It is much more common than previously suspected, recent studies show a prevalence of up to 1% of UK population (225). The inflammation of the small bowel leads to an alteration in the brush border with villous atrophy and a subsequent reduction in the activity of the lactase enzyme. It is well known that patients affected by coeliac disease have a higher incidence of lactose malabsorption. This is present in many patients with untreated coeliac disease giving rise to more frequent and more watery stools. In well-treated coeliac disease, lactose malabsorption is not more common than the general population (226). In addition, a high prevalence of coeliac disease has been observed in patients with a positive HBT compared to healthy controls (227). Here, lactase deficiency seemed to be the only manifestation of coeliac disease. As a result, serologic screening for coeliac disease in all patients with a positive H_2-lactose breath test should be carried-out before beginning a milk-exclusion diet (227).

1.3.3: LACTOSE SENSITIVITY IN GI INFECTIONS

Lactose malabsorption may also occur as a result of acute infection with agents such as *rotavirus* which cause small intestinal injury and the resultant loss of the lactase-containing epithelial cells from the villi. The immature epithelial cells that

replace these are often lactase deficient, leading to secondary lactose deficiency and lactose malabsorption (228). *Giardiasis, cryptosporidiosis*, and other parasites that infect the proximal small intestine often lead to lactose malabsorption from direct injury to the epithelial cells by the parasite.

1.3.4: LACTOSE SENSITIVITY AFTER RADIOTHERAPY

Approximately four out of 10 people with cancer will have radiotherapy as part of their treatment. During the course of this treatment, partly because of mucosal damage, 80% of patients who receive abdominal or pelvic radiotherapy will develop gastrointestinal problems such as diarrhoea, abdominal cramps, tenesmus or faecal incontinence (229). Small bowel bacterial overgrowth and lactose intolerance [15%] may occur during radical pelvic radiotherapy and are likely to contribute to acute and also long term gastrointestinal symptoms in some patients (230).

1.3.5: LACTOSE SENSITIVITY AND RISK FOR COLORECTAL CANCER

In the Finnish population low lactase enzyme activity [C/C_{-13910} genotype], was found to be significantly associated with the risk of colorectal cancer [$p = 0.015$], with an odds ratio of 1.40 (231). It has been proposed that the change in the colonic flora from the products of lactose metabolism, i.e. butyrate and galactose protect against development of colorectal cancer. This finding has not been replicated in other populations.

1.3.6: LACTOSE SENSITIVITY IN INFLAMMATORY BOWEL DISEASE

Lactose sensitivity has been suggested as a cofactor that predisposes to a clinical attack of IBD or alternatively as an accompanying phenomenon of the disease. There have been several studies that have looked at the prevalence of lactose malabsorption in IBD- these have reported very variable findings. This could be ascribed to different study populations, with varying ethnic backgrounds, differing disease activity and a variety of different testing protocols and definitions. These studies are summarised in table 1.13 at the end of this discussion.

After Binder reported a 49% incidence of lactose malabsorption in patients with UC in 1966 based both on the results from lactose tolerance test and biopsies, increasing attention was paid to the correlation between these two conditions (232). In 1970, Gudmand-Hoyer (233) investigated the incidence of lactose malabsorption in 85 patients with ulcerative colitis and 71 patients with Crohn's disease by means of lactose tolerance tests and the measurement of disaccharidases in small intestinal mucosa. They found that just 9% with ulcerative colitis and 6% with Crohn's disease had lactose malabsorption. A control group displayed a similar incidence and as a result it was concluded that LM is not more prevalent in IBD (233). A British study in 1973 (234) found no difference in hypolactasia between patients with UC and healthy controls [12.5% vs. 9.5%] using jejunal biopsies and a disaccharide assay. However, they did highlight 5 patients who demonstrated a temporary reduction in lactase activity during an acute attack of UC.

A study from Denmark (235) of 120 patients with ulcerative colitis, reported the prevalence of lactose malabsorption based on a lactose tolerance test ± jejunal biopsy to be 9.2%, which is not significantly higher than that in the

normal Danish population. They found no difference according to age, sex or the severity of ulcerative colitis. Bernstein (236) reported no statistical difference of lactose intolerance between ulcerative colitis [44%] patients and matched controls [36%] using the HBT. When they analysed the results based on ethnicity of all participants, an abnormal HBT occurred in 68% of Jews compared with 23% in those of Northern European origin. LM was studied, with HBT, in 72 adults suffering from IBS, 20 UC patients, and in 69 healthy subjects by Sciarretta (237). The incidence of LM was 70% of the healthy subjects, 86% in IBS and 85% in UC, respectively. In the IBS and UC groups, symptoms occurred with a smaller rise in breath hydrogen; the authors presumed that this was in association with a greater individual sensitivity of the colon to distension.

In Pironi's study (238) of 37 adult patients with CD [19 with intestinal resection, and 18 without] and 67 healthy controls, again using the HBT, the prevalence of LM was increased to 49% in CD in comparison to 16% of controls. They also found a higher proportion of positive breath test in patients with CD who have undergone intestinal resection when compared to those who did not [58% vs. 33%]. LM also occurred at a lower dose of lactose in patients who previously had surgery. In addition, the prevalence was higher if the disease involved the small bowel when compared to involvement of large bowel alone [62% vs. 20%].

A study by von Tirpitz (88) enrolled 49 patients with CD and 24 controls, and also used the HBT to detect LM which was present in 32.7% and 20.8% subjects respectively. In addition, all individuals underwent endoscopy of the upper gastrointestinal tract, where biopsies were taken from the distal duodenum. In CD, duodenal lactase levels correlated to disease activity i.e. decreased lactase

levels were found during an acute exacerbation; however there was no obvious relationship with symptoms, site of disease or a history of previous surgery.

Mishkin (87) carried out a study to compare the prevalence of LM in 121 patients with CD and 139 with UC compared with 158 controls using hydrogen breath testing. The prevalence of LM was 58.2% in CD and 46.8% in UC. This was not significantly different to the 46.9% found in healthy controls. Analysis based on low ethnic risk for LM yielded interesting results where the control group had a prevalence of 29.2% compared with 40.0% in CD [$p < 0.025$] and 13.3% of UC patients [$p < 0.025$]. In the moderate ethnic risk group it was 68% in CD, 56% in UC & 65% in controls. In CD, the results based on the segment of bowel involved showed that 68.1% of patients with CD limited to the terminal ileum were lactose malabsorbers compared with 43.5% of patients with Crohn's colitis [$p < 0.05$]. In patients with UC, the extent of the disease did not have any effect on the result. Additional analysis according to anatomical location indicated that Crohn's disease of the proximal small bowel [duodenum, jejunum], terminal ileum, terminal ileum plus colon, and colon alone were associated with a LM prevalence of 100, 68.1, 54.5, and 43.5% respectively. Similar results based on the location of CD were seen in a Japanese study (239).

HBT were performed in 70 children and adolescents 20 with UC and 50 with CD by Kirschner (240). 29% of these patients demonstrated LM and the prevalence was not significantly different whether the diagnosis was UC [15%] or CD [34%]. With the exception of those with diffuse small bowel disease, the location of intestinal involvement with CD and the severity of clinical symptoms did not affect LM. Also, it was not more commonly seen in patients with IBD than in a group of children with recurrent abdominal pain. Significant differences

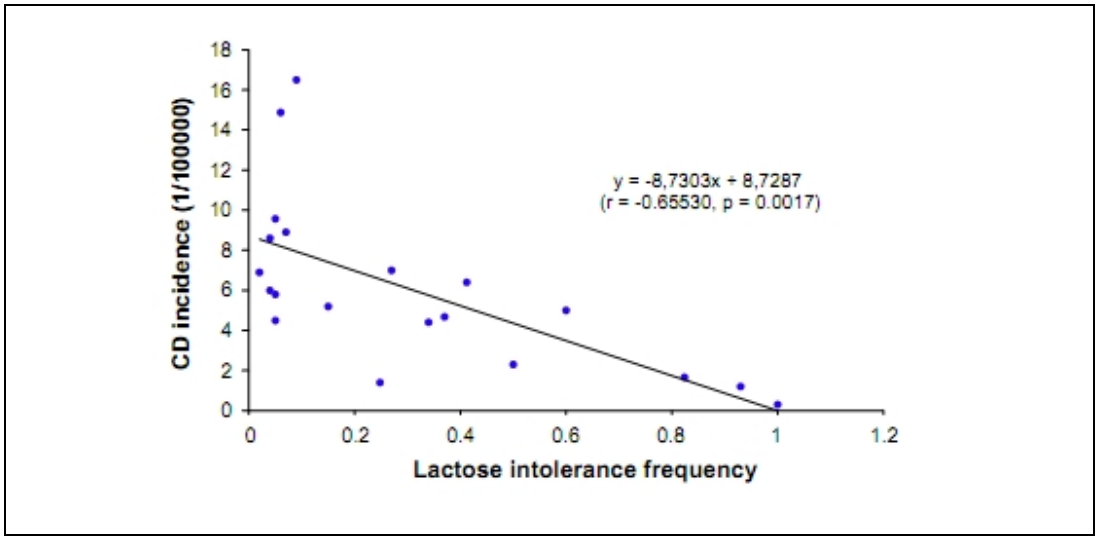
were again seen in the prevalence of LM between participants with different ethnic background. It was noted that there was no difference in the prevalence of LM between hospitalised participants compared to patients from the out-patient clinic.

The two single nucleotide polymorphisms C/C 13910 and G/G 22018 associated with the adult-type hypolactasia have been studied in 232 patients with IBD and 307 healthy controls in a German cohort (168). It revealed a frequency of 21.4% of these 2 genotypes, which is higher than previously reported in German subjects of 15% by Flatz which was based on the HBT (241). There was no significant detectable difference in the frequency of the C/C and G/G genotypes in patients with CD 21.7% & 22.3% compared to first-degree relatives 21.7% & 20.8%, patients with UC 20.3% & 20.3% and healthy individuals 21.4% & 21.4% respectively They concluded that the C/C and G/G genotype of adult-type hypolactasia is not associated with susceptibility to the pathogenesis of CD and UC (168). In contrast to this study a subsequent study from New Zealand of 333 individuals with CD and 612 healthy controls (242) showed that individuals homozygous for the T allele [T/T genotype] showed a significantly increased risk of having CD as compared with those homozygous for the C allele [OR = 1.61, 95% CI = 1.03-2.51]. Additionally, a significant increase in the frequency of the T allele was observed in CD patients [OR = 1.30, 95% CI = 1.05-1.61, p = 0.013], suggesting that the T allele encoding lactase persistence is associated with an increased risk of CD in this New Zealand Caucasian population. The T allele was also associated with early onset of disease,[<40 years Vs >40 years], the presence of ileal disease, the inflammatory phenotype of CD, a family history and previous bowel resection (242). Similarly Juste et al from Spain (243) also reported an

increased frequency of the T allele in CD patients [61.9%] compared with controls [47.1%, p = 0.0275].

IBD patients avoid dairy products more often than they may need, possibly due to incorrect patient perceptions and arbitrary advice from physicians (87, 236, 244 & 245). 80% of responding physicians stated that they recommend avoidance of milk products at some time to their IBD patients (236). Also, 66% patients reduced or eliminated milk products from their diet, but only 45% of these patients were lactose intolerant (236). In a study by Peroni in patients with CD, only 8% of the total with LM experienced symptoms of intolerance after the ingestion of 250 mls of milk and they concluded that this amount can be empirically taken in the daily diet of an adult with CD (238). There is a strong and highly significant negative correlation between raw country annual incidence of Crohn's disease and the frequency of lactase persistence in adulthood [p = 0.0017] (246), this is illustrated in figure 1.10.

Figure1.10: Association between Crohn's disease incidence and lactose intolerance taken from Juste 2010 (246).



The data included in this study was from 20 countries which are Belgium, Canada, China, Croatia, Denmark, Estonia, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Lebanon, Netherlands, New Zealand, Norway, Portugal, Saudi Arabia, Spain, Sweden, UK and USA. The data of incidence and prevalence of CD for each country from 1990-2005 was obtained from relevant medical literature, official national authorities and organisation with an interest in gastroenterology or IBD. It included both adult and paediatric populations. The LI frequency for each country was taken from published data in medical literature and official authorities. A similarly strong correlation between the country incidence of Crohn's disease and cattle heads per capita was also observed on a slightly larger sample [$p = 0.0013$] (246). Shrier et al. showed that an inverse correlation between yearly capita intake of dairy foods and national prevalence of lactose non-persistence status (247). In this study, there were statistically significant [$p \leq 0.05$] increases in risk for UC with dairy food consumption and a similar trend [$P < 0.1$] for CD (247).

Antigens from milk are homologous with proteins present in colonic mucosa which could stimulate the development of antibodies through antigenic cross reactivity. Alternatively, colonic antigens released from the inflamed mucosa of milk sensitive individuals could initiate the immunological reaction (248). Milk and milk products have been considered among the dietary factors that may initiate the IBD process, or exacerbate the disease once established (249, 250). It has been suggested that milk proteins may be pathogenic by acting as foreign antigens stimulating gut immunological responses; however this concept

of allergic response does not fit with the current concepts of IBD pathogenesis. Also, reports have been inconclusive and inconsistent in relation to antibody levels to various proteins in cow's milk (251-253).

In a study by Glassman (254), 35 patients with CD and 43 with UC were compared with a control population of 36 children without organic disease. They were surveyed to determine the frequency of symptoms compatible with cow's milk-protein sensitivity during infancy. The incidence of a history compatible with cow's milk sensitivity was 8.5% in patients with Crohn's disease and 2.8% in controls. Patients with ulcerative colitis had a significantly greater prevalence of symptoms, compared with the other patient groups [20.9% in UC, 8.5% in CD and 2.8% in the control group]. In addition, patients with a history of cow milk sensitivity, who subsequently developed UC, did so at an earlier age [6.68 vs. 10.62 years & $p < 0.02$] than those without this sensitivity. They concluded that there appears to be a potential relationship between early cow's milk sensitivity and the development of ulcerative colitis. In another study, 21 patients with UC and 9 with CD, none of whom had LM, were placed on milk-free diets. A beneficial effect was noticed in five of the patients with UC, and in three of those with CD. The mechanism is unknown but milk allergy is not responsible for the beneficial effect of a lactose free diet in patients with associated lactose malabsorption (233). In a study by Von Tirpitz (88), the prevalence of milk intolerance in healthy controls was 16.6% versus 46.9% in patients with CD, with a high frequency [83.3%] in patients with active disease [CD activity index >150].

Milk intolerance was correlated to the duration of IBD [p = 0.023] but not to its location in the GI tract or previous bowel resection (88).

Jejeunal villous atrophy was common during the active phase of UC (255) though this was not shown in other studies which have shown abnormal brush border lactase levels. (256) Also, there appears to be a normal permeability to lactose in patients with UC (257). A low content of several disaccharidases has been reported in UC and the levels of lactase, the most sensitive of the enzymes, may be particularly lowered (234, 258 & 259). The proportion of hypolactasics was low among the UC patients in clinical remission [2.8%], higher in patients with a mild attack [16%] and was higher still in patients with a severe attack [33%].

To summarise, the prevalence of LM ranges in reported studies varies from 6-58% in CD and 9-85% in UC [Table 1.13]. It should always be compared with the background incidence which varies with the ethnicity of the study population. LM in CD is more common if the disease involves the small bowel [68%] when compared to the large bowel [43%]. Any risk of developing IBD or affecting its activity that has been attributed to dairy foods may also be due to breakdown products, bacterial changes, or because a patient's lactase status may dictate their choice and quantities of dairy food they ingest. As stated earlier it should be remembered that these have used different definitions and diagnostic criteria in testing as well as a variety of patient types and disease activities. Very few have also recorded the symptoms generated after a lactose challenge, although this

could be influenced by subjectivity. The genetic studies have not conclusively shown a 'primary' association however, no-one has yet done the genotyping in parallel with breath testing to determine if any association could be related to the presence of the disease itself. This secondary association could have several explanations such as villous atrophy, an allergic reaction or immunological response to milk proteins, change and/or composition of colonic microbial flora in the presence of IBD or a chronic infection by an unknown pathogen. It is also thought that the lactose malabsorption in Crohn's disease may be determined by factors other than lactase enzyme activity, such as bacterial overgrowth and/or accelerated small bowel transit time (87).

Table 1.13: Summary of the studies looking at lactose malabsoprtion [LM] in inflammatory bowel disease.

	Tests Used	Patients & Numbers	% of LM	Comments Made
Gudmand-Hoyer 1970 (233) Denmark Hospital based	Blood Glucose & Biopsies	UC 85 CD71	9 6	Biopsies in 22 UC and 40 CD Results similar to controls
Pena 1973 (234) UK Hospital based	Biopsies & Assay	UC 72 Controls 21	12.5 9.5	Proportion of hypolactasics increased with severity of UC
Busk 1975 (235) Denmark Hospital based	Biopsy & LTT	120 UC	9.2	No difference between active and inactive disease
Kirschner 1981 (240) USA Hospital based	LHBT	20 UC 50 CD	15 34	Children & adolescents, No significant difference between UC & CD
Sciarretta 1984 (237) Italy Hospital based	HBT	69 HV 20 UC 72 IBS	70 85 86	Italian study all groups similar results no significant difference seen
Pironi 1988 (238) Italy Hospital based	HBT	CD 37 HV 67	49 16	Increased incidence if surgery [58%] vs. no surgery [33%]

Bernstein 1994 (236) USA Hospital based	HBT	UC 29 Controls 14	45 36	Family controls No difference seen
Mishkin 1997 (87) USA Hospital based	HBT	UC 139 CD 121 Controls 60	47 58 47	25 g of Lactose Crohn's of SB 100%, TI 70%, IC 55% & Colonic 44%
Von Tirpitz 2002 (88) Germany Hospital based	HBT	24 HV 49 CD	20.8 32.7	HBT was more positive if active CD 54% when compared 12.5% inactive CD
Bunning 2006 (168) Germany Community based	Genotype CC 13910	UC/controls 63/187 CD/Controls 166/120	22 vs. 22 21 vs. 22	Healthy individuals First degree relatives
Nolan 2010 (242) New Zealand Community based	Genotype CC 13910	CD/Controls 333/612	9.6 Vs. 13.4	Healthy individuals and first degree relatives. Increased frequency of T allele in CD Odds ratio 1.3 & p=0.013

1.4: SCOPE OF THE THESIS

Controversy still exists as to the prevalence and the role of lactose intolerance in inflammatory bowel disease. Studies report a wide variety of findings which range from a positive association between through to no relationship. In addition, this association is different based on the type of IBD [UC/CD] and also on the extent of the disease; it is especially common in small bowel CD. LI is conventionally diagnosed using breath test and or symptoms after a lactose challenge, recently genetic testing has become available. The hypothesis that the increased prevalence of lactose sensitivity in IBD is related to the lactase non persistence status and this may also be responsible for the high level of positive breath test results obtained in these patients after the lactose challenge. The results from these studies will help in understanding this complex interaction between lactose sensitivity and IBD and in addition has the potential to improve the lives of patients with these conditions. No one has yet performed a study involving the combination of genetic, breath testing and symptom analysis in a defined IBD cohort, all in clinical remission and compare the results obtained with healthy controls. Patients with UC and CD who are all in remission will be recruited together with healthy disease free volunteers to act as controls. The study will be limited to people of European Caucasian origin to remove any bias due to ethnic origin.

The overall objective of this dissertation is to determine the significance of Lactose Sensitivity in Inflammatory Bowel Disease. The specific questions to be addressed by this thesis are a] the frequency of the genetic polymorphism of lactase persistence in inflammatory bowel disease, b] the relationship between the genotype results with the breath testing as well as symptoms of lactose sensitivity

with breath test results, c] the benefits of lactose exclusion on the symptoms and quality of life reported in a small cohort of patients with Crohn's disease with proven lactose sensitivity, d] the presence of 'hidden' sources of lactose in the commonly used drugs to treat IBD and common GI disorders, e] the advantages or disadvantages in the techniques used for genotyping [Restrictive Fragment Length Polymorphism vs. Real Time Polymerase Chain Reaction] and breath testing [*Quintron MicroLyzer* vs. *Micro* H_2] in the diagnosis of lactose sensitivity.

To address the specific questions described above the following studies have been carried out:

1. **Lactose sensitivity and inflammatory bowel disease**

    a. **Lactase persistence status in patients with inflammatory bowel disease**

    The frequency of lactase persistence/non-persistence status will be determined in patients with UC and CD by analysing for the presence of C/T polymorphisms on chromosome 2. These results will be compared with a group of healthy volunteers. This will provide the necessary evidence to support the hypothesis that lactose intolerance in IBD is related to the individual's lactase non-persistence status.

    b. **The results of breath test and symptoms after lactose challenge and comparison with the genotype**

    Patients with ulcerative colitis and Crohn's disease along with healthy controls will be challenged with 50 grams of lactose. The results of

this will address whether the cause of intolerance to lactose is primary [i.e. genetic] or secondary to their disease state. In addition it will also provide information if the type of IBD has a role in the differences seen in the results. In addition, it will possible to see if there are any differences in these diseases by the type of breath gases produced [hydrogen or methane].

2. Role of minimal lactose diet on patients with Crohn's disease.

The proposal that dietary intervention with minimal lactose diet could lead to reduction in the symptoms experienced and improves the health related quality of life [HRQOL] will be tested in patients with Crohn's disease and lactose sensitivity. Patients with CD and LS will have their symptoms and HRQOL recorded whilst they are on their normal diet and then these will be compared with the results obtained on a minimal lactose diet.

3. **Lactose in the drugs commonly used to treat IBD and common GI disorders**.

Medications used primarily in the management of IBD and co-existent GI disorders will be analysed for lactose content using High Performance Liquid Chromatography [HPLC]. Lactose is thought to be present in most drugs in quantities that could cause symptoms and identify if there are lactose free alternatives available.

4. **To determine the optimal techniques which detect lactose malabsorption by breath testing [*Micro* H_2 compared with the *Quintron MicroLyzer*]?**

Since the development of hand held device to measure hydrogen, they have been used increasingly to diagnose lactose malabsorption. However, they are not accurate in measuring all of the gaseous products of lactose malabsorption [including methane]. This will test the hypothesis that using these devices will lead to under-reporting of malabsorption in clinical practice.

5. **The symptoms that occur after a lactose challenge are predictive of a positive breath test.**

Does an association exists between the gastrointestinal symptoms [abdominal pain, diarrhoea, bloatedness, borborygmi and nausea] developed after lactose challenge and a positive breath tests after 50g of lactose challenge. If there is an association then it may be possible to use the symptoms developed after lactose challenge in diagnosis of lactose intolerance. To test this hypothesis, the presence and number of GI symptoms will be recorded after lactose challenge and correlated to the results of the breath tests.

CHAPTER 2

MATERIALS AND METHODS

2.1: SUBJECTS

The two groups of subjects were recruited prospectively; patients with known IBD who were attending the gastroenterology clinic of Cardiff and Vale University Health Board and healthy volunteers from the staff who worked for the health board. None of the participants had previously been assessed for lactose sensitivity.

2.1.1: INFLAMMATORY BOWEL DISEASE PARTICIPANTS

Participants with UC and CD were diagnosed according to clinical, endoscopic, radiological and histological criteria. They were included if they were aged 18 years or more and of North European Caucasian origin. Patients with IBD were excluded if they had life threatening co-morbidity that was apparent from the initial assessment, those who were pregnant or regularly consumed alcohol above the recommended limits [more than 21 units of alcohol in a week in men and 14 units of alcohol in a week for women]. Patients with IBD were only included if they were in remission as defined by a Harvey-Bradshaw index (260) score of 4 or less in those with Crohns disease and by the Simple clinical colitis activity index (261) score of 3 or less in those with ulcerative colitis. These were chosen as they are simple to use, depend on clinical parameters only, can also be accurately self administered, correlate well with a more complicated disease activity index, and can be used to define relapse with high specificity & sensitivity. These scoring systems are given in tables 2.1 and 2.2 respectively.

Table 2.1: Harvey Bradshaw Index (260) to assess severity of Crohn's disease which is based on five items.

Features	Scores
General well being	0= very well, 1=slightly below par 2=poor, 3= very poor, 4= terrible
Abdominal Pain	0= none, 1=mild, 2=moderate, 3= severe
Number of liquid stools per day	
Abdominal Mass	0= none, 1=dubious, 2=definitive, 3= definitive and tender
Complications	Arthralgia, Uveitis, Erythema Nodosum, Apthous ulcers, Pyoderma Gangrenosum, Anal Fissure, New Fistula, Abscess [score 1 per item]

Table 2.2: Simple clinical colitis activity index (261) for assessment of severity of ulcerative colitis which is based on six items.

Features	Scores
Bowel frequency [day]	1-3= 0, 4-6=1, 7-9=2, >9=3
Bowel frequency [night]	1-3= 1, 4-6=2
Urgency of defecation	Hurry = 1, Immediately = 2, Incontinence = 3
Blood in stool	Trace = 1, occasional Frank = 2, Usually frank = 3
General well being	0= very well, 1=slightly below par, 2=poor, 3= very poor, 4= terrible
Extra-colonic features	1 per manifestation

The data collected from participants included: age, sex, smoking status, weekly alcohol consumption and family history of IBD. In addition the date of diagnosis, co-existing illness, current medications, nature and type of surgery for IBD was also recorded. This was obtained by direct questioning and reviewing the medical

notes of the participants. If the participant had a first degree relative [the individual's biological parents, direct siblings, or own children] who was afflicted with IBD then they were considered as having a positive family history. Both forms of IBD were sub-classified as per the extent of the disease. There are many different classifications in use and for this study the Montreal classification was used which is shown in tables 2.3 & 2.4.

Table 2.3: Crohn's disease based on Montreal classification (3)

Age of onset	Location	Behaviour
≤16 years [A1]	Ileal [L1]	Non structuring, non penetrating [B1]
17-40 years [A2]	Colonic [L2]	Stricturing [B2]
>40 [A3]	Ileo-colonic [L3]	Penetrating [B3]
Isolated Upper GI disease L4 this can be added to L1-3 if concomitant disease present. Add + P for peri-anal disease		

Table 2.4: Distribution of ulcerative colitis based on Montreal classification (3)

Distribution	Description
Proctitis [E1]	Involvement limited to the rectum
Left sided [E2]	Involvement limited to the proportion of the colon distal to the splenic flexure
Extensive [E3]	Involvement extends proximal to the splenic flexure, including pancolitis

2.1.2: HEALTHY VOLUNTEERS

The control group comprised of healthy volunteers, recruited from staff working in the hospital. They were included if they were aged 18 years or more and of North European Caucasian origin. They were excluded if they had gut symptoms such as abdominal pain, distension or bloatedness, and/or change in stool frequency or form on most days in the preceding 12 months. Those with inflammatory bowel disease, previous gastro-intestinal surgery, coeliac disease, known lactose intolerance or irritable bowel disease were also excluded. In addition participants who could not attend for the tests, or who had concurrent life threatening illness, consumed alcohol above the recommended limit or were pregnant, were also excluded. The data collected from healthy volunteers included: age, sex, smoking status, weekly alcohol consumption, co-existing illness, current medications and family history of IBD.

2.2: GENOTYPING

2.2.1: DNA EXTRACTION METHOD

Patient DNA was extracted from EDTA blood samples using Qiagen Kit [Qiagen Ltd, Sussex, UK], as per the manufacturers instructions and it was then diluted in 10 mmol/L Tris–HCl, 0.5 mmol/L EDTA at pH 9.0 and was used immediately or stored at -20°C. The purity of the DNA sample from each participant was analysed by determining the spectrophotometric absorbance of the sample at 260nm to that of 280nm and this $A_{260/280}$ ratio was greater than 1.7 (262).

2.2.2: REAL TIME POLYMERASE CHAIN REACTION FOR DNA ANALYSIS

Lactase genotype was determined by RT-PCR for C/T_{13910} lactase non-persistence using Assays-by-Design^{TM}. Service for custom SNP genotyping assays [Applied Biosystems Ltd., Cheshire, UK]. Designed sequences were subject to BLAST [Basic Local Alignment Search Tool], http://www.ncbi.nml.nih.gov/BLAST and Repeat Masker [http://repeatmasker.genome.washington.edu] in order to detect sequence similarities and repetitive elements respectively prior to submission for manufacture. The custom assay was designed to discriminate between the C/T 13910 lactase non-persistence polymorphism located in an intron of the MCM6 gene. Probes were as follows: Forward primer sequence coded JPWLPHCTL1-CTF:CTCTGCGCTGGCAATACAG; Reverse primer sequence coded JPWLPHCTL1-CTR: AAATGCAACCTAAGGAGGAGAGTTC; Reporter 1 sequence coded JPWLPHCTL1-CTV1 VIC; ATAAGATAATGTAGCCCCTGGC; Reporter 2 sequence coded JPWLPHCTL1-CTM1 FAM; ATAAGATAATGTAGTCCCTGGC. The reagent type manufactured was small scale, human, 40X concentration [part no. 4331349] and Taqman®Universal PCR master mix No Amperase UNG X2 [part no. 4324018] was used. 12.5µl of TaqMan® Universal PCR Master Mix No Amperase®UNG[X2], 0.625µl of 40X Assay mix was made up to 20µl with sterile water and added to each well of a 96 well plate [Applied Biosystems, Cheshire, UK]. 5µl of patient DNA was added and mixed prior to RT-PCR analysis. Each 96 well plate contained duplicate control samples and sample blanks in which no DNA was added. RT-PCR was performed using an Applied Biosystems 7500 Real Time PCR System and ABI Prism 7500 SDS software

[Applied Biosystems Ltd., Cheshire, UK]. Following a pre-run cycle, PCR amplification was performed for 10mins at 95˚C [1 cycle] then 15sec 92˚C, 1min 60˚C [40 cycles]. A post read run was then undertaken to determine allelic discrimination with automatic call [typically 98.43 – 99.9% quality was observed]. The assay clearly discriminated between allelic variants and sample blanks.

2.2.3: RESTRICTION FRAGMENT LENGTH POLYMORPHISM FOR DNA ANALYSIS

DNA sequences were obtained from the UCSC genome assembly version hg16 [http://genome.ucsc.edu/] based on the NCBI Build 34 produced by the International Human Genome Sequencing Consortium. C/T13910 polymorphisms are in an intron of the MCM6 gene at 13910. In the PCR reaction [50 μL], C/T13910 polymorphism primers were: MCMF2 – GGACATACTAGAATTCACTGCAA and MCMR2 – GGTTGAAGCGAAGATGGGACG. 25 pmol/L of each primer was added to a 50 μL PCR, 200 mmol/L deoxy-nucleotide triphosphates, 1.25 U Taq, 500ng DNA, Magnesium 1.5 mmol/L, buffer was added as per manufacturer's recommendations and sterile water to 50 μL. The PCR amplification was carried out as follows: HotStart 95°C for 15 min, followed by 35 cycles at 93°C for 40 s, 62°C for 60 s and 73°C for 100 s. Finally, for one cycle, the PCR was heated to 72°C for 7 min, then cooled to 4°C and either used immediately or stored frozen until further analysis. The amplification product was digested with BsmFI [3 U] as per the manufacturer's protocol for a minimum of 3 h at 65°C for the C/T13910 polymorphism. Following restriction digestion, the amplification product bands

were visualized under ultraviolet illumination using an ethidium bromide-impregnated agarose gel comprising 3% NuSieve and 1% regular agarose.

The results of all genotyping in both the methods were assessed independently and in duplicate. Controls of known lactase genotype and a blank in which no DNA was added were used to assure the quality of the PCR amplification in each run.

2.3: LACTOSE CHALLENGE TEST AND LACTOSE SENSITIVITY

The key clinical criterion for diagnosing lactose sensitivity in this study was the effect of an oral lactose challenge on breath hydrogen and methane, together with the appearance of gut and systemic symptoms.

2.3.1: LACTOSE CHALLENGE TEST

The subjects were given 50grams of lactose [Lactose powder BP: BN: M07001589 MS/13880/1 North Staffordshire Hospital Trust Pharmacological Services] dissolved in 300mls of water. Participants were told not to eat or drink anything other than water from midnight the night before the test which commenced at 9am. They were also told not to smoke for at least 4 hours before the test. In addition, they were advised to choose lactose-free food and avoid those foods that they recognise will produce gastrointestinal symptoms in the preceding 3 days. This was confirmed on the day of the test and in addition it was also ascertained that they had not received any antibiotic treatment or had bowel preparation for gastro-intestinal investigations during the preceding 4 weeks. Breath samples were obtained before the ingestion of the lactose and at 30 min

intervals for 3 hours and then one hour later. On each occasion, the subject exhaled into a polyethylene bag via a one way valve. Once the bag was fully inflated the collected sample was stored to be analysed. For the entire duration of the test they were not allowed to eat or drink except water. This was done to ensure that lactose and other sugars in food/drink do not affect the results of the breath test. They were also not allowed to smoke during the test period.

2.4: BREATH GAS ANALYSERS

The analysers used to measure hydrogen and/or methane by a static analyser *Quintron MicroLyzer Self Correcting Model SC* and a hand held device *Micro* H_2. The lactose breath test was defined as positive if the rise in hydrogen by ≥ 20 ppm and/or methane by ≥ 5 ppm above the lowest value respectively.

2.4.1: *QUINTRON MICROLYZER SELF CORRECTING MODEL SC*

The *Quintron MicroLyzer Self Correcting Model SC* [Quintron Instrument Co. Inc., Milwaukee, WI, USA] contains sensors that measure both breath hydrogen and methane values in the patients' samples after separation by gas chromatography. A thermal conductivity detector also measures carbon dioxide [CO2] in the sample and a correction factor is applied to account for any dilution of alveolar CO2 with dead space or inspired air during collection. The exhaled air was collected through a small one way valve into a 500 ml polyethylene bag, from which it is transferred though a sampling valve into a 50mls syringe and then injected into the analyser. The result obtained after analysis was recorded on a chart. The calibration of the *Quintron MicroLyzer* was carried out each time the analyser was used for the first time and then at hourly intervals with Microcan-

Disposable Calibration Gas from MicroGas UK Batch [3335/1006] which contains 100 ppm of Hydrogen, 50ppm of Methane and 6% Carbon Dioxide and balance Air [UN1956] 20 litres at 20°C and 300 psig.

2.4.2: *MICRO H_2*

The *Micro H_2* [Micro Medical Limited, Kent, UK] is a portable, hand-held, hydrogen monitor which is designed to give instant results. The measurements are obtained by exhaling through a 22-mm mouthpiece [Bedfont EC50-MP/200] for adults that is connected to the analyser. The *Micro H_2* was calibrated at monthly intervals using Microcan gas [MCG 100] which contains 50ppm of Hydrogen UK batch [3330/0075].

The *Quintron MicroLyzer Self-Correcting Model SC* is the standard used in the laboratory for the measurement of the break down products of lactose metabolism. Increasingly portable breath analysers are used in the diagnosis of lactose malabsorption in clinical practice. A study was carried out to compare the accuracy of portable breath hydrogen analyser Micro H_2 in the measurement of breath gases in the diagnosis of lactose malabsorption compared with Quintron MicroLyzer Self-Correcting Model SC and the results of this study are demonstrated in **Appendix 5**.

2.5: GUT AND SYSTEMIC SYMPTOMS

Gastro-intestinal symptoms [total = 7; abdominal pain, bloatedness, diarrhoea, flatulence/belching, borborygmi, constipation and nausea/vomiting], and systemic symptoms [total = 14; headache, memory impairment, loss of concentration,

dizziness, fatigue, muscle and joint pain, skin itching, rhinitis, asthma, increased micturition, heart palpitations, hot and cold, mouth ulcers, altered taste] were recorded by the participants [0= no symptoms, 10= severe symptoms] on a symptom chart for 48 hours [Table 2.5]. The symptom chart was not specifically validated for this study, but it was used in a previously published study (136) and is used as a part of the lactose breath test in the institution where the study was carried out. Patients and healthy volunteers were advised on how to fill the chart and assess the severity of any symptom. These were recorded before and after the lactose challenge at the time intervals indicated on the symptom chart. They were provided with a stamped envelope to return the chart after completion of the monitoring period.

Lactose sensitivity was defined as development of symptoms and / or a positive breath test after the lactose challenge.

If there is a correlation between the results of lactose breath tests and gastro-intestinal symptoms reported after a lactose challenge, then lactose sensitivity could be diagnosed by assessing symptoms only. In order to ascertain this, the sensitivity and specificity of the gastro-intestinal symptoms reported after lactose challenge was compared with a positive breath test after an oral lactose challenge. The results of such a study are shown in **Appendix 6**.

Table 2.5: Chart for recording symptoms after lactose challenge

SYMPTOMS CHART

Symptoms Severity: 0: No symptoms, 1-3: Annoying, 4 – 6: Discomfort, 6 – 8: Having to stop/sit down & 10: Severe

SYMPTOMS	Mins	30	60	90	120	150	180	4hr	5hr	6hr	7hr	8hr	9hr	10hr	11hr	12hr	**Sleep**	Day 1	Day 2	Day 3
Stomach pains																				
Diahorrea																				
Nausea/sickness																				
Bloated stomach																				
Rumbling stomach																				
Burping/ Flatulence																				
Constipation																				
Tiredness																				
Hiccups																				
Headaches																				
Palipitations																				
Rashes/Dry skin																				
Asthma																				
Hay fever																				
Muscle pain																				
Joint pain																				
Swelling feet/fingers																				
Itching																				
Dizziness																				
Light headedness/Lack of concentration																				
Feeling hot or cold																				
Mouth ulcers																				
Sore throat																				
Tingling feet/fingers																				
Irritable eyes																				
Bad taste																				
↑ Urine																				

2.6: APPROVAL

The study was approved by the Research and Development committee of Cardiff and Vale NHS Trust, Cardiff. Reference number: 05/CMC/3319.

The study was scrutinised by the South Wales Research & Ethics Committee, Cardiff, and Project Reference Number: 05/WSE04/74 & Approval Date on 21st June 2005.

Written informed consent was obtained from each participant prior to recruitment.

These materials and methods were used throughout the studies performed and they are described in further detail in the following chapters.

CHAPTER 3

COMPARISON OF A REAL-TIME POLYMERASE CHAIN REACTION AND RESTRICTIVE FRAGMENT LENGTH POLYMORPHISMS IN THE DIAGNOSIS OF GENETIC LACTOSE SENSITIVITY

3.1: AIM

This study aims to compare the techniques of Real Time Polymerase Chain Reaction [RT-PCR] and Restrictive Fragment Length Polymorphism [RFLP] for the diagnosis of genetic lactose intolerance.

3.2: INTRODUCTION

Polymerase Chain Reaction [PCR] is a revolutionary method developed by Kary Mullis in the 1980's (263-265). It is based on using the ability of DNA [Deoxyribonucleic acid] polymerase to synthesize a new strand of DNA complementary to the offered template strand. Because DNA polymerase can add a nucleotide only onto a pre-existing 3'-OH group, it needs a primer on to which it can add the first nucleotide. This requirement makes it possible to delineate a specific region of template sequence that needs to be amplified. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies [amplicons]. The applications of PCR are in cloning, genetic engineering or sequencing of DNA & RNA. Real-Time PCR [RT-PCR] as well as the Restrictive Fragment Length Polymorphism [RFLP] are techniques that are widely employed in the identification of DNA sequences.

RT-PCR (266, 267) is used to amplify and simultaneously quantify a targeted DNA molecule in "real time". RT-PCR is also called a quantitative polymerase chain reaction [qPCR] and is one of the most powerful and sensitive techniques that is available for gene analysis. It is used for a broad range of applications including quantitative gene expression analysis, genotyping, SNP analysis, pathogen detection, drug target validation and the measurement of RNA interference. RT-PCR data is collected during the exponential growth [log] phase

of PCR when the quantity of the PCR product is directly proportional to the amount of template nucleic acid. There are a number of techniques that are used to allow the progress of a PCR to be monitored. Each technique uses some kind of fluorescent marker which binds to the DNA - as the number of gene copies increases during the reaction so the fluorescence increases. This is advantageous because the efficiency and rate of the reaction can be seen. Intercalating fluorescent dyes such as SYBR green are the simplest and cheapest way to monitor a PCR in real-time. These dyes fluoresce only when bound to double-stranded DNA but their major disadvantage is a lack of specificity as this dye will report the amplification of any DNA not just the gene of interest. This can be resolved by employing fluorescent probes which are pieces of DNA complimentary to the gene of interest that are labelled with a fluorescent dye. The simplest and most commonly used type of probe is the Taqman-type probe. These probes are labelled with a fluorescent reporter molecule at one end and a quencher molecule [capable of quenching the fluorescence of the reporter] at the other. Therefore under normal circumstances the fluorescent emission from the probe is low. However during PCR, the probe binds to the gene of interest and becomes cleaved by the polymerase. Hence the reporter and quencher are physically separated and the fluorescence increases. An increase in reporter fluorescent signal is directly proportional to the number of amplicons generated & the cleaved probe provides a permanent record amplification of an amplicon (266-268).

RFLP involves fragmenting a sample of DNA by a restriction enzyme which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest (265, 269). The resulting DNA fragments are then separated by length via electrophoresis on ethidium bromide impregnated

agarose gel. The gel is then illuminated with an ultra violet lamp in a light box and an image is viewed. Ethidium bromide fluoresces reddish-orange in the presence of DNA, into which it has intercalated and these images are usually shown in black and white. The technique measures the amount of accumulated product at the end of the PCR cycles by comparing the intensity of the amplified band on a gel to standards of a known concentration and therefore gives 'semi-quantitative' result (265, 269 & 270).

Enattah and his team (108) analysed the region flanking the Lactase gene at 2q21 by genotyping - sequence analysis of the 47 kb region upstream of the LCT gene resulted in the identification of a total of 52 non-coding variants. Two of the variants, C to T-13910, and G to A-22018, showed complete co-segregation with lactase persistence. The C/T variant is located 13,910 base pairs from the initiation codon of the LCT gene, in intron 13 of the MCM6 gene, and the G/A variant 22,018 base pairs upstream of LCT, in intron 9 of MCM6. This enabled a genetic test for the detection of lactose persistence/non persistence i.e. CC equates to lactase non-persistence, CT and TT to lactase persistence.

3.3: METHODS

3.3.1: PATIENT POPULATION:

Samples from 48 participants who took part in the study outlined in Chapter 4 were included. They were all of Caucasian origin and included 10 healthy volunteers, 19 with Crohn's disease and 19 with Ulcerative colitis.

3.3.2: DNA EXTRACTION METHOD

Patient DNA was extracted from EDTA blood samples using a Qiagen Kit [Qiagen Ltd, Sussex, UK], diluted in 10 mmol/L Tris–HCl, 0.5 mmol/L EDTA at pH 9.0 and was then used immediately or stored at -20°C. The purity of the DNA sample from each participant was analysed by determining the spectrophotometric absorbance of the sample at 260nm to that of 280nm and this $A_{260/280}$ ratio was greater than 1.7 (262).

3.3.3: DNA ANALYSIS METHODS

Lactase genotype was determined by the RT-PCR and RFLP methods for C/T_{13910} lactase non-persistence/non persistence genotypes on the extracted DNA as described in chapter 2.

3.3.4: STATISTICS

Sensitivity, specificity and positive and negative predictive values were calculated in order to evaluate the RT-PCR method compared with the RPLF. Sensitivity [%] = true positives / [true positives + false negatives] X 100, Specificity [%] = true negatives / [true negatives + false positives] X 100, Positive predictive value [%] = true positives / [true positives + false positives] X 100 & Negative predictive value [%] = true negatives / [true negatives + false negatives] X 100.

3.4: RESULTS

48 participants had their DNA analysed by both methods. There were 24 men and 24 women. The age range was 19-86 years with a mean of 42 years, [41.6 for men and 43.2 for female]. The results obtained were CC in 5 cases [10.4%], 17 CT

[35.4%] & 26 TT [54.2%] by both methods i.e. the results were exactly the same in every participant by the 2 methodologies as shown in table 3.1 and summarised in table 3.2. This means that the sensitivity and specificity of the RT PCR with RPLF was 100%. Of those genotyped as CC by both methods 2 were male and 3 female, 7 male and 10 female were CT and of those found to be TT there were 15 males and 11 females. The computerised software reporting system gives a result that is clear and easy to interpret e.g. 13910_C for CC, 13910_T for TT or both for CT. The results as obtained by RT-PCR are shown in Figure 3.1 where each sample is represented by a block on the chart. In the RFLP method, each column on the agarose gel represents an individual sample - see figure 3.2. Each column is compared with a known sample of CC, CT or TT to read the results of the test sample.

Table 3.1: Results of allelic discrimination by RT-PCR and RFLP

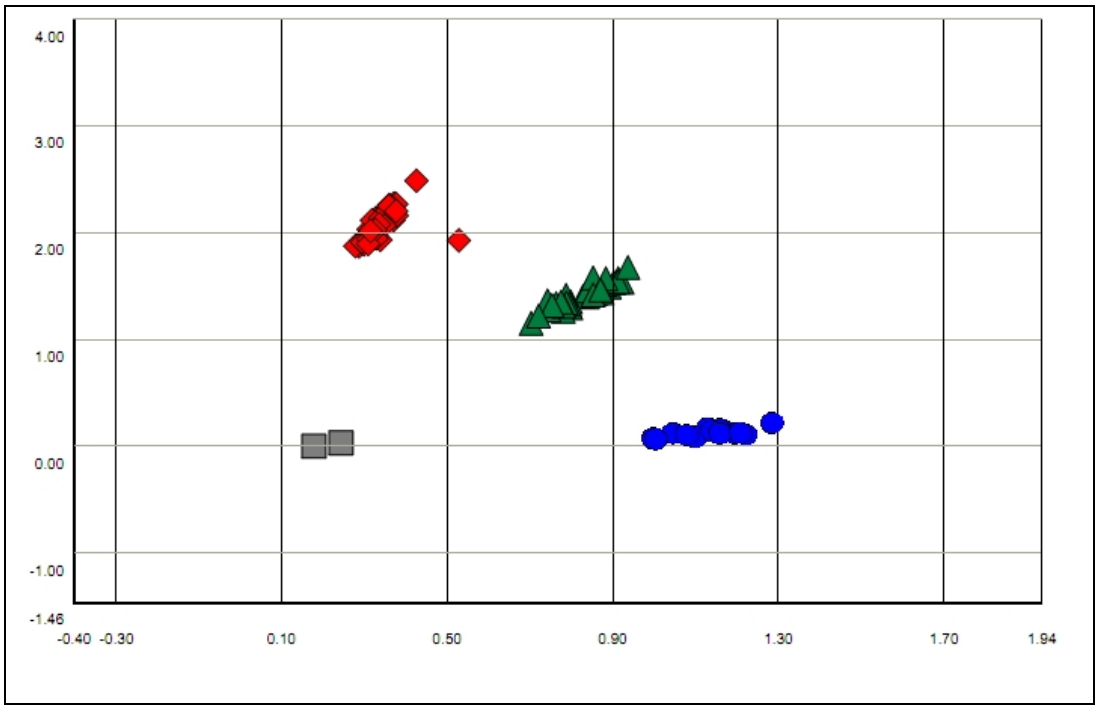
	Patient Number	Sex	RFLP	RT-PCR
Healthy Volunteers	1	M	TT	TT
	2	F	CT	CT
	3	M	TT	TT
	4	M	CT	CT
	5	F	TT	TT
	6	F	TT	TT
	7	F	TT	TT
	8	F	CT	CT
	9	M	TT	TT
	10	M	CT	CT
Ulcerative Colitis	1	M	TT	TT
	2	F	CT	CT
	3	M	TT	TT
	4	M	CT	CT
	5	F	TT	TT
	6	F	TT	TT
	7	F	TT	TT
	8	F	CT	CT
	9	M	TT	TT
	10	M	CT	CT
	11	F	CC	CC
	12	M	TT	TT
	13	F	CT	CT
	14	M	TT	TT
	15	M	CT	CT
	16	F	TT	TT
	17	F	TT	TT
	18	M	CC	CC

	19	F	CT	CT
Crohn's Disease	1	M	TT	TT
	2	F	CT	CT
	3	F	TT	TT
	4	M	CT	CT
	5	F	TT	TT
	6	M	TT	TT
	7	F	CC	CC
	8	F	CT	CT
	9	M	TT	TT
	10	M	CT	CT
	11	F	CC	CC
	12	M	TT	TT
	13	M	CC	CC
	14	M	TT	TT
	15	F	CT	CT
	16	M	TT	TT
	17	F	TT	TT
	18	M	TT	TT
	19	F	CT	CT

Table 3.2: Summary of the results comparing the allelic discrimination by RT-PCR and RFLP – number of patients are given in each cell.

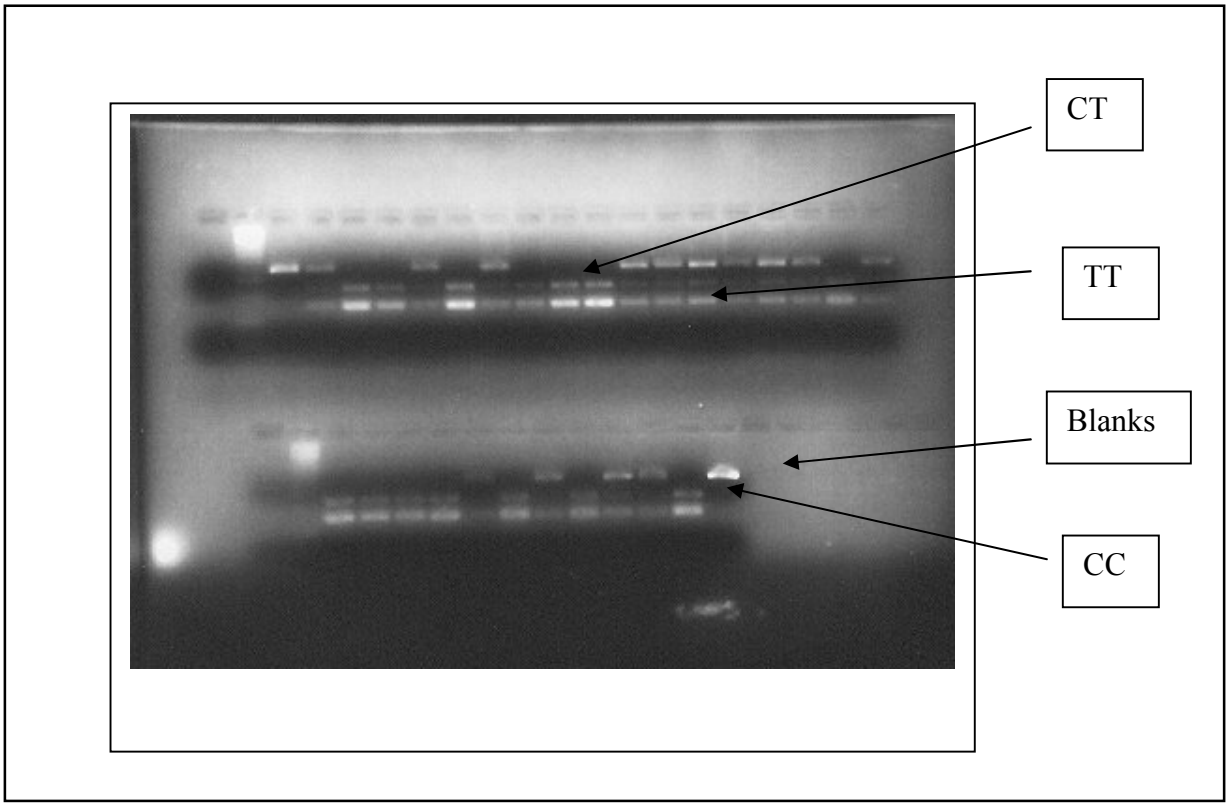
	RFLP	RT-PCR
CC	5	5
CT	17	17
TT	26	26

Figure 3.1: Allelic discrimination from individual patients using the RT-PCR method.



	Blue Circles: CC		Red Rhombus: TT
	Green Triangles: CT		Grey Squares: Blanks

Figure 3.3: Allelic discrimination as obtained by RFLP in 30 patients.



3.5: DISCUSSION

The results obtained by both methods were identical in all subjects that were included. The RFLP was the standard method used in the laboratory at the time that I did this work however there are several potential disadvantages of this technique. There may be poor precision, low sensitivity and a short dynamic range. The images may be low resolution and it affects size-based discrimination. It is also non-automated and results are not expressed as numbers. In addition, Ethidium bromide for staining is not very quantitative, and there is a need for

post-PCR processing (265, 266 & 269). 2 hours was the hands on time for 40 samples and the processing time was 12 hours.

In contrast, RT-PCR is a rapid, robust technique that is suitable for the screening of large numbers of samples, and is considerably less labour-intensive. The RT-PCR method is associated with an increased dynamic range of detection and there is no post-PCR processing. It is highly sensitive as it is capable of detecting a 2-fold change (267, 268, 271 & 272). The RT-PCR was easy to do, the number of steps involved was less, and it was less time consuming [3 hours vs. 12 hours] for the whole process from the start of the reaction to the generation of the results. RT-PCR could analyse 96 samples whereas 16 samples were analysed with RFLP method in a single setting. Costs for consumables and reagents per sample are higher for RT-PCR but this is balanced by less labour time. Hands-on time with this assay would be only 1 h for the analysis of up to 96 samples.

3.6: CONCLUSION

Real-time PCR assay provides a rapid means for the genotyping of LPH C/T 13910. Given the advantages as described above the RT PCR has now become the standard method for analysis of lactase polymorphisms in the laboratory where I undertook this work and will be used in the work described in this thesis.

CHAPTER 4

ASSESSMENT OF LACTOSE SENSITIVITY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE BY THE ANALYSIS OF LACTASE GENETIC POLYMORPHISMS, THE LACTOSE BREATH TEST AND SYMPTOMS.

4.1 AIM

The aim is to investigate the prevalence of lactose sensitivity in ulcerative colitis and Crohn's disease when compared to healthy volunteers.

4.2 INTRODUCTION

Sensitivity to lactose occurs as a result of reduced activity of the small intestinal brush border enzyme lactase phlorizin hydrolase [LPH] resulting in a deficient hydrolysis of lactose (95, 107 & 125). As a result lactose is metabolised in the large intestine by colonic bacteria, producing hydrogen, methane and a variety of other metabolites. The gases and bacterial metabolites cause abdominal (125, 126) and systemic symptoms (89, 127, 128 & 136). There have been several studies which have looked at the rates of hypolactasia and sensitivity to lactose; these have been detailed in section 1.3. Breath testing, after oral lactose, is widely used in clinical practice to assess LI. However, it has been shown that measurement of breath hydrogen/methane alone, without a record of symptoms, detects less than 50% of people who are lactose sensitive (95, 136). Some of the protocols for breath testing after lactose challenge record symptoms but there is limited information about their importance. There are only few studies evaluating the relationship between symptoms and the results of the breath test (136, 179, 180 & 273). These showed that a positive breath test is associated with a higher frequency and intensity of symptoms and concluded that recording symptoms with breath test results improved sensitivity and specificity in identifying people with lactose sensitivity. In the review of literature, I have detailed the known information regarding the genotyping and its correlation with primary adult type hypolactasia. The polymorphism involving a T to C change at position 13910 base

pairs on chromosome 2 exhibits a close association with hypolactasia (108) The three genotypes, CC, CT and TT respectively, correlate with the levels of lactase activity in intestinal biopsy samples and their lactase: sucrase ratio (108, 115). To date, there have not been any published studies in patients with IBD where the polymorphism which is associated with hypolactasia C/C_{13910}, has been correlated properly with lactose sensitivity by concurrently performing breath tests measuring both hydrogen and methane as well as analysing symptoms.

4.3: MATERIALS AND METHODS

4.3.1: SUBJECTS

This was a prospective study enrolling patients with known IBD from the gastroenterology clinics as well as healthy volunteers from hospital staff who had not been previously assessed for lactose sensitivity. The inclusion and exclusion criteria for both the groups to participate in the study are detailed in chapter 2. All patients and healthy volunteers were white Northern Europeans and over the age of 18 years. Milk allergy was also excluded in all patients and healthy volunteers by a negative blood test for IgE milk proteins using Phadia 250 [Phadia Ltd, Milton Keynes, UK]. Coeliac disease was also excluded in all patients by serological tests.

4.3.2: GENOTYPING

Lactase genotype was determined by RT-PCR for the C/T_{13910} polymorphism. The methodology is described in detail in Chapter 2.

4.3.3: LACTOSE CHALLENGE TEST AND LACTOSE SENSITIVITY

The diagnosis of lactose sensitivity was made by the effect of an oral lactose challenge on breath hydrogen/methane, and/or the appearance of symptoms. Participants were given 50 grams of lactose, the full details of the methods used in the breath testing is described in chapter 2. Both hydrogen and methane gas concentration was analysed by *Quintron MicroLyzer Self Correcting Model SC* again as described in Chapter 2. Raised basal hydrogen or methane, before lactose, was defined as > 2 standard deviation over the mean of the control group of HV. Raised hydrogen or methane, after lactose, was defined as ≥ 20 ppm or ≥ 5 ppm over the nadir for hydrogen or methane respectively

4.3.4: GUT AND SYSTEMIC SYMPTOMS

The presence of GI and systemic symptoms were recorded by the participants along with their severity before a lactose challenge and afterwards for up to 48 h in a symptom chart [Table 2.5] as described in chapter 2.

DNA analysis was undertaken blind, and precautions were taken to minimise bias in the analysis of symptoms and breath hydrogen and/or methane. The results of DNA, breath tests and symptoms were not available until the end of the study when the results were collated.

4.3.5: STATISTICAL ANALYSIS

Quantitative variables are given as mean ± standard deviation; results between groups are compared using t test & Mann-Whitney test. Categorical variables are given as total numbers and percentages; differences between groups were compared using Chi squared statistics. Comparison of the frequency of the DNA

and breath test results between patients groups, healthy controls was performed using Chi-squared statistics. The comparison between each sub-group was also performed using Chi Squared statistics. Differences in the mean age of patients with positive and negative breath test were compared by using the unpaired Student's t-test. The Cochran-Mantel-Haenszel test was used to compare the effect of T allele [lactase persistence] association with risk of IBD. P values less than 0.05 considered to be significant. The data was entered into statistical program SPSS version 12 [Chicago, USA] for analysis.

4.4: RESULTS

4.4.1: PATIENTS

A total of 165 patients with IBD were recruited [95 with UC, 70 with CD], all were in remission and on a range of treatments. In addition 30 healthy volunteers [HV] were recruited. Their basic characteristics are shown in table 4.1. The mean age of HV was 31± 11 years [mean ± SD], CD 47 ± 15.6 years and 48 ± 14 years for UC patients. The duration of the disease was 10.8 ± 10.1 years [mean ± SD], 9.1 ± 9 years for CD and UC subjects respectively. None of the HV took any medications, they were all non-smokers and no one had undergone any abdominal surgery. 96% UC patients were either ex-smokers or lifelong non smokers. However, 14% of CD patients were currently smokers. The 3 groups [HV, CD, & UC] were equally matched for sex distribution and family history of IBD, but not for age, smoking status, current medications and previous abdominal surgery.

Table 4.1: Patient details: Baseline characteristics of 195 subjects who participated in the study, this includes 70 with CD, 95 with UC & 30 HV. The results displayed as per the extent of disease in patients with UC and CD.

		Crohn's disease [CD]				**Ulcerative colitis [UC]**				**Healthy volunteers [HV]**
		Total	Ileal	Colonic	Ileo-colonic	**Total**	Extensive colitis	Left sided	Proctitis	
Number		**70**	18	28	24	**95**	35	46	14	**30**
Age range		**19-86**	24-86	22-70	19-72	**20-81**	25-78	20-81	29-66	**21-56**
Sex M:F		**32:38**	10:8	13:15	9:15	**51:44**	20:15	24:22	5:9	**15:15**
Duration of Illness [year]		**0.5-48**	0.5-39	0.5-30	1-48	**0.5-43**	0.5-43	0.5-43	3-22	**NA**
Family History of IBD		**14**	5	3	6	**16**	7	6	3	**2**
Smoking	Never/Ex	**32/28**	8/9	13/12	11/7	**51/40**	19/15	23/20	9/5	**30/0**
	Current	**10**	1	4	5	**4**	1	3	0	**0**
Current Medications	Yes/No	**54/16**	14/4	24/4	16/8	**82/13**	32/3	41/5	9/5	**0**
	5 ASA	**32**	6	19	7	**59**	26	28	5	**0**
	Thiopurines	**24**	8	8	8	**20**	12	6	2	**0**
	Steroids	**7**	2	2	3	**4**	2	2	0	**0**
	Rectal Therapy	**0**	0	0	0	**9**	0	6	3	**0**
Surgery	Yes	**17**	8	1	8	**1**	1	0	0	**0**

HV were significantly younger [p<0.001], were less likely to smoke [p=0.006], used fewer medications [p=0.001] & were less likely to have a history of previous abdominal surgery [p=0.001] when compared to those with IBD. There was no difference between patients with CD and UC with regards to age, sex distribution, disease duration, family history of IBD and smoking status. However, significantly more patients with CD had surgery [p=0.001] and less frequently were on medications [p=0.03] when compared to UC patients.

13 patients with UC were on no medications at the time of the study. 3 of these had extensive colitis including one patient who had previously had a total colectomy. The second was a 74 year old man who was previously on Azathioprine for 8 years and in remission for 7 years with normal colonoscopy on 2 occasions in this time period – he had elected to come off all his medications. The third was a 36 year old woman who did not want to take 5ASA's as she was in symptomatic remission for the previous 10 years and wanted to start a family. 5 patients with left sided colitis were not taking any medications when the tests for the study were carried out: 2 used enema's as required and another one [48 years old man] used short courses of oral steroids only when their condition flare up. The fourth was a 56 years old man who was in remission for 4 years and his thiopurine was stopped a year prior to entry into this study and finally the fifth patient was a 26 years old man who on rectal nicotine treatment. 5 patients with proctitis were not on any medications, 4 used 5-ASA suppositories and one used steroid enema's when their condition flared up.

The patient with UC and a prior colectomy, as mentioned above, only underwent genotyping and not the breath test. 17 patients with CD had previously under gone 24 surgical procedures which included 10 patients who had undergone

a right hemi-colectomy, 5 ileal resections, 4 stricutoplasties, 3 Seton placements, 1 fistula repair & 1 segmental colectomy.

4.4.2: GENETICS

The C to T polymorphism on chromosome 2 was analysed in all 165 patients [95 with UC; 70 with CD], and compared with 30 HV [Table 4.2].

Table 4.2: Genetic analysis results for patients with Crohn's disease, ulcerative colitis & healthy volunteers who were included in the study. It is sub-divided in patients with IBD as per the extent of the disease.

CC [homozygous for lactase non persistence], CT [heterozygous] & TT [homozygous] for lactase persistence.

		Genotype Result		
		CC	CT	TT
Healthy Volunteers [30]		**0 [0%]**	**15 [50%]**	**15 [50%]**
Inflammatory Bowel Disease [165]		**12 [7.3%]**	**58 [35.2%]**	**95 [57.5%]**
Ulcerative Colitis	**Total [95]**	**7 [7.4%]**	**35 [36.8%]**	**53 [55.8%]**
	Left Sided [46]	5	18	23
	Proctitis [14]	1	4	9
	Extensive colitis [35]	1	13	21
Crohn's Disease	**Total [70]**	**5 [7.1%]**	**23 [32%]**	**42 [60%]**
	Colonic [28]	1	10	17
	Ileal [18]	1	8	9
	Ileo-Colonic [24]	3	5	16

The results showed that 7% of IBD patients were CC, 35% and 58% were CT and TT respectively. No HV had the CC genotype and the CT/TT genotypes were equally divided. There were no significant differences of the genotype results between IBD and HV [$\chi^2 = 3.94$ & $p = 0.14$]. When the results of UC patients was compared to HV there was no significant difference [$\chi^2 = 3.34$ & $p = 0.19$] and similarly no difference was seen in CD [$\chi^2 = 4.14$ & $p = 0.13$ respectively]. In UC the genotypes results did not differ based on the extent of colitis [$\chi^2 = 2.63$ & $p = 0.6$] again there was no relationship between genotype and the segment of bowel affected by CD [$\chi^2 = 3.87$ & $p = 0.4$]. The 3 genotypes showed no statistically significant association based on age [$p = 0.38$], sex [$p=0.93$] and patient groups i.e. UC, CD & HV [$\chi^2 = 4.24$ & $p = 0.36$].

Additionally, the increased frequency of the T allele [table 4.3] that was observed in IBD patients was not significant [OR= 1.357, 95% CI = 0.62-2.9, $p = 0.44$], indicating that the T allele encoding lactase persistence was not associated with risk of IBD. Similar results were also seen in the T allele frequency when IBD patients were sub-divided by the type of IBD i.e. CD or UC.

Table 4.3: The frequency of the C & T alleles in healthy volunteers and patients with IBD. CMH [Cochran Mantel Haenzsel] Trend

Genotype Allele	**Patient Groups**		**Odds Ratio [Confidence Interval]**	**χ^2_{CMH}=0.589 p= 0.44**
	HV	**IBD**		
T Allele	15	95	1.00	
C Allele	15	70	1.36 [0.62-2.9]	

4.4.3: EFFECT OF LACTOSE CHALLENGE ON BREATH HYDROGEN AND METHANE

Breath hydrogen and methane were analysed, just before and up to 4 hours after a 50g oral lactose challenge. All the 30 healthy volunteers who had genotyping done completed this part of the study and their details are as in table 4.1. Several patients with IBD were not able to take part in the lactose challenge test so, the total number of people who were analysed with the breath test following lactose challenge were 110 [59 with UC and 51 with CD] and their base line characteristics are shown in table 4.4. Therefore, 55 [33%] patients with IBD could not take part in the lactose challenge, the reasons for non-attendance were: 10 [18%] requested withdrawal from this part of the study, 20 [37%] could not get time off from work and 25 [46%] did not respond to multiple appointment letters.

Of the IBD cohort who underwent the LTT, 50.7% were women [n=71] with an average age of 45.1 years [range 20-86 years] and 49.3% were men [n=69] with an average age of 44.9 years [range 19-81]. The mean age of patients with CD was 48.2 ± 16.2 years [mean ± SD] and 50.4 ± 15 years for UC patients. The duration of the disease was 10.9 ± 10.5 years [mean ± SD], 9.8 ± 9.2 years for CD and UC subjects respectively. The 3 groups [HV, CD, & UC] were equally matched for sex distribution and family history of IBD, but not for age, smoking status, current medications or previous abdominal surgery. Once again HV were significantly younger [$p<0.001$], less likely to smoke [$p=0.004$], used fewer medications [$p=0.0001$] & less likely to have a history of previous abdominal surgery [$p=0.005$] compared to those with IBD. There was no difference between patients with CD and UC with regards to age, sex distribution, and duration of disease, family history of IBD or smoking status.

Table 4.4: Patient details: Baseline characteristics of 110 subjects who had a lactose breath test this includes: 70 with Crohn's disease [CD], 95 with Ulcerative colitis [UC]. For UC & CD, they are also shown as per the extent of disease. The details of the healthy volunteers [HV] are same as outlined in table 3.1.

		Crohn's disease [CD]				**Ulcerative colitis [UC]**			
		Total	Ileal	Colonic	Ileo-Colonic	**Total**	Extensive colitis	Left sided	Proctitis
Number		**51**	15	20	16	**59**	24	25	10
Age range		**22-86**	31-86	22-70	22-70	**20-81**	25-73	20-81	32-66
Sex M:F		**24:27**	8:7	10:10	8:8	**30:29**	14:10	13:12	3:7
Duration of Illness [year]		**0.5-48**	0.5-39	1-48	1-48	**0.5-43**	0.5-43	0.5-43	3-22
Family History of IBD		**7**	3	3	1	**13**	5	5	3
Smoking	**Never/Ex**	**24/22**	6/8	10/9	8/5	**31/25**	9/14	15/8	7/3
	Current	**5**	1	1	3	**3**	1	2	0
Current Medications	Yes/No	**40/11**	11/4	17/3	12/4	**52/7**	22/2	22/3	8/2
	5 ASA	**17**	3	7	7	**44**	19	20	5
	Thiopurines	**23**	7	8	8	**12**	6	4	2
	Steroids	**2**	1	0	1	**2**	1	1	0
	Rectal Therapy	**0**	0	0	0	**5**	0	2	3
Surgery	Yes	**11**	4	1	6	**0**	0	0	0

However, significantly more patients with CD had surgery [p=0.001] and were less frequently on medications [p=0.02] than those with UC. 7 patients with UC who took part in the lactose breath test were on no medications [for full details see section 4.4.1]. 2 with extensive colitis were on no medications this included the 74 year old man and 36 year old woman previously described in section 4.4.1. Three patients with left sided colitis were not taking any medications – these included a patient who was on thiopurines until a year previously but stopped as he was in prolonged remission, the second who only used 5-ASA enema's during exacerbations and the third was on rectal nicotine treatment again as detailed in section 4.4.1. Similarly 2 patients with proctitis were not on any medications, they used 5ASA suppositories only when their condition flared up. 11 patients with CD had previously under gone 13 surgical procedures which included 6 who had undergone a right hemi-colectomy, 2 Seton placement, one fistula repair, 2 ileal resections, 2 stricutoplasties & 1 segmental colectomy.

The results for all the 140 subjects are shown in table 4.5; figures 4.1 & 4.2. There was a considerable variation in the absolute value in both the basal breath gases and the maximum after lactose. The maximum breath hydrogen ranged from 0 – 227 ppm, and the maximum methane ranged from 0 – 157 ppm, the lowest maximum breath hydrogen or methane values being 22 and 7 respectively [Figs. 4.1 and 4.2]. Interestingly, a significant number of IBD patients had raised basal hydrogen and/or methane before the lactose challenge [Table 4.5; Figs.4.1 and 4.2], 12% of UC patients having raised basal methane, compared with only 2 % in patients with CD, there being no relationship with large or small bowel disease distribution. None of the healthy volunteers showed a significant increase in hydrogen or methane levels after the lactose challenge. All

subjects with high basal breath hydrogen or methane exhibited a gradual fall to levels < 20 ppm or < 5 ppm respectively during the 4 h after ingestion of lactose.

Table 4.5: The effect of a lactose load on breath hydrogen and methane.

110 Patients with IBD and 30 healthy volunteers ingested 50 g lactose, after an overnight fast, and breath hydrogen and methane measured every 30 min for 3h and then an hour later. Results are expressed as parts per million [ppm], and the percentage of each group calculated. SD [standard deviation].

	Breath analysis	**CD [n=51]**	**UC [n=59]**	**HV [n=30]**
Basal [before lactose load]	Hydrogen range [ppm]	0 - 60	0 - 55	0 - 9
	Methane range [ppm]	0 - 19	0 - 132	0 - 19
	High hydrogen [> 2 SDs over mean]	41.0	42.0	14.3
	High methane [> 2 SDs over mean]	2.0	12.0	7.1
Maximum within 6 h after lactose load	Hydrogen range [ppm]	0 – 227	0 – 217	0
	Methane range [ppm]	0 – 157	0 -157	0
	Number with high hydrogen only [% > 20 ppm]	31.0	6.8	0
	Number with high methane only [% > 5 ppm]	16.0	17.0	0
	Number with both high hydrogen and methane [%]	2.0	3.0	0
	Total positive either or both hydrogen and methane[%]	49.0	26.8	0

Figure 4.1: The breath hydrogen levels before and after a lactose challenge.

Patients and healthy volunteers were given a 50 g oral dose of lactose, and breath hydrogen measured 30 min for 3h and then an hourly later. Results were plotted as parts per million [ppm] for basal values before the lactose challenge, and maximum values after the lactose challenge.

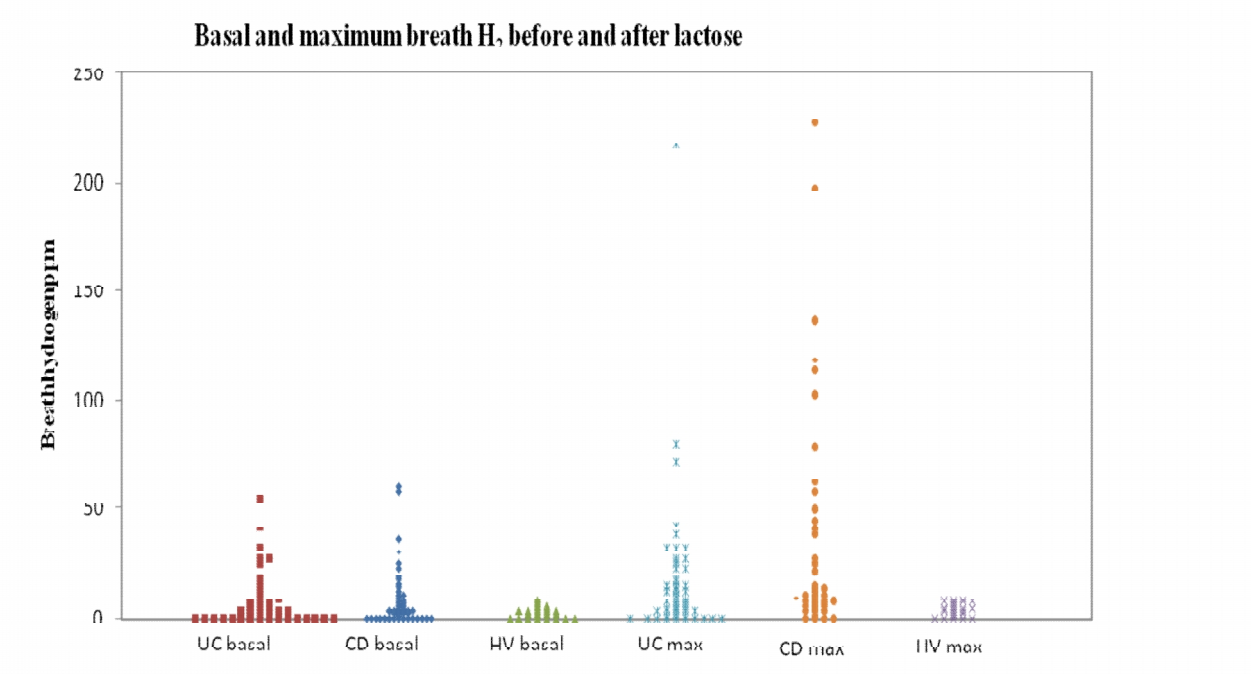
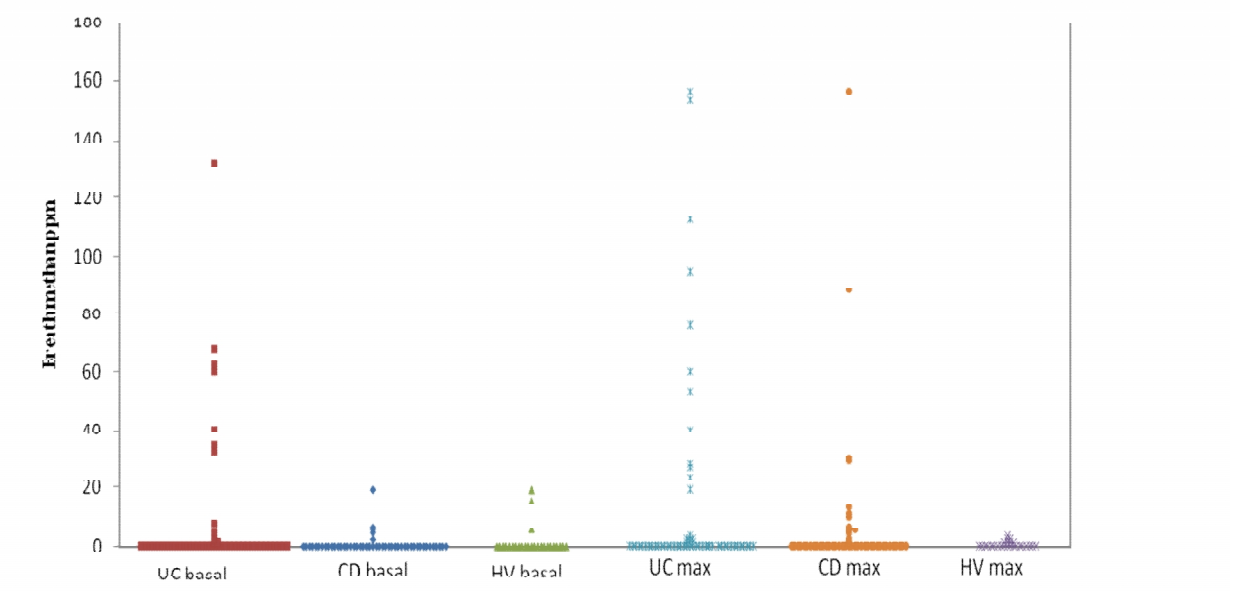


Figure 4.2: The breath methane levels before and after a lactose challenge.

Patients and healthy volunteers were given a 50 g oral dose of lactose, and breath methane measured 30 min for 3h and then an hourly later. Results were plotted as parts per million [ppm] for basal values before the lactose challenge, and maximum values after the lactose challenge.



A substantial number of IBD patients i.e. 41 [37%] had raised hydrogen and/or methane after lactose [Table 4.5; Figs 4.1 and 4.2] and this was statistically significant when compared to healthy volunteers [$\chi^2 = 15.81$ & $p = 0.00007$]. 69 [63%] with IBD had a negative breath test as did all the 30 healthy volunteers. The mean age of patients with a positive test was 49.6 ± 19.1 years [mean ± SD] and negative test 43.1 ± 15.3 years which did not differ significantly [p=0.31]. Similarly, no statistically significant association was found between gender and the result of the breath test [p=0.65], nor was any association found with the

smoking status [p=0.62], use of medications [p=0.5] or history of GI surgery [p=0.49].

When the results of IBD are divided by the type of IBD, a positive breath test occurred in 16 [27%] of UC patients compared with 26 [49%] who had CD. These differences were highly significant when CD was compared to HV [χ^2 = 21.27 & p <0.0001] and also when UC was compared to HV [χ^2 = 9.92 & p = 0.0016]. The rise in both the gases was also significantly higher in CD patients when compared with UC patients [χ^2 = 5.61 & p = 0.018]; these are shown below in the table 4.6. Raised breath gases [hydrogen and/or methane] occurred in 73%, 56% & 33% of patients with CD affecting the ileum, ileo-colon and colon respectively. The rise in breath gases was highly significant [χ^2_{CMH} = 9.11 & p = 0.003], with a statistically greater number in those patients with CD affecting the ileum compared to both ileo-colonic and colonic disease, additionally ileo-colonic disease was significantly higher when compared to colonic disease [χ^2 = 4.94 & p = 0.026]. In contrast, the prevalence of high breath hydrogen in patients with UC affecting the whole colon [extensive], left colon and rectum [Proctitis] was not statistically different - 25%, 24% & 30% respectively. Breath hydrogen was raised in 2 out of 3 smokers with UC and 3 out of 5 smokers with CD. This should not be due to any acute effects of smoking, as patients were instructed not to smoke immediately prior to the breath sample being taken. The breath test was positive in 41 patients with IBD, this was on the basis of a significant rise in hydrogen in 20 patients [18%], but importantly, a substantial number 18 [16.4%] of patients had raised breath methane, with no detectable rise in breath hydrogen. In 3 [2.7%] there was a rise in both the gases. Based on the sub-type of IBD, 16 [27%] cases of UC had a positive breath test result, which was based on rise in hydrogen in 4

[25%], methane in 10 [62.5%] and both gases in 2 [12.5%]. 25 [49%] patients with CD had a positive breath test, which was based on the rise in hydrogen in 16 [64%], methane in 8 [32%] and both gases in one patient [4%]. These are shown in table 4.5. The breath test in UC patients was positive with a significant production of methane compared to hydrogen [$\chi^2 = 4.6$ & $p=0.03$] in contrast with CD patients where an opposite effect was seen i.e. more hydrogen than methane and this was also significant [$\chi^2 = 5.13$ & $p= 0.024$]. There was no statistically significant difference in the gases produced based on the segment of the bowel in either form of IBD.

The comparison of genotype and the results of the breath test are shown in table 4.7. As previously reported (95, 136), all patients of genotype CC [100%] had a significantly raised maximum breath hydrogen or both hydrogen and methane, none had just a raised breath methane. In comparison significantly raised breath gases were seen in 34% of those CT and 31% of those TT. The CT and TT genotypes were more likely to have a positive breath hydrogen and/or methane in CD than UC and HV [$\chi^2 = 20.01$ & $p<0.0001$]. There was no difference in the positivity of the breath gases in the genotypes CT or TT when analysed by the disease extent in UC. However, in CD both CT and TT genotypes, there was a significant difference when the CD affected just the ileum compared with that involving the ileo-colonic region when compared to colonic involvement alone [$\chi^2 = 7.2$ & $p=0.027$]. These results show that both breath hydrogen and methane must be measured if a correct assessment of lactose sensitivity is to be made in IBD patients.

Table 4.6: Breath Test results for patients with Crohn's disease, ulcerative colitis & healthy volunteers before and after lactose challenge up to 4 hours. It is sub-divided in patients with IBD as per the extent of the disease.

A positive test is defined as raised hydrogen or methane, after lactose, was defined as ≥ 20 parts per million or ≥5 ppm over the nadir for hydrogen or methane respectively.

Patient Group	Sub Type	Positive Test	Negative Test
Healthy Volunteers	**Total [n=30]**	0	30 [100%]
IBD	**Total [n=110]**	**41 [37%]**	**69 [63%]**
Crohn's Disease	**Total [n=51]**	**25 [49%]**	**26 [51%]**
	Ileal [n=15]	11 [73%]	4 [27%]
	Ileo-colonic [n=16]	9 [56%]	7 [44%]
	Colonic [n=20]	5 [33%]	15 [67%]
Ulcerative Colitis	**Total [n=59]**	**16 [27%]**	**43 [73%]**
	Extensive [n=24]	7 [29%]	17 [71%]
	Left-sided [n=25]	6 [24%]	19 [76%]
	Proctitis [n=10]	3 [30%]	7 [70%]

Table 4.7: The rate of positive breath tests seen after an oral lactose challenge in healthy volunteers [HV], patients with ulcerative colitis [UC] and Crohn's disease [CD] based on genotype results and sub-classified based on the extent of the disease.

Groups	Sub Types	Genotype		
		CC	CT	TT
HV[n=30]		0/0	0/15 [0%]	0/15 [0%]
IBD [n=110]		**8/8 [100%]**	**11/32 [34.4%]**	**22/70 [31.4%]**
Ulcerative Colitis [n=59]	**Total**	**4/4 [100%]**	**4/16 [25%]**	**8/39 [21%]**
	Proctitis	1/1 [100%]	0/1 [0%]	2/8 [25%]
	Left sided	2/2 [100%]	2/9 [22%]	2/14 [14%]
	Extensive	1/1 [100%]	2/6 [33%]	4/17 [24%]
Crohn's Disease [n=51]]	**Total**	**4/4 [100%]**	**7/16 [37.5%]**	**14/31 [42%]**
	Ileal	1/1 [100%]	4/6 [67%]	6/8 [75%]
	Ileo-colonic	3/3 [100%]	1/3 [33%]	5/10 [50%]
	Colonic	0/0	2/7 [29%]	3/13 [23%]

4.4.4: GUT AND SYSTEMIC SYMPTOMS FOLLOWING LACTOSE CHALLENGE

After the oral lactose challenge, GI [abdominal pain, distension, borborygmi, flatulence, diarrhoea, constipation, nausea], and systemic symptoms [headache, muscle and joint pain, tiredness, a range of allergies, such as eczema, sinusitis, rhinitis and asthma, increased micturition, and heart palpitations] were recorded

for 48 hours. A substantial number of patients with IBD exhibited these symptoms after the lactose challenge - 69% for UC and 73% in CD as shown in Table 4.8. 39% of UC and 47% of CD patients reported both gut and systemic symptoms but less than 20% reported only GI symptoms in both the types of IBD. Furthermore, 13% and 6% with UC or CD respectively recorded systemic symptoms without any apparent GI symptoms [Table 4.9]. The gut and/or systemic symptoms reported as per the results of the genotype is also shown in table 4.9

Table 4.8: Symptoms experienced by participants after lactose challenge.

Patients with ulcerative colitis [n=59] or Crohn's disease [n=51] and Healthy Volunteers [n=30] were given an oral dose of 50 g lactose. The presence of gut [abdominal pain, distension, borborygmi, flatulence, diarrhoea, constipation, nausea], and systemic symptoms [headache, muscle and joint pain, tiredness, a range of allergies, such as eczema, sinusitis, rhinitis and asthma, increased micturition, and palpitations] were recorded, together with their severity on a scale of 0 – 10.

None of the healthy volunteers exhibited symptoms

Symptoms	Ulcerative colitis [n=59]	Crohn's disease [n=51]
No	18 [31%]	14 [27%]
Yes	41 [69%]	37 [73%]

Table 4.9: The relationship between symptoms and genotype

Patients with ulcerative colitis [n=59] or Crohn's disease [n=51] were genotyped as described in Chapter 3 and given an oral dose of 50 g lactose. Breath hydrogen and methane were recorded every 30 min for 3 hours and then an hour later. The presence of gut [abdominal pain, distension, borborygmi, flatulence, diarrhoea, constipation, nausea], and systemic symptoms [headache, muscle and joint pain, tiredness, a range of allergies, such as eczema, sinusitis, rhinitis and asthma, increased micturition, and palpitations] were recorded, together with their severity on a scale of 0 – 10. Results were expressed as total number of participants within each clinical group.

Disease	Genotype	Both symptom [GI or systemic]	GI symptoms only	Systemic symptoms only	No symptoms reported
UC	CC [n=4]	4	0	0	0
	CT [n=16]	3	3	1	9
	TT [n=39]	16	7	7	9
	All [n=59]	**23 [39%]**	**10 [17%]**	**8 [13%]**	**18 [31%]**
CD	CC [n=4]	4	0	0	0
	CT [n=17]	5	5	2	5
	TT [n=30]	15	5	1	9
	All [n=51]	**24 [47%]**	**10 [20%]**	**3 [6%]**	**14 [27%]**

The recording of both breath hydrogen and methane, with GI and systemic symptoms, increased the detection of lactose sensitivity from 31% to 73% in CD and in UC from 10% to 69%, compared with an assessment using raised breath gases alone [Table 4.10]. Furthermore, UC patients exhibiting the most symptoms after the lactose challenge had raised breath methane, but not elevated breath hydrogen.

Table 4.10: The diagnosis of lactose sensitivity based on breath gases and symptoms after an oral lactose load.

110 inflammatory bowel disease patients and 30 healthy volunteers were given an oral dose of 50 g lactose, as described in Chapter 2. Each individual filled in a questionnaire, recording gut and systemic symptoms, scoring the severity from 1 – 10. Patients with positive symptoms were then assessed as to whether they had a positive breath test. **None of the healthy volunteers exhibited symptoms**. Results were expressed as total number of participants and as a % of the total number of patients within each clinical group.

Breath test result	**Ulcerative colitis [n=59]**	**Crohn's disease [n=51]**
Positive hydrogen [> 20 ppm]	6 [10%]	15 [31%]
Positive hydrogen [> 20 ppm] & methane [5 ppm]	16 [27%]	25 [49%]
Positive hydrogen [> 20 ppm] & methane [5 ppm] & positive symptoms	41 [69%]	37 [73%]

All patients with the CC genotype had significantly elevated breath hydrogen and/or methane levels after lactose challenge and also had both GI and systemic symptoms [Table 4.9]. A significant number of UC patients with the TT genotype [13%] exhibited only systemic symptoms after lactose. This compared with only 6% of CD patients who were TT genotype. There were no other major correlations of symptoms with genotype. The numerical range of GI and systemic symptoms reported by the IBD patients after the lactose challenge ranged from 0 to 20. Interestingly, UC patients who exhibited most gut symptoms, i.e. 3 or more, and had the highest elevated breath methane, in the range 28-157 ppm, after a lactose load, compared with patients with Crohn's, who showed much lower or no elevation in breath methane after lactose [$p<0.03$]. There was no obvious correlation between the breath test results and symptoms after the lactose load in patients when comparing those who had previous surgery for CD with those who had no previous abdominal operation.

Information about the GI and systemic symptoms after the 4 hour monitoring period was collated for the next 44 hours [symptoms recorded for total duration of 48 hours]. None of the healthy volunteers reported any symptoms. Diarrhoea after the lactose challenge was a common symptom, in 20 patients the diarrhoea continued until the second day after the lactose load. Similarly 18 patients documented abdominal bloating and 15 abdominal pains up to 24 hours after lactose load whilst 14 nausea and 11 borborygmi reported up to 12 hours. Among the systemic symptoms headache and tiredness continued for 48 hours in 18 and 12 patients respectively. The timing of both gut and systemic symptoms often correlated closely. For example, several patients suffered both diarrhoea and headache for at least 48 h. There was a trend suggesting CC genotype patients

symptoms persisted longer when compared to CT and TT though this was not statistically significant.

New or additional symptoms were reported in several patients after the monitoring period and these included both GI and systemic ones. Among the GI symptoms, diarrhoea occurred in 6, bloatedness in 9, flatulence in 17, abdominal pain in 5, nausea in 8 and constipation in 3 patients. Muscle aches and joint pain started in 15, sore throat in 5, lack of concentration in 7, dizziness in 5, palpitations in 5, headache in 9, and tiredness in 13 patients. The CC patients reported new symptoms which were muscle aches and joint pain more often than CT and TT individuals. The rest of the systemic symptoms were reported more frequently by the other genotypes. There was no difference in the intensity or frequency of the symptoms expressed after 4 hours based on the breath test result or on the type of gas produced [methane or hydrogen].

4.5: DISCUSSION

The results show that sensitivity to lactose occurs in a high proportion [approximately 70%] of patients with IBD, even when they are in remission. The reason for the incidence being higher than that previously reported is because of the comprehensive tests used. These are a combination of three parameters - the C/ T polymorphism on chromosome 2, both breath hydrogen and methane measurement after an oral lactose dose and a record of both gut and systemic symptoms up to 48 h after the lactose load. The results from this study show genetic polymorphism analysis alone is insufficient in identifying all patients who are lactose sensitive suggesting that it is predominantly a secondary phenomenon related to the presence of IBD.

There have been several studies that have investigated whether there is a correlation between the CC/CT/TT genotype and different GI disorders (165, 274-280). An increased prevalence of lactose sensitivity has been reported in Crohn's disease when using breath testing (87, 88) but this has not been shown to arise from a genetic basis. Given the fact that only 7% of IBD patients in my study were the CC genotype, the results confirm previous reports (246, 281) that this genotype is not associated with IBD. A high incidence of IBD was seen in patients who were TT [Table 4.2], these results do not support an increased risk of IBD in people who have the T allele when compared with C allele, as has been reported elsewhere (242, 246). As in previous studies (95, 136), substantial numbers of the other two genotypes, CT and TT were found to be lactose sensitive as they had raised breath hydrogen and/or methane as well as symptoms. In the current study, only 7% of IBD patients were CC, 35% and 58% being CT or TT respectively – this is in contrast to the study of IBD patients from Germany by Buning (168) where 21% were CC, 56.8% CT and 22.2% TT. However, my results are similar to a study also from Cardiff, where the frequencies of genotypes in a cohort of IBS with lactose sensitivity were 14.5% CC, 39% CT and 46.5% TT (136). The higher percentage of CC in this study of IBS patients is to be expected as these patients had been specifically referred to a food intolerance clinic because of possible lactose sensitivity. My results also confirm the findings of studies where all patients with the CC genotype are lactose sensitive (165, 274-276, 282 & 283). I have found that this cohort develop multiple GI and systemic symptoms, as well as having higher hydrogen levels and symptom severity after lactose challenge. If genotyping is done first, those who are CC do not need the

oral lactose challenge test, thereby avoiding major symptoms, which these patients are known to suffer (128, 129 & 136).

A positive breath test occurred in patients with IBD more frequently than in healthy volunteers. This lactose sensitivity is therefore due to the presence of the disease itself rather than a genetic predisposition. Crohn's disease was associated with a higher breath test positivity compared to UC. In CD, the results were higher if the disease involved the ileum [73%] than the ileo-colon [56%] and lowest in pure colonic disease [33%]. However UC patients also had a higher frequency of positive breath tests when compared to healthy volunteers with no effect based on the extent of disease. These results are similar to a study by Mishkin (87) for Crohn's disease but not for Ulcerative Colitis. However, other publications have shown similar results to my work. In a recent study from Australia (284) a high prevalence of lactose malabsorption was shown by the hydrogen breath test in 148 patients with IBD [92 CD, 56 UC] – it was 42% in CD and 40% in UC, compared with 18% in 71 HV. They also showed a higher incidence of lactose malabsorption of 68% in ileal CD compared to 39% in ileo-colonic and 18% in isolated colonic disease. Again, no difference was seen when the results was examined based on the extent of disease in UC as was seen in my study. Measurement of both hydrogen and methane may account for the slightly higher rates of positive breath test results that I found.

My study included CD patients who had surgery which could be considered as a confounding variable. However, of the 51 patients with CD who underwent lactose breath testing, it was positive in 6 [54%] of the 11 patients who had previous surgery for CD compared with 20 [50%] of the 40 who did not have any surgery – a non-statistically significant difference.

Other factors such as smoking, non-compliance with dietary restriction, GI transit and bacterial overgrowth may lead to high hydrogen and methane at baseline [i.e. prior to lactose challenge]. An early rise of hydrogen [90-120 min] has been used to indicate bacterial overgrowth, rapid oro-caecal transit or a combination of these factors - differentiating between these may be problematic. In my study, this early rise in breath gases was not taken as positive test to identify lactose malabsorption. Instead, a sustained rise in breath gases or rise in breath gases after this period was interpreted as a positive result. In addition, considerable variations in the basal levels of hydrogen and methane could be due to residual starches in the colon or from incomplete fasting. To counter this, baseline gases were measured prior to the lactose challenge to act as a comparator. In the patients who have elevated baseline gases, levels fell during the test probably due to digestion of products in colon. In some patients the high levels of either of these gases was seen before the lactose load – this is possibly from slow digestion of food consumed the day before. These levels fell over the next 90-120minutes and in order to improve the assessment of positivity during the breath test, I used the lowest level of the breath gases [the nadir] as the baseline against which any rise was measured, which was the method originally suggested by Flatz et al in 1984 (285). In a study by He (286), a lactose challenge demonstrated a faster oro-caecal transit in LI when compared to those who are lactose tolerant and it was hypothesised that this could be a direct effect of lactose on intrinsic factors regulating intestinal motility rather than the effect of osmotic load of lactose.

Elevated levels of breath hydrogen have been reported in IBD (287) I have shown high breath methane levels with or without an elevated breath hydrogen. This elevation in breath hydrogen and/or methane in a significant number of both

UC and CD patients are consistent with an on-going carbohydrate malabsorption, even when the disease is in apparent remission. Whilst carbohydrate malabsorption could be expected in small bowel Crohn's, the findings here also shows that diseases of the large bowel i.e. colonic Crohn's disease and UC also lead to malabsorption of lactose. Lactase is only found in small intestine therefore, it is understandable why small bowel disease like Crohn's disease may lead to the malabsorption of lactose by causing inflammation of the mucosa which leads to loss of enzyme lactase and therefore problems in hydrolysing lactose. However, large bowel disorders like UC and CD also cause lactose malabsorption. There is no definitive explanation identified so far to explain this. It is likely in some patients with CD of the large bowel they may have associated small bowel disease which is not identified by current tests. One of the possibilities for LM in large bowel IBD is that, this could result from the higher prevalence of lactase non-persistence status in colonic disease compared to healthy population, but this is not the case from the data available from the studies published on this topic. Several possibilities could explain this abnormality in large bowel inflammatory disorders. The number of intestinal cells expressing lactase may be reduced or they may have a defective function despite lactase persistence status; the result would be an inadequate hydrolysis of lactose. These could result from the bacterial by products [similar to the model proposed for symptoms i.e. the 'bacterial toxin' hypothesis], drugs used to treat IBD or as a consequence of cytokines produced by inflammation in the large bowel. Colonic inflammation could also lead to colonisation of the small bowel by unidentified micro-organisms. Jejeunal villous atrophy was common during the active phase of UC (255). The other mechanism which could play a role is an increased intestinal

transit resulting in reduced contact time between lactose and lactase. The products that arise from cleavage of lactose are taken up via the sodium-activated glucose transporter SGLT1. This transporter is inhibited by tri- and tetra-saccharides, such as stachyose and raffinose, found particularly in many root vegetables and soya (288). Eating carbohydrates with these non-metabolisable sugars causes significant amounts of sugars reaching the bacteria in the large intestine which produce large amounts of gas causing symptoms. These observations may be due to co-existing changes in the small bowel intestinal lining, a different bacterial population, changes in transit times or other mechanisms and need to be explored further. The lactose sensitivity in IBD cannot be solely attributed to any significant ongoing inflammation, since all of my patients were apparently in remission based on recognised clinical indices. It would, however, be interesting to have checked faecal calprotectin levels or confirmed mucosal healing to provide further objective evidence of remission.

The rise in breath methane may have an important implication for the pathogenesis of IBD or it could have occurred as an epiphenomenon but again warrants further investigation., The pathogenesis of IBD may involve an inappropriate activation of the intestinal mucosal immune system in predisposed hosts (289), so it is possible for this immune response to be elicited in response to methanogenic bacteria, disruption of tolerance to these organisms or these bacteria may generate metabolic toxins. Higher methane levels were associated with IBS, healthy volunteers than with IBD patients in some previous studies (290-292). The difference that was seen in this study could be due to the way healthy volunteers were selected.

Methane production is limited to only a few species of micro-organisms which are called methanogens e.g. *Archaea*. This group of micro-organisms are widely distributed in natural environments and include *Methanobrevibacter smithii*, *Methanobacterium ruminatum* and *Methanosphaera stadtmanae* (293-295) and halophilic *Archaea* [genus Halobacteriaceae] (296). *Methanobrevibacter smithii* is the predominant archaeal species present in the human large intestine (297, 298) and is responsible for almost all CH_4 produced in the intestine (292, 299 & 300). whilst *Methanosphaera stadtmanae* is another common one isolated (301). Methane is produced by utilising substrates such as hydrogen, CO_2, acetate, formate, methanol and methylamines, this process is called methanogenesis. There is a complex interaction between H_2 and CH_4 production which is not fully understood. Methanogenic micro-organisms are able to convert H_2 to CH_4 within the colon (292, 299, 300).

Measurement of breath methane is an indirect means of determining methane production (302). Despite the absence of CH_4 in the breath of some subjects, methanogenic micro-organisms can be cultured from faeces in many of them (292). Methane production occurs primarily in the left colon in 54% of normal subjects (292) and hydrogen is produced primarily in the right colon. The development of high concentrations of methanogens depends on a continuous supply of high H_2 concentrations from exogenous or endogenous sources that exceeds the capacity of removal. The advantage of methanogenesis to the host is that it lowers the pressure that would normally be exerted by a given amount of H_2 because 4 litres of H_2 are used to produce 1 litre of CH_4 (303). It has been shown that as many as 40-50% of people are methane producers (304, 305). In some subjects, at least part of the hydrogen is used to produce methane (304, 306). A

study by Bjorneklett showed the prevalence of CH_4 production in a group of 120 healthy subjects, determined by a single midday breath sample, was 44%, with no significant difference between sexes and no correlation with age (304, 307).

Methane has been shown to reduce small bowel transit time. In a study with healthy subjects by Cloarec, methane production was associated with reduced oro-caecal transit time [111 Vs 68 min] when compared with subjects not producing methane. In another study (308) of patients with IBS patients and animal models, methane was shown to slow small intestinal transit and this effect was caused by triggering non-propulsive or segmental contractions in the small bowel. The effect of methane on colonic motility has not been studied but its production has been shown to be elevated in constipation predominant IBS (290, 291, 309-311).

Both luminal and breath methane production in IBD patients has previously been reported to occur at lower levels than controls (290, 291). In a study by McKay methane excretion was detected in 54% of healthy controls, 53% of non- gastrointestinal patients and 32% of GI patients (292). Patients within the GI disease group with IBD had significantly lower methane detection; 13% for CD and 15% for UC. In another study by Pimentel, methane production in ulcerative colitis and Crohn's disease was reported to be almost non-existent (312). This could be due to the effect of diarrhoea which occurs in active IBD and is known to reduce or eliminate methane production and lower the incidence of methanogens (290, 312). In contrast to these studies, higher levels of breath methane have been detected in patients with colonic polyposis as well as cancer of the colon than in healthy patients (313). In a study by Scanlan et al (314) using PCR to amplify methanogenic DNA in faecal samples, the frequency of

methanogens ranged 45-50% in HV, colorectal cancer, in those who had polyps removed and IBS groups. In the same study, patients with IBD were also studied. There were significantly fewer patients who had methanogenic micro-organism - 30% for Crohn's disease and 24% for ulcerative colitis compared to the control group of 48%. The difficulty in all these studies is that they have included IBD patients in different phases of illness [i.e. active disease, remission etc]. It is not known whether there is a difference based on the activity of the disease and if the effects of methane or the organisms that produce it influence the disease process. It could be postulated that this may be the case. In a recent study of Boros (315), methane was shown to confer a protective effect on the oxidative stress and inflammation in ischemic and reperfusion induced intestinal injury in canines. Sulphate reducing bacteria have been proposed to be involved in the aetio-pathogenesis of IBD and colon cancer (316, 317). These bacteria consume some of the hydrogen produced in fermentation (318) and in vitro data has shown that sulphate reducing bacteria out compete methanogens for hydrogen (319). This limited substrate range could be a factor in competition between methanogens and the sulphate reducing bacteria may play a role in IBD activity and complications. The presence of such a bacterial population is also dependent on host factors, diet, intestinal transit time and other environmental factor.

Patients who suffered from diarrhoea after the lactose challenge often started to experience it several hours, even a day, after the lactose would have disappeared from the intestine, as was also reported in a previous study (128). In fact, 50% of the patients with CD, and over 75% of those with UC, who had the diarrhoea after the lactose challenge, still had this 48 h later; long after the lactose would have gone from the intestine. This supports the 'bacterial metabolic toxin'

hypothesis [chapter 1.2] i.e. the mechanism causing diarrhoea involves cell signalling, analogous to the diarrhoea in gut infections (95, 128, 135 & 139), and is not simply an osmotic effect of the lactose.

The ingestion of sugars other than lactose can induce gut and systemic symptoms in patients with IBD. They can also occur due to anticipation or as an independent event. The questionnaire about the symptoms was given to the participants to complete at base line for documentation of their symptoms prior to consumption of lactose and administered at regular time intervals as per the protocol. This questionnaire was not validated for this purpose but has been utilised in a previously published study by Waud et al (136). It is also used as a part of breath test in the institution where my study was carried out. None of the healthy volunteers exhibited symptoms after the lactose challenge suggesting that these symptoms in patients are less likely to be due to a 'nocebo' effect (320), although this should be explored further. The nocebo effect is a phenomenon in which inert substances or mere suggestions of substances actually bring about negative effects in a patient or research participant. It is the opposite phenomenon to the placebo effect which is more widely understood. Like the placebo effect, it is thought to be brought about by a combination of pavalovian conditioning and a reaction to expectations. During the recruitment phase of the study, I encountered just 2 patients in clinic who had been diagnosed with lactose sensitivity and were on a restricted diet - these were not recruited into the present study to avoid bias, and potential nocebo effects. A weakness of my study was the lack of a placebo element to breath testing as all were done after a standardised lactose load. This makes it difficult to quantify the significance of the symptoms that were reported by the subjects.

None of the healthy volunteers showed features of lactose sensitivity. They did not have had any gut symptoms, such as abdominal pain, distension or bloatedness, and/or change in stool frequency or form on most days in the preceding year. This could potentially exclude anyone with possible lactose intolerance [LI]. I acknowledge that the inclusion/exclusion criteria used to recruit healthy volunteers could have contributed to the results seen here. The HV were also not on any medications when compared to IBD participants. The lactose in medications could have been a cause of the symptoms in some of the lactose sensitive individuals. The criterion for recruitment was to avoid these confounding variables, but they could also lead to selection bias. When setting up this study we elected to use IBD patients who were attending clinics so that they can participate in various components of the study. The advantage of recruitment from a data base which is based on a community [i.e. those attending hospital clinics and those who are not] is to ensure that the cohort is more representative of the local population. This will also include patients in different stages of the disease process. By recruitment from the clinics alone, you are likely to recruit those who are conscious of their health needs, highly motivated and are able to attend.

Smoking has no direct or indirect casual effect on LS therefore the fact that all of the healthy volunteers are non-smokers will not have any effect on the result. The age difference between healthy volunteers and IBD patients should also have no bearing on the results. All were over the age of 18 years, there is evidence that intestinal lactase activity does not continue to decline with age after childhood, because there were no differences in the prevalence of hypolactasia between older and younger adults (321). There are very few studies to date looking at the effect of age on symptom tolerance. One described there was no

difference in the symptoms between the age groups of over 65 years and 20 to 40 years (188). Some parts of the world have high incidence of lactose intolerance. This confounding variable needs to be matched to exclude any bias and this is the reason why my study is focussed on Caucasian population.

It can be concluded that it is essential to record both gut and systemic symptoms when assessing lactose sensitivity after an oral lactose challenge. The results are restricted to the Caucasian population and they should be interpreted with caution when the test is carried out in non Caucasian subjects. The study is also limited to IBD in apparent remission using clinical criteria and may be different with greater activity.

My results show the value of genotyping, measuring both breath hydrogen and methane for up 4 h after an oral lactose challenge, as well as recording both gut and systemic symptoms for up to 48 hours.

4.7 CONCLUSIONS

This study shows that there is a much higher prevalence of lactose sensitivity in all types of IBD when compared to healthy volunteers. This has been shown when the disease is in remission. The question that now arises is what active disease will do to this sensitivity as it would be expected to make it worse. The results argue strongly for a full clinical trial to investigate the effect of removing lactose from the diet in patients – a small pilot study of this is outlined in the next chapter.

The fact that subjects report symptoms up to 48 hours after a lactose challenge cannot be solely due to its osmotic effect and would give temporal support for the ‘bacterial toxin’ hypothesis. The role of putative toxins and the

need to further exclude subjective factors when reporting symptoms after a lactose challenge both need to be tested further. The raised breath methane, particularly in ulcerative colitis is intriguing and the potential role of *Archaea*, in the pathogenesis of IBD should also be explored.

CHAPTER 5

A PILOT STUDY OF THE AVOIDANCE OF LACTOSE IN THE DIET OF PATIENTS WITH CROHN'S DISEASE AND LACTOSE SENSITIVITY.

5.1: AIM

In chapter 4, a substantial number of people with IBD were identified as having lactose sensitivity on the basis of genotyping, breath test and symptoms. The aim of this chapter is to determine whether there is any relationship between inflammatory bowel disease and lactose sensitivity by exploring if the avoidance of lactose in the diet of patients with IBD and lactose sensitivity, can lead to improvements in symptoms.

5.2: INTRODUCTION

Diet is thought to play a role in the immuno-pathogenesis of inflammatory bowel disease, whether this is primary due to formation of antibodies to antigens from diet or secondary to intestinal inflammation is not clearly known yet. Review of some of the dietary factors implicated in the pathogenesis of IBD is in section 1.1.2.3.3.

Dietary factors have been proposed to be involved in the pathogenesis of IBD and dietary modification has also been used as a treatment. The precise role of enteral nutrition in inflammatory bowel disease [IBD] remains to be defined. Enteral nutritional support in addition to normal food is indicated in undernourished patients with CD or UC to improve nutritional needs. In children with active CD, enteral nutrition is often used as a first line therapy. However, a recent Cochrane meta-analysis (322) of ten trials showed no statistically significant difference between CD patients treated with elemental and non-elemental diets. Enteral nutrition therapy in active UC has not been adequately evaluated. Exclusion diets have been shown to prolong remission in Crohn's disease (323, 324). In a study of 40 participants with CD by Riordan et al (323)

they instructed participants to introduce one new food daily, excluding any that precipitated symptoms. They observed sensitivity to corn in seven patients; wheat, milk and yeast in six; egg, potato, rye, tea and, coffee in four; and apples, mushrooms, oats and chocolate in three. IgG4-guided exclusion diets have resulted in significant symptomatic improvement in symptoms and a fall in inflammatory markers in some studies and this approach may be useful in certain patients. The advice provided by doctors to their patients is very variable, some tell their patients to avoid dairy products at the time of diagnosis, but others do not consider dairy products play a role in the IBD symptom management (244). In addition IBD patients also avoid dairy products by their own choice or due to a lack of clear advice from their doctors.

Milk and related lactose containing products are thought to cause problems by patients with inflammatory bowel disease especially those with CD – many of these may alter their diet accordingly. Lactose metabolism occurs in the small bowel which is the primary site of inflammation in many patients with CD and this leads to secondary LS. Milk is also reported commonly by patients IBD as causing symptoms on reintroduction. The reasons for this being, the increased prevalence of lactose intolerance in IBD compared to controls and the allergy to milk proteins, both of which are discussed in section 1.3. The prevalence of dairy sensitivity in IBD patients is thought to be in the range of 10-20% (250, 325).

In a recent study from New Zealand (326) using a dietary questionnaire, patients with CD perceived worsening of symptoms if they consumed dairy products such as cream, ice-cream, cheese and milk. In addition symptoms were perceived less when CD patients consumed cow's milk compared to the other dairy products. These patients felt yoghurt was more tolerable compared to above

mentioned dairy products. The authors felt the lactose content of the dairy products did not influence the self-reported symptoms, but they did not collect the quantity of the dairy food that is required to cause symptoms. This also shows variations in symptoms experienced based on the type of dairy product consumed. Interestingly, an increased frequency in self reported symptoms in CD patients was noticed if they had colonic disease compared to ileal disease. The consumption of dairy products made no difference to CD symptoms for the majority of participants in this study.

Studies have attempted to identify dietary risk factors in the expression of IBD, but the overall conclusion appears equivocal, at least in part because of serious methodological limitations and inconsistencies. Dietary habits are usually recalled either before the onset of the disease or as what is being currently eaten. However, the present diet may not necessarily reflect the previous one, elimination diets are difficult to follow-up due to a high drop-out rate and patients seem to have difficulty in identifying foods that trigger symptom exacerbation.

Despite these factors, the results of the previous chapter, where a large proportion of IBD patients had evidence of lactose sensitivity, naturally leads to an enquiry of what will happen if such patients exclude lactose in diet. An initial pilot is presented here in a cohort of patients with small bowel Crohn's disease in clinical remission and documented lactose sensitivity.

5.3: METHODS

5.3.1: STUDY POPULATION

20 participants were initially invited to take part in this study from those who took part in the study outlined in chapter 4. The study population were all of Caucasian origin and were patients with Crohn's disease in clinical remission as defined by a score of ≤ 4 on the Harvey Bradshaw Index. They were all diagnosed with lactose sensitivity based on a positive breath test and symptoms following a 50g lactose challenge.

They were asked to fill a food diary which outlined a complete record of what was eaten each day [Appendix 1] and a daily symptom diary [Appendix 2] which consisted of the following questions Wellbeing [0= very well, 1=slightly below par, 2=poor, 3= very poor, 4= terrible], Abdominal Pain [0= none, 1=mild, 2=moderate, 3= severe], Number of loose stools in a day and as well as recording other symptoms like flatulence, bloatedness, nausea, vomiting, rash, headache, fatigue, muscle and joint pain, palpitations, itching etc. Both diaries were kept daily for 2 weeks whilst the participants ate their normal diet. At the end of this period they completed a health related quality of life questionnaire [HRQOL]. The short inflammatory bowel disease questionnaire [SIBDQ] was chosen for this and is given in Appendix 3 (327). The license for its use in this study was obtained from McMaster University [Copyright ©1989, McMaster University Hamilton, Ontario, Canada]. Each item is scored on the seven point graded scale from 1 [a severe problem] to 7 [not a problem], giving an absolute SIBDQ score range from 10 [poor HRQOL] to 70 [optimum HRQOL].

Participants were then asked to go on a minimal lactose free diet for 4 weeks during which time they maintained a daily food diary. The minimal lactose free diet information sheet that is used within clinical practice in the health board where this study was carried out was provided to all participants [Appendix 4]. During the last 2 weeks on this diet they completed the symptom diary again along with another SIBDQ at the end of this period. They were provided with a pre-paid envelope to return the food and symptom diaries together with the HRQOL questionnaire at the end of the 2 week period on normal diet and then after a minimal lactose free diet. The symptoms and SIBDQ scores whilst eating a normal diet were compared to those recorded during the minimal lactose free diet. Written informed consent was obtained from each participant. The study was approved by the South East Wales Research Ethics Committee.

5.3.2: STATISTICS

Quantitative variables are given as mean ± standard deviation and categorical variables are given as total numbers and percentages. Comparison of the group during normal diet and during lactose free diet was analysed by Wilcoxon Signed rank test. P values less than 0.05 were considered to be significant. The data was entered into the statistical program SPSS version 12 [Chicago, USA] and was used for analysis.

5.4: RESULTS

20 suitable participants with CD were invited to take part in this study. Three participants did not respond to the invitation letter. Of the 17 who consented to take part in the study 3 asked to drop out, as they felt the diet was too restrictive for themselves and their family. In 3 cases, forms were only returned whilst on their normal diet and this was incomplete in one. In one case only information whilst on a lactose free diet was available. Complete information was therefore only available in 10 participants.

The age range of these 10 participants was from 27-86 years and the average age was 58.8± 21 years. There were 6 women aged 60.3 ± 18.2 years and 4 men 56.5 ± 27.6 years. The duration of the disease was 14.8 ± 10.8 years with range from 1-39 years. 7 never smoked and 3 were ex-smokers.

All the ten participants had CD involving the small bowel. In 6 participants the disease was limited to small bowel and the remaining 4 had both large and small bowel disease. 2 participants were not taking any medications but the remaining 8 were on medication for their CD of which 3 patients were on Azathioprine, 4 were on 5-ASA preparations and one was on a combination of 5-ASA and Azathioprine. 5 had no surgery and 5 had surgery for CD which included 3 patients with a previous right hemi-colectomy, one ileal resection and one partial colectomy. During the study period, no changes were made to the treatment of their CD. The baseline characteristics of the participants are summarised in table 5.1 including the results of the genetic and breath tests. All the 10 participants in this study had a positive breath test and symptoms after lactose challenge test. The breath test was positive in seven with a rise in H_2, in two a rise in CH_4 and one had a rise in both the gases after the lactose challenge.

During the baseline fortnight when they were asked to continue with their normal diet, all participants consumed milk which totalled 2.3±1.35 litres and ranged from 250mls to 5.2 litres. In addition to this, patients consumed lactose within a range of other foodstuffs and drugs. During the minimal lactose diet trial period only 3 participants consumed milk; 30mls in one case whilst the other two took 1.5 and 1.72 litres respectively of lactose free milk.

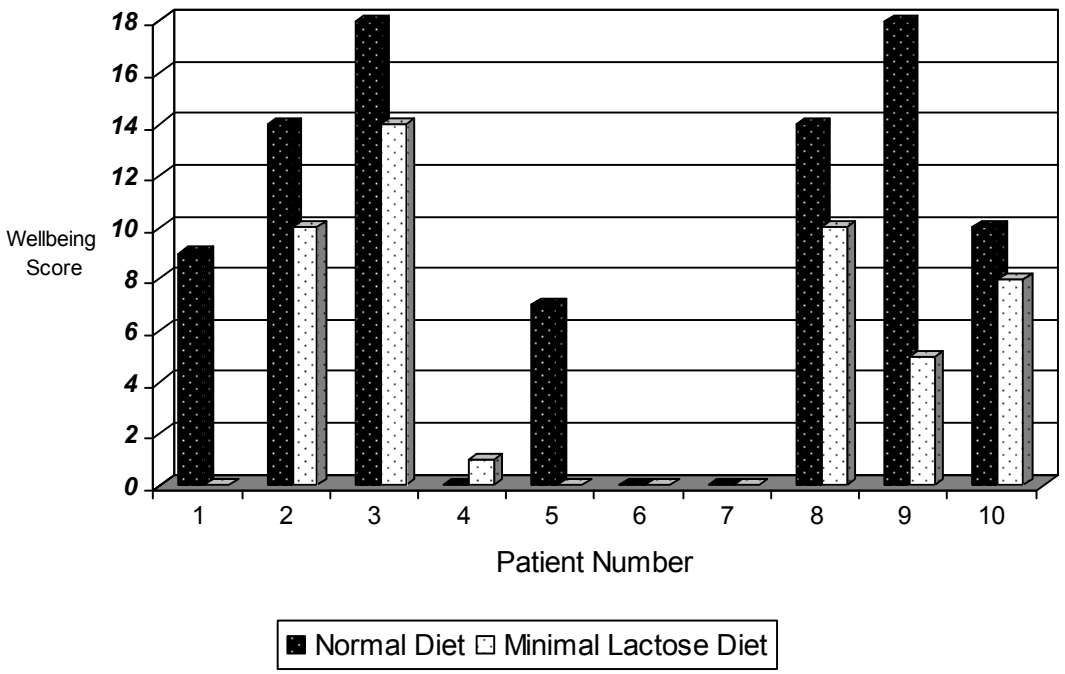
Table 5.1: Details of individual participant in the study

Patient Number	Age [years]	Sex	Duration of Disease [years]	Extent of Crohn's disease	Smoking Status	Genotype	Breath Test	Drugs	Previous Surgery
1	86	M	20	SB	Ex	TT	H & M	Y	N
2	69	F	21	B	N	TT	H	Y	Y
3	27	M	12	B	N	CC	H	Y	N
4	80	F	7	SB	Ex	TT	H	N	N
5	38	F	13	B	Ex	CT	H	N	Y
6	73	M	17	SB	N	CC	H	Y	Y
7	69	F	5	B	N	TT	H	Y	Y
8	37	F	1	SB	N	CT	M	Y	N
9	69	F	39	SB	N	TT	H	Y	Y
10	40	M	13	SB	N	CT	M	Y	N

Sex: M male, F female, Extent of CD: SB small bowel, B;-both large and small bowel, Genotype: TT lactose persistence, CT Heterozygote's & CC lactose non-persistence, Breath test: positive for gas produced, H [hydrogen> 20ppm] and M [methane >5ppm], Smoking status: Ex- Past smokers & N Never smoked. Y Yes & N No

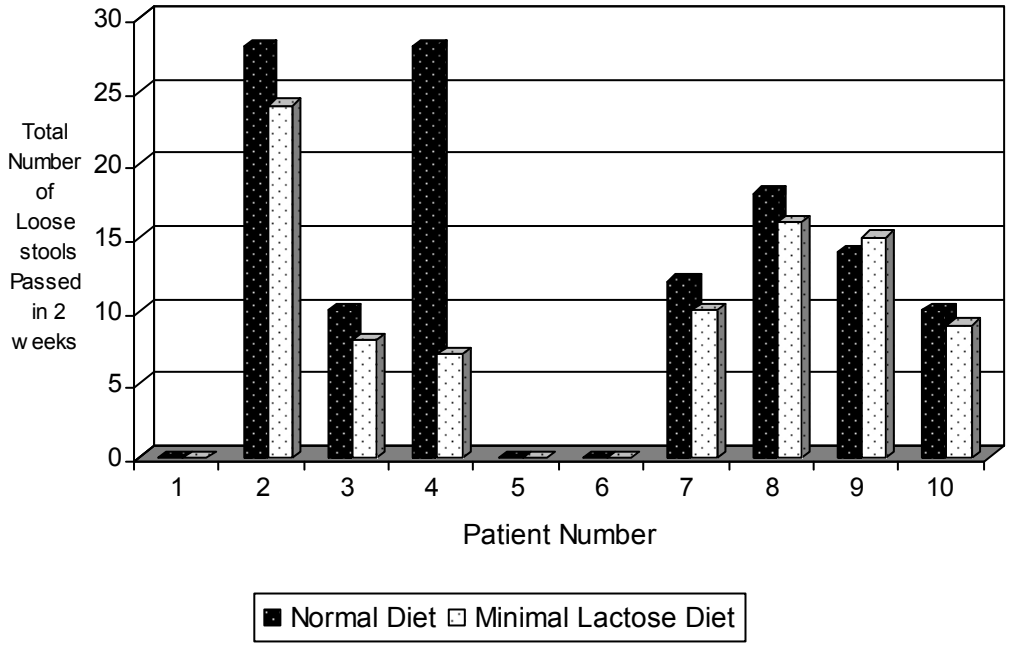
The ‘wellbeing’ score was compared during the normal diet with that recorded when on a lactose restricted diet – wellbeing is better if the score is lower. The sum total of daily wellbeing scores of each individual in the two week period on their usual diet averaged 9 [SD±7.2] with a range of 0-18 and this was 4.8±5.3, with a range of 0-14 on minimal lactose free diet. The difference between the 2 diets reached statistical significance p=0.017. There was an improvement in 7 cases [by 2-13 points], no change in 2 however, both had a baseline score of zero and worsening in one patient by a single point. These are illustrated in figure 5.1.

Figure 5.1: The sum total of wellbeing score of each participant during the two weeks on normal diet compared with period on minimal lactose diet. The perceived wellbeing is greater with lower scores



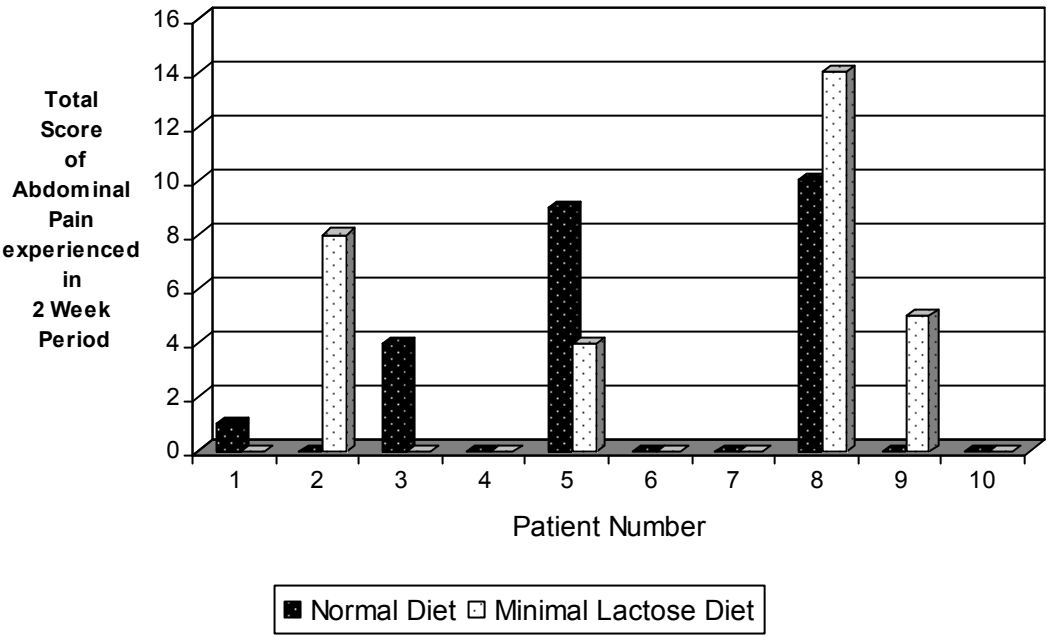
The total number of loose stools passed by each individual during the two week period ranged from 0-28 with an average of 11.8 [SD±10.5] during the normal diet which improved with a reduced lactose intake to 9.1 [SD±7.9] with a range of 0-24. There was an improvement in 5 patients [range 2-21 occasions]; no change in 3 [all had no episodes of loose stool at baseline assessment] and again worsening in two patients- these are shown in figure 5.2. These changes did not reach statistical significance.

Figure 5.2: The total number of loose stools passed by each participant during the two weeks on normal diet compared with period on minimal lactose diet.



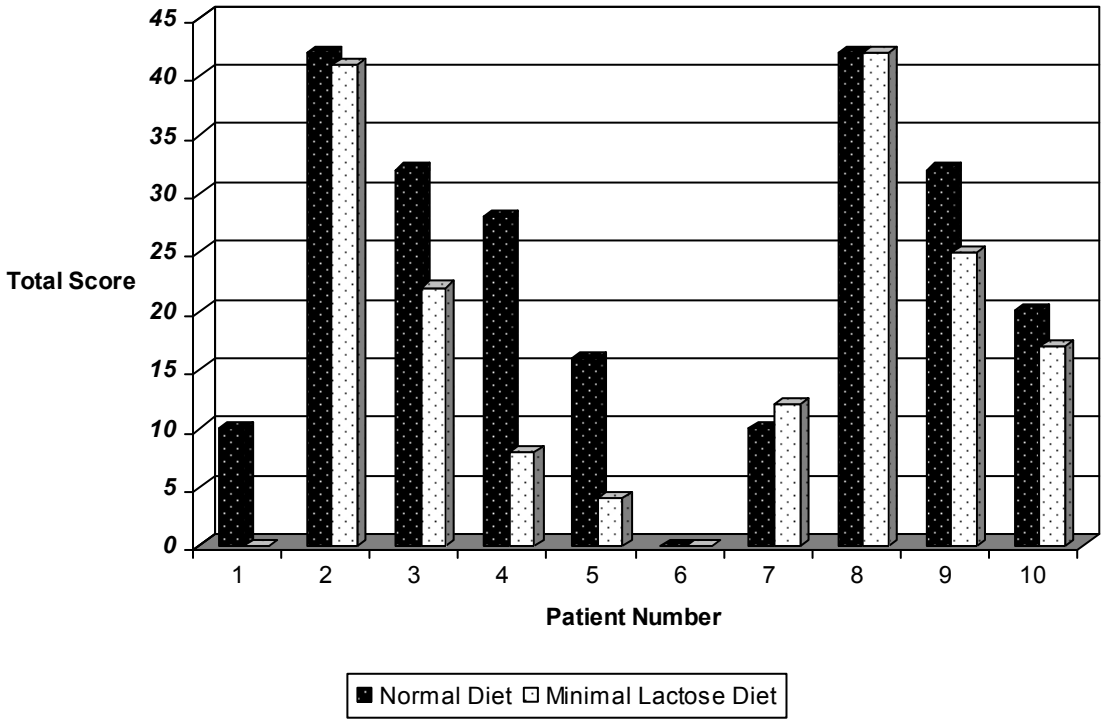
The number of days when abdominal pain [figure 5.3] was experienced was not significantly different between both diets. Higher scores indicate increased number of days with pain or increased severity of pain. The abdominal pain was slightly worse whilst on the minimal lactose free 3.3 [SD±5.3] when compared to normal diet 2.4 [SD±4].

Figure 5.3: The total score of abdominal pain experienced by the participants daily during the two weeks on normal diet compared with period on minimal lactose diet. Higher scores reflect increased number of days with pain with or without severity of pain.



The daily score of wellbeing, abdominal pain severity and number of loose stools passed each day were added up and the total score obtained during the 14 day period whilst eating a normal diet – this was compared to the total score obtained whilst on a minimal lactose diet. The average score on the normal diet was 23.2 [SD±14.3] with a range 0-42 and scores while on the minimal lactose diet was 17.2 [SD±15.6] with a range of 0-42. The score was therefore lower on a minimal lactose diet and this was statistically significant p=0.028. There was an improvement in 7 participants, no difference in 2 cases and worsening in just one patient. The mean improvement in scores was 5.5 [SD±7.5]. The changes in each individual are shown in the figure 5.4.

Figure 5.4: The sum total of daily wellbeing score, abdominal pain score and number of loose stools passed during the 14 day period of each participant on normal diet compared with period on minimal lactose diet. The lower the total score, the better the participant feels.



The SIBDQ scores whilst on the normal diet were 48.6 [SD±6.7] which improved on the minimal lactose free diet to 55.1 [SD±5.2] – higher scores reflect an improved HRQOL. The score improved in all participants which was therefore statistically significant [p=0.005]. The increase was by a minimum of 2 and a maximum of 10 points with a mean of 6.5 [SD ±2.5] points. The changes in each individual SIBDQ scores are shown in the figure 5.5. These results are also summarised in table 5.2. They suggest that there is an improvement in quality of life by switching to a minimal lactose diet in this small cohort.

Figure 5.5: The Short Inflammatory Bowel Disease Questionnaire [SIBDQ] score of each participant at the end of the two weeks on normal diet compared with period on minimal lactose diet. The higher the SIBDQ score, the better the HRQOL.

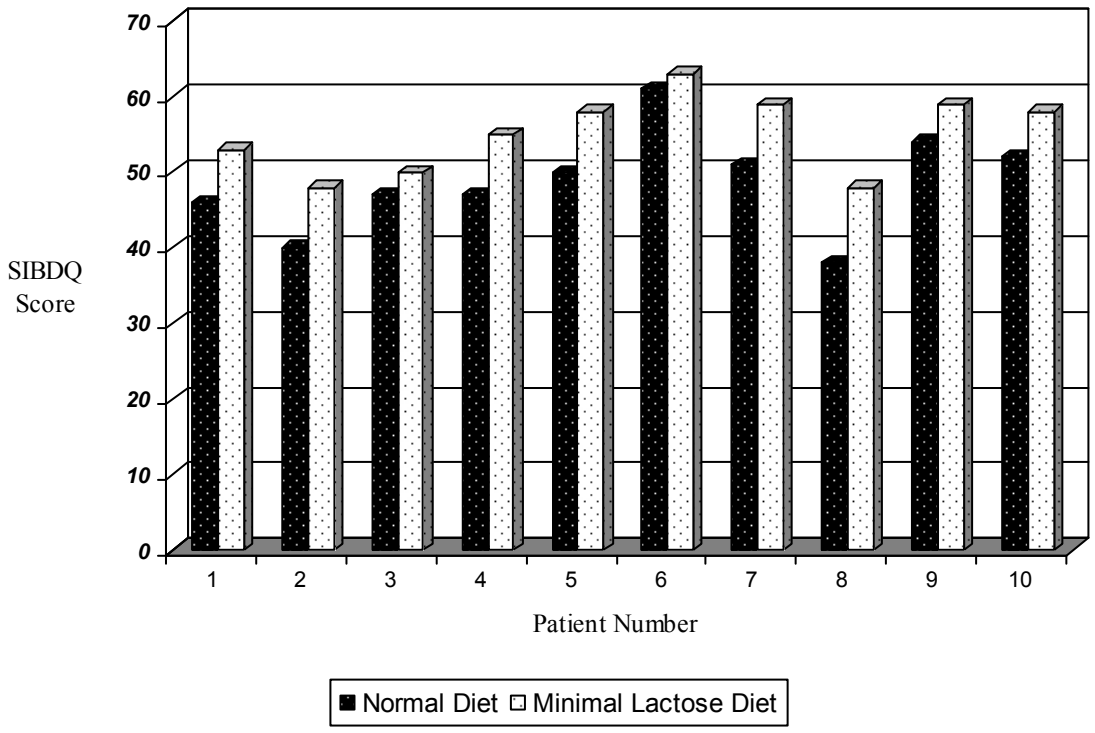


Table 5.2: The well being, abdominal pain, total no of loose stools passed and the SIBDQ scores whilst on normal diet and on minimal lactose free diet.

*Statistically significant difference, SD Standard deviation, *p* based on Wilcoxon Signed Rank Test.

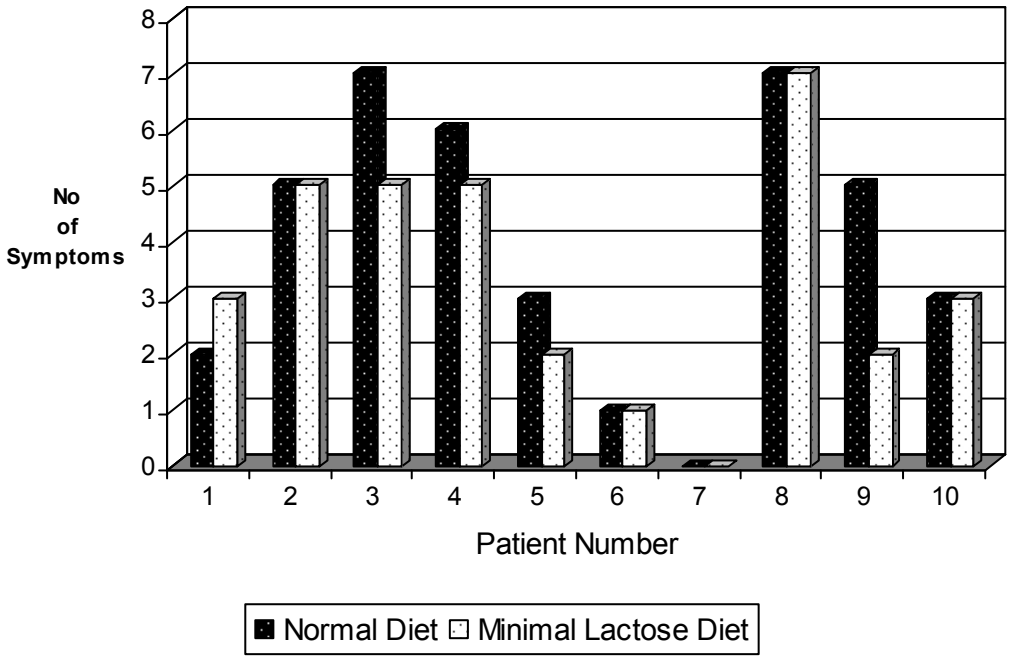
The average of the sum total of daily scores of abdominal pain and well being reported by the patients during the 2 week period on normal diet and whilst on minimal lactose free diet. The average of the sum total of the number of episodes of loose stools passed each day during the same period.

	Normal Diet		Minimal Lactose Free Diet		*p* value
	Average±SD	Range	Average±SD	Range	
Well Being*	9±7.2	0-18	4.8±5.3	0-14	0.017
Abdominal pain	2.4±4	0-10	3.3±5.3	0-16	0.4
No of loose Stool	11.8±10.5	0-28	9.1±7.9	0-24	0.147
SIDBQ score* [HRQOL]	48.6±6.7	38-61	55.1±5.2	48-63	0.005

The most common symptoms that were reported were flatulence, tiredness, bloatedness, nausea, muscle & joint aches, but also itching, dizziness, pins & needles and headache. The number of symptoms reported by each participant ranged from 1-7 symptoms whilst on normal diet compared to 1-4 symptoms on minimal lactose free diet [figure 5.6]. The average number of symptoms during normal diet was 3.3 ± 2.2 and on minimal lactose free diet was 2.5 ± 1.2. The number of symptoms experienced by each individual is lower whilst on minimal

lactose diet though the differences in symptoms experienced by the individuals was not significant [p=0.084].

Figure 5.6: Total number of symptoms experienced by each participant whilst on their normal diet to when they are on the minimal lactose diet.



The percentage of participants experiencing the symptoms is shown in figure 5.7. If the results are looked at more closely, the total number of days that the participants experienced these symptoms is lower during the period when they were on minimal lactose diet 136 versus. 96 days [figure 5.8].

Figure 5.6: Percentage of patients experiencing symptoms on a normal and a minimal lactose diet

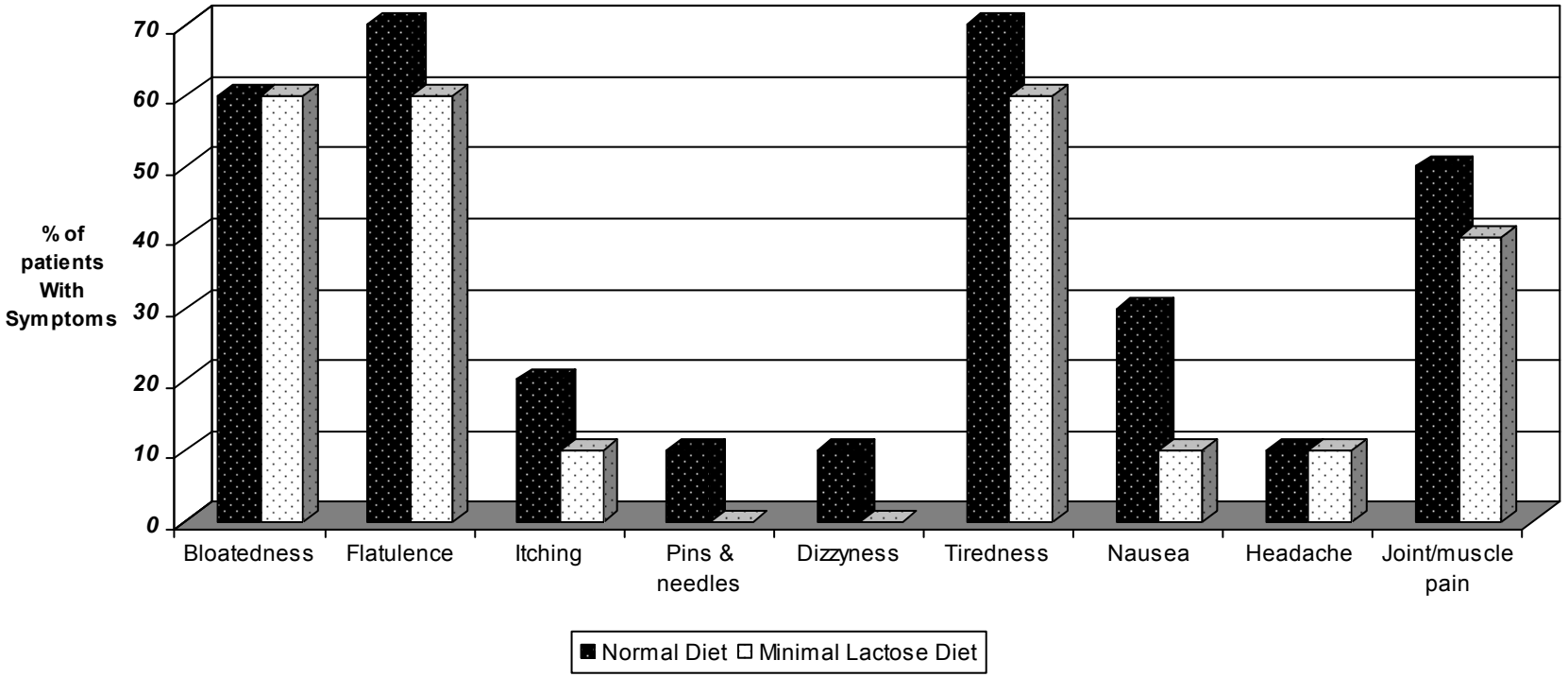
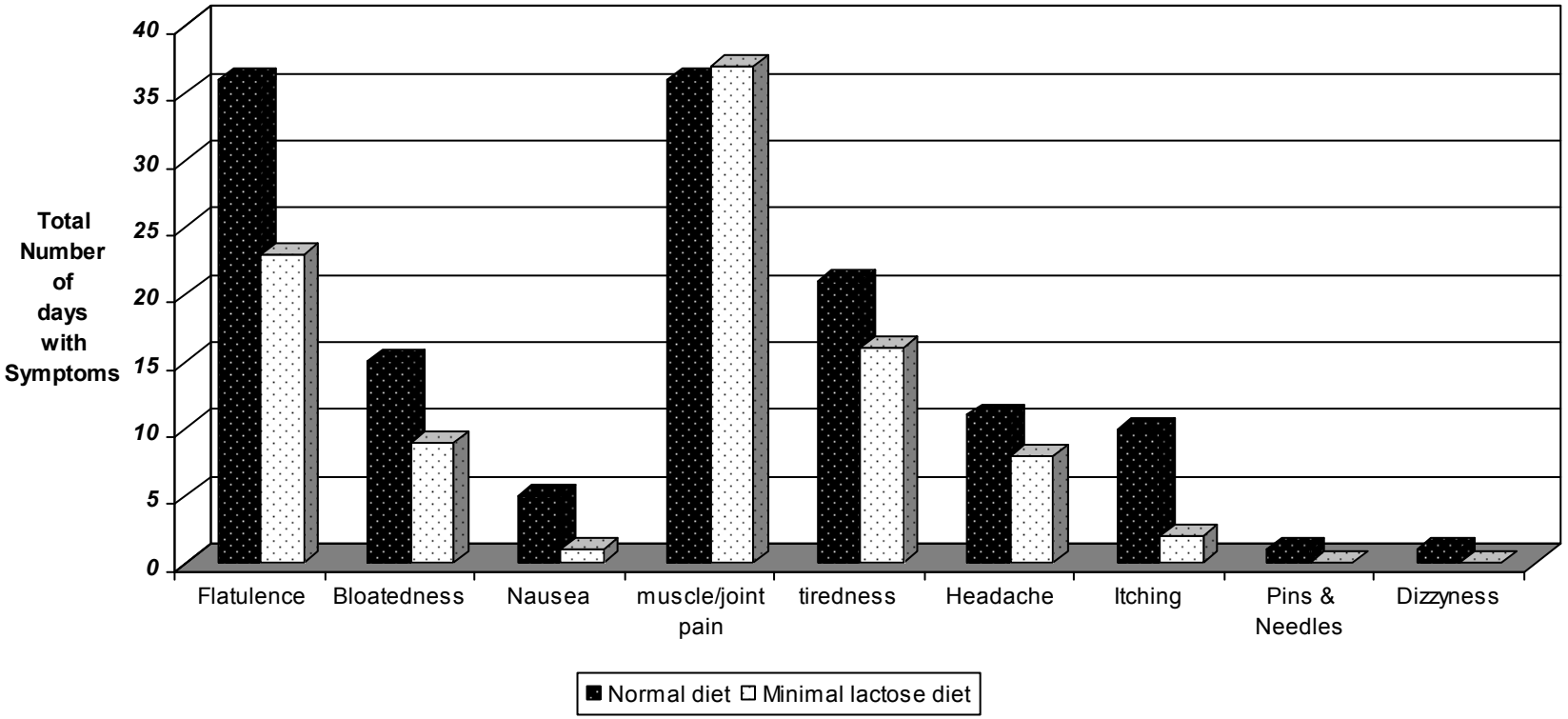


Figure 5.8: Total number of days symptoms experienced on their normal diet and whilst on a minimal lactose diet



5.5: DISCUSSION

The results in a very small number of patients with small bowel Crohn's disease in remission and known lactose sensitivity [all of whom had symptoms and a positive lactose breath test as indicated by a rise in breath H_2 and/or CH_4] shows that a minimal lactose free diet leads to an improvement in symptoms and health related quality of life scores. There was a significant improvement in general wellbeing, number of loose stools and quality of life scores but not abdominal pain in this pilot. Whilst these findings could be explained by an incidental temporal change in the activity of the disease itself which is characterised by waxing and waning of symptoms, this was not readily apparent. Adherence to the diet is very important for both the success of this pilot, and to support its efficacy. It is important to determine whether the dietary changes were specifically responsible for reduction in symptoms, rather than improvement being a reflection of a non-specific placebo response or the desire of patients to give the 'correct' answers to the questions.

The results of the ten participants who completed the study protocol were analysed [per protocol analysis] rather than an intention to treat analysis which is useful when subjects are entered into randomised studies. There are only a very small number of patients who completed the study and of course great caution should be taken before extrapolating the results into wider clinical practice. Whether a randomised control trial carried over a longer period of time would provide similar results is to be seen. It is also unknown if those with lactose sensitivity with active CD would benefit from this dietary change long term. Finally it is not known if a

minimal lactose free diet will produce similar results in patients with CD who is lactose sensitive compared with lactose tolerant individuals.

There are no published well constructed placebo controlled or randomised studies evaluating the effect of lactose free diet [nor a diet which is sucrose/fructose free] in patients with IBD. The specific carbohydrate diet [SCD] strictly limits complex carbohydrates [disaccharides and polysaccharides] and eliminates refined sugar in diet and there has been only a single published case report of two patients indicating symptomatic improvement following the SCD (328). In a randomised control trial (329), 20 patients with Crohn's disease were treated for an average of 18 months with a low carbohydrate diet [refined sugar excluded] compared to a high carbohydrate diet [refined sugar-rich]. There was an improvement in activity indices in patients who were on low carbohydrate diet. A recent pilot study suggests that reduction of FODMAP's [fermentable oligosaccharides, disaccharides, monosaccharides and polyols] in the diet offers an efficacious strategy for patients with IBD who have concurrent functional gut symptoms (330). The most common symptoms described by these patients were abdominal pain, diarrhoea, bloating and wind. These were also the symptoms that were most likely to respond to dietary intervention, suggesting that a reduction in dietary FODMAP's may be an effective therapeutic option in such patients.

According to the Food Standards Agency of UK [FSA] and the European food standards agency [EFSA] rules of 2005, pre-packed food sold should demonstrate on its label if it contains milk. The term "lactose-free" in EU legislation has only been defined for infant and follow-on formula as ≤ 10 mg/100 kcal and no such threshold

exists for adult population. The EU, requested the EFSA Panel on Dietetic Products, Nutrition and Allergies to deliver a scientific opinion on lactose thresholds in lactose intolerance. The panel recommended that a single threshold of lactose for all lactose intolerant subjects cannot be determined owing to the great variation in individual tolerances (331). The food labelling to indicate the absence or reduction of lactose or milk is not regulated. The practice regarding labelling terms and associated threshold levels varies across the EU for e.g. in UK no threshold levels are present, in Ireland "lactose free" means no lactose present whilst in Scandinavia it is less than 10mg of lactose per 100g of the final product In some countries the manufacturers have set their own unofficial thresholds for making such claims. In a research conducted by the FSA (332) they found that the words 'dairy', 'milk' and 'lactose' used in labelling is far from clear. The consumers, health professionals and food businesses understood differently the meaning of terms, 'dairy free', 'milk free' and 'lactose free'. As a result, any patient who is embarking on a lactose free / reduced lactose diet needs specific advice about how to avoid the inadvertent ingestion of lactose containing foods.

The limitations to my study primarily centred around the fact that this is a small pilot study with just 10 participants, but all were defined as lactose sensitivity and were in remission. The participants included different subtypes of CD i.e. 6 with small bowel disease and 4 with both large and small bowel disease. It is not known if the benefits seen here would be reproducible if this was analysed in a larger group with Crohn's disease or if they had different segments of bowel affected and / or active inflammation. The improvements seen were within a limited period of

observation and whether the improvement is sustained over a longer duration of time is not clear. Also, symptoms were self reported by the participants and this may contribute to some bias.

Both the food diary to record what was consumed by the participants and the diary used to record symptoms were designed for this study i.e. they have not been validated before. The symptom diary is a modification of the Harvey-Bradshaw index which includes its first 3 questions. The minimal lactose free diet leaflet which was used is the standard leaflet used in the local health board where my study was conducted. This is given to patients who are lactose sensitive and would like to modify their diet. This has been used for at least 5 years and was the same leaflet given to patients in a previous study carried out by Waud et al (136). On this basis it was felt to be appropriate to use for this study. Patients were instructed on the dietary principles and provided with minimal lactose free diet information leaflet. There was no formal input for the dietetic team whether their support and guidance would have had any influence on the results is not known. In addition due to factors discussed in the earlier sections [perception, labelling, terminology and thresholds] setting up a study to examine the effect of the presence or absence of lactose in diet is extremely difficult.

5.6: CONCLUSION

Decreased lactose intake appeared to benefit this small group of patients with CD in remission who are diagnosed with lactose sensitivity. It gives encouragement for further large scale trials where patients are initially assessed for lactose sensitivity

and appropriate patients are commenced on minimal lactose free diet with careful documentation of symptoms and quality of life scores. The benefit of decreased lactose intake in patients who are lactose sensitive and have active IBD should also be assessed. In addition, the contribution from the ingestion of lactose unwittingly taken from other sources such as that contained in drug preparations, should also investigated – see chapter 6.

CHAPTER 6

QUANTIFYING THE 'HIDDEN' LACTOSE IN DRUGS USED FOR THE TREATMENT OF GASTRO-INTESTINAL CONDITIONS

6.1: AIM

To quantify the amount of lactose used as an excipient in medications that may be taken for the treatment of inflammatory bowel disease and other gastro-intestinal disorders. In addition, to assess if alternative 'lactose-free' medications are available for patients with hypolactasia and co-existing GI conditions

6.2: INTRODUCTION

Excipients are defined as the constituents of the pharmaceutical form that is taken by or administered to the patient, other than the active substance (333). The excipients are said to have no effect on the action of active ingredients present in the medication and are inert (333). The purpose of their presence is to improve the appearance and palatability of the drug. In addition they may exert some effect on the bioavailability and stability of the product. In general they make up the bulk of the mass or volume of drug formulations. They are listed in the Medicines Compendium [MC] but not in the British National Formulary [BNF] and are not generally quantified. Lactose is one of the most widely used excipients by the pharmaceutical industry (334). There are many reasons for its popularity as lactose is perceived to be inert, relatively inexpensive, and non-toxic. It is also chemically stable and has no tendency to react with the active ingredient or other components of a formulation (334). Lactose is also freely soluble in water and it is very palatable providing sweetness without any aftertaste (333, 334). Lactose has a long history of being utilised within many formulations world-wide including dry powder inhalers, tablets, capsules and sachets. Lactose was found in 20% of medical prescriptions drugs and in 6% of over the

counter medications (335). Montalto et al looked in the Italian Physician's Desk Reference and found that there were at least 950 of the 2900 available oral drugs [33%] contained lactose as an excipient (336). Amongst these, capsule/tablet formulations contain, when specified, no more than 400 mg of lactose. Sometimes, labels of these medications warn that their administration is not recommended in people with lactase deficiency (336).

Many of the drugs used in the treatment of inflammatory bowel disease contain lactose as an excipient. Patients with IBD also have co-existent gastrointestinal conditions such as dyspepsia, irritable bowel syndrome [IBS] etc and may use drugs to control symptoms that contain lactose as an excipient. Of course, they may also take medications for non-GI ailments. In those patients with concurrent hypolactasia, the use of medications that contain lactose may be of clinical significance.

6.3: METHODS

Medications used for the treatment of GI disorders or symptoms were identified from the BNF [Vol. 53 March 2007]. The presence of lactose in each formulation was assessed by referring to the 2007 edition of the MC. A selection of these medications was obtained from the hospital pharmacy and analysed for lactose content using high performance liquid chromatography [HPLC]. HPLC was performed using a Kontron HPLC system. Carbohydrates were separated using a Thames Resek Pinnacle II™ Amino [NH_2] 5µ 150x4.6mm HPLC column [cat. 9217565] preceded by a

Phenomenex guard column [containing NH_2 [amino, aminopropyl] 4mmx3.0mm ID cartridge filter [cat. KJ 0-4282]. The column was pre-heated to 35°C using a Jones chromatography column heater. The carbohydrates were detected using a Shodex RA101 refractive index detector set at 32 or 64μRIU at 35°C. Mobile Phase was 75% acetonitrile 25% HPLC water [v/v] filtered through a 0.45μ nylon filter and degassed prior to use. The mobile phase flow rate was 1.0 mls/min. α-Lactose [L-3625], D [-] Glucose [G7021], Fructose [F2543], Sucrose [S-7903], Maltose [M-5885] was purchased from Sigma. HPLC grade Acetonitrile [A/0626/17] and HPLC water [W/0106/17] was purchased form Fisher Scientific Ltd. 0.08M Phosphate buffer was prepared at pH 7.3, pH 4.6 and pH 4.0 by dissolving 1.4g anhydrous dibasic sodium phosphate [Na_2HPO_4] and 9.68g monobasic sodium phosphate monohydrate [$NaH_2PO_4.H_2O$] in 750mls of HPLC water. The pH was checked and adjusted as necessary. The buffer solution was then made up to 1 litre with HPLC water.

6.3.1: SAMPLE PREPARATION

Pharmaceutical preparations in tablets, powders or capsule form were received in the laboratory within their original packing. They were crushed with a pestle and mortar and then transferred to a universal container where they were rehydrated or diluted in 5mls of phosphate buffer pH 7.3. This was then mixed on a flat bed rotor and incubated overnight at 4ºC or until dissolved. The sample mixture was then centrifuged for 10 minutes at 2500rpm before the supernatant was filtered through a 0.45μ filter. Two ml of the supernatant was mixed with 2ml of acetonitrile [v/v] and

mixed. The mixture was then left overnight at 4°C, before microfuging it at 13,000rpm for 5 minutes and filtering the supernatant through a 0.45µ filter. It was then passed through a previously primed LC-NH_2 SPE tube [As per manufacturers instructions, Cat.504483 Supelco] using positive pressure [flow approximately 1ml/12seconds]. Finally the filtrate was dispensed into HPLC auto sampler vials and analyzed for lactose content.

The concentration of lactose and other sugars including glucose, fructose, sucrose and maltose was determined by reference to the calibrator peak height. The peak data was integrated using Kroma 2000 chromatography software and quality control was performed with known carbohydrate solutions (337). In order to calculate the carbohydrate concentration the following formula was used. Sugars, µg/g = [Test Peak Height/Standard Sugar Peak Height] x buffer added to drug in µl/ [amount of sample injected x Sugar Concentration µg]/test portion weight in grams.

6.4: RESULTS

I selected drugs used in the treatment of IBD that were identified in the MC as having lactose present or absent. Drugs from each of the sub-sections listed in the Gastro-Intestinal chapter of the British National Formulary [BNF] were also selected and their lactose content analysed. As some medications are available from multiple manufacturers, selections of drug preparations were obtained for assessment. In addition to those used in GI disorders, I also analysed drugs that can be used for a variety of symptoms including abdominal ones e.g. analgesics and anti-depressants.

A total of 71 preparations were obtained and analysed, of these 31 [43.7%] preparations contained lactose whilst 40 [56.3%] were lactose free. The results of this HPLC based methodology correlated exactly with the summary of product characteristics in the MC i.e. it detected lactose in all formulations which were stated to contain it and did not measure lactose in those where it was not mentioned. To ensure that the methodology was robust and the results obtained were reproducible, three drugs were randomly selected and re-analysed on three different occasions. This showed consistency with the quantity of lactose detected per tablet for prednisolone [Pfizer] 31±1 mg, azathioprine [GlaxoSmithKline] 71±1 mg & colofac [Solvay] 95±2 mg. 17 drugs used in the treatment of IBD were tested - of those 8 contained lactose but was not identified in the other 9. In preparations where lactose was detected, it ranged from 28.9 mg [equivalent to 0.6mls of milk] in a single 2.5 mg tablet of methotrexate [Cyanamid] to 600mg of lactose [equivalent to 12.7mls of milk] in a 3mg capsule of budenofalk [Dr Falk]. The maximum amount of lactose that patients may consume per day from the ingestion of a single drug used at its maximum recommended dosage in the BNF for the treatment of IBD was 1200mg [equivalent to 26mls of milk] with asacol MR 4800 mg a day. In other preparations where lactose was detected, it ranged from just 4mg [equivalent to 0.2mls of milk] in a single 40mg capsule of losec to 125mg [equivalent to 2.7mls of milk] in immodium [Janssen-Cilag] 2mg. The maximum amount of lactose that patients may consume per day from the ingestion of GI drugs ranged from 4mg/day [0.2mls of milk] in losec 40mg to 10.2 grams/day [equivalent to 216mls of milk] when the maximum BNF quoted daily dosage, 150ml, of lactulose is ingested. The chromatogram produced by the

standard sugars is shown in figure 6.1, loperamide 2mg [Tillomed] with lactose is shown in figure 6.2, imodium instant melts [McNeil UK] which also has loperamide with no lactose in figure 6.3 and figure 6.4 shows codeine phosphate 30mg [Teva UK] which contains lactose.

Figure 6.1: Separation of standard sugars by high performance liquid chromatography, time at which the sugars appear during chromatography is shown on the X-axis and the amplitude is shown on Y-axis which depends on the quantity of the sugar present.

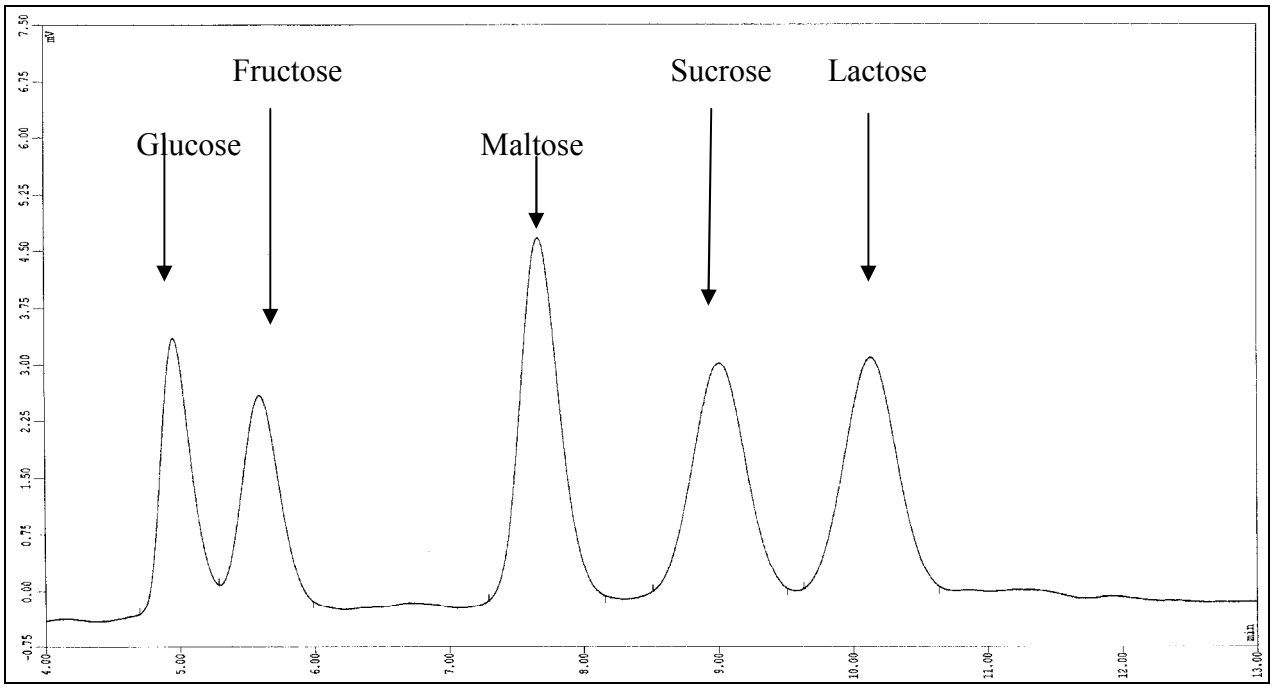


Figure 6.2: A chromatogram showing Loperamide [Tillomed] containing lactose, on the X-axis is time at which these sugars appear during chromatography and Y-axis is the amplitude which depends on the quantity of the sugar preset.

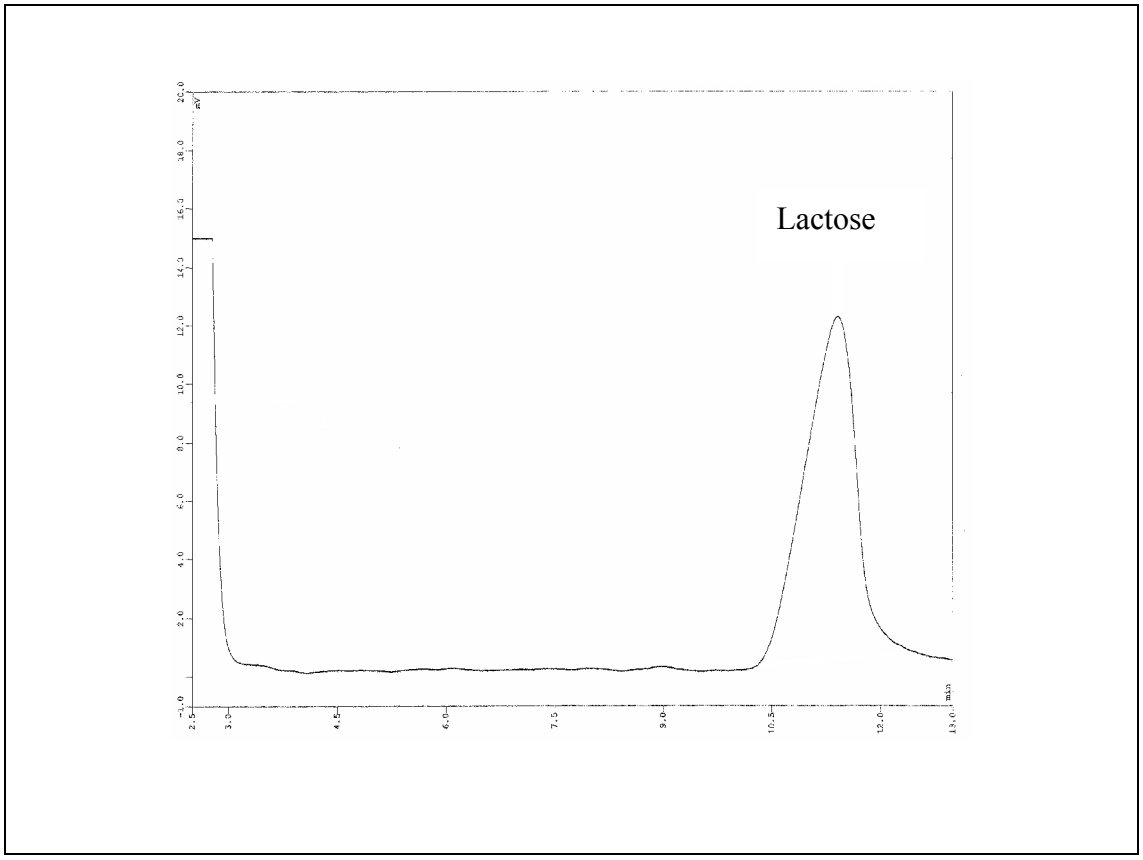


Figure 6.3: A chromatogram showing another formulation of Loperamide Imodium Melts [Mc Neil UK] without lactose, on the X-axis is time at which these sugars appear during chromatography and Y-axis is the amplitude which depends on the quantity of the sugar preset.

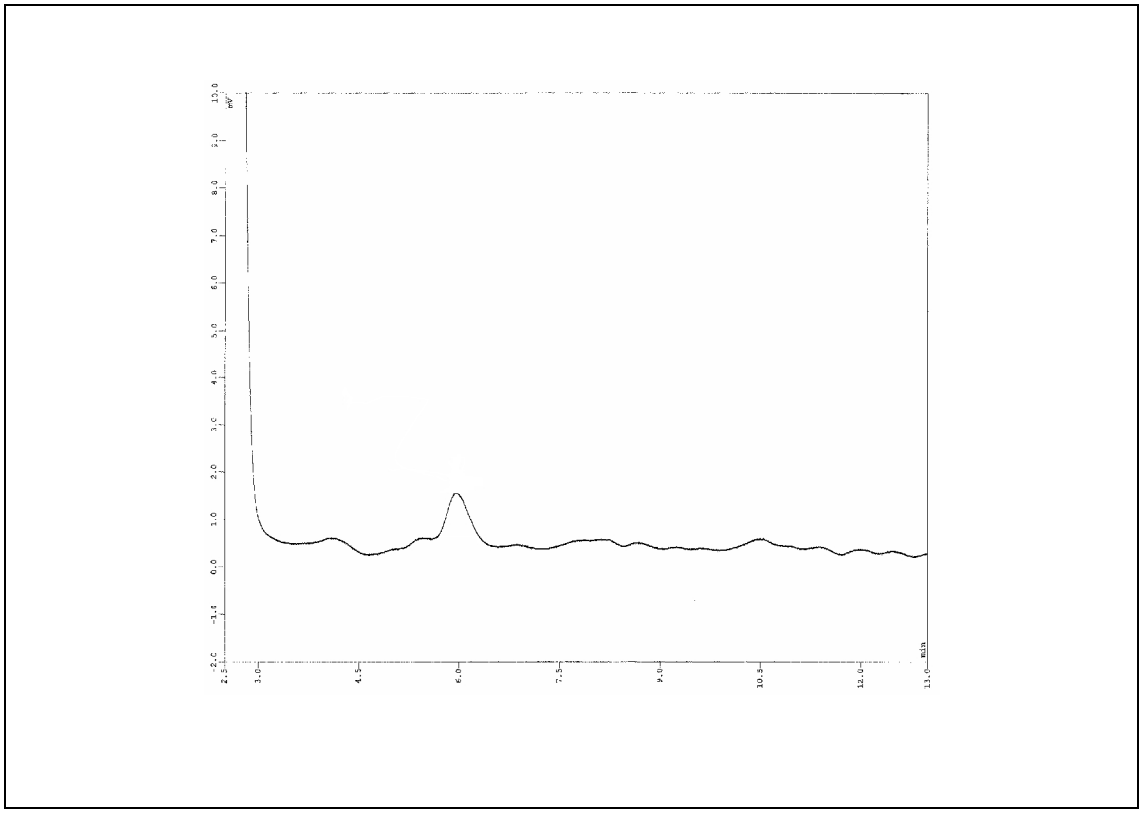
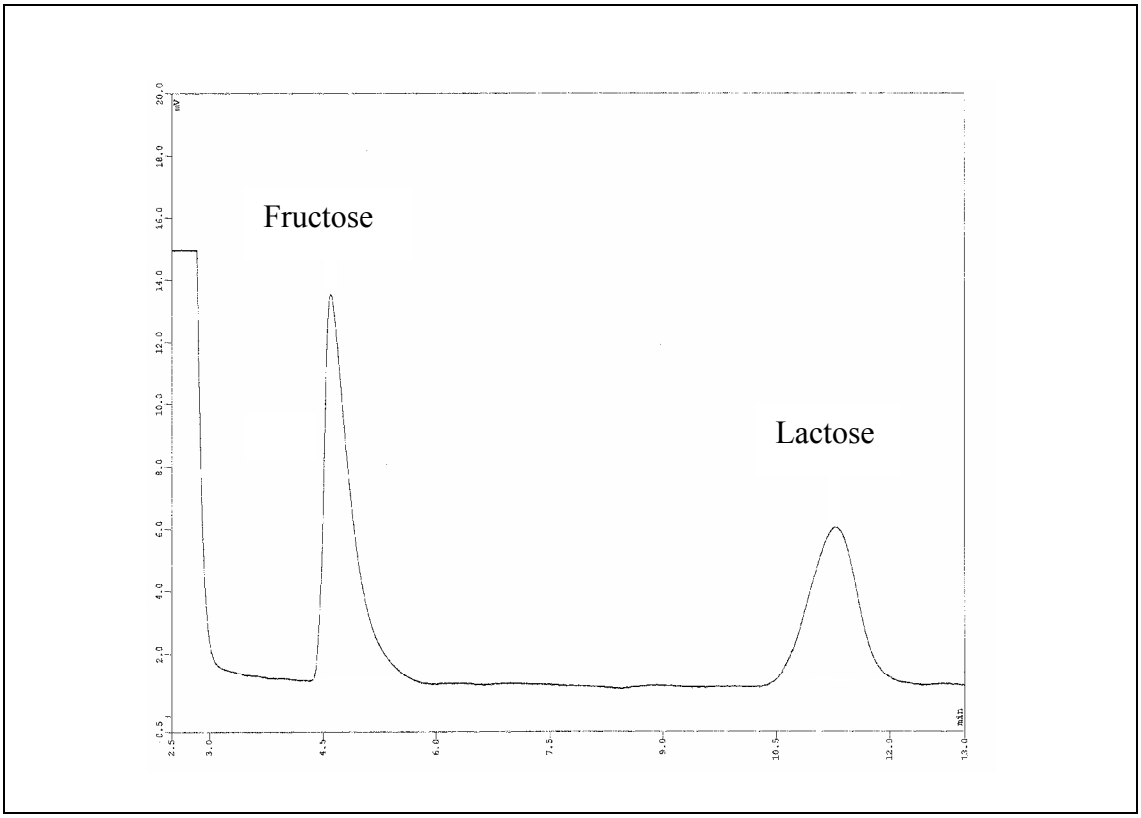


Figure 6.4: A chromatogram showing Codeine Phosphate [Teva UK] containing Lactose in addition has Fructose, on the X-axis is time at which these sugars appear during chromatography and Y-axis is the amplitude which depends on the quantity of the sugar preset



The results are presented in tables 6.1 to 6.6 using similar subgroups as the BNF i.e. based on the actions and indications of a drug. For those with lactose present, the results are summarised for each formulation in tables 6.1 to 6.4, giving potential daily intake of lactose if maximum recommended daily dosages is ingested. For each indication, lactose free alternatives were identified and these are listed in Tables 6.5

& 6.9. The results show that lactose is present in drugs used for all GI symptoms but lactose free alternatives are available for every indication. By taking the maximum recommended daily dosage, it is possible to be exposed to quite large amounts of lactose through the ingestion of drugs alone – this is in addition to what is ingested in the diet. The tables show that 1,000mg per day of lactose [equivalent to 21.2mls of milk] would be taken if 16mg of Imodium [Janssen-Cilag] were ingested for diarrhoeal or functional abdominal conditions ; 450mg [9.5mls milk] of lactose would be ingested if 80mg Domperidone [Winthrop] was taken as an anti-emetic ; 1,800mg [38.1ml of milk] if 9mg of Budenofalk [Dr Falk] was taken for active IBD or the treatment of microscopic colitis and by taking 150ml of Lactulose a patient would ingest 10,200mg of lactose [216mls milk] each day.

Table 6.1: Antispasmodics and drugs altering GI motility that contain lactose

Group Name	Generic name	Brand Name & Dosage	Manufacturer	Lactose per tablet [mg]	Maximum no of tablets per day	Lactose present if maximal daily dose ingested [mg]	Equivalent amount of milk[mls]
Antimuscarinics	**Dicycloverine Hydrochloride**	Merbentyl 10mg	Florizel	74	6	444	9.4
	Propantheline Bromide	Pro-Banthine 15 mg	Concord	38	8	304	6.4
Other Antispasmodics	**Mebeverine Hydrochoride**	Colofac 135 mg	Solvay	95	3	285	6.0
		Mebeverine Hydrochloride 135 mg	Merck Generics	99	3	297	6.3
Anti-motility Drugs	**Codeine Phosphate**	Codeine Phosphate 30mg	Teva UK	46	8	368	7.2
	Loperamide Hydrochloride	Imodium 2mg	Janssen-Cilag	125	8	1000	21.2
		Imodium 2mg	Tillomed	108	8	864	18.3

Table 6.2: Antacids & Ulcer healing Drugs that contain lactose

Group Name	Generic name	Brand Name & Dosage	Manufacturer	Lactose per tablet [mg]	Maximum no of tablets per day	Lactose present if maximal daily dose ingested [mg]	Equivalent amount of milk[mls]
Proton Pump Inhibitors	**Omeprazole**	Losec 40 mg	AstraZeneca	4	2	8	0.2
	Lansoprazole	Zoton Fastab 30 mg	Wyeth	28	2	56	1.2
Anti-emetics	**Domperidone**	Domperidone 10 mg	Winthorp	56	8	450	9.5
	Metoclopramide	Metoclopramide 10 mg	APS	71	3	213	4.5
	Prochlorperazine	Prochlorperazine 5mg	IVAX	70	6	420	8.9
Others	**Vitamins**	Valupak Multivitamins	BR Pharmaceuticals	38	2	76	1.6

Table 6.3: Drugs for IBD that contain lactose

Group Name	Generic name	Brand Name & Dosage	Manufacturer	Lactose per tablet [mg]	Maximum no of tablets per day	Lactose present if maximal daily dose ingested [mg]	Equivalent amount of milk[mls]
Aminosalicylates	**Mesalazine**	Asacol MR 400mg	Proctor & Gamble	75	6	450	9.5
		Mesren MR 400mg	Ivax	77	6	462	9.8
Corticosteroids	**Budesonide**	Budenofalk 3mg	Dr Falk	600	3	1800	38.1
	Prednisolone	Delta-cortil 5mg	Pfizer	31	12	372	7.9
		Prednisolone 2.5mg	Alpharm	56	24	1344	28.5
Immuno-suppressants	**Azathioprine**	Imuran 50mg	Glaxo SmithKline	71	3.5	248.5	5.3
	6 Mercapto-purine	Puri-Nethol 50mg	Glaxo SmithKline	61	2	122	2.6
	Methotrexate	Methotrexate 2.5mg	Cyanamid	28.9	6	173.4	3.7

Table 6.4: The other drugs that may be used in GI patients that contain lactose

Group Name	Generic name	Brand Name & Dosage	Manufacturer	Lactose per tablet [mg]	Maximum no of tablets per day	Lactose present if maximal daily dose ingested [mg]	Equivalent amount of milk[mls]
Laxatives	Bowel cleansing solutions	Picolax	Ferring	4	2 sachets	8	0.2
	Senna	Senokot 7.5 mg	Reckitt Benckiser	16	4	64	1.4
	Lactulose	Lactulose	Novartis	340mg/5ml	150ml	10200	216.1
	Methylcelluose	Celevac 500 mg	Shire	27.7	12	332	7.0
	Bisacodyl	Dulco-Lax 5 mg	Boehringer-Ingelheim	41	2	82	1.7
Ursodeoxycholic acid	Ursodeoxycholic acid	Destolit 150mg	Norgine	78	7	546	11.6
Others	Pancreatin	Pancrex V Tablets	Paines & Byrne	54	45	2430	51.5
	Tricyclic antidepressants	Amitryptiline 10mg	Teva UK	43	20	860	18.2
		Allegron 10mg	King Pharmaceuticals	38	15	570	12.1
	Selective serotonin reuptake inhibitors	Citalopram 20mg	Ranbaxy	45	3	135	2.9

Table 6.5: Antispasmodics and drugs altering GI motility that are lactose free.

Group Name	Generic name	Brand Name & Dosage	Manufacturer
Antispasmodics	Mebeverine hydrochloride	Colofac MR	Solvay
	Alverine Citrate	Spasmonal	Norgine
	Hyoscine Butylbromide	Buscopan	Boehringer Ingelheim
	Peppermint Oil	Colpermin	Pharmacia
		Mintec	Shire
Anti-motility Drugs	Loperamide	Imodium Instant Melts	McNeil UK
	Co-Phenotrope	Lomotil 2.5/0.025	Goldshield

Table 6.6: Antacids & Ulcer healing Drugs that are Lactose free.

Group Name	Generic name	Brand Name & Dosage	Manufacturer
H_2-**Receptor antagonists**	Ranitidine	Zantac	GlaxoSmithKline
	Cimetidine	Dexcel 200mg	Dexcel
	Nizatidine	Axid	Flynn
	Famotidine	Pepcid	MSD
Complexes	Sucralfate	Antepsin	Chugai
Prostaglandin analogues	Misoprostol	Cytotec	Pharmacia
PPI	Pantoprazole	Protium	Altana
	Rabeprazole	Pariet	Janssen-Cilag, Eisai
	Esomeprazole	Nexium	AstraZeneca
	Lansoprazole	Zoton	Wyeth
	Omeprazole	Losec MUPS	AstraZeneca
Anti-emetics	Prochlorperazine	Buccastem	Reckitt Benckiser

Table 6.7: Lactose free alternatives for the drugs used in the management of IBD

Group Name	Generic name	Brand Name & Dosage	Manufacturer
Aminosalicylates	Mesalazine	Pentasa 500mg tablets	Ferring
		Ipocol 400mg	Sandoz
		Salofalk 250mg tablets	Dr Falk
	Balsalazide	Colazide 750 mg	Shire
	Olsalazine	Dipentum 250/500mg	Celltech
	Sulfasalazine	Salazopyrin-EN 500mg	Pharmacia
	Budesonide	Entocort CR 3mg	AstraZeneca
Immunosuppressant's	Ciclosporin	Neoral 100mg	Novartis

Table 6.8: Lactose free drugs that may be used in the treatment of other GI disorders.

Group Name	Generic name	Brand Name & Dosage	Manufacturer
Laxatives	Docusate Sodium	Dioctyl 100mg	Schwarz
	Macrogols	Movicol	Norgine
Ursodeoxycholic acid	Ursodeoxycholic acid	Ursofalk 250mg	Dr Falk
		Ursogal	Galen
Bile acid sequestrants	Colestyramine	Questran	Bristol-Myers Squibb
Pancreatin	Pancreatin	Creon 10,000 & 25,000	Solvay
		Pancrex V 125mg capsules	Paines& Byrne

Table 6.9: Other lactose free drugs that may be used in IBD and patients with GI disorders.

Group Name	Generic name	Brand Name & Dosage	Manufacturer
Analgesics	Paracetamol	Paracetamol 500mg	Tesco
		Paracetamol 500mg caplets	Value Health
	Co-Codamol	Co-Codamol 8/500	Alpharm
		Co-Codamol 30/500	CP
	Co-Dydramol	Co-Dydramol	M&A Pharmaceuticals
Selective Serotonin Re-uptake Inhibitors	Fluoxetine	Fluoxetine 20mg	Tillomed
	Paroxetine	20mg	Sandoz

6.5: DISCUSSION

These results clearly show that lactose is present in medications prescribed for the treatment of IBD and also a wide range of GI disorders. This could lead to symptoms of lactose intolerance in susceptible individuals. Doctors may not know that the medicines they prescribe contain lactose as the details of the excipients in medicines are not available in the BNF [the most trusted guide used by doctors in the UK] (338). Although the European Commission guidelines of 2003 state that by law, manufacturers must list the excipient in the patient leaflets; they are however not required to quantify the amount present. A summary of the product characteristics of drugs are listed in the Medicines Compendium; this is used by pharmacists to find information about excipients but all the versions of the drug are not listed in MC or ECM [electronic version of the compendium of medicine]. It should also be noted that neither the MC nor the ECM are widely used by doctors and allied health professionals - and many maybe not even aware of their existence. I feel that the presence and quantity of lactose should be displayed in a prominent place to alert both the prescriber and the patient e.g. in the BNF or the drug packet.

Clinicians may not feel that the amount of lactose present in drug formulation is enough to contribute towards the symptoms of a patient. However sensitivity to lactose and the severity of symptoms it causes, vary widely in patients. Petrini et al. observed two lactase-deficient women with Graves' disease who experienced severe diarrhoea after ingestion of propylthiouracil [amount of lactose not specified] and methimazole [about 60 mg of lactose] (339). Brandstetter et al. described the case of a young woman with lactase deficiency who complained of gastrointestinal symptoms after inhalation of a capsule of

cromolyn sodium containing 20 mg of lactose (340). Lieb et al. described two patients with lactose malabsorption in whom abdominal cramps and diarrhoea developed after a medication with lithium carbonate and flurazepam hydrochloride [unspecified dosages of lactose] was started (341).Several other medications have also been implicated to cause lactose sensitivity in susceptible individuals (339-344).

Due to variations in tolerance to lactose by individuals, a single threshold for all lactose sensitive individuals cannot be determined. Despite this it has been widely reported that most individuals can tolerate up to 11.8g of lactose per day [250ml of milk] (192, 331 & 345). Studies have shown that symptoms can be precipitated by consumption of 3 to 5g of lactose (345-347) but in highly sensitive individuals it has been reported that the symptoms may occur after ingestion of as little as 200mg of lactose (347). Also, different types of medications have been shown to cause lactose sensitivity and this may affect compliance (130, 339-344). The reported threshold in very susceptible individuals could easily be crossed by taking a single 3mg tablet of budenofalk [Dr Falk] which contains 600mg of lactose. Patients often take more than one medication for their condition and so these may have a cumulative exposure. I have identified that the ingestion of certain medications for GI medications, either alone or in combination may result in a patient consuming of over 10 grams of lactose a day in addition to that taken in their normal diet. However, none of the medications used primarily for IBD alone or in combination would result in intake greater than 10g per day of lactose but it is worth re-iterating that they may take lactose containing drugs for other GI or co-existent illnesses e.g. hypertension.

The drugs used for treatment of IBD like 5-ASA preparations and budesonide are formulated to deliver the active ingredients topically to the distal small bowel and colon after oral ingestion. This is achieved in by a pH dependent release mechanism or through bacterial degradation. I have shown that some of these preparations contain lactose and as they remain intact in the upper GI tract they will also deliver undigested lactose to the colon where they could give the symptoms of LI. Of the nine 5-ASA drug preparations used in the treatment of IBD that were tested, two contained lactose and lactose was not identified in the other seven. The preparations where lactose was detected, were asacol MR [400mg] 75mg of lactose [equivalent to 1.6mls of milk] and mesren MR [400mg] 77mg of lactose [equivalent to 1.6mls of milk] in a single tablet The maximum amount of lactose that patients may consume per day from the ingestion of 5 ASA drugs used in the treatment of IBD was 1200mg [equivalent to 26mls of milk] with Asacol MR 4800 mg a day. The other 7 preparations without lactose pentasa 500mg, ipocol 400mg, salofalk 250mg, colazide 750mg, dipentum 250 & 500mg and salazopyrin-EN 500mg. Since the completion of this study the formulation of asacol MR 400 mg has been changed by the manufacturer and it has now become lactose free.

Improved lactose handling occurs when oro-caecal transit time is prolonged. Drugs which affect gastro-intestinal motility either directly or as a side effect could therefore have an influence on the digestion and absorption of lactose. In clinical practice it is very common to find lactose malabsorbers who believe that the ingestion of a very small amount e.g. in white coffee can produce gastrointestinal symptoms. Frequently encouraged by information on the internet etc or advised by their family physicians, they start a restrictive lactose-free diet.

Moreover, in some recent scientific publications, authors suggest caution with these medications or, even, advise to avoid them completely (342, 348 & 349). The onset of gastrointestinal symptoms after ingestion of a lactose containing drug in lactose maldigesters has been described but only in a limited number of case reports (339-341, 344), and there are no large population or randomised control studies. Drugs that impair mucosal function or modifies its structure may have an effect on lactase expression or on its activity. The broad spectrum antibiotic neomycin has been shown to reduce lactase activity (350). Fixed food eruptions have been caused by lactose identified after oral administration of four unrelated drugs (130). Lactose was shown to induce bronchospasm in asthmatics (351).

The primary form of therapy for lactose sensitivity after diagnosis is to adjust the amount of lactose containing foods in the diet accordingly. The results here show that patients on a restricted diet may inadvertently take medications that contain lactose in amounts that could cause symptoms. The details of excipients in medications are not readily accessible by doctors and other health professionals. They may also be unaware that lactose free alternatives are available. It should be noted that liquid preparations of most drugs are lactose free and provide another alternative form of treatment in those with known lactose sensitivity. The clinical management of LS may be confused by not taking into account “hidden” lactose in foods and medications. Symptoms may occur in individuals, which could be contributed by this “hidden” lactose, this is often overlooked by healthcare professionals (348). But it should be recognised that there is lack of evidence [clinical studies, trials or case reports] which

demonstrates lactose present in these medications at small doses either causes symptoms or exacerbation of IBD.

6.6: CONCLUSION

Assessment of lactose derived from medications should be considered in addition to dietary sources for both primary and secondary hypolactasic patients. In such patients the use of alternative 'lactose free' medications may avoid exacerbating symptoms in GI disorders such as IBD or IBS. I propose that lactose free medications should be prescribed to patients with a high risk of LS due to ethnicity, in those whose symptoms worsen with no objective evidence of disease deterioration, and in those who develop new symptoms after commencing medications. Ideally, in these groups of patients, tests to diagnose LS should be carried out to aid both dietary advice and the prescription of medications (136). The significance of the presence of lactose in the medications need to be further ascertained in a randomised control trial but in the interim, observational data should be collected from patients with lactose sensitivity and GI conditions to see if they have symptomatic benefit from using lactose free alternatives.

CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTION

The discussion which follows draws together the important findings presented in this dissertation. An attempt is made not to repeat the detailed discussions which accompany each chapter, but instead to identify salient points and assess their overall significance with some suggestions about future work.

The results demonstrate that sensitivity to lactose occurs in a high proportion [approximately 70%] of patients with IBD, both ulcerative colitis and Crohn's disease - this was demonstrated in cohorts who were in remission. The hypothesis that lactose sensitivity in IBD is related to lactase non-persistence status is not true, as in this study, only 7% of IBD patients had the CC genotype [which correlates very closely with primary lactase non-persistence]. 35% and 58% were CT or TT which are associated with lactase persistence. These results were similar to the control group. Although there was a high incidence of IBD in patients who were TT, the current results do not support any obvious increased risk of IBD in people who have the T allele, compared with the C allele, as has been previously reported. All patients with the CC genotype were lactose sensitive, this concurs with the findings in previous studies. No differences in genotype results were seen based on which segment of bowel was affected in either form of IBD.

A significantly greater prevalence of positive breath tests after a lactose challenge was seen in Crohn's disease, as well as ulcerative colitis when compared to healthy controls. The breath test was significantly positive in CD patients who had ileal disease when compared to those with ileo-colonic or pure colonic disease but no such effect was seen based on the distribution of the disease in ulcerative colitis. A significant number of patients with IBD were

shown to produce methane with or without a rise in hydrogen after lactose challenge – to my knowledge this is the first time that this has been reported.

The results also show that genetic polymorphism analysis alone is not sufficient to identify all patients who are lactose sensitive. The reason for the higher incidence of lactose sensitivity in IBD patients reported here is because of the comprehensive tests used. The quality of the study is further improved by avoiding patients who have previously been assessed or primarily referred for tests to diagnose lactose sensitivity. Small bowel disease would be expected to lead to carbohydrate malabsorption, but my study shows that large bowel disorders can also lead to malabsorption of lactose, the mechanism of this is unclear. It should also be remembered that all the study population were in remission and that it is therefore possible that increased disease activity may have an additional effect.

Patients with a positive breath test had a significantly greater number of symptoms and higher intensity scores. This supports the need for recording symptoms as a part of the protocol for breath testing after lactose challenge. Several patients had persistent or new symptoms after the 4 hour monitoring period. Patients who suffered from diarrhoea after the lactose challenge often started experiencing this several hours, even a day, after the lactose would have disappeared from the intestine. In fact, 50% of the patients with CD, and over 75% of those with UC, who had the diarrhoea after the lactose challenge, still had this 48 h later. This supports the hypothesis, that the mechanism causing diarrhoea involves cell signaling, analogous to the diarrhoea in severe gut infections, and is not simply an osmotic effect of the lactose.

A pilot study was undertaken in a small cohort of patients with Crohn's disease who were in remission and known lactose sensitivity. It showed that a minimal lactose diet leads to an improvement in both symptoms and health related quality of life scores. The improvements seen were within a limited period of observation and it is now important to determine whether this is sustained over a longer duration of time. The benefits of excluding lactose in patients with active IBD also merits a carefully constructed clinical trial where patients again are formally tested by genotyping, breath testing with symptom analysis following a lactose challenge before they are included.

The results here have shown that lactose is present in numerous medications prescribed for the treatment of IBD and a wide range of co-existent GI disorders. The amount of lactose that is present in medications could lead to lactose sensitivity in susceptible individuals either on its own or in combination with food. Doctors may not know that the medicines they prescribe contain lactose as the details of the excipients in medicines are not easily available. They may also be unaware that lactose free alternatives are available. The presence and quantity of lactose should be displayed in a prominent place to alert both the prescriber e.g. in the BNF and the patients e.g. within the information leaflet contained in the drug packet. An informed decision can then be made particularly when there is known sensitivity to lactose.

The Real-Time PCR and Restrictive fragment length polymorphism method yielded exactly the same results in the evaluation of the lactase genotype. The former technique is rapid and robust as well as being considerably less labour intensive technique and is suitable for the screening of large numbers of samples. It is also easy to do and when compared with the RFLP, has less

number of steps and therefore a lower chance of errors. Since my work, this has become the laboratory standard technique for this genotyping.

The breath samples after lactose hydrogen breath test was assessed by two different breath analysers. The results here show that the hand held *Micro* H_2 analyser would have diagnosed 95% of those who produce significant quantities of hydrogen after a lactose challenge. It would however, only diagnose 53.9% of those who malabsorb lactose as it cannot detect methane, unlike the *Quintron MicroLyzer*. Since the diagnosis of lactose malabsorption should require the measurement of hydrogen and methane, this device cannot be recommended to be used on its own. A handheld device should now be developed to measure expired methane similar to hydrogen. The advantages associated with such a device will allow us to the freedom to perform near patient testing.

The laboratory standard to diagnose lactose sensitivity in any patient should be based on comprehensive tests which include genetic testing, measurement of breath hydrogen and methane for 4 hours, and a full record of symptoms for up to 48 hours. In those who have the CC genotype, advice should initially be given to remove all lactose from their diet, including that hidden in foods and drugs. Those who are CT or TT should undergo a lactose challenge test, where both breath hydrogen and methane are measured, together with a record of gut and systemic symptoms. Those diagnosed lactose sensitive should go on a lactose free diet and then be reassessed.

What are the future research needs for understanding and managing lactose sensitivity and its role in inflammatory bowel disease? Future prevalence studies should be derived from population-based samples that include adequate distributions across ages and ethnic variation in order to assess the effects of these factors. From my current work the question arises as to what active disease will do to this sensitivity to lactose – this should ideally be assessed by comparing paired observations from individual patients when in different phases of illness i.e. remission and relapse. Large scale trials are also needed where patients with proven lactose sensitivity and inflammatory bowel disease, whether active or in remission, are recruited to assess the benefits of eliminating dietary lactose.

It is also important to perform further work to ascertain the true significance of the abdominal and systemic symptoms generated after a lactose challenge. In particular it should be determined whether these symptoms, which form part of the definition of lactose sensitivity, are due to subjective or placebo effects. To do this, blinded challenges with and without lactose are needed.

All the current methods for diagnosing problems with lactose digestion have limitations. The optimal test would be able to assess and quantify the true functional lactase capacity in an individual in a non-invasive manner this may be possible with new innovations such as positron emitting tomography.

Apart from further work to determine the significance of lactose sensitivity in IBD, there is another novel finding from my work that definitely merits further research. The discovery of high methane in the breath samples of patients with IBD before and after the ingestion of lactose needs to be further

explored. As this gas is only produced by *Archae*, the possibility that they may play a pathogenetic role in IBD now needs to be investigated.

The further investigation of the interplay between inflammatory bowel disease, lactose sensitivity and the so called 'bacterial metabolic toxin' hypothesis, may yet give very valuable insights into the pathogenesis of this condition and open up new treatment options.

CHAPTER 8

APPENDICES

APPENDIX 1

DIET PILOT FOOD DIARY

Name: Trial number:

Dates:

First week Second week:

Third week Fourth Week:

Instructions: Please read carefully.

Please write everything down: Keep your form with you all day, and write down everything you eat or drink. A piece of candy, a can of pop, cup of tea, or a small doughnut may not seem like much but these all add up!. Don't depend on your memory at the end of the day. Record what you're eating as you go.

Be specific: Make sure you include "extras," such as gravy on your meat or cheese on your vegetables. Do not generalize. For example, record chips as chips, not as potatoes. Estimate amounts: If you had a piece of cake, estimate the size [2" x 1" x 2"] or the weight [3 ounces]. If you had a vegetable, record how much you ate [1/4 cup]. When eating meat, remember that a 3-ounce cooked portion is about the size of a deck of cards.

	Breakfast	Morning Snacks	Lunch	Afternoon Snacks	Dinner	Evening Snacks	Milk consumed 1 cup= 200mls
Monday							
Tuesday							
Wednesday							
Thursday							
Friday							
Saturday							
Sunday							

APPENDIX 2

DIET PILOT SYMPTOM DIARY

	Well Being 0= very well, 1=slightly below par, 2=poor, 3= very poor, 4= terrible	Abdominal Pain 0= none, 1=mild, 2=moderate, 3= severe	No of Liquid stools	Others Symptoms Examples: Rashes, Headache, Fatigue, Muscle and joint pain, Palpitations, Itching, Flatulence, Bloatedness, Nausea etc
Monday				
Tuesday				
Wednesday				
Thursday				
Friday				
Saturday				
Sunday				

APPENDIX 3

Short IBD Questionnaire** [SIBDQ]

This questionnaire is designed to find out how you have been feeling during the last 2 weeks. You will be asked about symptoms you have been having as a result of your IBD, the way you have been feeling in general, and how your mood has been.

1. How often has the feeling of fatigue or of being tired or worn out been a problem for you during the last 2 weeks? Please choose an option from [Systemic]
    a. All of the time
    b. Most of the time
    c. A good bit of time
    d. Some of the time
    e. A little of the time
    f. Hardly any of the time
    g. None of the time

2. How often during the last 2 weeks have you had to delay or cancel social engagement because of your bowel problem? Please choose an option from (Social]
    a. All of the time
    b. Most of the time
    c. A good bit of time
    d. Some of the time
    e. A little of the time
    f. Hardly any of the time
    g. None of the time

3. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would have liked to have done in the last 2 weeks? Please choose an option from [Social]
    a. A great deal of difficulty, activities made impossible
    b. A lot of difficulty
    c. A fair bit of difficulty
    d. Some difficulty
    e. A little difficulty
    f. Hardly any difficulty

g. No difficulty; the bowel problem did not limit sports or leisure activities

4. How often during the last 2 weeks have you been troubled by pain in the abdomen? Please choose an option from (Bowel]
    a. All of the time
    b. Most of the time
    c. A good bit of time
    d. Some of the time
    e. A little of the time
    f. Hardly any of the time
    g. None of the time

5. How often during the past 2 weeks have you felt depressed or discouraged? Please choose an option from [Emotional]
    a. All of the time
    b. Most of the time
    c. A good bit of time
    d. Some of the time
    e. A little of the time
    f. Hardly any of the time
    g. None of the time

6. Overall, in the last 2 weeks, how much of a problem have you had passing large amounts of gas? Please choose an option from [Bowel]
    a. A major problem
    b. A big problem
    c. A significant problem
    d. Some trouble
    e. A little trouble
    f. Hardly any trouble
    g. No trouble

7. Overall, in the last 2 weeks, how much of a problem have you had maintaining or getting to the weight you would like to be? Please choose an option from [Systemic]
    a. A major problem
    b. A big problem
    c. A significant problem
    d. Some trouble
    e. A little trouble
    f. Hardly any trouble
    g. No trouble

8. How often during the last 2 weeks have felt relaxed and free of tension? Please choose an option from [Emotional]
    a. All of the time
    b. Most of the time
    c. A good bit of time
    d. Some of the time
    e. A little of the time
    f. Hardly any of the time
    g. None of the time

9. How often during the last 2 weeks have you been troubled by a feeling of having to go to the bathroom even though your bowels were empty? Please choose an option from [Bowel]
    a. All of the time
    b. Most of the time
    c. A good bit of time
    d. Some of the time
    e. A little of the time
    f. Hardly any of the time
    g. None of the time

10. How much of the time during the last 2 weeks have you felt angry as a result of your bowel problem? Please choose an option from [Emotional]
    a. All of the time
    b. Most of the time
    c. A good bit of time
    d. Some of the time
    e. A little of the time
    f. Hardly any of the time
    g. None of the time

APPENDIX 4

MINIMAL LACTOSE DIET SHEET

WHAT IS LACTOSE?

Lactose is a sugar found naturally in the milk of mammals. Lactose is also found in foods prepared with mammal's milk. Lactase is an enzyme that plays a vital role in breaking down lactose in order that your body can absorb it more effectively.

HOW DO I CONTROL MY LACTOSE INTAKE?

Lactose intolerance is very individual and the amount tolerated by one person may cause symptoms for another. Similarly, the type of lactose containing products that individuals are able to tolerate varies. Dietary control of individual symptoms largely depends on each person knowing, through trial and error, how much lactose and in what form, their body can handle lactose-containing foods. Sensitivity to lactose also changes over time and with changes in your general health status. Finding out how much lactose you are able to tolerate can help you to live with the condition and provides a greater variety in your diet.

RESTRICTING YOUR LACTOSE INTAKE

Initially you may wish to restrict your lactose strictly in order that you alleviate uncomfortable symptoms. After a trial period under the supervision of your consultant you may be able to gradually be reintroduced.

After this time with guidance you may be able to gradually increase the amount of lactose in your daily diet and trial different lactose containing foods. If your original symptoms reappear you know that you have exceeded your tolerance level and therefore you must stick to a level of intake slightly below that which causes symptoms.

The minimal lactose diet for lactose intolerance and excludes foods most likely to cause symptoms. This diet must be followed strictly for 3-4 weeks. If your symptoms do not improve a review with the consultant is needed. Your symptoms may not be caused by lactose intolerance and other possibilities will need to be explored.

Following this further advice, if the low lactose diet is unsuccessful at relieving symptoms, after the trial period it is essential that the dietary restrictions do not continue unnecessarily. Unnecessary restrictions can lead to nutrition shortcomings or even fail to detect other illness.

If your symptoms have improved the next step is to establish your tolerance levels. Challenge your tolerance regularly to see if your tolerance has improved. When testing your tolerance to your usual cow's milk, drink less than one cupful along with a meal or snack. If you experience symptoms, wait until these resolve and then trial an even smaller amount.

Knowing your tolerance level should mean that you could include some diary products in your diet. Remember these are important sources of calcium and protein and therefore should not be limited unless it is essential to do so. After you have established the range of foods and tolerance levels you can handle, please contact your consultant to ensure that your new diet is nutritionally adequate.

WHAT TO LOOK FOR ON FOOD LABELLING

The following ingredients, if listed, on a product label indicate that the food contains lactose and therefore should be avoided. Ingredients include: -

Milk [cows, goats, sheep]	Artificial Cream	Buttermilk
Milk Solids	Cheese	Butterfat
Non-milk Solids	Yoghurt Animal Fat	Lactose
Skimmed Milk Powder	Margarine	Milk Powder
Cream	Butter	Curd & Whey

Remember to look for lactose containing foods as well e.g. butter, cream, and cheese. It is important to realise that differing brands vary considerable with the ingredients that are used in their products, therefore also read the food labels carefully. If you are in any doubt about a product - avoid it and then contact your consultant to see if it is suitable. Certain ingredients are not a source of lactose despite their name e.g. lacto albumin, lactate, lactic acid, casein, non-diary creamer, milk protein.

Your consultant can support you in making appropriate food choices, identifying your tolerance levels, planning meals and in ensuring that your diet is well balanced and nutritionally adequate. Vitamin and mineral supplements may be necessary - if you are concerned in any way contact your consultant.

WHAT ABOUT MEDICATIONS?

Lactose can be used in both prescription and over the counter medication. Ask your consultant or local pharmacist if you are concerned about your medication. Do not stop taking your medication without first consulting your doctor.

VARIETY

Reducing the lactose content of your diet causes some limitations in your dietary variety. It is important to try and consume a varied diet using the foods that are suitable for use. This will help to ensure that you are getting adequate amounts of vitamins and minerals.

MILK ALTERNATIVES

Soya and rice milk is lactose free. Lactolite and Lactard are milk alternatives, which have very low lactose content. With all of these products it is important to try and use a calcium-fortified version. Many lactose-containing foods are essential sources of calcium within the diet. It is important to include alternative sources of calcium. Yoghurts, which contain active bacterial cultures for the yoghurt making, contain lactase enzymes, which support the lactose digestion. Therefore, yoghurt may be tolerated and provides a valuable source of calcium.

MINIMAL LACTOSE DIET

	FOODS ALLOWED	FOODS EXCLUDED
Breads & Cereals	Bread / cakes; biscuits. Breakfast cereals [check ingredients]. Rice; flour, semolina; sago	Milk breads, buns and cakes – check ingredients. Prepared mixes e.g. muffins, pancakes
Milk & Dairy Products	Soya and rice milk. Reduced lactose milks e.g. "Lactolite" or "Lactcid". Hard cheeses. Live yoghurt.	Milk – fresh, dried, evaporated, condensed, skimmed, sterilised. Cottage cheese, cheese spreads. Other creams. Fromage Frais.
Fats & Oils	Butter [up to 30 g / day]. Tomor, Flora, Outline & other margarine free from ingredients * Salad oil, lard, cooking oil., olive oil	Excess butter or margarine. Margarine [check ingredients]
Desserts	Soya desserts / soy ice-cream [check label]. Homemade pies and desserts. Some commercial cakes & biscuits. Packet jellies.	Puddings and sauces made with milk. Ice-cream, commercial cakes and biscuits containing milk.
Meat, Fish, Poultry, Eggs	Plain beef, chicken, fish, lamb, pork etc. Some sausages. Eggs.	Creamed or crumbed meats or fish. Some sausages, meat pies. "Ready" cooked meats.
Fruit	All fruits – fresh, tinned or frozen	None.

Vegetables	Potato and all vegetables cooked without milk or butter.	Instant potato, white sauce for vegetables unless made with soy milk.
Soups	Clear soups, bouillon, vegetable soup, home-made soups using known ingredients. Some packet soups.	Cream soups unless made with soy milk. Packet soups with lactose. Some stock powders and stock cubes.
Drinks	Tea, coffee, cocoa, Fruit squashes, soft drinks.	Horlicks, Ovaltine, Bournvita, Milo. Non-dairy creamers containing lactose, drinking chocolate.
Miscellaneous	Sucrose, white sugar, brown sugar, icing sugar, fructose, glucose. Vegemite, Marmite Peanut butter, jams,marmalade. Salt, pepper, spices, seasonings. Boiled sweets, fruit gums etc Proper mayonnaise. Lactose free chocolate	Artificial sweeteners with lactose. Fudge, caramels, toffees, chocolate. Salad dressings. Most crunchy snack products e.g. Wotsits, Cheese Puffs etc.

SUGGESTED DAILY MEAL PLAN

Breakfast

Cereal and fruit juice or soya milk or reduced lactose milk and sugar.

Egg and bacon. Bread and butter and marmalade.

Black tea or coffee and sugar [herbal or lemon tea].

Mid Morning

Black tea or coffee and sugar. Biscuits – check ingredients.

Lunch

Meat, fish, egg, cheese. Salad or vegetables. Bread and butter or potatoes.

Fruit or soya dessert.

Mid Afternoon

Black tea or coffee and sugar. Cake – check ingredients.

Evening meal

Meat, fish, egg, cheese. Salad or vegetables. Bread and butter or potatoes.

Soya milk pudding, milk free sponge or pastry.

Bedtime

Black tea or coffee and sugar.

APPENDIX 5

COMPARISON OF A PORTABLE BREATH HYDROGEN ANALYZER [MICRO H_2] WITH THE *QUINTRON MICROLYZER* IN MEASURING BREAKDOWN PRODUCTS OF LACTOSE METABOLISM.

5.1: AIM

The aim of this study was to compare the accuracy of a portable breath hydrogen analyser [*Micro H_2*] with that of a widely used stable laboratory model [*Quintron MicroLyzer Self-Correcting Model SC*] in measuring the breakdown products of lactose metabolism. This would simplify the detection of lactose sensitivity in clinical practice [as described in the preceding chapters].

5.2: INTRODUCTION

The most commonly used methods to diagnose hypolactasia rely on the indirect measurement of breakdown products following an oral dose of lactose. The breath hydrogen test is based on the fact that bacteria in the colon ferment undigested lactose, causing the release of hydrogen, which is then absorbed through the intestine wall into the blood circulation. In some people, the hydrogen is converted to methane by methanogenic bacteria in the colon. The hydrogen and methane generated is absorbed by the cells in the colonic mucosa and then transported by the blood to the lung and then exhaled. An increased concentration of at least 20 parts per million (352) in exhaled hydrogen or an increase of 5 ppm of exhaled methane following lactose ingestion, as compared with the pre-test value is generally accepted as the threshold to reveal lactose maldigestion (126). The combined measurements of the exhaled concentrations of hydrogen and methane provide more reliable indicators (304) of the fermentation processes in the large intestine than results based on measurements of breath hydrogen alone.

It is desirable to find an easy, cheap and reliable method of diagnosing lactose maldigestion. A rapid breath hydrogen analyzer [Sensistor AB, Linkoping,

Sweden] to detect lactose malabsorption was described first by Berg in 1985 who used the hydrogen in expired air to give a voltage change that can be transformed into a 'ppm' value from a calibration curve (353). Rumessen used an exhaled H_2 monitor [Gas Measurement Instruments Ltd, Renfrew, Scotland] which detects the gas using an electrochemical cell comprising of working, reference and counter electrodes. The potential of the working electrode is held constant against the reference electrode. H_2 is oxidized at the working electrode generating a current which is proportional to the amount of H_2 diffusing; the signal is converted to give a digital display (354).

Since then, different devices have been constructed to facilitate measuring breath hydrogen levels in the clinical setting. These include hand held devices that give an instant result and those based in laboratories where bags of exhaled breath are taken to be analysed after completing the lactose challenge. *Bedfont EC 60* Hydrogen monitor and the newer *Bedfont Gastro*$^+$ [Bedfont Scientific Ltd, Maidstone, UK] use an end tidal sampling system which allows diffusion of expired air directly into the electrochemical sensor. The electric output from the sensor is fed to a liquid crystal display which displays results in parts per million. Another device which is commonly used in the measurement of expired hydrogen is the *Micro* H_2 [Micro Medical Limited, Kent UK] which also provides an instant value for breath hydrogen using a sensor drift detection technique. A study comparing the portable breath hydrogen analyser *Micro* H_2 with a *Quintron MicroLyzer, Model DP* [Quintron Instrument Company, Milwaukee USA] which uses gas chromatography technique where expired gas is collected in a bag and taken to this machine in a lab and analysed was carried out by Peuhkuri et al

(273). It showed a 100% concurrence between the two methods for the diagnosis of lactose maldigestion using breath hydrogen analysis in 44 cases. They concluded that the *Micro H_2* appeared as reliable for measuring breath hydrogen concentrations as the *Quintron MicroLyzer* in order to diagnose hypolactasia (273).

5.3: METHODS

5.3.1: SUBJECTS

The study population consisted of 134 subjects, 55 patients with Ulcerative Colitis, 49 with Crohn's disease & 30 Healthy volunteers. The study populations were all of Caucasian origin. All IBD patients were in clinical remission at the time of the study as determined by a Harvey-Bradshaw index (260) score of 4 or less in those with CD and by the simple clinical colitis activity index (261) score of 3 or less in those with UC. The study was approved by the South East Wales Research Ethics Committee.

5.3.2: BREATH HYDROGEN ANALYSERS

As a reference analyser for detecting the breakdown products of lactose metabolism in exhaled air, the *Quintron MicroLyzer Self-Correcting Model SC* [Quintron Instrument Co. Inc., Milwaukee, WI, USA] was chosen as this was the analyser which is used as the standard in the laboratory currently where I was based as described in chapter 2.

The *Micro H_2* [Micro Medical Limited, Kent, UK] a portable, hand-held, hydrogen monitor which is designed to give instant results was used for comparison, again as described in chapter 2. The technical data for both the analyzers is summarised in Table 5.1. The calibration of both analyzers was carried out with Microcan-Disposable Calibration Gas [MicroGas UK Batch 3335/1006] which contains 100 ppm of Hydrogen, 50ppm of Methane and 6% Carbon Dioxide and balance Air [UN1956] 20 litres at 20°C and 300 psig for the *Quintron MicroLyzer*. The *Micro H_2* was calibrated using Microcan gas [MCG 100] which contains 50ppm of Hydrogen [UK batch 3330/0075].

Table 5.1: Technical data of the *Micro H_2* monitor, and the *Quintron MicroLyzer SC*, according to the manufacturer's manual.

	Micro H_2 Hydrogen Monitor	***Quintron MicroLyzer SC***
Range	0-500ppm	0-500ppm
Sensitivity	1ppm	±2ppm
Dimensions	17X6X2.6cms	45X28X31cms
Weight	0.16 kg	7.2 kg
Power Supply	Single 9 V PP3	220 V
Calibration Gas	H_2 50 ppm	$H_2$100 ppm, CH_4 50 ppm & CO_2 6%
Sample size	Single breath	20mls of breath [minimum]
Results Display	Liquid Crystal Display	Liquid Crystal Display

5.3.3: EXPERIMENTAL DESIGN

Participants were told not to eat or drink anything other than water from midnight the night before the test which commenced at 9am. They were also told not to smoke for at least 4 hours before the test. In addition, they were advised to choose lactose-free food and avoid those foods that they recognise will produce gastrointestinal symptoms in the preceding 3 days. They had not received any antibiotic treatment or had bowel preparation for gastro-intestinal investigations during the 4 weeks before the study. The subjects were given 50grams of lactose [Lactose powder BP: BN: M07001589 MS/13880/1 North Staffordshire Hospital Trust Pharmacological Services] dissolved in 300mls of water. Breath samples were obtained before the ingestion of the lactose and then at 30 min intervals for 3 hours and then an hour later. On each occasion, the subject exhaled into the *Micro* H_2 and then straight afterwards they breathed into a polyethylene bag via a one way valve. Once the bag had fully inflated, the collected sample was stored to be later analysed by the *Quintron MicroLyzer* which measured both breath hydrogen and methane values. The test was defined as positive if the rise in hydrogen or methane or both is 20 ppm and 5 ppm above the baseline/nadir, respectively.

5.3.4: STATISTICS

Sensitivity, specificity and positive and negative predictive values were calculated in order to evaluate the *Micro* H_2 method compared with the breath hydrogen results from the *Quintron MicroLyzer*. Sensitivity [%] = true positives / [true positives + false negatives] X 100, Specificity [%] = true negatives / [true negatives + false positives] X 100, Positive predictive value [%] = true positives /

[true positives + false positives] X 100 & Negative predictive value [%] = true negatives / [true negatives + false negatives] X 100. The Bland-Altman plot, or difference plot, was used to compare the two measurement techniques (355). In this method, the differences between the two techniques are plotted against the averages of the two techniques. Horizontal lines are drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference ± 1.96 times the standard deviation of the differences. The data was entered and analysed by statistical software SPSS version 12 [Chicago, USA].

5.4: RESULTS

Out of the total 134 participants, there were 68 females and 66 males in the study. The age range was 19-86 years and their mean age was 44.98years. The mean age for male patients was very similar to female participants 44.9 versus 45 years and the age range was 20-86 years for male and 19-81 years for female participants.

The results obtained with *Quintron MicroLyzer* which was taken to be the standard, showed 39 [29.1 %] participants had a positive test and therefore 95 [70.9%] had a negative result. The test was positive in 18/66 [27.3%] male and 20/68 [30.9%] females participants. Of the 39 who had a positive test as determined by the results from the *Quintron MicroLyzer*, 19 [48.7%] were positive for Hydrogen [H_2] production, 17 [43.6%] were positive for methane [CH_4] and 3 [7.7%] were positive for both H_2 & CH_4.

The results obtained with *Micro H_2* analyser, which is only able to detect hydrogen, showed that 21 [15.7%] participants had a positive test and 113 [84.3%] had a negative test. The test was positive in 8/66 [12.1%] men and 13/68

[19.1%] females participants. The *Micro H_2* picked up 21/22 [95.5%] cases that the *Quintron MicroLyzer* had detected significant rises in breath hydrogen. However, it missed one person i.e. 1/22 [4.5%] of cases. This was a 26 year old female with ulcerative colitis for 9 years. The rise in Hydrogen from nadir was 10 ppm with *Micro H_2* but was 28 ppm with *Quintron MicroLyzer*.

If the results of lactose metabolism were just based on hydrogen detection alone then the *Micro H_2* had a sensitivity of 95.5%, a specificity of 100%, and a positive predictive value of 100% and a negative predictive value of 99.1%.

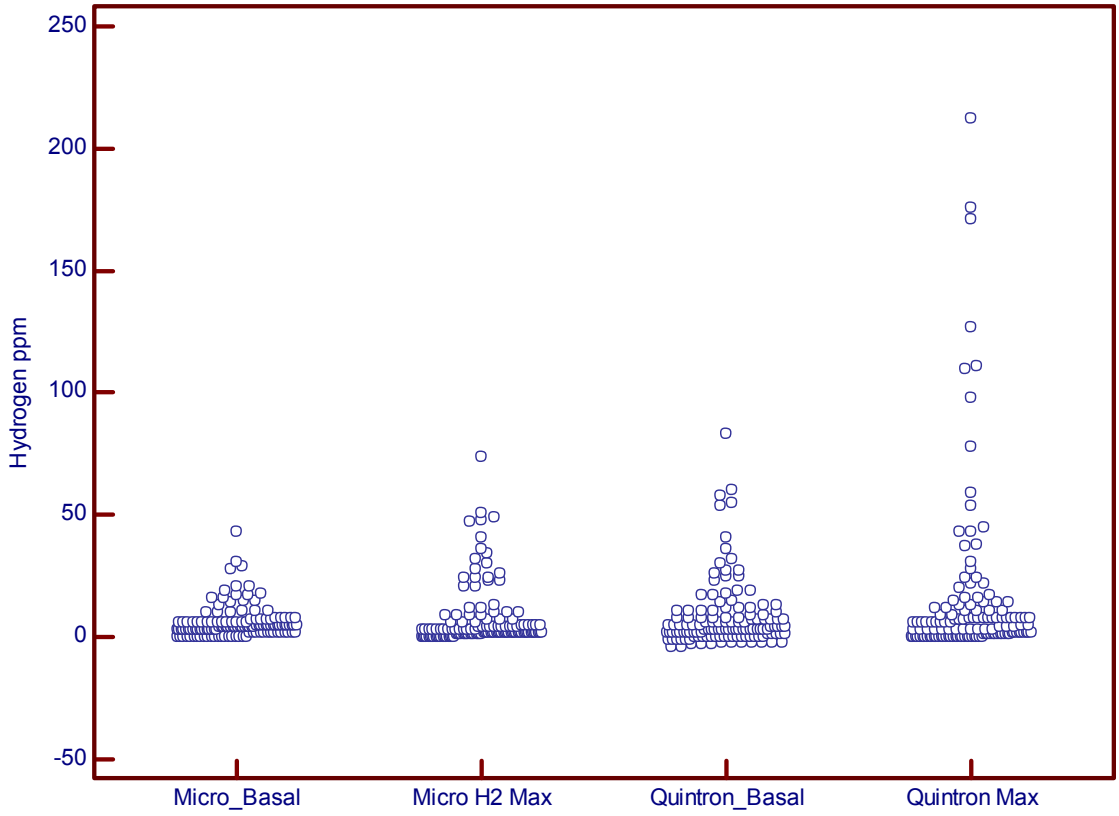
However, the gases produced by metabolism of lactose are both hydrogen and methane. The *Micro H_2* does not measure methane levels and so it would have missed 17 other cases where there was a significant rise in the levels of this gas after lactose ingestion. This means that in the subjects studied, 17 [43.6%] cases with results from the *Quintron MicroLyzer* suggestive of hypolactasia, were due to methane production and would not have been picked up if only this handheld device was used. The *Micro H_2* therefore has an overall sensitivity of 53.9%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 83.2%. The results are summarized in table 5.2.

Tables 5.2: Comparison of *Micro H_2* results with *Quintron MicroLyzer* in measuring the breakdown products of lactose metabolism- the number of patients [not percentage] is given for each of these tests.

Micro H_2	***Quintron MicroLyzer***		
	Positive	Negative	Total
Positive	21	0	21
Negative	18 [17 CH4 & 1 H_2]	95	113
Total	39	95	134

The amount of H_2 detected by the *Micro H_2* ranged from 0 ppm to 78 ppm when compared to 0 ppm to 227 ppm with the *Quintron MicroLyzer*. The highest increase in the breath hydrogen concentration over the baseline after ingestion of lactose was extremely variable and ranged from 4 to 78 ppm measured with the *Micro H_2*, or from 8 to 217 ppm measured with the *Quintron MicroLyzer*. The increase in hydrogen from the basal values to the highest recorded ones during the test is also shown in figure 5.1. The amount of methane detected was 0 ppm to 157 ppm with *Quintron MicroLyzer* with the highest rise in methane ranged from 0 ppm to 157 ppm.

Figure 5.1: Basal and Maximum Hydrogen rise between *Micro H_2* and *Quintron MicroLyzer*.



The maximum rise in hydrogen from the base line or nadir detected by *Micro H_2* was 74 ppm [range 21-74] for a positive test and 13 ppm for a negative test [range 0-13]. The mean peak rise in hydrogen detected by the *Micro H_2* for a positive test from base line or nadir detected was 34.5±13.99. The maximum rise in hydrogen from the base line or nadir detected by the *Quintron MicroLyzer* was 212 ppm [range 20-212] for a positive test and 17 ppm for a negative test [range 0-17]. The mean peak rise in hydrogen and methane detected by the *Quintron MicroLyzer* for a positive test from base line or nadir detected was 42.1±54.3 ppm and 36.8 ± 40.6 ppm. The results are shown in table 5.3.

Table 5.3: Summary of Hydrogen range and mean rise in participants with a positive and negative test with the *Quintron MicroLyzer* compared to the handheld *Micro* H_2. [SD Standard deviation, ppm Parts per million, NA not applicable]

		Quintron MicroLyzer	***Micro H_2***
Hydrogen Range	Positive test	0-227 ppm	0-78 ppm
	Negative test	0-17 ppm	0-13 ppm
Mean peak rise in Hydrogen ± SD	Positive test	71.6 ± 56.9 ppm	33.5±13.7 ppm
	Negative test	4.4 ± 4.6 ppm	2.98±2.9 ppm
Methane range	Positive test	5-157 ppm	NA
	Negative test	0-4 ppm	NA
Mean peak rise in Methane ± SD	Positive test	42.3 ± 46.3 ppm	NA
	Negative Test	0.35 ± 0.9 ppm	NA

For all the participants, the *Quintron MicroLyzer* gave higher levels of hydrogen than the *Micro* H_2 15.4 ± 33.95 ppm Vs 7.5 ± 12.5 ppm respectively, and the mean difference between the results of the *Quintron MicroLyzer* and the *Micro* H_2 was 7.87 ± 24.2 ppm. The rise in hydrogen in the 2 tests matches each other though the magnitude of rise as expected from the results above is smaller with *Micro* H_2. These are shown in the figure 7.2. The rises in both positive and negative cases are similar between the two analysers. The intra-individual correlation between the *Micro* H_2 and the *Quintron* is shown in the figure 5.3.

Figure 5.2: Mean rise in hydrogen during positive and negative test detected by *Micro* H_2 compared with *Quintron MicroLyzer* after a challenge with 50 grams of lactose

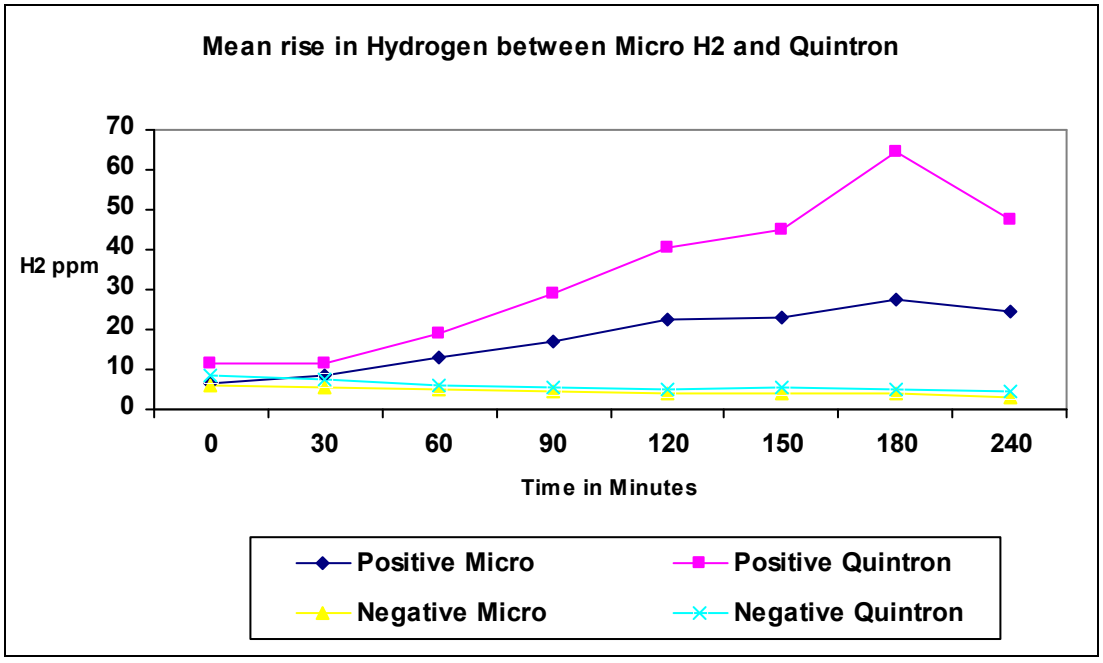
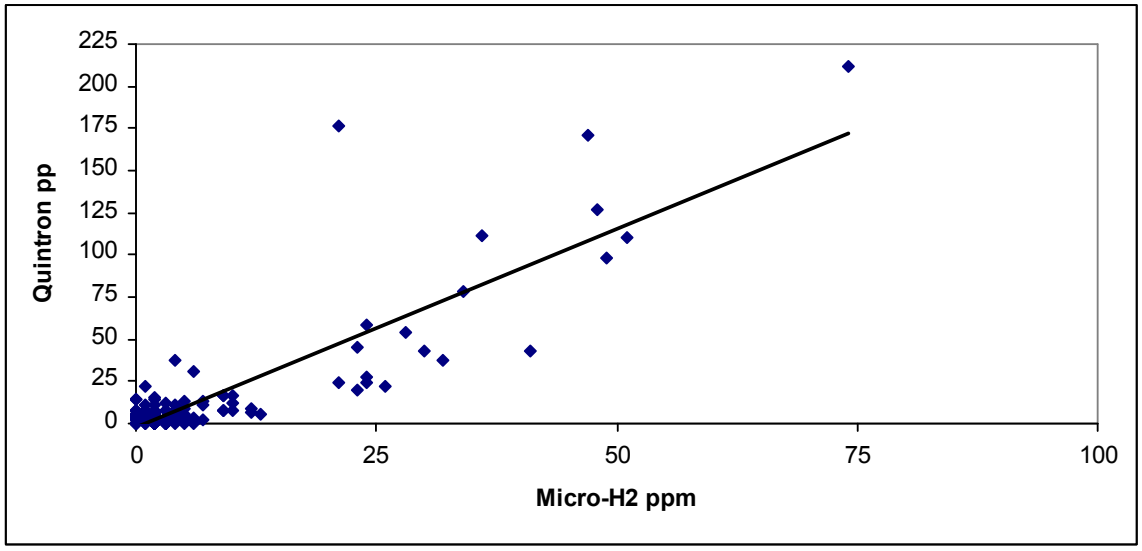
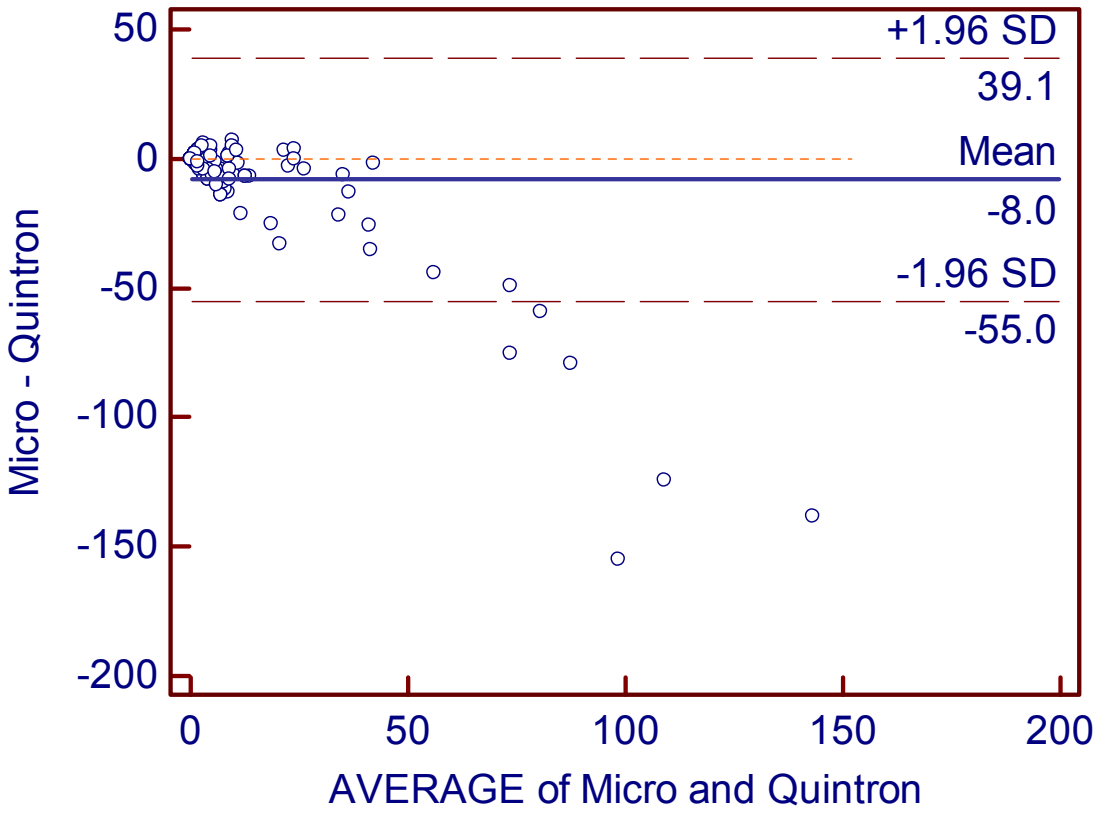


Figure 5.3: Comparison of the *Micro H_2* and the *Quintron MicroLyzer* hydrogen monitors in measuring the maximum increase of breath hydrogen concentration [ppm] in 134 volunteers after lactose challenge. The 'line of linearity' is plotted on the figure.



The Bland Altman's plot [figure 5.4] shows the two methods [*Micro H_2 & Quintron MicroLyzer*] are interchangeable in the diagnosis of lactose malabsorption if the test is based on hydrogen production alone (355) The absolute values of the difference between the *Micro H_2* and *Quintron MicroLyzer* were significantly related to the average level, with a Spearman's rank correlation r=-0.587and significance level $p<0.0001$.

Figure 5.4: Bland Altman's Plot showing *Micro H_2* with *Quintron MicroLyzer* are interchangeable in the diagnosis of lactose malabsorption if was based only on the detection of hydrogen in the expired breath sample.



5.5: DISCUSSION

The results from these two systems were very similar for the detection of a significant rise in breath hydrogen after a standard lactose challenge. The handheld *Micro H_2* analyser would have only missed one case where the hydrogen levels were found to have increased by a significant level when recorded on the *Quintron MicroLyzer*. The results did however; show a difference in the magnitude of the increase in hydrogen levels after a lactose load in the same subject when the two systems were compared with much higher values obtained from the *Quintron MicroLyzer*.

The *Micro H_2* analyser that was used has the ability to measure only exhaled hydrogen rather than both hydrogen and methane which is possible with the *Quintron MicroLyzer*. As a result the handheld system, although easier to use with an instant result, gives restricted information on the fermentation processes in the large intestine. CH_4 is produced in the human intestine chiefly by H_2-utilising flora and so the adequate assessment of gut bacterial carbohydrate fermentation requires parallel measurement of both breath H_2 and CH_4. The use of hydrogen excretion as the only means to quantifying carbohydrate malabsorption is not correct and flawed because methane-producing patients are likely to have a higher 'false negative' results after an oral load of lactose (356). It has been shown that as many as 40-50% of people can be identified as methane producers (304, 305). In some subjects, at least part of the hydrogen is used to produce methane, but in most hydrogen-producing subjects, part of the hydrogen is dissolved into the blood stream and exhaled through the lungs (304, 306). H_2 and CH_4 are only produced by bacteria, and carbohydrates are the primary substrate for their

production, the presence of either gas in breath will signal the breakdown of carbohydrates in intestinal tract. Methanogenic bacteria are able to convert H_2 to CH_4 within the colon (292, 299, 300). There is a complex interaction between H_2 and CH_4 production which was discussed in chapter 4.

In the study by Peuhkuri there were 44 volunteers [34 female and 10 male] with no gastro-intestinal disease. Our study is a larger study with 134 participants, with nearly equal sex distribution [66 males and 68 females] and also involving healthy volunteers and disease groups although they were all in remission. Peuhkuri et al showed that the diagnoses were the same in 100% of the cases with the two breath hydrogen analysers, the *Micro H_2* and the *Quintron MicroLyzer* (273). In contrast, my study shows that the *Micro H_2* would diagnose 95% of those with hydrogen production but would only diagnose 53.9% of the total number of cases that would be diagnosed using *Quintron MicroLyzer* this is due to the inability of the *Micro H_2* to measure breath methane concentrations.

The *Micro H_2* gave higher peak levels of hydrogen than the *Quintron MicroLyzer* 51.8 ppm [SD 86.0 ppm] and 32.3 ppm [SD 49.2 ppm] respectively in Peuhkuri's study, and the mean difference between the results of the *Micro H_2* and the *Quintron MicroLyzer* was 19.5 ppm [SD 42.6 ppm] (273). In my work the *Micro H_2* gave lower peak levels of hydrogen than the *Quintron MicroLyzer* 33.5 ppm [SD 13.7 ppm] and 71.6 ppm [SD 56.9 ppm] respectively, and the mean difference between the results of the *Quintron MicroLyzer* and *Micro H_2* was 41.2 ppm [SD 46.7 ppm]. The results from the *Quintron MicroLyzer* were clearly higher than *Micro H_2* meter. There are several potential reasons why this may have differed from the Peukhuri paper. Firstly, I used the *Quintron MicroLyzer*

Self-Correcting Model SC which in addition measures CO_2 and corrects the results for expired CO_2 when compared to *Quintron MicroLyzer DP model* used in the study by Peukhuri. This reduces a source of error in trace-gas analyses which is contamination of the alveolar sample with dead space air during its collection. This is based on the concept that CO_2 is present in alveolar air at a constant concentration, while it is absent in room air. If alveolar air is erroneously mixed with room air, the concentration of CO_2 will be reduced so will be the trace gases present. By knowing the degree to which CO_2 was diluted, a correction is applied to calculate true alveolar concentration of the gas.

The higher values may also result from the different concentration of gases used to calibrate the analyzers: 100ppm of H_2 with *Quintron MicroLyzer* and 50ppm of H_2 with *Micro H_2*. Also, the sampling techniques differ between the analysers and this may have an effect on the hydrogen concentrations measured. The *Micro H_2* measures the hydrogen concentration of total breath and shows the highest peak value after the end of the breath. Even though the subjects were allowed to exhale slowly through the mouthpiece of the *Micro H_2* analyser, methods of blowing may be varied. With the *Quintron MicroLyzer*, the gases are collected in a bag and then hydrogen is measured from the collected breath and the whole breath sample is used in the analysis. However it should be noted that my comparison was conducted in a standardised fashion using a uniform protocol to exclude any known sources of error. A set lactose load was used with a predetermined sampling schedule. All participants were nil by mouth except water for at least 9 hours before the test. They were also not allowed any medications, told not to smoke for at least 4 hours before the test and smoking was not allowed

during the test. All those with IBD had their disease in remission as determined by standard criteria. During the lactose breath test the participants were in the hospital under direct supervision and they were not allowed to leave the room. This ensured that no one smoked during the test process. The CO_2 concentration in the alveolar air is at a constant concentration and it would have changed if the participant has smoked. The detection of CO_2 concentration by the *Quintron MicroLyzer* is an advantage and this will help in picking up participants if they have smoked during the test, if they had smoked prior to coming to the test their CO_2 concentration at baseline will be elevated and would fall to much lower levels during the test and therefore have not been compliant with the instructions.

The *Micro H_2* is simple to use, transportable, can be taken to the patient and gives results instantaneously. It is also cheaper to buy and has lower maintenance costs. The *Quintron MicroLyzer* needs to be calibrated before it is used each time. Every sample takes at least 2 minutes to analyse and it takes about 30 minutes for the analysis of all the samples taken from one person which leads to higher labour costs. On the other hand, a potential advantage of the *Quintron MicroLyzer* is that the patients could be sent home and they can collect their samples unsupervised. This could free up time for both the physicians and technicians where they could see more patients, in addition the samples collected can be analysed at later date.

The *Micro H_2* did correctly pick up the rise in hydrogen in 95.5% of cases compared to *Quintron MicroLyzer*. In clinical practice you could argue that the breath samples to detect lactose malabsorption should be analysed first by a hand held device and if the results are suggestive of malabsorption i.e. a raised

hydrogen, then no further analysis is needed. Those samples that are negative could then have separate, stored samples assessed later by the *Quintron MicroLyzer* for both hydrogen and methane. This way the two analysers could be used complementary to each other which have benefits like ease of use and reduced laboratory time and cost in diagnosis of lactose malabsorption.

Measurement of breath hydrogen and methane, together with lactase genotype, should now form the current best practice for investigation of lactose sensitivity (136). A handheld device should be developed to measure expired methane similar to hydrogen, with the advantages associated with a hand held device; this would be of great help when formally assessing a patient for lactose sensitivity.

5.6: CONCLUSION

The results show that the *Micro* H_2 was not accurate in diagnosing lactose malabsorption because of its inability to measure methane levels. It was, however, reliable in measuring breath hydrogen concentrations alone after an oral dose of lactose. The fact that it cannot measure breath methane concentrations is a serious weakness, because this inability leads to an under diagnosis of lactose sensitivity. I would therefore advocate, where possible, using the *Quintron MicroLyzer* with analysis of both hydrogen and methane levels to determine hypolactasia in clinical practice. The two analysers could be used complementary to each other i.e. if the samples are analysed first by a hand held device and the results are not suggestive of malabsorption, then they should be then assessed by the *Quintron MicroLyzer*

for confirmation. This strategy has the benefits like ease of use and reduced laboratory time and cost in diagnosis of lactose malabsorption.

APPENDIX 6

CORRELATION BETWEEN THE RESULTS OF LACTOSE BREATH TESTS AND GASTRO-INTESTINAL SYMPTOMS

6.1: AIMS

The aim of this study was to evaluate the sensitivity and specificity of gastro-intestinal symptoms when compared with a positive breath test after an oral lactose challenge. If there is a good correlation then lactose sensitivity could perhaps be diagnosed by just assessing symptoms after the ingestion of lactose and negate the need to obtain breath samples.

6.2: INTRODUCTION

Lactase-deficient individuals are unable to cleave lactose into glucose and galactose, as a result, lactose reaches the large intestine where it is metabolized by the colonic flora. The high osmotic load caused by lactose in the small intestine and the bacterial metabolites that are produced appear to be the cause of the symptoms associated with lactose malabsorption. In clinical practice breath tests are often used to diagnose lactase deficiency. The specificity of the breath test varies between 89-100% whilst its sensitivity ranges from 69-100% (126). Although the breath tests are the most frequently used investigation to diagnose LI there is very limited information available on the sensitivity and specificity of symptoms which develop after a lactose challenge. Indeed, there are only a couple of studies evaluating the relationship between the symptoms and the result of the breath test. A study by Hermans (179) evaluated the severity of 4 symptoms [bloating, flatulence, abdominal distension and diarrhoea] in 309 consecutive patients with suspected lactose malabsorption. Blood glucose and H_2 concentrations in breath samples were measured after a 50g lactose challenge. During the 4 hours of the test, patients were asked to score their symptoms semi-

quantitatively as 0 [no complaint], 1 [moderate complaint] or 2 [severe complaint]. The sum total of the scores for each of these four symptoms was used to calculate the total symptom score [TSS]. A positive breath test was defined as a rise in hydrogen after lactose challenge if the H_2 concentration rose by 20 ppm above baseline and was seen in 40% of patients. An increase in the TSS by one point was associated with a significant increase [$p<0.05$] in the mean peak hydrogen concentration. The mean TSS of patients with a positive breath test [TSS=1.7] was significantly [$P<0.001$] higher than the mean score of patients with a negative breath test [TSS=0.96]. In addition, the authors noted that the peak hydrogen concentration was higher in patients with higher a TSS when compared with those with a low TSS. Another study suggested a strong association between the number of GI symptoms and a positive H_2 breath test. In this study by Beyerlein(180), the intensity of five GI symptoms [nausea, abdominal pain, borborygmi, bloating and diarrhoea] was recorded every 15 min up to 3 h after a challenge with 50g of lactose. 1127 patients [72% females] participated and the test was considered positive if the rise in hydrogen was by ≥20 ppm above baseline. A positive test occurred in 376 [33%] patients - 21% of patients with one symptom, 40% of patients with two symptoms, 44% of patients with three symptoms, 67% of patients with four symptoms and 82% of patients with five symptoms. Intensity of the symptoms was significantly higher for each symptom in the positive group. They concluded that evaluating symptoms that developed after the ingestion of a 50 g lactose load can be used as a simple screening test to select patients who need to be referred for lactose intolerance testing.

6.3: METHODS

Lactose breath test was carried out and the samples were analysed using *Quintron MicroLyzer* as described in chapter 2. Samples were collected at baseline and then for the next 4 hour period after a 50g lactose challenge as described in chapter 2. At the time of sampling, patients were asked to rate five symptoms [abdominal pain, nausea, bloating, borborygmi and diarrhoea] using a 10-point scale [0 – no symptoms to 10 – severe] on a symptoms chart [Chapter 2]. For each patient, the maximal intensity of each of the five symptoms was defined as the highest symptom intensity value recorded during the duration of the breath test. The test was considered positive if the hydrogen concentration in the exhaled air exceeded 20 ppm or methane exceeded 5ppm above baseline or nadir during the test period. A symptom was considered positive if the patient reported an increase above baseline in the severity of the symptom during the four hour monitoring period.

6.3.1: STATISTICAL ANALYSIS

Quantitative variables are given as mean ± standard deviation and categorical variables are given as total numbers and percentages. The chi-squared test was utilized to analyse differences between proportions. Differences in the mean age of patients with positive and negative breath test were compared by using the unpaired Student's t-test. Correlations between variables were quantified by calculating the Spearman rank correlation coefficients. P values less than 0.05 were considered to be significant. All sensitivity, specificity, predictive values and likelihood ratios (13) were calculated by using the absence of the specific symptom or the absence of any symptom as reference [=test negative]. The chi-

squared test for trend was use to evaluate if the increase in the number of symptoms is associated with increased positivity of breath test. The data was entered into Statistical program SPSS version 12 [Chicago, USA].

6.4: RESULTS

The results from 140 participants which includes 110 patients with IBD [59 UC & 51 CD patients], and the 30 healthy volunteers were available for analysis. 41 participants [29.3%] had a positive breath test indicative of lactase deficiency and 99 [70.7%] had a negative breath test. The mean age of patients with a positive test was 49.6 ± 19.1 years and for those with a negative test was 43.1 ± 15.3 years which did not differ significantly [p=0.31]. Similarly, no statistically significant association was found between gender and the result of the breath test [p=0.65]. None of the HV had a positive breath test, 16 [27.2%] of UC and 25 [49%] of CD had a positive breath test. The full details of the participants and the implications of this are discussed in chapter 4, section 4.5.3 and page 95.

6.4.1: SENSITIVITY AND SPECIFICITY OF INDIVIDUAL SYMPTOMS

After the lactose challenge, 49 [35%] patients reported nausea, 61 [44%] bloating, 56 [40%] diarrhoea, 51 [36%] borborygmi and 59 [42%] abdominal pain. Diarrhoea was reported by 25 [61%] with a positive breath test and 31 [31.3%] with a negative breath test. Nausea was reported by 19 [46%] with a positive breath test and 30 [30.3%] with negative breath test. Borborygmi was reported by 23 [56%] with a positive breath test and 28 [28%] with negative breath test. Abdominal pain was reported by 25 [61%] with a positive breath test and 34

[34.3%] with negative breath test. Bloatedness was reported by 25 [61%] with a positive breath test and 36 [37%] with negative breath test. Diarrhoea & borborygmi [45%] were the most sensitive symptoms and the most specific was diarrhoea [81%]. Borborygmi had the highest negative predictive value at 72% and diarrhoea had highest positive predictive value of 61.4%. The table 6.1 shows the sensitivity, specificity, likelihood ratios and predictive values of each symptom.

Table 6.1: Sensitivity, Specificity, Positive Predictive Value [PPV] and Negative Predictive Value [NPV], Likelihood Ratio [LR] positive and negative for each individual symptoms following lactose challenge.

Symptom	Sensitivity [%]	Specificity [%]	PPV [%]	NPV [%]	Positive LR	Negative LR
Abdominal pain	42	80	61	66	2.2	0.7
Diarrhoea	45	81	61	69	2.3	0.7
Bloatedness	41	80	61	64	2.0	0.7
Nausea & Vomiting	39	76	46	70	1.6	0.8
Borborgymi	45	80	56	72	2.2	0.7

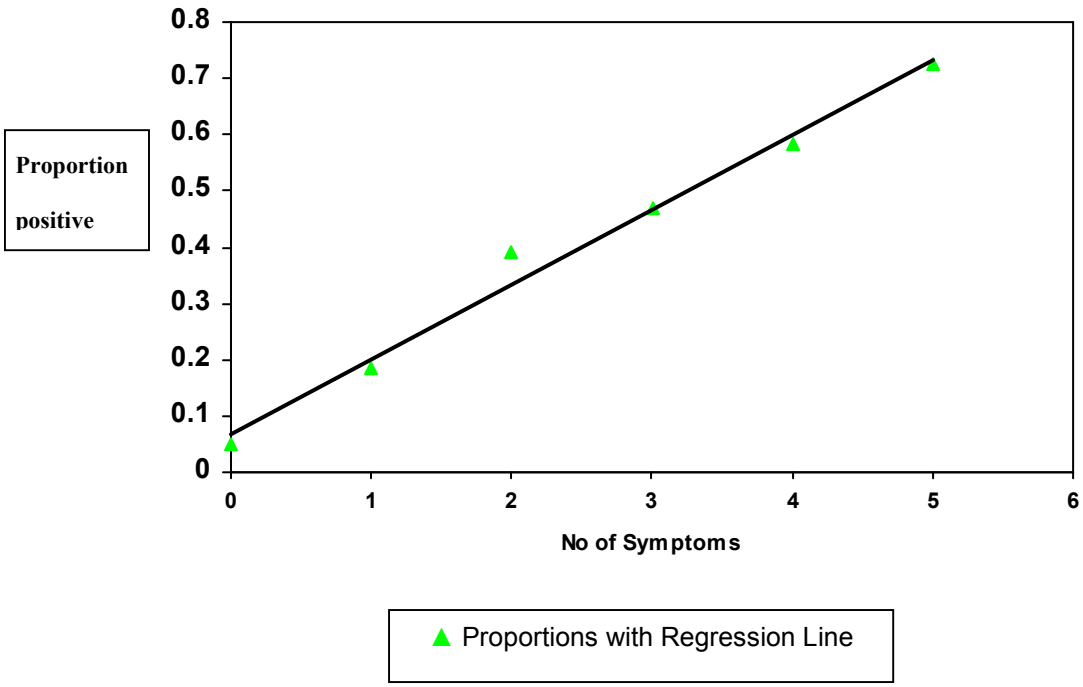
6.4.2: SENSITIVITY AND SPECIFICITY OF COMBINATIONS OF SYMPTOMS

Thirty nine patients [27.9%] patients did not develop any symptoms during the test. In this group of patients, 95.9% had a negative breath test. The proportion of patients with a positive breath test increased with the number of symptoms developed after the ingestion of lactose from 18.4 % in those developing only one symptom to 73% in those developing all five symptoms as shown in table 6.2 and shown as a scatter plot of proportion of positive breath test based on the number of symptoms in figure 6.1. This trend of increased positivity of the breath test with number of symptoms was statistically significant [χ^2 for trend=31.74 & $p<0.0001$].

Table 6.2: Patients with positive and negative breath tests based on the number of symptoms developed after lactose challenge and showing proportion of positive breath test.

No of symptoms	Positive Breath Test	Negative Breath Test	Totals	Proportion Positive Breath Test
0	2	37	39	0.051
1	7	31	38	0.184
2	9	14	23	0.391
3	8	8	17	0.471
4	7	5	12	0.583
5	8	3	11	0.727
Totals	41	99	140	0.293

Figure 6.1: Scatter plot of proportion of positive breath test based on the number of symptoms developed after lactose challenge in all patients. Proportions are shown on Y axis and number of symptoms on X axis with a regression line.



There was a strong positive correlation between the number of symptoms and percentage of patients with a positive lactose breath test [$r = 0.468$; $P < 0.01$]. There was no difference when the results were assessed by the disease state [i.e. UC Vs CD]. Sensitivity, specificity, PPV, NPV, positive and negative likelihood ratio's of one, two, three, four and five symptoms to identify a positive breath test are shown in Table 6.3.

Table 6.2: Sensitivity, Specificity, Positive Predictive Value [PPV] and Negative Predictive Value [NPV], Positive and Negative Likelihood Ratio [LR] of one, two, three, four and five symptoms following lactose challenge.

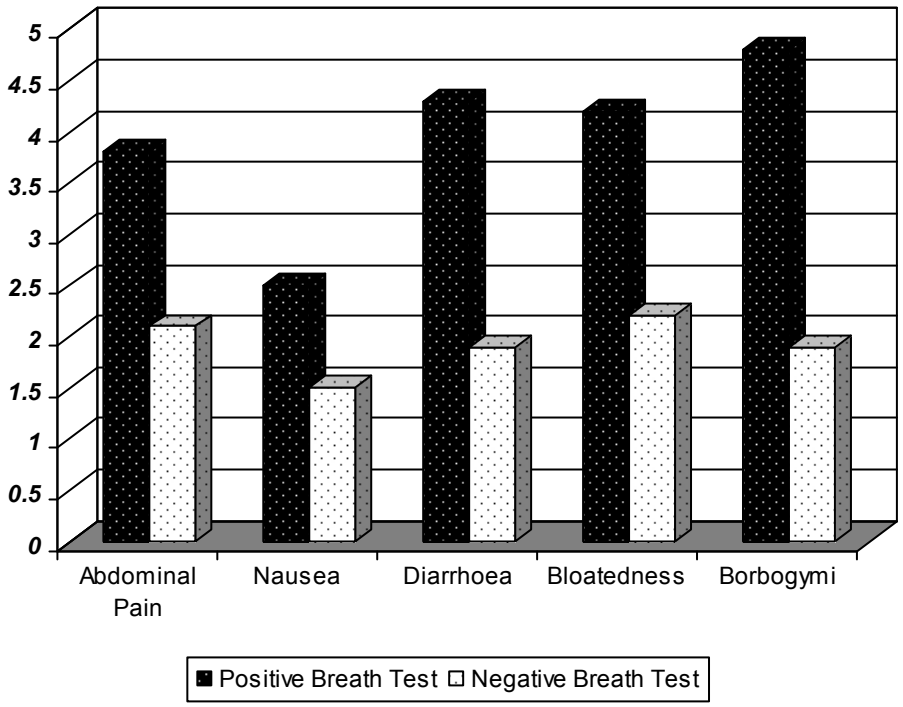
No of Symptoms	Sensitivity [%]	Specificity [%]	PPV [%]	NPV [%]	Positive LR	Negative LR
One [n=38]	78	54	18	95	1.7	0.4
Two [n=23]	82	73	39	95	3	0.3
Three [n=17]	81	82	53	95	4.6	0.2
Four [n=12]	77	88	58	95	6.5	0.3
Five [n= 10]	78	92	70	95	10.3	0.2

Patients developing zero [no] symptoms [n=39] were used as reference [2 with a positive breath test and 37 with negative breath tests]

6.4.3: INTENSITY OF SYMPTOMS BASED ON BREATH TEST RESULTS

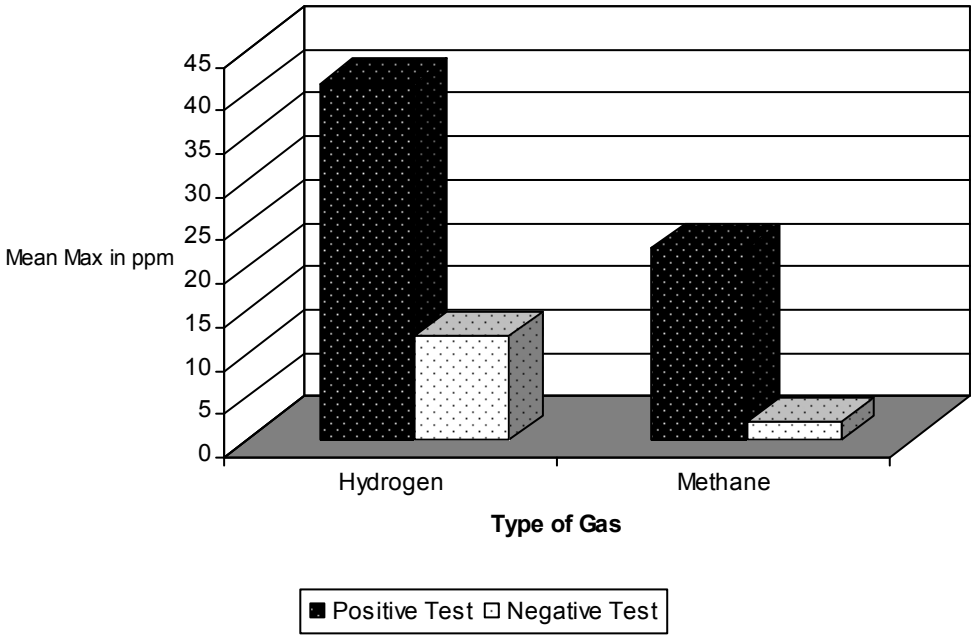
Patients with a positive breath test reported a higher intensity of symptoms when compared with patients with a negative breath test. The differences in symptom intensity [Figure 6.2 below] were statistically significant [$p<0.05$] for all five symptoms: abdominal pain [3.8 vs.2.1], nausea [2.5 vs. 1.5], bloatedness [4.3 vs. 1.9], diarrhoea [4.2 Vs 2.2] and borborygmi [4.8 vs. 1.9]. These were significant for both forms of IBD. Such relationship could not be established in healthy volunteers as none of them had a positive test. The sum of subjective symptom scores was higher in the 41 lactose intolerant subjects when compared to the 99 lactose tolerant subjects. The median score was 14 [7-17] in the former and 4 [1-13] in the latter. This was statistically significant with a p value of < 0.032.

Figure 6.2: Mean maximal symptom intensity score in those with a positive and negative breath test following lactose challenge. Symptoms are given on the X axis and mean maximal intensity score is shown on Y axis.



When the breath test was positive after a lactose challenge, the rise in hydrogen from baseline or nadir ranged from 22 ppm to 227 ppm and for methane this ranged from 7 ppm to 157 ppm. The test was positive in 41 cases where the mean maximal rise in hydrogen and methane was 41 ppm and 22 ppm respectively. The test was negative in 99 cases with a mean maximal rise in hydrogen and methane was 12 and 2 ppm respectively and these are shown in figure 6.3.

Figure 6.3: Mean maximal rise in hydrogen or methane recorded from base line or nadir with a positive and negative breath test in individuals following lactose challenge.



6.5. DISCUSSION

The results show that the symptom of diarrhoea had the best sensitivity, specificity and positive predictive value whilst borborygmi had the best negative predictive value [72%] for a positive breath test after a standard lactose load. Lactose malabsorption based on a breath test after the ingestion of lactose in the participants was found to be 18.4% if they developed one symptom and 70% if all the five symptoms were reported. This trend of increased positivity of the breath test with the number of symptoms was statistically significant [$p<0.0001$].

This study reports the sensitivity and specificity of abdominal pain, nausea, bloating, borborygmi and diarrhoea in response to a lactose challenge when compared to a positive breath test. This information is important as symptoms of lactose intolerance overlap with features of irritable bowel syndrome and, thus, discrimination between these two disease entities may be difficult (224). The results here show some similarities but some differences to the study by Beyerlein (180) who found that bloating had the best sensitivity [71%] and NPV [82%], while they found that diarrhoea had the best specificity [90%] and PPV [66%] to identify patients with a positive breath test. Beyerlein (180) found that LM was present in 21% of subjects who developed one symptom and 80% who had all five symptoms. Symptom intensity was significantly higher for each symptom in the positive group. In another study by Peuhkuri (273) the sum of a subjective symptom score was higher in the lactose maldigesters [18 subjects] than in the lactose digesters [26 subjects]. These data indicate that evaluation of symptoms developed in response to the ingestion of lactose could be used as a simple screening test for lactose intolerance.

Although I have suggested earlier on in the thesis that the standard investigation for lactose sensitivity should initially be genotyping, it is not widely available and so most centres rely on breath testing. Studies comparing symptom evaluation and genetic testing against lactose breath are warranted. From this analysis, a pragmatic approach could be to consider other diagnoses in subjects who do not develop any symptoms after a 50g lactose challenge as my work suggests the likelihood of a positive test is only 5%. Breath testing may not be necessary in patients who develop all five symptoms as the likelihood of them having a positive test is high [73%]. Recommending these patients to strictly

adhere to a lactose-free diet and re-evaluating their symptom pattern after 4–6 weeks could be tried first. Those patients with persisting symptoms on a confirmed lactose-free diet should be further evaluated. In patients improving on a lactose-free diet, formal documentation of low lactase activity may be appropriate given the major lifestyle and diet changes implied by a life-long diagnosis of lactose intolerance. Lactose breath testing should be recommended for patients who develop one to four symptoms after the ingestion of 50 g lactose. Although patients developing one symptom are more likely to be negative and patients developing four symptoms are more likely to be positive, testing should be performed given the implications on lifestyle and diet.

This study has several limitations. The PPV and NPV for individual symptoms and their combination depend on the prevalence of lactase deficiency in the examined population – this was not present in any of the healthy volunteers who were selected on the basis that they had no symptoms or condition. The results here are restricted to a Caucasian population and they should be interpreted with caution in subjects of other ethnicity. The proposed approach for patients who developed symptoms after the ingestion of lactose should be further evaluated in a clinical setting. The cohort of IBD patients were all in remission and again it is unclear if these results could be extrapolated if they have active disease. Finally, of crucial importance was the lack of a placebo component which would have helped to see if the symptoms and results seen here are truly related to lactose.

6.6: CONCLUSION

This study shows a positive correlation between the number of GI symptoms and a positive breath test. Evaluating symptoms developed after ingestion of 50 g lactose can be used as a simple screening test to select patients who need to be referred for lactose intolerance testing but this needs to be confirmed in a clinical setting.

CHAPTER 9

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