

LACTOSE, LACTASE, AND BOWEL DISORDERS

Reducing hypolactasia-related gastrointestinal symptoms by improving
the digestibility of lactose

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ACADEMIC DISSERTATION

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*Ahmi, ahmi, nauti, nauti!
Vatsaan saapuu puhkutahti.
Vatsan seinä pullistuu,
masun rauha mullistuu.
Kautta kovan karhunkallon:
olo on kuin ilmapallon.
Ellei masu enää jousta
saatan vaikka ilmaan nousta.*

Elina Karjalainen: Uppo-Nallen kootut runot

Eat up, little bear, and eat your fill
Till your stomach blows up and you're almost ill!
The walls of my tummy grow smooth and round,
And I can't stand up and I can't sit down.
I feel all over as tight as a drum,
From my hard bear head to my soft bare tum.
And I'll swell and swell till I think very soon
I'll just take off like a huge balloon!

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List of original publications

- I Peuhkuri K, Poussa T, Korpela R. Comparison of a portable breath-hydrogen analyser (Micro H₂) with a Quintron MicroLyzer in measuring lactose maldigestion, and the evaluation of a Micro H₂ for diagnosing hypolactasia. *Scand J Clin Lab Invest* 1998;58:217-224.
- II Peuhkuri K, Vapaatalo H, Nevala R, Korpela R. Influence of the pharmacological modification of gastric emptying on lactose digestion and gastrointestinal symptoms. *Alim Pharmacol Ther* 1999;13:81-86.
- III Peuhkuri K, Vapaatalo H, Nevala R, Korpela R. The temperature of a test solution influences abdominal symptoms in lactose tolerance tests. *Scand J Clin Lab Invest* 2000;60:75-80.
- IV Peuhkuri K, Nevala R, Vapaatalo H, Moilanen E, Korpela R. Ibuprofen augments gastrointestinal symptoms in lactose maldigesters during a lactose tolerance test. *Alim Pharmacol Ther* 1999;13:1227-1233.
- V Peuhkuri K, Hukkanen M, Beale R, Polak J M, Vapaatalo H, Korpela R. Age and continuous lactose challenge modify lactase protein expression and enzyme activity in gut epithelium in the rat. *J Physiol Pharm* 1997;48:719-729.

General abbreviations

6-keto PGF _{1α}	6-keto-prostaglandin F _{1α} (metabolite of prostacyclin)
ANOVA	Analysis of variance
BMI	Body mass index; weight (kg):height (m) ²
CGMP	Cyclic guanosine 3',5'-monophosphate
CI	Confidence interval
COX-1, COX-2	Cyclo-oxygenase isoenzymes
fB-gluc	Blood glucose (fasting concentration)
H ₂	Hydrogen (breath)
IBS	Irritable bowel syndrome
LPH	Lactase (phlorizin hydrolase)
NO	Nitric oxide
NOS	Nitric oxide synthase
NO _x	Nitrate + nitrite (metabolite of nitric oxide)
PGE ₂ -M	Prostaglandin E ₂ -M
SEM	Standard error of mean
U-gal	Urine galactose concentration
VAS	Visual Analogue Scale

Abstract

The ability to digest lactose depends on the expression and activity of intestinal enzyme, lactase. In Finland, 17% of the population suffers from hypolactasia, resulting in lactose maldigestion. Symptomatic lactose maldigestion is called lactose intolerance. The aim of this study was to investigate factors which may either improve or impair the digestibility of lactose and will thus affect the development of maldigestion symptoms, and also the reliability of lactose intolerance diagnosis.

The possible regulatory role of gastric emptying in the digestibility of lactose was studied in 18 maldigesters. In an oral lactose tolerance test, delaying gastric emptying with propantheline improved tolerance to lactose by 26% ($p=0.03$), as measured by the reduced area under the 12-h gastrointestinal symptoms score curve, compared to the placebo. In a study with 9 lactose maldigesters, the temperature of the test solution used in the test modified symptoms but had only minor effects on the other maldigestion indicators. The effects of a cold test solution were more intense than those of a hot one, and the former reduced flatulence ($p=0.01$) and abdominal bloating ($p=0.04$) compared with the room temperature solution.

Inflammation markers were investigated in eight lactose maldigesters. In the urinary excretion of prostacyclin metabolite (6-keto-prostaglandin $F_{1\alpha}$) a moderate increase of about 30% ($p=0.17$), was seen following lactose intake. Ibuprofen, a nonselective inhibitor of cyclo-oxygenases, tended to inhibit this increase ($p=0.02$). In none of the other indicators of inflammation used (e.g. nitric oxide production, blood leucocyte count) were any differences observed compared with the controls.

In an experimental model, the effect of dietary lactose on the expression or activity of lactase was tested for seven days with 8-week-old rats on a lactose-containing diet. About 40% induction of lactase was noticed in the lactose-fed rats compared with the controls ($p=0.04$), especially in the proximal and middle parts of the jejunum.

In the diagnosis of lactose intolerance, it was found that any one of the laboratory variables used (breath hydrogen, blood glucose and urine galactose tests) was more reliable than self-diagnosis. Only a third of the self-diagnosed subjects proved to be real lactose maldigesters, and about the same proportion of the previously tested subjects were, in reality, lactose digesters, indicating either a high incidence of secondary hypolactasia or incorrect previous diagnoses.

It was thus shown that rather than being of inflammatory origin, lactose intolerance is caused, at least partly, by motility disorders. This reduction of motility could be the result of any factors, dietary or otherwise, which retard gastric emptying and/or reduce intestinal motility. The continuous intake of lactose further improves tolerance to lactose by increasing the expression and activity of the lactase, at least in rats. Finally, we suggest that practical details, such as the temperature of the test solutions, should be re-estimated in testing tolerance to lactose.

1. Introduction

Lactose is a disaccharide consisting of glucose and galactose. It is found in milk and other dairy products. The concentration of lactose in human milk is about 7% whereas in cow's milk it is about 5% (Palmiter 1969). The ability to digest lactose depends on the presence of an enzyme (lactase) located in the small intestinal brush border. The monosaccharides are then absorbed by active transport.

If the activity of lactase is low in the relation to the amount of lactose ingested, the lactose cannot be hydrolysed to its components, resulting in maldigestion. This situation is called *hypolactasia* (*lactase nonpersistence*, *lactase restriction*) and means that there is low lactase activity in the jejunal mucosa. The term for the opposite of hypolactasia is *normolactasia* (*lactase persistence*), and is applied to those with moderate or high ('normal') lactase activity. *Lactose maldigestion* and *lactose malabsorption* are terms to describe a poor lactose hydrolysing capacity. The term *lactose intolerance* should only be used for a clinical entity, describing symptomatic lactose maldigestion.

Reasons for lactose maldigestion can be classified as congenital lactase deficiency (almost total lack of lactase, *alactasia*), hypolactasia ('general' lactose maldigestion) and secondary hypolactasia (due to reversible injury in the gastrointestinal tract).

Lactase activity has a typical genetically-determined pattern during mammalian life. This activity increases in late gestation and remains at a high level during early childhood, whereafter it declines to the lower adult level (Sahi and Launiala 1978, Flatz 1987). Hypolactasia may develop as a secondary condition in people already suffering from other gastrointestinal diseases such as celiac disease or enteritis (see Ushijima *et al* 1995, Gudmand-Høyer and Skovbjerg 1996).

The decline of lactase activity from the high infant level to the lower adult level is the normal physiological pattern in about 75% of the world adult population, as reviewed by Sahi (1994). The maintenance of high lactase levels occurs in only a few populations, mainly in northern Europe. In Europe the incidence of hypolactasia ranges from about 10% up to 70%. In Finland the incidence is less than 20% (see Sahi 1994).

The inability to digest lactose will not always result in symptoms of intolerance (abdominal pain, bloating, flatulence and diarrhoea) if lactose is consumed. The terms *lactose intolerance* and *lactose maldigestion* are often used as if they were

synonymous, but they are not, in fact, the same. There are subjects who have a low lactase hydrolysing capacity, i.e. they are hypolactasians but are still asymptomatic after an oral dose of lactose (Rosado *et al* 1987, Scrimshaw and Murray 1988, Carraccio *et al* 1998, Teuri *et al* 1999, Peuhkuri *et al* 2000b).

The most commonly used methods of diagnosing hypolactasia are the indirect measurements of the breakdown products of lactose following an oral dose. Reduced increase in blood glucose concentration, increase in exhaled breath hydrogen or excreted urinary galactose all indicate hypolactasia (reviewed by Arola 1994).

The use of laboratory methods for diagnosing lactose intolerance (i.e. symptomatic lactose maldigestion) is not sufficient. During a lactose tolerance test the development of gastrointestinal symptoms must always be recorded side by side with the laboratory results. Unhydrolysed lactose is transported to the colon and fermented by colonic bacteria into short-chain fatty acids and gases (hydrogen, carbon dioxide, and methane). The development of gastrointestinal symptoms depends on the balance between the production and the removal of these fermentation products. If the disposal capacity is exceeded, excessive rectal gas and/or abdominal distension occur, as reviewed by Villako and Maaros (1994). A hypolactasian subject who suffers no gastrointestinal symptoms during the test is *not* lactose intolerant.

In the study of Teuri *et al* (1999) we showed the correlation between the blood glucose concentration and gastrointestinal symptoms to be fair, between the concentration of expired breath hydrogen and symptoms to be moderate, and between the concentration of urinary galactose and symptoms to be good. In practice, this means that if the blood glucose concentration alone is measured during the tolerance test, as is the case in most Finnish health care centres (Peuhkuri *et al* 2000a), the number of incorrect diagnoses is likely to be significant.

In oral lactose tolerance tests the most widely used test dose in Finland is 50 g lactose dissolved in 200 - 400 ml water (Peuhkuri *et al* 2000a). Newcomer *et al* (1978) demonstrated that the majority of hypolactasian subjects develop gastrointestinal symptoms following this dose. This equals the lactose found in a whole litre of milk, and even normolactasians may suffer gastrointestinal symptoms after such a large dose. The minimum dose of lactose needed to cause notable symptoms differs between individuals. Most maldigesters are reportedly able to tolerate 12 g lactose if it is consumed with a meal (Suarez *et al* 1995, Vesa *et al* 1996). However, there appear to be no well-controlled trials in which a more natural dose of lactose (20-50 g) has

been consumed in smaller doses divided between the meals, which is the usual pattern of lactose intake.

The prevalence of lactose intolerance seems to be overestimated, and many people who describe themselves as intolerant, whether tested by professionals in official health care or not, are actually lactose digesters (Peuhkuri *et al* 2000b). Similar results have been shown by other authors, too (Johnson *et al* 1993a, Suarez *et al* 1995, Saltzman *et al* 1999). Discussion of the principles for testing lactose tolerance and possible affecting factors is very much needed.

Even though hypolactasia is fairly common in Finland, milk and other dairy products are essential part of the Finnish food culture. This has inspired to several series of studies on lactose intolerance, for example, of genetics (Sahi 1974a), diagnostic methods (Arola 1988a) and gastrointestinal symptoms (Vesa 1997).

The aim of this study was to investigate factors which might possibly improve the digestibility of lactose in the small intestine, thus affecting the development of maldigestion symptoms, and also affecting the reliable diagnosis of lactose intolerance.

2. Review of literature

2.1. The lactase enzyme and its regulation

The brush border of the intestinal epithelium (microvilli) contains glycoproteins which are responsible for the hydrolysis and absorption of dietary sugars. Lactose, the disaccharide of milk, consists of galactose joined to glucose by β 1,4-glycosidic linkage (Figure 2.1). Before absorption this β 1,4-glycosidic linkage must be hydrolysed by a microvilli enzyme called lactase (lactase-phlorizin hydrolase [LPH]).

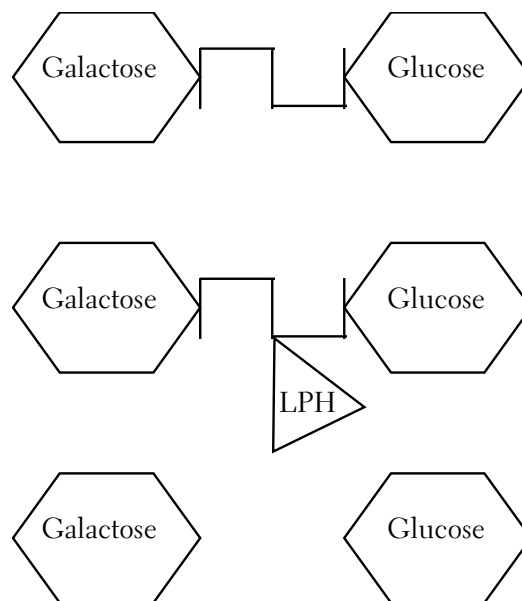


Figure 2.1. Lactose, which is made up of galactose and glucose molecules joined by β 1,4-glycosidic linkage, is hydrolysed by lactase (LPH).

Lactase has two properties: lactase activity (β -galactosidase EC 3.2.1.23) and glucosidase activity (EC 3.2.1.62). The lactase site splits lactose and cellobiose, and some animal enzymes of this group also hydrolyse β -D-fucosides and β -D-glucosides. The glucosidase activity hydrolyses phlorizin, glycosylceramides and other aryl- or alkyl- β -glycosides, as reviewed by Keller *et al* (1993). However, lactose is the only substrate of significant nutritional importance.

Despite these two activities, lactase is a single polypeptide. It is synthesized as a large precursor of molecular weight, 205-245 kDa, and then processed to a mature enzyme of varying molecular weight, from 120-130 kDa in the rat to 160 kDa in the human, as shown in Figure 2.2 (Naim 1993). The variations in the molecular weights are probably due to the different glycosylation patterns in the various species (Naim 1993). The primary structure of a lactase molecule consists of 1927 amino acids in

humans, 1926 amino acids in the rabbit and 1928 amino acids in the rat (see Keller *et al* 1993). Unlike sucrase-isomaltase, which is attached to the brush border membrane from the N-terminal end of the protein chain, lactase is anchored by a short hydrophobic C-terminal segment (see Keller *et al* 1993).

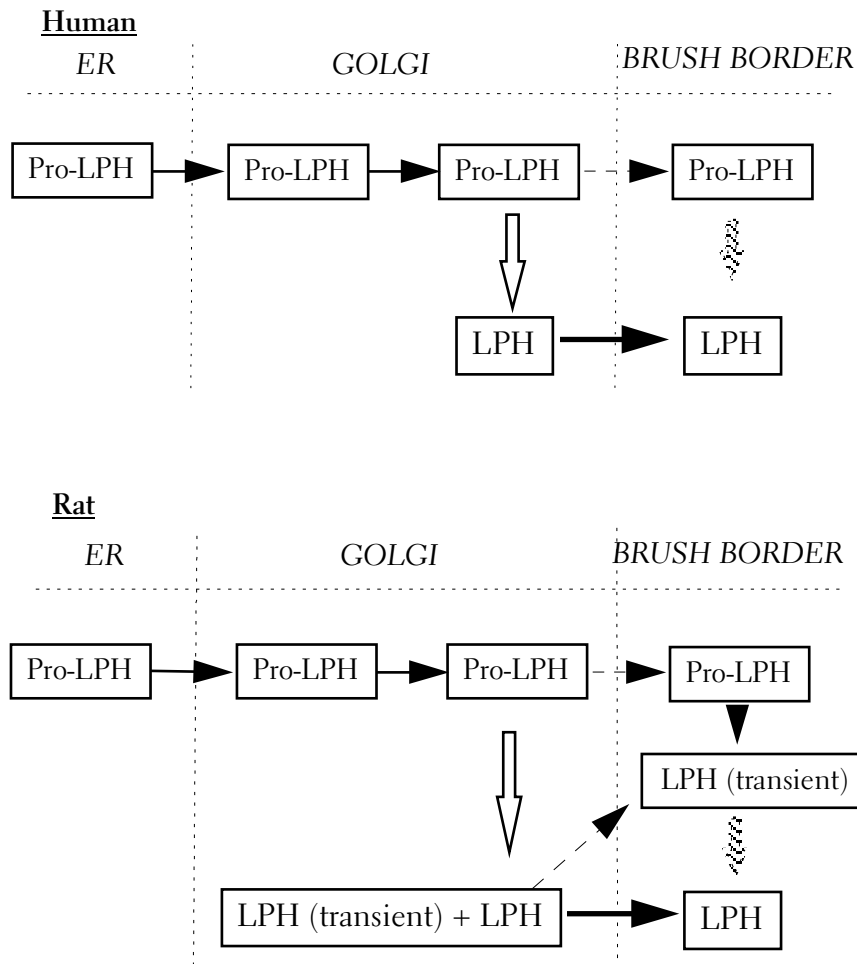


Figure 2.2. Synthesis of lactase in humans and rats. In humans, the lactase precursor (Pro-LPH) is synthesised and glycosylated in the endoplasmic reticulum (ER). In the Golgi it is converted to a complex glycosylated molecule and is cleaved by trypsin-like proteases (\Downarrow). The final lactase (LPH) is transported to the brush border membrane. Some Pro-LPH molecules may not be cleaved and are transported to the brush border membrane and may be cleaved there by luminal proteases (\Downarrow). In the rat, Pro-LPH has two potential cleavage sites and may be cleaved to transient or final LPH in the Golgi. In the brush border membrane, uncleaved Pro-LPH and transient LPH are further cleaved to LPH (Naim 1993).

Lactase exists only in mammals, but in other living organisms there are compounds which are related to it. Lactase-related β -glycosidases and phospho- β -glucosidases have been found even in eubacteria and fungi (see Keller *et al* 1993).

2.1.1. Endogenous regulation

The relative activity of lactase is genetically determined and controlled by two alleles at a single gene locus (Swallow and Harvey 1993). The lactase gene has been located on chromosome 2q (Kruse *et al* 1988, Harvey *et al* 1993). In adults genetic polymorphism determines a high or low messenger RNA (mRNA) expression and activity (Harvey *et al* 1995). The mechanism for this polymorphism is not known but it is presumed that sequence differences in the gene determine whether or not lactose is down regulated (Wang *et al* 1998). Persistence of lactase (as measured by a lactose tolerance test) is dominant to non-persistence (hypolactasia) (Sahi *et al* 1973, Flatz 1987, Swallow and Harvey 1993). The activity in jejunal mucosa is less than 7 units/g protein in lactase nonpersistent subjects, and over 35 units/g protein in lactase persistent subjects, as reviewed by Arola (1994). The level of lactase activity in the heterozygotes is approximately half that of the persistent homozygotes (Swallow and Harvey 1993) but sufficient in most cases to hydrolyse fully a 50 g lactose load in a lactose tolerance test.

Most of the lactase activity in the rat develops late in gestation and stays at a high level from just before birth till the time of weaning. After that, within a few weeks the activity declines to the low levels of adulthood (Büller *et al* 1989). In the adult rat lactase mRNA and protein are abundant only in the middle segment of the intestine and are barely detectable in the duodenum and the ileum (Rings *et al* 1993). Studies on rats have suggested that intestinal lactase activity further declines in old age (Lee *et al* 1997).

The situation in humans is more complex. In the proximal small intestine of adults with high lactase activity, lactase protein and activity are present in all villus enterocytes. In hypolactasia lactase is patchily distributed on the villus enterocytes, and even enterocytes in the very same villus differ from each other (Maiuri *et al* 1992, Rossi *et al* 1997).

The genetically programmed down-regulation of the lactase gene is detectable in children from the second year of life (Wang *et al* 1998). In the Finnish population, the usual age of the onset of the clinically significant decline of activity varies within the 5-20-year range (Sahi *et al* 1972). Several factors have been implicated as the cause of lactase decline at weaning or in human hypolactasia. The reduction of (pre)pro-lactase synthesis has been associated with adult type hypolactasia (Witte *et al* 1990, Sterchi *et al* 1990, Lloyd *et al* 1990). A slow processing of the protein has also been reported (Witte *et al* 1990, Sterchi *et al* 1990). The major control mechanism is

now thought to be at the level of mRNA (Rossi *et al* 1997, Wang *et al* 1998), but the heterogeneity of mRNA/activity ratio of lactase, even in a homogenous population, probably indicates that other mechanisms, besides transcriptional regulation, may be involved (Rossi *et al* 1997).

In lactase nonpersistence the activity of lactase in adults is about 5-10% of that found in early childhood (see Büller and Grand 1990). A very rare condition is congenital lactase deficiency (CLD) with an almost total lack of lactase (0-2% of activity of the enzyme at birth) (Savilahti *et al* 1983). CLD is part of the so-called Finnish disease heritage and the estimated incidence in the Finnish population is 1:60,000 births (Savilahti *et al* 1983). The gene locus for congenital lactase deficiency is found to be separate from but near to the lactase-phlorizin gene (Järvelä *et al* 1998).

2.1.2. Exogenous regulation

Milk and lactose

The role of milk, and lactose as a component of milk, in modifying the expression of the lactase protein and its activity has been intensely studied during the past decades. At weaning, when diet is changed from a milk-based to a mixed adult diet, the small intestine undergoes functional maturation, as shown in many experimental studies with rats. Lactase activity decreases and its longitudinal distribution is modified, while the activity of other enzymes, such as sucrase-isomaltase (EC 3.2.1.10-48), increases. Intestinal maturation in rats depends on an intrinsic ontogenic programme (Duluc *et al* 1994) and on hormonal changes at weaning (Paul and Flatz 1983, Freund *et al* 1991). Nutritional changes have been shown to accelerate or delay the enzymatic decline and to modify the distribution of lactase mRNA in the small intestine (Lebenthal *et al* 1973, Duluc *et al* 1992, Nsi-Emvo *et al* 1994).

Experimental studies of the role of milk and lactose can be divided into two groups according to the age and the weaning stage of the animals. There have been numerous experiments to prevent the physiological decline of lactase expression and activity, by continued nursing, or by adding lactose to the diet immediately after weaning (Table 2.1). The diet of control groups varies from conventional rat pellets to a mixed diet with other di- or monosaccharides in the place of lactose. It is impossible to compare the actual doses of lactose because of the inadequate descriptions of the methods. The length of experimental periods varies from a few extra days nursed to several months with added lactose in the diet. The conclusion, however, is quite clear: dietary lactose, no matter what its source or the length of the

test period, cannot prevent the physiological decline of lactase activity. In most of the studies, however, the reduction in activity is smaller in the lactose treated groups compared to the control groups on lactose-free diets.

Table 2.1. Effects of lactose or prolonged nursing on the expression and/or activity of lactase in preweaned experimental animals.

Species, age or weight	n	Dose and source of lactose	Length of experiment	Lactase A= activity, E=expression	Reference
Rat, Sprague-Dawley, male, age ?	23	25% lactose / corn starch in diet	6-11 wk	A +25%	Fischer 1957
Rat, Wistar, 30 - 60 d Germ-free / Conventional	44	Lactose, glucose or maltose (70.5 g / 100 g solids)	4-8 wk	A +40% compared with glucose after 30 d	Reddy <i>et al</i> 1968
Rat, Sprague-Dawley, 40-50 g	16	5-60% lactose / lactose- free	7 wk	A +	Cain <i>et al</i> 1969
Rat, Wistar, 4 wk	<100	10% lactose / lactose-free	24 ,32 and 40 wk	A +	Bolin <i>et al</i> 1971
Rat, albino, 2 wk	84	Lactose / glucose 8% of total energy	2-16 wk	A ±	Sriratanaban <i>et al</i> 1971
Rat, age ?	40	Prolonged nursing / conventional lactose-free rat diet	2-4 wk	A +120%	Lebenthal <i>et al</i> 1973
Rat, Wistar, 1 d	40	30% lactose / glucose + galactose	2-11 wk	A ±	Leichter 1973
Rat, Sprague-Dawley, 21 d	30	Powdered cow's / rat's milk 20% of diet (w/w) (1.6 - 2.5 g milk /day) / no milk	0-10 wk	A ±	Becker <i>et al</i> 1974
Pig, Chester White / Hampshire, 5 mo	48	30% lactose (from dried whey) / corn starch in diet	3 wk	A ±	Ekström <i>et al</i> 1976
Rat, 0-27 d	8	Prolonged nursing	4 wk	A & E +25%	Sakuma <i>et al</i> 1996
Rat, Sprague-Dawley, 0-27 d	<50	Prolonged nursing	4 wk	A & E ±	Motohashi <i>et al</i> 1997

+ = Increase ; ± = no change / effect

The number of experimental studies in which weaned adult animals were treated with a lactose-containing diet is smaller than that of experiments with preweaned animals (Table 2.2). The aim of these studies was to increase the low adult level of lactase activity in order to regain the higher level of sucklings. The conclusion of these studies was that dietary lactose can increase lactase activity to a level double the

normal low adult level at the most, thus being about one fifth of the activity found in suckling animals.

Table 2.2. Effects of dietary lactose on expression and/or activity of lactase in weaned experimental animals.

Species, age /weight	n	Dose of lactose (g / day)	Length of experiment	Lactase activity	Reference
Rat, Wistar, female, 3 - 5 mo	34/ 55	Lactose / glucose 30% of diet	2-31 wk	+ >50% in jejunum with lactose and + 50% with glucose	Bolin <i>et al</i> 1969
Monkey, adult	7	Lactose 20% (dry weight of diet)	7 wk	+ >30% in jejunum, + 70% in ileum	Wen <i>et al</i> 1973
Rat, 2 mo	33	Dose ? goat's milk	1-3 d	+ 100% in 24 h, no change after 72 h Added progesterone injections increased activity further	Goldstein <i>et al</i> 1974
Rat, Sprague-Dawley, female, 2 mo	16	40% of energy as lactose / sucrose force fed	1 wk	+ 50% with lactose compared with low-carbohydrate diet +100% with sucrose compared with low-carbohydrate, high-fat diet	Goda <i>et al</i> 1984
Mouse, Swiss albino, age ?, 32-33 g	48	Milk, fermented milk or yoghurt 30% of diet (w/w)	0.5-2 wk	+ >25% in prox and >65% in distal jejunum after 3 d with fermented milk and yoghurt ± with milk diet	Thoreux <i>et al</i> 1998

+ = Increase ; ± = no change / effect

In human studies there have been only a small number of subjects in whom lactase activity was measured from small intestinal biopsies before and after the study period. None of the studies showed any increase in lactase activity after a daily oral load of lactose with increasing doses for ten days to 12 months (Cuatrecasas *et al* 1965, Newcomer and McGill 1967, Kreusch *et al* 1969, Gilat *et al* 1972). The number of subjects participating in these studies was three, two, 50 and ten respectively.

All these experimental (Tables 2.1 and 2.2) and human studies (Cuatrecasas *et al* 1965, Newcomer and McGill 1967, Kreusch *et al* 1969, Gilat *et al* 1972) to investigate those factors affecting or controlling the physiological decrease in lactase activity after childhood showed that dietary lactose did had minor effects. The same conclusion was drawn from a different angle in a study in which suckling mice were fed by transgenic α -lactalbumin-deficient females that produced lactose-free milk (Jost *et al* 1998). The feeding pattern was thus physiological. In spite of the lactose-free milk, the level of lactase activity and the longitudinal distribution of mRNA for

lactase were unchanged compared to suckling animals nourished with normal lactose-containing milk, indicating that the effect of dietary lactose is small. There are no experiments to show how much lactase activity should increase in order to significantly reduce gastrointestinal symptoms in lactose intolerant subjects.

Other dietary components

The possible effect of other dietary carbohydrates on lactase has mainly been studied with rat models. A high carbohydrate diet for one week, with corn starch forming 70% of the total energy and with no added lactose, increased disaccharidase activity (lactase, maltase and sucrase) in the rat jejunum (McCarthy *et al* 1980, Leichter *et al* 1984). Messenger RNA levels for lactase were elevated in rats fed a sucrose-enriched diet (Goda and Takase 1994) within 12 h of a carbohydrate intake (Goda *et al* 1999). This rapid accumulation of mRNA is thought to suggest that dietary sucrose enhances the efficiency of the transcription of the lactase gene.

Oat saponins (a mixture of avenacosides A and B) *in vitro* inhibited lactase activity (Önning and Asp 1995). This was not shown in *in vivo* studies in rats, probably due to far lower concentrations of saponins in their diets (Önning and Asp 1995). Saponins are thought to combine with the lactase enzyme and in this way to reduce the activity. Concentrations of saponins found in oat products probably have no effect on lactase activity in humans. Tannins have also been shown to reduce the lactase activity in rats fed on a diet containing 4 g tannins /kg body weight (Thomsen and Tasman-Jones 1982).

As shown by McCarthy *et al* (1980), a high fat diet is connected with low disaccharidase activity. Recently it was demonstrated that the saturation of dietary fats influences the activity of intestinal disaccharidases (Kaur *et al* 1996). Diets rich in saturated fats (coconut oil) increased lactase activity in adult rats compared to the control diet (commercial rat pellets). During a polyunsaturated fat diet (corn oil) or a fish oil diet lactase was not induced compared with the control diet. In piglets (Dudley *et al* 1994) the effect of the fat saturation level was the same as in rats.

Conflicting results of the effects of alcohol intake on lactase activity have been reported. In an experimental study with adult rats after three months of ethanol consumption (30% in drinking water [v/v]) lactase activity decreased (Rodriguez-Castilla *et al* 1996). The same effect has been seen in previous *in vivo* (Baraona *et al* 1974) and *in vitro* models (Dinda *et al* 1979), and also in one human study with alcoholic men (Perlow *et al* 1977). On the other hand, there are conflicting results in

other experiments where no differences in lactase activity were found (Leichter 1987, Rodriguez-Castilla *et al* 1996). In fact, low concentrations (1-3%) increased lactase activity, at least in the epithelial cell line (Nano *et al* 1990).

Manipulating intestinal microflora by adding a probiotic (Probios®, Pioneer Hi-Bred International Ltd, Johnston, Iowa, USA) to the diet of new-born piglets increased lactase activity in the piglets at three weeks old, but in the post-weaning period the differences in activity diminished and after about three months disappeared altogether (Collington *et al* 1990). Oral treatment of adult volunteers with lyophilized *Saccharomyces boulardii* for two weeks increased lactase activity, as measured by intestinal biopsy, by almost 80% compared to the basal activity (Buts *et al* 1986). The same effect was seen in adult rats on both viable and killed *Saccharomyces boulardii* doses for five days (Buts *et al* 1986). No morphological alteration of the intestinal mucosa was found either in humans or in rats. The possible reason for this yeast-induced increase could be the stimulation of protein synthesis at a translational level or the interference of proteolytic events of mature lactase by yeast cells, as Buts *et al* (1986) concluded.

Reports from the field of dietary components that may modify lactase expression and activity are fragmentary and unconsolidated. Only the surface of the subjects seems to have been scratched. Dietary patterns are changing, and the actual role of such factors as the whole diet or new dietary elements and functional foods on the capacity of lactase to digest lactose has nor been examined. There is a definite need for further investigation.

Stress factors

Lactose maldigestion may be caused by several diseases associated with injury in the small intestinal epithelium, such as chronic inflammatory bowel disease and Crohn's disease; infectious gastroenteritis, whether of viral (rotavirus), parasitic (giardiasis) or bacterial origin; and immunorelated injury such as sensitivity to gluten or immunodeficiency syndrome (HIV), as reviewed recently by Ushijima *et al* (1995) and Gudmand-Høyer and Skovbjerg (1996). A disease-induced decrease in lactase activity is usually temporary and can occur at any age. This is called *secondary hypolactasia* or *secondary lactose maldigestion*.

As seen above, lactase activity is depressed in celiac disease. The small intestinal mucosa from patients with celiac disease in remission synthesizes brush border membrane hydrolases like a normal ('healthy') mucosa. When challenged with

gluten at a standard dose of 0.5 g/kg/day for one month, the tissue showed only slight mucosal damage, but the biosynthesis of brush border membrane hydrolases was reduced to the same level as in untreated celiac disease (see Lentze *et al* 1991). The lactase enzyme is said to be one of the slowest enzymes to recover (see Lentze *et al* 1991).

Lactase activity in adult rats has been increased by starvation for 48 h (Freund *et al* 1989) and 72 h (Leichter *et al* 1987). However, in obese lactose persistent human subjects, fasting resulted in the reduction of lactase activity (Knudsen *et al* 1968). Feeding lactose or glucose after a two-week fast in a two-subject trial did not increase lactase activity to the pre-fast level (Knudsen *et al* 1968). There seem, however, to be no differences in lactase activity/g mucosal protein between genetically obese mice (C57BL/6Jobob) and their lean controls, though there was difference in sucrase activity (Flores *et al* 1990). Total lactase activity was greater in obese mice because of their greater intestinal mass.

Drugs

The field of drug interactions on lactase expression and activity has scarcely been explored. Theoretically, any drug that impairs mucosal function or modifies its structure may have an effect on lactase expression and/or activity.

It has been common knowledge since the late 50s that the broad spectrum antibiotics such as neomycin, oral chlortetracycline and chloramphenicol reduce lactase activity (Faloon *et al* 1958, Sharma and Majudmar 1970). In a more recent study a low dose (i.p. 0.25 mg/g body weight) of actinomycin, which inhibits the transcription of genes, slightly increased lactase activity, but a high dose (i.p. 1.5 mg/g body weight) reduced this activity in hamsters *in vitro* (Andres *et al* 1985). A sucrose-induced increase in lactase mRNA can be reversed by the injection of actinomycin D (50 µg/kg body weight) in rats (Goda *et al* 1999).

Derivates of 1-deoxynojirimycin and acarbose, which are α -glucosidase inhibitors, strongly inhibit sucrase activity without significantly affecting lactase (Lembcke *et al* 1985, Samulitis *et al* 1987). These types of α -glucosidase inhibitors have been developed for the treatment of metabolic and gastrointestinal disorders such as diabetes, obesity and the dumping syndrome (see Berger 1992).

In a series of short- and long-term experiments, Gill and co-authors showed the inhibitory effect of two widely-used histamine H₂-receptor antagonists, ranitidine (0.1

- 1 mg/kg body weight) and cimetidine (0.003-2 mg/g body weight), on lactase activity in mice *in vivo* and *in vitro* (Gill *et al* 1989, Gill *et al* 1990, Gill *et al* 1991). They speculated that this adverse effect resulted from the chemical structure of these drugs and their interaction with the lipids of cell membranes, but this needs to be confirmed.

There are few studies of other drug-induced cases of the reduction or induction of lactase. Colchicine, which was previously used to treat acute gout, administered orally (50 µg/day) reversibly halved lactase activity in rats (Hudson and Smith 1986). In organ culture, the glucocorticoid agent dexamethasone stimulated the production of lactase in rats, but this effect was totally overturned by incubating the culture with cycloheximide (0.5 g/ml), a protein synthesis inhibitor (Hudson and Smith 1986). In hamsters, however, a dose of 1.5 mg/g body weight of cycloheximide produced no change in enzymatic activity *in vitro* (Andres *et al* 1985). The immunosuppressive agent cyclosporin A retarded normal maturation of the small intestine at the end of weaning, thus retaining lactase activity longer and delaying the physiological increase of sucrase and maltase activity in the rat (Cummins *et al* 1989).

As the studies referred to above show, further research is needed into drug-based modifications of small intestine digestion and capacity to absorb nutrients. Drugs affecting intestinal motility and thus the net absorption of lactose will be discussed later.

2.2. Metabolism of lactose in the human body

In lactose-digesting subjects, after the β 1,4-glycosidic linkage between glucose and galactose has been hydrolysed by lactase, monosaccharides are actively transported through the epithelial cell. Galactose is absorbed more efficiently than glucose. Glucose enters the body glucose pool, but galactose is first metabolised to glucose, mainly in the liver (Leloir 1951) (Figure 2.3). The regulating enzyme of this pathway is UDP-galactose 4-epimerase. If galactose escapes hepatic metabolism, then it will either be metabolised by the enterocytes or be excreted in the urine (Henderson *et al* 1982). Galactose concentrations in urine are about 10 times higher than those in blood (Tengström 1968).

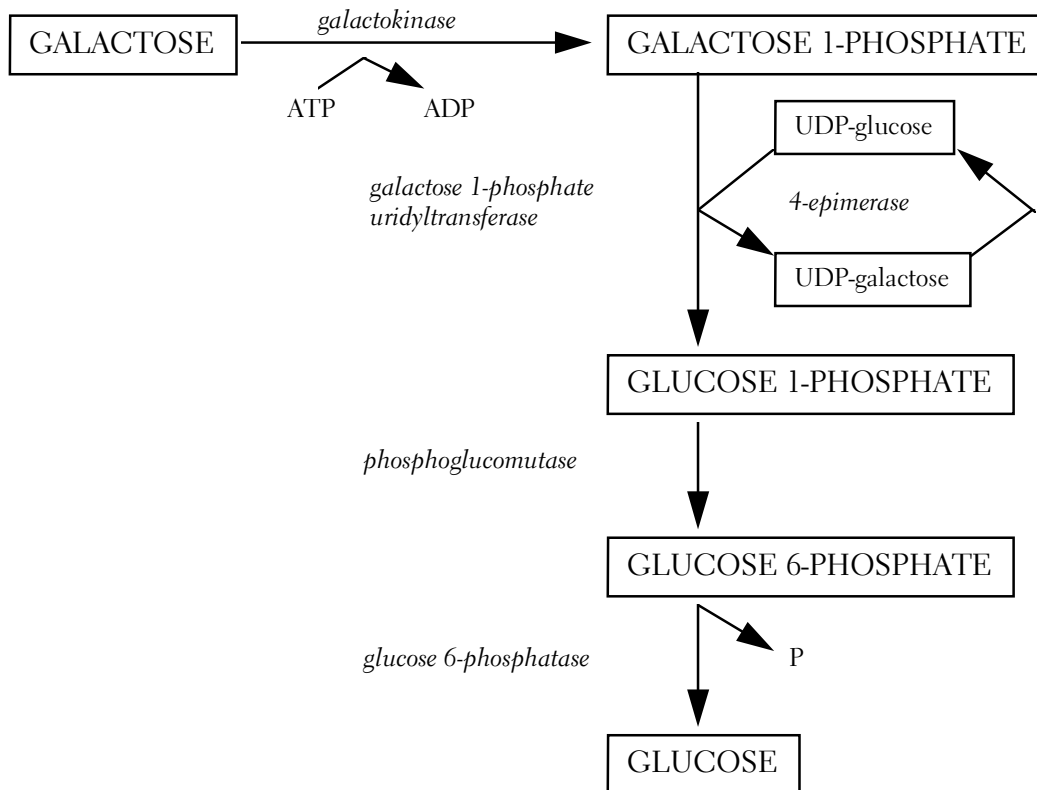


Figure 2.3. Galactose metabolism to glucose.

In lactose-maldigesting subjects immediately following a lactose challenge, an increased peristalsis was observed in jejunoscopy, at the same time as the mucosa became hyperemic and edematous (Banai *et al* 1984). Increased quantities of unhydrolysed lactose are present in the distal small intestine and the colon. The result of this is a significant osmotic pressure. Water and electrolyte secretion into the lumen increases. This osmotic flow will continue until equilibrium is reached (Launiala 1968).

The human colon epithelial cells do not absorb lactose as such. The colonic flora in each person is relatively stable but differs markedly between individuals (see Arola and Tamm 1994). The factors affecting the composition and activities of the colonic flora, and which could account for inter-individual variations, are largely unknown. One preliminary study showed that high concentrations of *Escherichia coli* tended to be associated with gas production in lactose maldigesters (Rautio *et al* 1999). Colonic bacteria, some of which have β -galactosidase activity, will metabolise a proportion of the lactose-producing short-chain fatty acids (SCFA) e.g. acetate, butyrate, propionate. Some of these acids, especially butyric acid, are absorbed by the colonic mucosa, to be used as substrate for the mucosa cells, but if the amount of SCFA exceeds absorption capacity, the residue is excreted in the stools, which will become acidic. The motor response of the colon to SCFA is complex. It seems that rather

than inducing colonic transit time, at high concentrations SCFA may inhibit colonic motility and thus participate in the adaptation of the colon to its contents (Cherbut *et al* 1997).

The bacterial fermentation of lactose also produces gases such as carbon dioxide (CO₂), hydrogen (H₂) and methane (CH₄). There are also large inter-individual variations in the activities of the flora that produces or consumes hydrogen. Excessive gas production causes abdominal distension, pain, borborygmi and flatulence. Excessive gas production and accumulation are strongly related to subjective symptoms (Hermans *et al* 1997). In a study with lactose maldigesters, the subjective symptoms did not correlate to the amount of malabsorbed lactose or to the volume or the rate of gas accumulation per se, but rather to altered intestinal transit and increased perception of luminal distension (Hammer *et al* 1996). These gases diffuse into the portal circulation and their concentrations in exhaled air will increase and can be used as an indicator of maldigested lactose.

The development of symptoms depends on the capacity of the colon to remove and use lactose and its fermented intermediary metabolic products. If this capacity is exceeded, gastrointestinal symptoms will develop. Women seem to be more liable than men to produce symptoms from similar amounts of malabsorbed lactose (Krause *et al* 1997).

The bacterial colonic adaptation of lactose maldigesters to a continued intake of milk or lactose (Johnson *et al* 1993b, Hertzler and Savaiano 1996, Briet *et al* 1997) or of totally unabsorbable carbohydrate lactulose (Flourie *et al* 1993, Florent *et al* 1985) has been reported. This may be due to changed acidity in the colon caused by unhydrolysed lactose. The metabolic production of gases is reduced with decreased acidity (Perman *et al* 1981, Holtug *et al* 1992), suggesting more effective fermentation. An increase in faecal β -galactosidase activity has also been found after continuous ingestion of lactose (Hertzler and Savaiano 1996). Rather than metabolic adaptation, Briet *et al* (1997) suggested that improved tolerance was just a placebo effect, because clinical improvement was also observed in the control group, which received sucrose.

2.3. Motility and lactose-induced gastrointestinal symptoms

2.3.1. Neural and hormonal control of gastrointestinal motility

The purpose of mixing and propulsive movements in the gastrointestinal system is to increase the contact of the luminal contents with the mucosal surface and to move the chyme along the tract. These movements are a result of the contraction and relaxation of the smooth muscle cells, which are arranged along the gastrointestinal tract longitudinally, obliquely (as in the stomach) and circularly (see Moffet *et al* 1993) (Figure 2.4).

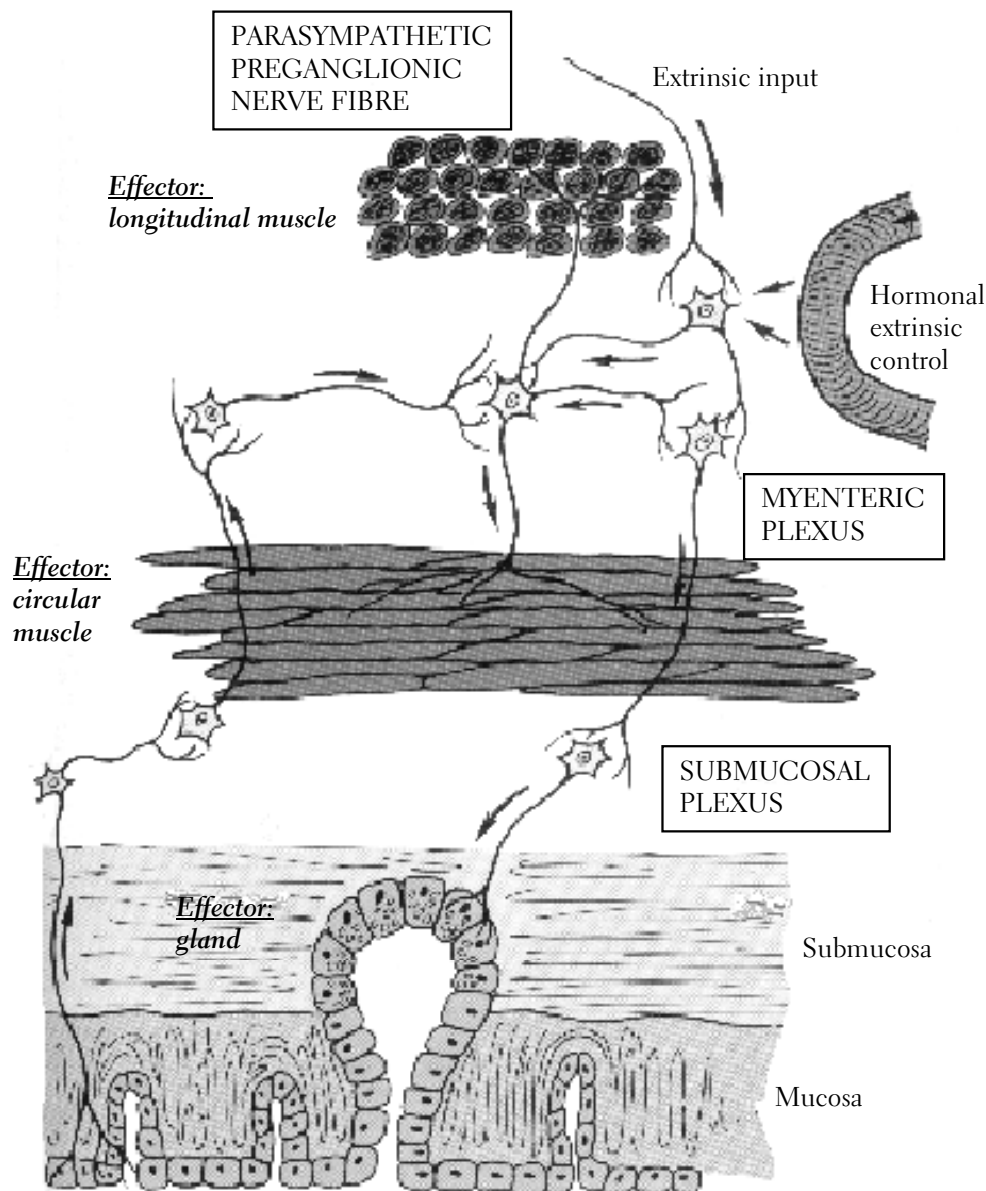


Figure 2.4. Enteric nervous system based on Moffet *et al* (1993). Even though the myenteric plexus primarily controls muscle contraction and the submucosal plexus controls secretion, they are extensively interconnected.

The main categories of movement are peristalsis and segmentation (see Moffet *et al* 1993). Peristalsis is composed of waves of contraction and relaxation of the longitudinal and circular muscle layers, resulting in the movement of chyme along the length of the tract. It is strongest in the swallowing pattern of the oesophagus, moderately strong in the stomach, and relatively weak in the intestine. Segmentation is the contraction activity of the circular muscle layer in order to mix the intestinal contents. The relationship and connection between propulsive and mixing movements is still under investigation (see e.g. Stevens *et al* 1999, Wood 1999).

Gastrointestinal motility is regulated by myogenic, neural and hormonal factors. In a fasting state the migrating myoelectric complex (MMC) passes along the intestine with the intense rhythmic contractions of the circular muscle (Szurszewski 1969, see Kunze and Furness 1999). It is followed by periods of less intense activity and rest. MMC clears the stomach and the small intestine of food remnants, intestinal secretions and other contents. It has been called the 'intestinal housekeeper' (Vantrappen *et al* 1977). The fasting motility pattern is interrupted by food and changed to continuous, irregular contractility.

The interdigestive MMC activity and the fed state activity are generated by the enteric nervous system and modified by extrinsic nerves. The enteric nervous system consists mainly of two plexuses (see Kunze and Furness 1999) (Figure 2.4). The myenteric plexus (Auerbach's) is located between the longitudinal and circular muscle layers, and the submucosal plexus (Meissner's) is in the submucosa. The enteric nervous system covers the whole gastrointestinal tract from the oesophagus to the anus. The myenteric plexus mainly controls gastrointestinal movements, and the submucosal plexus is responsible for gastrointestinal secretion and local blood flow. Even though the enteric nervous system is an independent controller of the gastrointestinal system, the stimulus from parasympathetic and sympathetic systems can further activate or inhibit gastrointestinal functions. In addition, there are some reflexes from the gut that transmit signals for long distances in the gastrointestinal tract, such as gastrocolic reflex and enterogastric reflex (see Moffet *et al* 1993).

Understanding of different neurotransmitters released by the nerve endings is increasing (see Kunze and Furness 1999, Lindberg 1999). In addition to the classical neurotransmitters acetylcholine and noradrenaline a number of other transmitters are also known. In many studies to investigate possible regulators of MMC, such as insulin, opioids, calcitonin, motilin, and nitric oxide, some of these factors have been shown to act via the enteric nervous system and others via neural connections from

the brain (see McConalogue and Furness 1994, Lindberg 1999). Nitric oxide may act as a messenger directly on the smooth muscle cells in regulating fasting intestinal motor activity (Russo *et al* 1999).

The motility effects of gastrointestinal hormones are minor to their secretory effects (Moffet *et al* 1993). Cholecystikinin is one with at least a moderate motility effect. It is secreted by the mucosa of the duodenum and the jejunum in response to dietary fats, fatty acids and monoglycerides. It releases bile into the small intestine by increasing the contractility of the gall bladder. It also moderately inhibits stomach motility and thus slows the emptying of the stomach, as does the gastric inhibitory peptide. Secretin is another gastrointestinal hormone with some slight effect on motility. It has mild inhibitory influences on most of the gastrointestinal tract motility. Motilin is released when the pH of the duodenal chyme is over 4.5. It facilitates digestion by increasing the strength of gastric contractions and the tone of the pyloric sphincter.

Visceral hypersensitivity

Visceral hypersensitivity has been recognized as being responsible for both motor alterations and abdominal pain in the pathophysiology of functional digestive disorders, particularly in the IBS (see Mayer and Raybould 1990, Mayer and Gebhart 1994, Bueno *et al* 1997). The role of afferent nerve pathways from the gut to the central nervous system have been emphasized, and it seems that in some IBS patients the pain threshold or response may be altered, and normally non-painful distension is sensed as being painful.

There are only a few studies on the sensitivity to pain of lactose intolerants. Whitehead *et al* (1990) compared tolerance to stepwise distension of a balloon in the rectosigmoid and to holding one hand in icy water, in irritable bowel patients, lactose maldigesters and asymptomatic controls. The lactose maldigesters had the lowest tolerance to icy water and the second lowest tolerance to balloon distension. On the other hand, in a recent study with previously carefully tested lactose maldigesters, an increased tolerance to ischemic pain was noticed compared to the asymptomatic healthy controls (Ylitapio 1997). In a questionnaire study these lactose maldigesters reported more frequent stomach pains than the controls, and the pain was more disturbing than to the controls. According to these studies it is possible that at least the lactose maldigesters possess reduced tolerance to visceral pain (visceral hypersensitivity), even though reports on tolerance to experimental pain produced by balloon distension or ice cold water have been conflicting.

2.3.2. Exogenous factors modifying intestinal motility and the digestion of lactose

Dietary components and nutrient content of the diet

Gastric emptying. The inhibition of gastric peristalsis, and thus the slowing of gastric emptying, by chemical or mechanical stimulation of the mucosa of the duodenum is called enterogastric inhibitory reflex. As there are receptors in the stomach and the duodenum which respond to volume, to osmotic pressure, to acids, fats, fatty acids and amino acids, and which thus control gastric emptying (see Cooke 1975, Malagelada 1990) many dietary manipulations have been carried out. By increasing viscosity or osmolality, and by increasing the energy, fat or carbohydrate content of test meals, gastric emptying has been delayed (Holt *et al* 1979, Foster *et al* 1980, Shafer *et al* 1985, Sandhu *et al* 1987, Vist and Maughan 1995).

It has been suggested that by delaying gastric emptying and thus increasing substrate mucosal contact time, the amount of undigested lactose can be reduced. This was investigated in studies where lactose was ingested in the form of milk with varying energy and fat contents (Welsh and Hall 1977, Dehkordi *et al* 1995, Vesa *et al* 1997a, Vesa *et al* 1997b), or as yoghurt (Marteau *et al* 1990, Arrigoni *et al* 1994, Mahe *et al* 1994), or with added ingredients such as chocolate (Welsh and Hall 1977, Dehkordi *et al* 1995), lactic acid bacteria (Dehkordi *et al* 1995), starch (Vesa *et al* 1997a), and fibre (Nguyen *et al* 1982), or as a part of a test meal (Solomons *et al* 1985, Martini and Savaiano 1988). All these factors are known to modify gastric emptying and thus the conclusion that lactose digestion is improved by retarding gastric emptying is justified. However, the relationship between pure lactose and gastric emptying, without the possible interference of the contents of milk or other dietary components, osmolality, viscosity or the consistence of diet has not been well documented.

The recent study of Barnet *et al* (1999), which is published only as an abstract, showed that previous exposure to lactose affected gastric emptying. The investigators speculate that this supports the inhibitory role of the intracolonic fermentation of lactose in the control of gastric emptying, which may explain frequent upper gastrointestinal symptoms in lactase nonpersistence.

Intestinal motility. Many factors that are known to delay gastric emptying also reduce intestinal motility and transit time. In many studies it is actually hard to distinguish the dietary effect on gastric emptying and on intestinal motility.

There are many studies investigating the role of dietary components in the interdigestive (postabsorptive) MMC. Food interrupts it and changes the motility pattern to a postprandial state. Even intravenously, certain nutrients, at least amino acids (Gielkens *et al* 1999), may modulate the interdigestive cycle of MMC. In one experimental study, the postprandial disruption of the MMC depended much more on the physiochemical composition of the diet than on its volume or energy content (De Wever *et al* 1978). In an other study, however, the caloric value of a meal regulated the duration of the fed state activity in the human small intestine without a 'physiological ceiling' of calories, at least for the normal caloric range per meal (220 - 1100 kcal) (Schönfeld *et al* 1997).

Of the dietary components, lipids are shown to have a stronger inhibitory effect on MMC than glucose, peptides or a mixed meal, when perfused with duodenal canula (Schang *et al* 1978) or eaten by conscious dogs (De Wever *et al* 1978, Eeckhout *et al* 1984). The infusions of nutrients (Schmid and Ehrlein 1993) or ethanol (Charles and Phillips 1995) in the proximal jejunum of dogs showed the same kind of change in the motility pattern as infusions in the upper parts of the intestine.

Coffee is said not to promote intestinal motility, as reviewed by Boekema *et al* (1999). Even if caffeine perfusion studies resulted in a net jejunal and ileal fluid secretion, no effect on small bowel transit time could be observed (Wald *et al* 1976). Nevertheless, Aranda-Michel and Giannella (1999) advise anyone suffering from diarrhoea to avoid caffeine-containing products because caffeine increases cyclic AMP levels and thus promotes the secretion of fluid, and may worsen the diarrhoea.

In the late postprandial state, the lower small intestine also regulates the proximal gastrointestinal motor function. In humans, intra-ileal perfusions with carbohydrates (Layer *et al* 1990, Gröger *et al* 1991, Layer *et al* 1993) or fats (Layer *et al* 1990, Layer *et al* 1993), simulating the late postprandial state, induce changes in the motility pattern of the intestine from the fed state to the interdigestive state, by activating MMC. These results are interesting because some of the carbohydrates, such as lactose, may be malabsorbed and thus reach the lower parts of the intestine under normal physiological conditions. However, in an experimental study with dogs, carbohydrate infusion of starch and glucose in a ratio of 3:1 in the proximal colon did not affect intestinal motility (Tohno *et al* 1995).

A very interesting study would be the intra-ileal perfusion of lactose and the subsequent measurement of its possible action on gastric emptying and upper gastrointestinal motility. In the study of Barnet *et al* (1999) this model is partly tested,

but without the measurements of intestinal motility. There may also be a connection, other than the osmotic diffusion of water, between undigested lactose in the contents of the proximal intestine and the motility of upper parts of the intestine.

The consistency of the diet affects not only gastric emptying but also intestinal motility. In healthy humans the liquid part of a test meal (polyethylene glycol PEG 4000) appeared in ileal aspirates 1-2 h postprandially and always earlier than the solid part of the test meal (beans) (Kerlin and Phillips 1983). Ileal flow was shown to increase postprandially and to remain at a high level for at least 3 h.

In conclusion, dietary contents modify gastric emptying and alter intestinal motility on the whole length of the small intestine. The role of dietary delay on gastric emptying and lactose digestion has been well demonstrated. The effect of lactose on MMC and thus on intestinal motility has not been studied.

Intestinal inflammation

In many intestinal inflammatory diseases in which symptoms resemble those found in lactose intolerance, the endogenous synthesis of prostaglandins increases (see Hawkey and Rampton 1985, Rask-Madsen 1986). Prostaglandins are synthesised via the cyclo-oxygenase pathway (constitutive COX-1 and inducible COX-2) from arachidonic acid. They stimulate the contraction of the gastrointestinal smooth muscle and provoke many inflammatory responses such as vasodilation, vascular permeability and hyperalgesia (see Ooms and Degryse 1986, Rask-Madsen 1986, O'Loughlin *et al* 1991, Barrett and Bigby 1995).

If lactose-induced gastrointestinal symptoms are, at least partly, caused by local intestinal inflammation, the possible increase in the endogenous synthesis of prostaglandins after an oral load of lactose should be prevented by the inhibitors of prostaglandin synthesis and thus should reduce gastrointestinal symptoms. This has been investigated in only a few studies, with conflicting results. Premedication with 900 mg acetylsalicylic acid did not reduce lactose-induced symptoms in 12 lactose maldigesters (Flatz and Lie 1982). In previous studies, however, the inhibition of prostaglandin synthesis by acetylsalicylic acid (900 mg), indomethacin (25 mg), or ibuprofen (400 mg) reduced symptoms caused by incompatible food in three out of six patients (Buissert *et al* 1978). In two case reports, Lieb (1978, 1980) describes how a dose of 975 mg acetylsalicylic acid removed milk- and coffee-induced gastrointestinal symptoms.

Nitric oxide is another mediator which has been connected with inflammatory intestinal diseases. The substrate for the synthesis of this gaseous mediator is L-arginine, and its formation is catalysed by nitric oxide synthases (NOS). It modifies normal intestinal motility as described above as well as in an inflamed intestine such as in cases of Crohn's disease (Boughton-Smith *et al* 1993). The enhanced production of nitric oxide has been found in inflammatory-induced tissues (see Stark and Szurszewski 1992, Lefebvre 1995). The inducible form of NOS (iNOS) seems to protect the intestine from inflammatory injuries (McCafferty *et al* 1997). Prostaglandins and nitric oxide, both mediating the normal and inflamed motility of the intestine, seem even to co-operate, at least in regulating the immune response to injury (see Wallace 1996). The possible role of nitric oxide in lactose-induced symptoms has not been studied, as far as we know.

To conclude, prostaglandins and nitric oxide mediate inflammatory responses in the intestinal tract. The possible local intestinal inflammation caused by unhydrolysed lactose, and the role of prostaglandins and nitric oxide in the development of gastrointestinal symptoms, both need to be investigated in more detail.

Emotional factors

The connection between the severity of gastrointestinal symptoms experienced in lactose intolerance and stressful life events has not been properly clarified. However, symptoms related to the passage of food through the gastrointestinal tract, such as abdominal pain and diarrhoea, are among the most commonly reported effects of acute life stress in patients with irritable bowel syndrome (IBS) (see Stam *et al* 1997). The symptoms of lactose intolerance resemble those of IBS, and on the basis of symptoms alone it is difficult to distinguish between these two states.

Patients with IBS often have exaggerated responses of gut motility in the small intestine (Camilleri *et al* 1989) and the colon (Fukudo *et al* 1993) in stressful situations. Colonic motility has been shown to increase after a stressful test, both in healthy controls and in IBS patients. Repeated tests, however, increased colonic motility only in the IBS patients (Narducci *et al* 1985). Cognitive therapy studies suggest that stress management procedures can alleviate the symptoms of IBS, including diarrhoea (Camilleri *et al* 1989).

The possible role of stressful life events and the expression of a changed intestinal motility pattern have been studied in healthy young men playing video games (Ditto *et al* 1998). The experimental stress reduced intestinal mean transit time, measured

by a lactulose breath hydrogen test, by about 25 min compared with a relaxed situation. This was correlated with change in an index of cardiac sympathetic activity (Ditto *et al* 1998). However, psychological stress in medical students during final examinations did not change the orocecal transit time measured by an excretion of lactulose breath hydrogen test (Harris and Martin 1994). Gastrointestinal symptoms were also unchanged by the stress, except for abdominal pain, which increased. In healthy subjects gastric antral motor activity recorded with real-time ultrasonography was reduced by mental stress, but not in patients with functional dyspepsia who had reduced motility at baseline (Hveem *et al* 1996).

It is possible that the responses of gastrointestinal motility may vary depending on the stressor, as suggested by Rao *et al* (1998). They found that both psychological stress induced by a dichotomous listening test and physiological stress induced by keeping a hand in cold water enhanced colonic motor activity. Psychological stress affected stool propulsion and colon transit, whereas in healthy humans physical stress was associated more with delayed gut transit time (Rao *et al* 1998).

In experimental studies with dogs, acoustic stress for 1-2 h had no effect on fasting jejunal MMC, but prolonged the pattern of irregular contractions after a meal (Gue *et al* 1987, Gue *et al* 1989). When laboratory rats were partially or totally immobilised (restraint stress) their motility patterns were changed both in a fasted state and after a meal (Wittmann *et al* 1990). In the colon the changed motility pattern continued for three days. In rats colonic motility seems to be a more sensitive indicator of stress than the motility of the small intestine (Stam *et al* 1995). Autonomic pathways are thought to mediate stress effects on intestinal transit and motility (see Stam *et al* 1997), although responses are individual (Stam *et al* 1999).

To conclude, stressful stimuli modify visceral sensory and motor responses, a fact which is based mainly on experimental studies and the studies of irritable bowel disease. In IBS patients, stress increases the severity of gastrointestinal symptoms. Even though the pathophysiology of lactose intolerance differs from that of IBS, the symptoms do not, and thus it can be assumed that a stressful life also impairs, at least to some extent, the gastrointestinal symptoms of lactose intolerance.

Drugs

Drugs which either deliberately or as an adverse-effect influence gastrointestinal motility may also have an influence on the digestion and absorption of lactose. Gastrointestinal prokinetics increase gut wall contractions, enhancing propulsive

movements. They are used for the treatment of upper gastrointestinal disorders such as gastro-oesophageal reflux disease (see Horn 1996, Tonini 1996). Metoclopramide and cisapride are considered 'old' prokinetics. Metoclopramide has been used in the treatment of vomiting of different etiologies and for a wide range of functional and organic gastrointestinal disorders (see Koch-Weser 1981). Rather than having an antagonist effect on dopamine D₂ receptors, metoclopramide seems to act via 5-HT₄ (serotonin) receptors, to stimulate gastric emptying and enhance lower oesophageal sphincter pressure (see Tonini 1996). Cisapride is a serotonin (5-HT₄) agonist, enhancing the release of acetylcholine and thus increasing lower oesophageal sphincter pressure and accelerating gastric emptying (see Horn 1996, Tonini 1996).

New prokinetic drugs are now being used in clinical studies. The 5-HT₄ agonist prucalopride (R093877) has been found to accelerate colonic transit (Bouras *et al* 1999). Erythromycin is a macrolide antibiotic which disturbs gastrointestinal motility (see Alvarez-Elcoron and Enzler 1999). Its new structural analogues avoiding antibacterial properties are classified as motilin agonists and have been tested for inducing gastric contractions and reducing the motility of the small intestine (e.g. Sarna *et al* 1991, Caron *et al* 1996).

Some other antibiotics, such as the amoxicillin-clavulanate combination, may also in some cases disturb gastrointestinal motility, as shown by Caron *et al* (1991). Rather than disturbing the normal intestinal microflora, Caron *et al* speculated that this changed motility was due to the release of an intraluminal mediator such as motilin or to direct interaction with some aminobutyric receptors in the myenteric plexus. In addition to these, motility effects have been reported with somatostatin analogues (Dueno *et al* 1987, see Bueno *et al* 1997), the calcium channel blocker nifedipine (Konrad-Dalhoff *et al* 1991), gastric acid suppressants ranitidine, famotidine, nizatidine and omeprazole (see Bortolotti 1999), and the prostaglandin E₂ analogue enprostil (Nicholl *et al* 1989).

As an adverse effect, many drugs may induce constipation. These drugs include agents from many categories such as analgesics, antacids (calcium and aluminium), anticholinergics, antidepressants, antihypertensives, diuretics, iron preparations, and neuroleptics (Tedesco *et al* 1985).

Laxatives enhance bowel movements by stimulating motility, retaining intraluminal fluid by osmotic mechanism, or by increasing luminal contents. Recent studies have shown that the pharmacological effects of some laxatives are mediated by prostaglandin release, increased mucosal permeability, epithelial injury, and the

increased release of nitric oxide (see Izzo *et al* 1998). In addition to interest in the role of NO in the mechanisms of laxatives and in other effects of NO, a great deal of attention has been paid to the investigation of NO and the inhibitors of its synthesis in regulating gut motility (see Tonini 1996). The inhibition of NOS may, however, result in undesired side-effects because of the widespread targets of NO.

Antidiarrhoeal agents reduce the number of bowel movements and diminish fluid loss. The most useful agents for slowing intestinal transit in acute diarrhoea are opiate derivatives such as loperamide, diphenoxylate and bismuth subsalicylate (see Aranda-Michel and Giannella 1999). New peripherally-acting opiates such as fedotozine have been developed and tested as antidiarrhoeal agents and for the treatment of visceral hyperalgesia (Read *et al* 1997, Delvaux *et al* 1999).

Motility and sensory disturbances play an important role in the pathophysiology of the symptoms of IBS. In fact, the drugs used to alleviate acute symptoms of IBS are an overall collage of those agents affecting gut motility (see Bueno *et al* 1997, Paterson *et al* 1999). Constipation may be relieved with laxatives and diarrhoea with antidiarrhoeal agents. Attempts to resolve pain have been made by using drugs that reduce gut spasm, such as anticholinergics and smooth muscle relaxants, or prokinetics, to normalise the motility pattern of the intestine (see Paterson *et al* 1999), even if a clear relationship between symptoms and gastrointestinal motility has not been demonstrated (see Klein 1988). Potential drugs for modifying visceral hyperalgesia include opioid agonists (especially κ agonist fedotozine), serotonin antagonists (5-HT₃ and 5-HT₄ receptor antagonists), and possibly somatostatin and somatostatin analogues (see Farthing 1998). No medication has been shown to alleviate abdominal bloating or distension (see Paterson *et al* 1999).

There is only one published study, as far as we know, that describes the influence of a motility-affecting drug on the absorption of lactose. Lactose maldigesters were pre-treated with 8 and 12 mg loperamide on different days and lactose absorption was measured by using a breath hydrogen test (Szilagyi *et al* 1996). The loperamide-induced prolongation of intestinal transit time by about 30 min improved tolerance to lactose, as measured by reduced breath hydrogen concentration and diminished gastrointestinal symptoms. These results should, however, be confirmed in a double-blind placebo-controlled study.

To summarise, the primary cause for lactose intolerance, an unbalanced intake of lactose in relation to the activity of lactase, cannot be treated, at least so far, with motility-reducing or other drugs. Many drugs, however, have a prokinetic or

antimotility action, and their use may either worsen or relieve the symptoms of lactose intolerance.

2.4. Diagnosis of lactose maldigestion and related gastrointestinal symptoms

A variety of methods has been used for diagnosing lactose maldigestion. Precise standards do not exist. An intestinal biopsy is the only direct way of measuring lactase activity. This cannot, however, be regarded as a standard test for measuring tolerance to lactose, because mucosal lactase activity varies along the small intestine (Maiuri *et al* 1992, Rossi *et al* 1997) and thus lactase activity in one biopsy, from a restricted part of the small intestine, does not necessarily represent the total lactase activity of the whole small intestine. There seem not to be consistent cut-off values for hypolactasia. Varying activities between 5-18 U/g mucosal protein have been proposed (see Brummer *et al* 1993). The measurement of lactase activities from a biopsy is laborious, unpleasant for the patient, and can be performed only in hospitals.

In Finnish health care centres, measuring blood glucose concentration after an oral load of 50 g lactose is the most commonly used method for testing tolerance to lactose (Peuhkuri *et al* 2000a). At regular intervals, usually of 20 min, for up to 1-2 hours after the intake of a lactose solution, blood samples are taken and the glucose concentration is determined. A dose of 50 g lactose (in small children 2 g/kg body weight) is necessary for the blood test (see Arola 1994). Most laboratories regard a glucose increase of less than 1.1 mmol/l as an indication of lactose maldigestion. The test is simple to perform and the equipment needed is found in every clinical laboratory. However, the sensitivity and specificity of the test have been questioned because of the great inter-individual variations in gastric emptying and glucose metabolism (see Newcomer *et al* 1975).

Some laboratories use a lactose tolerance test with ethanol to improve the sensitivity of the test. Usually there are no measurable amounts of galactose in the blood or the urine, because absorbed galactose has been quickly metabolised to glucose in the liver (see Figure 2.3). In the tolerance test this change into glucose is inhibited with ethanol (50-150 mg/kg), thus enabling the measurement of the galactose concentration in the blood or the urine. 40 min after the ingestion of 50 g lactose, one capillary blood sample is taken. A galactose concentration below 0.3 mmol/l is considered an indication of lactose maldigestion (Isokoski *et al* 1972). Many scientists have questioned the need to use ethanol for measuring galactose in the blood and the urine in lactose tolerance tests, and modified methods without ethanol ingestion

have been described (Arola 1988b, Grant *et al* 1989, Buttery and Ratnaïke 1995). In a recent study, in 3-hour pooled urine samples, the sensitivity of a lactose tolerance test without ethanol was 100% and specificity was 94%, (Alvarez-Coca *et al* 1996).

The measurement of a breath hydrogen response after an oral load of lactose is based on the principle that unhydrolysed lactose is fermented by the colonic microflora, producing hydrogen and methane. Gases are diffused into the blood circulation and excreted in expired air (Levitt 1969). A dose of 25 g (in children 1 g/kg body weight) has recently been demonstrated as being sufficient in a breath hydrogen test (see Hamilton 1998). This dose produces almost the same response in breath hydrogen concentration as the larger dose of 50 g. The hydrogen concentration is analysed either by gas chromatography or by an electrophysical sensor (Bartlett *et al* 1980). Breath samples are taken at regular intervals of 30 min after the ingestion of lactose until the hydrogen concentration exceeds the baseline value by at least 20 ppm, indicating lactose maldigestion, or for up to three hours (see Hamilton 1998). A recent study by Karcher *et al* (1999) suggested that a better cut-off value would be 10 ppm because the patients with only moderately increased hydrogen excretion (10-20 ppm) may not be lactose deficient at all, as Karcher *et al* speculated.

The simultaneous measurements of breath methane may increase the reliability of a tolerance test because some types of methanogenic bacteria in the colon convert colonic hydrogen to methane. Bjørneklett and Jenssen (1982) found that 44% of 120 healthy subjects were methane producers. Thus, there seem to be hydrogen producers, methane producers and those who produce both hydrogen and methane. There do not seem to be even rough estimations of the number of pure methane producers, i.e. the subjects who produce no hydrogen at all. If breath methane alone is analysed, an increase of about 12 ppm is considered an indication of lactose malabsorption without reference to the hydrogen response (see Hamilton 1998). The breath methane test alone has lower sensitivity and specificity than the breath hydrogen test and cannot replace the latter (Myo-Khin *et al* 1999).

The lactose breath hydrogen test is considered to be fairly reliable in detecting lactose maldigestion (see Arola 1994, Hermans *et al* 1997, Hamilton 1998). There are some factors that may possibly increase breath hydrogen excretion and may thus reduce the reliability of the test, as reviewed by Arola (1994) and Hamilton (1998). The influence of these factors is reduced when labelled lactose is used. After the ingestion of ¹³C-labelled lactose (non-radioactive isotope), the digestion and absorption of lactose can be detected as the cumulative concentration of labelled

CO₂ in the breath (Newcomer *et al* 1975, Hiele *et al* 1988, Vantrappen *et al* 1992). Nowadays there is no risk of radiation, as there was in an earlier modification of the use of a ¹⁴CO₂-labelled malabsorption test (Salmon *et al* 1969, Sasaki *et al* 1970). In a recent study by Koetse *et al* (1999), the sensitivity and specificity of a combined ¹³CO₂/H₂ breath test were 85% and 65% respectively.

The relationship varies between these tolerance tests and gastrointestinal symptoms. There is a stronger association between the amount of hydrogen excreted and gastrointestinal symptoms, whereas that between the increase in glucose concentration and the symptoms of lactose is less evident (Newcomer *et al* 1975, Hermans *et al* 1997, Teuri *et al* 1999). The blood glucose test is the most commonly used in Finland (Peuhkuri *et al* 2000a).

2.5. Current methods of reducing lactose-related gastrointestinal symptoms

It seems probable that the development of gastrointestinal symptoms caused by maldigested lactose depends on the function of the whole gastrointestinal tract, not just on the digestive and absorptive capacity of the upper small intestine.

Firstly, according to several studies, small doses of lactose, of up to 10-12 g, are tolerated by many lactose maldigesters without gastrointestinal symptoms (Suarez *et al* 1995, Vesa *et al* 1996). If a daily dose of lactose is divided into several small portions, to be ingested throughout the day, the possibility of symptoms will decrease. This will lead to an improved balance between the hydrolysing capacity of the enzyme and the load of ingested lactose.

Secondly, other components of food ingested with lactose, as discussed above, delay gastric emptying and intestinal motility, and this has been shown to improve tolerance to lactose. If lactose is ingested in a reasonable quantity as a part of meal, the possibility of symptoms, and/ or their severity, may decrease.

Thirdly, it may be possible in the future to reduce lactose-induced gastrointestinal symptoms simply by supporting the function of the colon and its bacteria.

Finally, the possibility of secondary lactose intolerance, induced either by gastrointestinal or other diseases or by their treatments, drugs, a stressful lifestyle or any other factor, should not be overlooked.

3. Aims of the study

The aim of this study was to clarify factors which may improve, pharmacologically or otherwise, the digestibility of lactose in order to reduce lactose-induced gastrointestinal symptoms.

More specifically, the present study had the following aims:

1. To prove the accuracy and usefulness of a simple, portable breath hydrogen analyser (Micro H₂) in oral lactose tolerance tests (I).
2. To evaluate whether the gastric emptying rate (II) or the temperature of the test solution (III) affects the development of lactose-induced symptoms.
3. To investigate the possible role of acute inflammation and inflammatory mediators, such as prostaglandins and nitric oxide, in the development of lactose-induced symptoms (IV).
4. To assess whether, in an experimental model, lactase expression and activity are inducible by dietary lactose in the intestinal epithelium (V).

4. Subjects, study designs, materials and methods

4.1. Human studies (Studies I - IV)

4.1.1. Subjects and study designs

All the subjects were adult volunteers, aged from 18 to 66 years. They were recruited mainly among the staff of the Institute of Biomedicine of the University of Helsinki, Finland, and their families and friends. In Study I subjects were also recruited by a local internet news group [rfc977] (puhe.terveys) which is mainly read by students and staff from Helsinki University of Technology, and through announcements among staff and students of the University of Helsinki. Additional subjects for Study IV were recruited by inviting all those who had participated in previous lactose intolerance studies (Vesa *et al* 1996, Teuri *et al* 1999) and had shown positive test results, indicating hypolactasia.

In Study I the inclusion criteria were either diagnosed lactose intolerance or a strong personal suspicion of it. Healthy subjects were invited as controls. All the volunteers, lactose intolerants as well as healthy controls, followed the whole test procedure of the oral lactose tolerance test. Of the volunteers in Studies II-IV, subjects were included if they showed positive test results, indicating hypolactasia, in two out of three lactose tolerance tests (the Golden Standard, see below). The tests used in Studies I - IV are shown in Table 4.1. Ten volunteers were excluded because they proved to be lactose digesters (II-IV). The final study group consisted of 72 subjects. Some of them volunteered for more than one study, so the total number of subjects in studies I-IV was 89 including those who withdrew or were excluded.

None of the subjects had received antibiotic (I-IV) or NSAIDs (IV) treatment during the 2 weeks preceding the studies, and they did not use any medication during the studies themselves. The subjects were interviewed by a physician, an authorized nutritionist, or a laboratory nurse, and they filled in a health questionnaire. Only those subjects who were seen to be healthy, with no recent history of gastrointestinal disturbances other than lactose intolerance, were included.

The first human study (I) was carried out as a method comparison study comparing two diagnostic instruments. The other human studies were carried out as randomised, double-blinded (II, IV), three - (II - III) or four-period (IV) cross-over trials with a 1-week wash-out period between the interventions. In each study in identical sessions the subjects were given lemon-flavoured test solutions, either 50 g

lactose or 50 g digestible disaccharide sucrose in order to compare the effects of the two, in 300 ml water. The sampling sites for the biochemical measurements of the lactose breakdown products and other biochemical indicators and the recording of gastrointestinal symptoms were carried out according to the schedule of measurements (Figure 4.1).

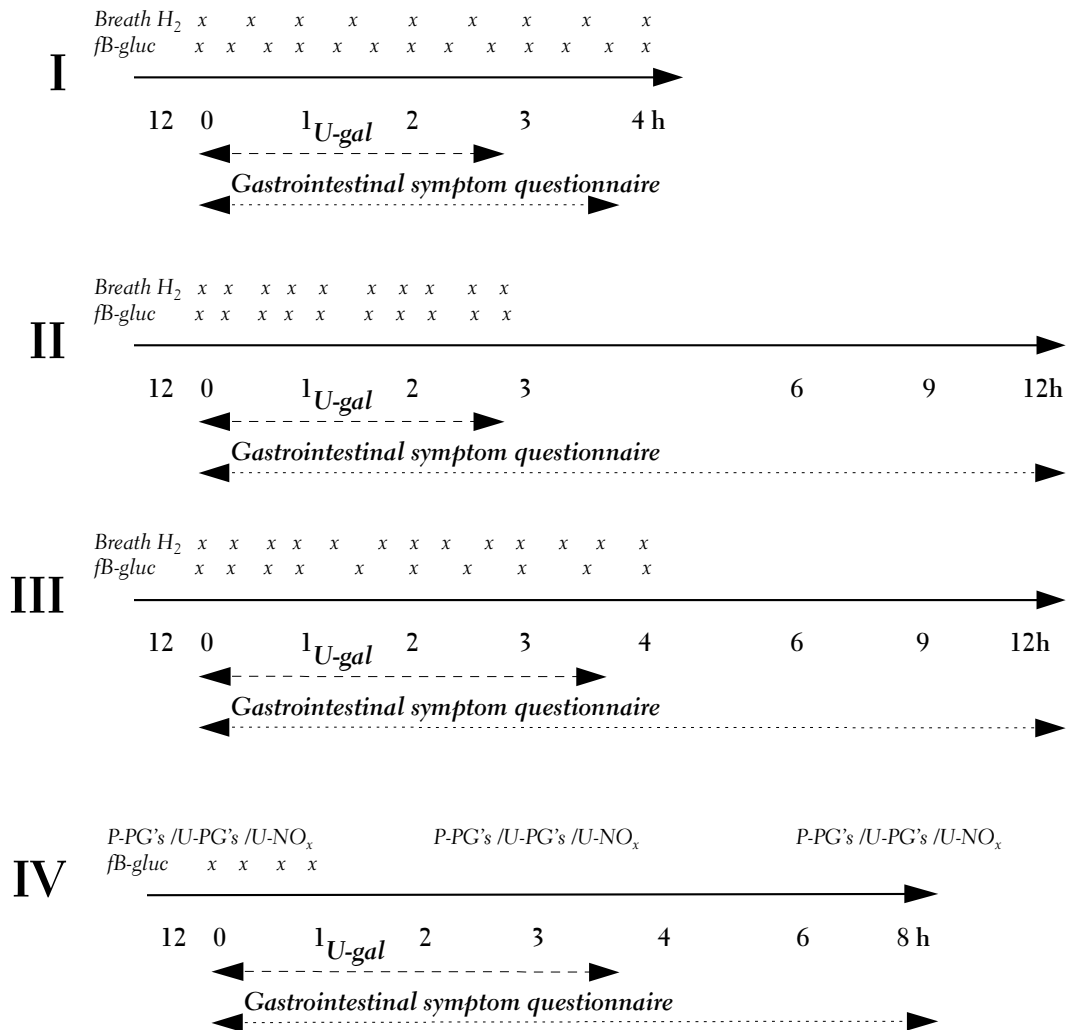


Figure 4.1. Schedule of measurements in each test session, Studies I-IV. There was a one-week wash-out period each time before the next test session. Breath H₂ = breath hydrogen, fB-gluc = blood glucose, U-gal = urine collection for galactose measurement; P-PG = plasma prostaglandin E₂ metabolites; U-PG = urine prostaglandin E₂ metabolites; U-NO_x = urine nitric oxide metabolites.

Study I was designed to compare a small portable breath hydrogen analyser with the more commonly used equipment, and to compare both these with other possible test variables used in lactose tolerance tests.

In order to modify the gastric emptying rate (II), subjects ingested either propantheline (as bromide, 15 mg), metoclopramide (as hydrochloride, 10 mg) or an identical placebo (methylcellulose), all in capsule form an hour before the test solution. The doses of these drugs were chosen on the basis of previous studies and of the therapeutic single dose recommendation (Dollery 1991, Massicotte *et al* 1996).

In Study III, to investigate the role of the temperature of the test solution on the digestibility of lactose and the subjects' tolerance to it, the lactose was served either cold (2-3° C), at room temperature (20-21° C), or hot (55-58° C).

In order to test the role of prostaglandins (IV), the subjects ingested either 600 mg ibuprofen capsule to inhibit the synthesis of prostaglandins, or an identical placebo (methylcellulose) capsule with the test solution. In Study IV, to test the role of nitric oxide (NO), the subjects were on a low-nitrate diet for 48 h before the test days. All the capsules used in Studies II and IV were identical apart from the active ingredient, and were prepared at the University Pharmacy (University of Helsinki, Finland).

Table 4.1. Study designs and subjects.

	Study I	Study II	Study III	Study IV
Interventions to be compared	Breath hydrogen analysers for diagnosing hypolactasia	L + propantheline L + metoclopramide L + placebo	L cold L room-temperature L hot	L + ibuprofen L + placebo S + ibuprofen S + placebo
Study design	Method comparison	Three-period cross-over	Three-period cross-over	Four-period cross-over
Subjects (f/m)	34/10	18/0	10/0	9/0
Age, mean (range) years	32 (18-66)	34 (20-64)	39 (26-55)	38 (24-63)
BMI, mean (range) kg/m ²	23 (19-33)	23 (18-42)	25 (20-42)	22 (18-27)
Smokers	5/44	3/18	3/10	1/9
Lactose tolerance tests used:				
Breath hydrogen	+	+	+	-
Blood glucose	+	+	+	+
Urine galactose	+	+	+	+
Symptom questionnaire (h)	4	12	12	8

L = Lactose, S = Sucrose, BMI = Body Mass Index

4.1.2. Dietary instructions and study diets

The subjects were instructed to choose lactose-free food items for the 24 h preceding the test days (Studies I - III) and to avoid alcohol, smoking and foods that commonly

produce gastrointestinal symptoms, such as beans, peas or fried foods, for the same period in order to diminish gastrointestinal symptoms induced by foods other than the test solution and to improve the reliability of the breath hydrogen measurements (Brummer *et al* 1985). In Study IV, in addition to instructions for lactose restriction, alcohol consumption and smoking, subjects were instructed to choose a low-nitrate diet for 48 h preceding the test days in order to clear the plasma of exogenously derived nitrate.

At fixed intervals from the beginning of the test procedure, the subjects in Studies II-IV were served a standard lactose-free lunch (after 3 h), an afternoon snack (after 6 h) and in Study II, dinner (after 9 h) (Table 4.2). In Studies III and IV the subjects were instructed to choose dinner from a selection of lactose-free items. This type of meal had been used previously in carbohydrate malabsorption studies (Rumensen *et al* 1990, Teuri *et al* 1999). Smoking was not allowed until the end of the test day, nor were coffee, tea or any other food items other than those provided.

Table 4.2. Content of meals served during the Studies II - IV.

Food	Study II	Study III	Study IV
Lunch 750 kcal (3.1 MJ)			
minced meat	+	+	+
spaghetti			+
rice + carrots	+	+	
wheat bread + margarine	+	+	+
blueberry puree			+
lettuce + canned peach	+	+	
juice	+	+	+
banana			+
Afternoon snack 400 kcal (1.7 MJ)			
wheat bread + margarine	+	+	+
cheese	+	+	
banana + juice	+	+	+
decaffeinated coffee	+	+	
Dinner 750 kcal (3.1 MJ)			
minced meat + spaghetti + cheese	+		
tomato	+		
wheat bread + margarine	+		
banana + juice	+		

4.1.3. Methods used for diagnosing hypolactasia and lactose intolerance

The Golden Standard diagnosis for hypolactasia and lactose intolerance (I-IV)

The lactose tolerance test was carried out by measuring the breakdown products of lactose following an oral dose - increase in exhaled breath hydrogen, unaltered concentration of blood glucose, increased excretion of urinary galactose - and the development of gastrointestinal symptoms. Between 7 and 9 a.m, after an overnight fast (10-12 h), the subjects were given 50 g lactose in 300 ml water, to be ingested in 5 min.

To avoid false positive or negative results in these studies, a Golden Standard diagnosis was established for this series of studies. The Golden Standard is a combination of the three diagnostic indicators: breath hydrogen, blood glucose and urine galactose. At least two positive indicators were considered sufficient to indicate hypolactasia. If lactose maldigestion was confirmed and gastrointestinal symptoms were considered to be at least moderate, the subject was diagnosed as being lactose intolerant.

Breath hydrogen measurement (I - III)

All the breath hydrogen measurements were carried out using a portable electrochemical hydrogen analyser, Micro H₂ (Micro Medical Limited, Chatman, UK). Measurements were obtained by blowing (70 s) end-alveolar air, using 22 mm mouthpieces. Breath measurements were carried out as shown in Figure 4.1. An increase of ≥ 20 ppm was considered to be an indication of lactose maldigestion (see Arola 1994). In Study I, as a reference analyser for hydrogen production in exhaled air, the Quintron MicroLyzer, Model DP (Quintron Instrument Co. Inc., Milwaukee, WI, USA) was used.

Blood glucose and urine galactose measurements (I - IV)

All blood glucose concentration measurements were taken with the Glucometer Elite (Bayer Diagnostics, Lungby, Denmark). Before each new lot, of blood strips the equipment was auto-calibrated with a test strip of the new lot according to the instructions of the manufacturer. The accuracy of this blood glucose test method has been verified in earlier studies (Harrison *et al* 1996). Blood samples were aspirated directly from a finger tip according to the study designs, as shown in Figure 4.1. An increase in blood glucose concentration of 1.1 mmol/l or less was considered an indication of lactose maldigestion (see Arola 1994).

The subjects collected urine for the first three (I, II and IV) or four (III) hours, and urinary galactose was assayed spectrophotometrically using a commercial enzyme kit. If the 3- or 4-hour urinary galactose excretion was less than 20 mg, this was considered to be an indicator of lactose maldigestion (see Arola 1994). This method is considered to be a reliable, quantitative, non-invasive technique for assessing profiles of 'whole' intestinal lactase activity (Bjarnason *et al* 1990).

Symptom questionnaires (I-IV)

The severity of gastrointestinal symptoms was assessed by the subjects themselves on a scale of 0 to 10 (0 = no symptoms, 10 = severe symptoms) in a questionnaire which was slightly modified from previously used models (Vesa *et al* 1996, Teuri *et al* 1999). The subjects filled this in at the baseline before the intervention, and every 60 min for the first three or four hours after the intake of lactose, and then at 6 h, 9 h and 12 h (Figure 4.2). In Studies I and IV the recording of symptoms was completed at the end of four and eight hours respectively from the ingestion of the test solution.

Pain	None		Only slight		Fairly severe		Moderate		Severe		
Baseline	0	1	2	3	4	5	6	7	8	9	10
1 h	0	1	2	3	4	5	6	7	8	9	10
2 h	0	1	2	3	4	5	6	7	8	9	10
3 h	0	1	2	3	4	5	6	7	8	9	10
4 h	0	1	2	3	4	5	6	7	8	9	10
5 - 6 h	0	1	2	3	4	5	6	7	8	9	10
7 - 9 h	0	1	2	3	4	5	6	7	8	9	10
10 - 12 h	0	1	2	3	4	5	6	7	8	9	10
Later, when?	0	1	2	3	4	5	6	7	8	9	10

Figure 4.2. An example of a numeric symptom questionnaire. Subjects were asked to mark the presence and severity of symptoms hourly.

The subjects assessed the intensity of flatulence, abdominal pain, abdominal bloating, borborygmi, nausea, headache, and the hardness of the stools. The sum of the most common lactose-induced symptoms (see Villako and Maaros 1994) - flatulence, abdominal pain, abdominal bloating, borborygmi, and in Studies I and IV, loose stools - was calculated for each time point. For the sum of four symptoms the possible range was 0 to 40 and for the five symptoms the range was 0 to 50. If each symptom was calculated individually for the 12 h follow-up, the maximum score for each symptom would be 120. This long period of recording symptoms is important because of individual variability in the time of gastric emptying and intestinal transit and thus the development of gastrointestinal symptoms.

In Study II, in order to evaluate the usefulness of the numerical grading of symptoms, a visual analogue scale (VAS) (Figure 4.3) was used besides the numerical rating scale to see whether there were differences between these two methods. At the beginning of the test procedure subjects were given a numerical symptom questionnaire to be filled in during the next 12 h. After each of the first four test hours they were given another form, a new VAS questionnaire to be filled in, which they returned as soon as they had done so. After Study II the results of these two query methods were compared. The results did not differ. The 3-h symptom score (flatulence, abdominal pain, abdominal bloating, borborygmi) of the placebo period was 39.0 ± 4.7 in the numerical rating scale and 39.1 ± 4.6 in the VAS scale. The subsequent work of measuring the results with the VAS questionnaire is very laborious, and mainly for this reason the numerical rating scale questionnaire was chosen.

No pain	Moderate pain	Severe pain

Figure 4.3. An example of the VAS symptom questionnaire for one time point.

4.1.4. Gastrointestinal transit time (II)

Gastrointestinal transit time was measured using a carmine dye mixed with the lactose solution. The appearance of the dye was assessed visually in the stools by the subjects themselves (Read *et al* 1980, Marlett *et al* 1981).

4.1.5. Urine and plasma samples for inflammatory markers (IV)

The subjects collected urine for the 12 h before the test and over the next eight hours, divided into three aliquots for measurement of the excretions of prostaglandin E_2 and prostacyclin metabolites (PGE_2 -M and 6-keto prostaglandin $F_{1\alpha}$ respectively), and indicators of nitric oxide production (nitrite and nitrate and cyclic GMP). Venous blood samples for separating plasma were taken for prostaglandin E_2 metabolite (PGE_2 -M) measurements, before the drug and carbohydrate intake (baseline) and 3 and 8 h after the baseline.

Plasma and urine levels of PGE_2 metabolites were determined by using commercial radioimmunoassay kits following the instructions of the company. For measuring the indicators of nitric oxide production, nitrate was reduced to nitrite and determined spectrophotometrically by a commercial test kit based on the Griess reaction. For the

cyclic GMP determinations, as another indicator of nitric oxide production, the urine samples were measured by radioimmunoassay as described by Axelsson *et al* (1988). The cyclic GMP antiserum has been described by Lahteenmaki *et al* (1998).

4.2. Animal experiments (V)

4.2.1. Experimental animals and study design

Female Wistar rats from the breeding colonies of the Laboratory Animal Centre, Helsinki University, Finland, were housed at 23°C with a light-dark cycle of 12 h.

Study V was designed to investigate the possibility that lactase protein expression and its activity can be induced by lactose. The expression of lactase protein in the gut and its possible induction was evaluated by immunohistochemical and biochemical techniques in 8-week-old rats (total number 24, divided into four groups) after a lactose challenge lasting seven days. The lactose-challenged rats received either 3%, 10% or 20% lactose-containing water or tap water (controls), *ad libitum*. The fluid intake was measured daily by weighing the drinking bottles.

4.2.2. Intestinal samples

The animals fasted overnight before they were killed by decapitation under CO₂ anaesthesia. Two-cm-long samples for determination of lactase activity and expression were taken. Samples were from the proximal part of the stomach and the oesophagus, the middle of the duodenum, the proximal, middle and distal parts of the jejunum, and the proximal part of the ileum.

4.2.3. Biochemical determinations

Lactase activity

Lactase enzyme (EC 3.2.1.23) activity was assayed spectrophotometrically by the method of Dahlqvist (1964). Lactase activity was expressed as units (one μmol of lactose hydrolysed per minute at +37°C) per gram of total protein present in the homogenates. The protein content of the homogenates was assayed by the method of Lowry *et al* (1951).

Lactase expression by immunohistochemical methods

Paraformaldehyde (1%) fixed samples were immunoperoxidase labelled and incubated with monoclonal antibodies raised against rat lactase. Optical density

measurements were used in order to obtain a semi-quantitative measurement of lactase protein expression.

4.3. Ethics

The designs of Studies I - IV were approved by the Ethical Committee of the Institute of Biomedicine, University of Helsinki, Finland. The design of Study V was approved by the Animal Experimentation Committee of the Institute of Biomedicine, University of Helsinki, Finland. Studies II and IV were also approved by the National Agency for Medicine, Finland.

4.4. Statistical analyses

In Study I, which was to compare the portable breath hydrogen analyser, the method of Bland and Altman (1986) was used to assess the agreement between the two breath hydrogen tests (Micro H₂ vs Quintron).

In Study II, a mixed-effect ANOVA model was used to analyse the sum of symptom scores. Pair-wise *post hoc* analyses were carried out using repeated measures contrast analysis. The distributions of the symptom scores of individual symptoms in Studies II and III and the urine and the plasma variables in Study IV were skewed. Friedman's nonparametric analysis of variance was therefore applied to compare the differences between the interventions. The Wilcoxon matched pairs test was used for pairwise comparisons. In Study II, Spearman's rho correlation was calculated between the total transit time and gastrointestinal symptoms.

In Study V, statistical significance was determined by analysis of variance (ANOVA), and multiple comparisons were made by the Dunnett's *post hoc* test.

Sensitivity, specificity, and positive and negative predictive values were calculated to evaluate the breath hydrogen, blood glucose and urine galactose as diagnostic variables. The comparisons were made with the Golden Standard. Cohen's Kappa values and percentages of agreement were calculated to evaluate the agreement between any two diagnostic variables.

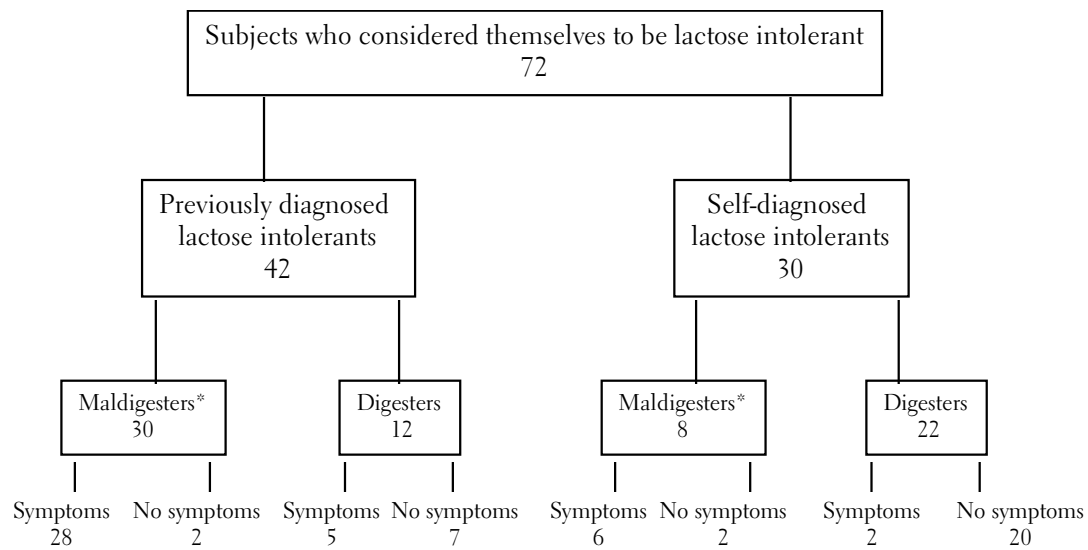
Results are expressed as Mean \pm SEM, as Mean (range) or as percentage and 95 % CI. All statistical analyses were carried out using the SPSS statistical package (Release 7.5.1 / 8.0, SPSS Inc., Chicago, IL, USA).

5. Results

5.1. Comparison of diagnostic methods for detecting lactose maldigesters

The final number of participants in Studies I-IV was 72. Overall, there were 89, but some of the subjects volunteered for more than one study and 10 withdrew or were excluded for various reasons.

Of the 72 participants 42 had previously been diagnosed as being lactose intolerant. These diagnoses were made by official health professionals, or else the subjects were diagnosed in earlier lactose intolerance studies by our group (Vesa et al 1996, Teuri et al 1999). Thirty were self-diagnosed lactose intolerants. A third of the previously diagnosed and two thirds of the self-diagnosed subjects proved to be lactose digesters according to the Golden Standard diagnosis (see Chapter 4) (Figure 5.1). About one tenth (4/38) of the maldigesters were asymptomatic and about 20% (7/34) of the digesters were symptomatic according to the oral lactose tolerance test (Figure 5.1).



* Positive findings for at least two of the three variables (breath hydrogen, blood glucose, or urine galactose excretion)

Figure 5.1. Studies I-IV. Distribution of previously diagnosed lactose intolerant and self-diagnosed lactose intolerant subjects, according to laboratory findings and symptoms (symptom score ≥ 12 / 3-4 h), after ingestion of 50 g lactose.

In Study I, in order to test the usefulness of the portable breath hydrogen analyser Micro H₂, altered breath hydrogen concentration was measured with the two analysers, the Micro H₂ and the Quintron MicroLyzer. Even though the highest

increase in the exhaled breath hydrogen over the baseline varied greatly (44-366 ppm for Micro H₂ and 27-187 ppm for Quintron MicroLyzer), the diagnoses were 100% identical. Since the Micro H₂ proved to be reliable for detecting lactose malabsorption, all the data of breath hydrogen concentrations presented here is measurements taken with the Micro H₂.

In addition to proving the usefulness of the Micro H₂ breath hydrogen analyser, the data of Study I made it possible to compare the three diagnostic variables (breath hydrogen, blood glucose and urine galactose) for detecting lactose maldigesters. In order to increase the number of subjects the comparison of the test variables was re-made with subjects participating in Studies I-III. Each subject is considered once only, even if she participated in more than one study. If this was the case, the first study in which she participated was chosen for this comparison. In the end the final number of subjects in Studies I-III was 70, of whom 35 proved to be lactose maldigesters according to the Golden Standard.

When these three variables for diagnosing hypolactasia were compared, none of them alone recognised all the maldigesters or all the digesters (Table 5.1). The number of misdiagnosed maldigesters was most obvious if the blood glucose test was used on its own (3/35), and the largest number of lactose digesters (5/35) was misdiagnosed by the urine galactose test (Table 5.1). The sensitivity (the ability of the test to recognise lactose maldigesters) of the breath hydrogen test was 94% (33/35), of the blood glucose test, 91%, (32/35), and the urine galactose test, 97% (34/35) (Table 5.2). The specificity of the tests (the ability to recognise lactose digesters), was 94%, 91%, and 86% respectively (Table 5.2).

Table 5.1. Studies I-III. Combination of results of breath hydrogen, blood glucose, and urine galactose for detecting lactose maldigesters (LM according to the Golden Standard).

Breath hydrogen	fB-glucose	Urine galactose		
		Positive***	Negative	Total
Positive*	Positive**	29 ^{LM}	1 ^{LM}	30
	Negative	3 ^{LM}	2	5
	Total	32	3	35
Negative	fB-glucose			
	Positive	2 ^{LM}	3	5
	Negative	5	25	30
	Total	7	28	35

Positive if * ≥ 20 ppm

** increase ≤ 1.1 mmol/l

*** ≤ 20 mg/3-4 h

Table 5.2. Studies I-III. Diagnostic characteristics of breath hydrogen, blood glucose, urine galactose tests, and symptoms (symptom score ≥ 12 / 4 h) compared with the Golden Standard. $n=70$.

	Breath hydrogen		fB-glucose		Urinary galactose		Symptoms	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Sensitivity	94	88–100	91	84–100	97	93–100	89	82–98
Specificity	94	88–100	91	84–100	86	78–96	77	67–89
Positive predictive value	94	88–100	91	84–100	87	79–97	79	69–91
Negative predictive value	94	88–100	91	84–100	97	93–100	87	79–97

Sensitivity (%) = [true positives / (true positives + false positives)] \times 100

Specificity (%) = [true negatives / (true negatives + false negatives)] \times 100

Positive predictive value (%) = [true positives / (true positives + false positives)] \times 100

Negative predictive value (%) = [true negatives / (true negatives + false negatives)] \times 100

The percentages of agreement and Cohen's kappa for the combination of breath hydrogen, blood glucose and urine galactose tests and gastrointestinal symptoms can be seen in Table 5.3. Differences between the test pairs were small and no statistical significances were found. Thus, none of the combinations is superior for detecting lactose maldigestion.

Table 5.3. Studies I-III. Percentages of agreement and Cohen's kappa for the pairwise comparisons of breath hydrogen (H_2), blood glucose (fB-gluc) and urine galactose (U-gal) and gastrointestinal symptoms (symptom score ≥ 12 / 4 h). $n=70$.

	Percentage of agreement		Cohen's kappa		
	%	95% CI	κ	95% CI	
H_2 vs fB-gluc		86	78–96	0.71	0.55–0.88
	U-gal	86	78–96	0.71	0.55–0.88
	Symptoms	83	74–94	0.66	0.48–0.83
fB-gluc vs U-gal		83	74–94	0.66	0.48–0.835
	Symptoms	77	67–89	0.54	0.35–0.74
U-gal vs Symptoms		77	67–89	0.54	0.34–0.74

The diagnostic limit for a clinically significant symptom score was ≥ 12 . The sensitivity, specificity, positive predictive value, and negative predictive value of the symptom questionnaire in Studies I-III can be seen in Table 5.2. The best percentage of agreement with Cohen's kappa for gastrointestinal symptoms and a positive test variable was with the breath hydrogen test (Table 5.3). Of the maldigesters, 83% (31/35) with a positive breath hydrogen test had an increased symptom score. On the other hand, about 20% of the lactose digesters with negative breath hydrogen had significantly increased symptoms. The percentage of agreement of the symptoms and

both the blood glucose and the urine galactose tests was 77% (95% CI 67 to 89) and Cohen's kappa for both these was 0.54 (Table 5.3).

In Studies II and IV there was a health questionnaire in which the subjects answered questions about gastrointestinal symptoms and their correlation to milk and dairy products (unpublished data). These questions were answered before the test and the diagnosis of lactose intolerance. Both the lactose maldigesters and the digesters experienced flatulence as the most uncomfortable gastrointestinal symptom (Table 5.4). In the oral lactose tolerance test after 50 g lactose, 1/27 of these maldigesters was asymptomatic whereas 4/7 of the lactose digesters were symptomatic with at least a 12-point symptom score.

Table 5.4. Studies II and IV. Gastrointestinal symptoms that were reported as the most uncomfortable, i.e. number of subjects reporting the order of discomfort, by lactose maldigesters (n=28) and digesters (n=7).

	Great discomfort	Some or slight discomfort	No discomfort
Maldigesters			
<i>Flatulence</i>	18	10	0
<i>Abdominal bloating</i>	10	16	1
<i>Abdominal pain</i>	17	7	3
<i>Diarrhoea</i>	8	10	9
<i>Constipation</i>	3	11	7
Digesters			
<i>Flatulence</i>	4	2	1
<i>Abdominal bloating</i>	3	2	2
<i>Abdominal pain</i>	1	4	1
<i>Diarrhoea</i>	1	4	2
<i>Constipation</i>	2	4	2

5.2. Modification of gastric emptying and the temperature of the test solution

In Study II gastric emptying was retarded with a single oral dose of propantheline (as bromide, 15 mg) and speeded up with metoclopramide (as hydrochloride, 10 mg). The most obvious result of modified gastric emptying was the diminishing of gastrointestinal symptoms after the ingestion of propantheline (Figure 5.2). The symptom score (flatulence + borborygmi + abdominal bloating + abdominal pain) was significantly reduced by propantheline compared to the metoclopramide, after 1 h and 2 h, or compared to the placebo after 6 h and 9 h (Figure 5.2). The acceleration of gastric emptying by metoclopramide did not affect the symptom score (Figure 5.2) or any single symptom (Table 5.5) compared with the placebo.

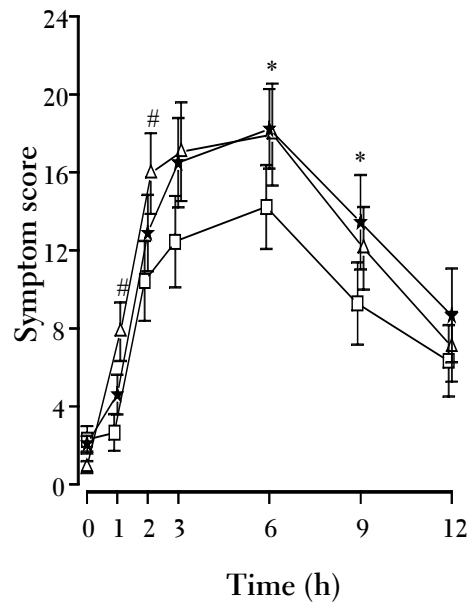


Figure 5.2. Study II. Gastrointestinal symptom scores of lactose maldigesters after an oral lactose dose with either propantheline (as bromide, 15 mg) (□), metoclopramide (as hydrochloride, 10 mg) (△) or a placebo (★). Results are expressed as mean \pm SEM, $n=18$. * $p<0.05$ propantheline compared with the placebo, # $p<0.05$ propantheline compared with metoclopramide.

The nature of lactose-induced gastrointestinal symptoms can be clearly seen in Figure 5.2. During an oral lactose tolerance test it takes a few hours for clinically significant symptoms to develop. Symptoms last several hours and their severity diminishes more slowly than it takes to develop, making the symptom curve skew to the right. Except in the case of abdominal bloating, the same shape as the overall symptoms curve can be seen in each individual symptom. With abdominal bloating, regardless of the intervention, reduction in the severity of the bloating was much slower than in any other symptom.

The 12-h sum of symptom scores for flatulence, borborygmi, abdominal bloating and pain can be seen in Table 5.5. Propantheline reduced abdominal bloating compared to the placebo. Compared to metoclopramide, propantheline reduced both abdominal bloating and abdominal pain.

Table 5.5. Study II. The 12-h sum of symptom scores of flatulence, borborygmi, abdominal bloating and abdominal pain, during the 12 h follow-up in lactose maldigesters after oral ingestion of 50 g lactose with either propantheline (as bromide, 15 mg), metoclopramide (as hydrochloride, 10 mg) or a placebo. Mean (min to max), n=18.

12-h Symptom scores	Placebo	Propantheline	Metoclopramide	Friedman p-value ¹
Flatulence	29 (6 to 51)	25 (6 to 52)	29 (10 to 55)	0.09
Borborygmi	21 (0 to 51)	17 (4 to 45)	20 (0 to 51)	0.16
Abdominal bloating	22 (0 to 53)	16 (0 to 51) ^{P,M}	21 (0 to 44)	0.02
Abdominal pain	14 (0 to 37)	11 (0 to 44) ^M	14 (0 to 40)	0.19

¹ Significance of treatment effect in a non-parametric Friedman analysis

^M p<0.05 compared to metoclopramide

^P p<0.05 compared to the placebo

The retarding of gastric emptying did not modify the total gastrointestinal transit time as measured with carmine dye. Transit time varied greatly (1.5 h - 72 h) independent of the treatment, because of large interindividual variations. There was no correlation between transit time and the development of gastrointestinal symptoms following the ingestion of lactose. Spearman's rho correlation values were very near zero, varying between -0.11 and 0.22.

In Study III the temperature of the lactose solution affected each of the symptoms differently. Due to these fluctuations, the symptom score of flatulence + borborygmi + abdominal bloating + abdominal pain was of little use in estimating the effect of the test solutions. The effects of a cold test solution were more intense than the effects of a hot solution, compared with the room temperature solution. The cold test solution tended to increase abdominal pain (p=0.09) and reduced flatulence and bloating compared with the room temperature solution, expressed as the 12-hour symptom scores (Table 5.6).

Table 5.6. Study III. The sum of symptom scores of flatulence, borborygmi, abdominal bloating and pain during the 12-h follow-up in lactose maldigesters after a room temperature, a cold and a hot solution of 50 g lactose. Mean (min to max), n=10.

12 -h Symptom scores	Room temperature 21-22° C	Cold 2-3° C	Hot 55-58° C	Friedman p-value ¹
Flatulence	31 (20 to 44)	24 (7 to 38) ^R	31 (10 to 52)	0.01
Borborygmi	19 (1 to 35)	19 (0 to 32)	20 (3 to 38)	0.25
Abdominal bloating	27 (17 to 34)	23 (8 to 32) ^R	30 (11 to 42)	0.02
Abdominal pain	12 (0 to 30)	18 (0 to 33)	17 (5 to 39)	0.08

¹ Significance of treatment effect in a non-parametric Friedman analysis

^R p<0.05 compared to the room temperature test solution

5.3. Measurement of inflammatory markers following the ingestion of lactose

The purpose of Study IV was to investigate the possible role of inflammation in provoking gastrointestinal symptoms following the ingestion of lactose. The enhanced or reduced production of prostaglandins as a signal for possible inflammation were measured as urine and plasma $\text{PGE}_2\text{-M}$ excretions. The synthesis of prostaglandins via inducible COX-2 increases with inflammation. In the urinary excretion of $\text{PGE}_2\text{-M}$ an increase of about 30% in the excretion was noticed after the lactose intake compared with the sucrose intake (Figure 5.3). Ibuprofen tended to inhibit this increase. The same increase and its inhibition by ibuprofen was also seen with the urinary excretion of another metabolite of prostaglandins, 6-keto prostaglandin $\text{F}_{1\alpha}$, but with both these variables, $\text{PGE}_2\text{-M}$ and 6-keto $\text{PGF}_{1\alpha}$, interindividual variation was considerable and no statistically significant differences in the inhibition by ibuprofen were remarked (Figure 5.3). In the plasma $\text{PGE}_2\text{-M}$ excretion there were no differences between the lactose and the sucrose intakes, with or without ibuprofen.

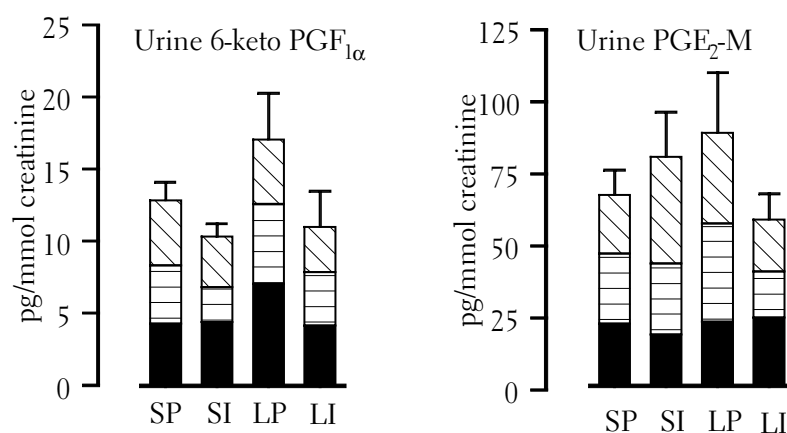


Figure 5.3. Study IV. Excretion of the prostaglandin metabolites $\text{PGE}_2\text{-M}$ and 6-keto $\text{PGF}_{1\alpha}$ in the urine during the 8-h collection in 3-h, 6-h and 8-h aliquots, in lactose maldigesters ($n=9$) after an oral lactose (L) or sucrose (S) solution with either oral ibuprofen (I) or a placebo (P). Mean + SEM. ■ = 3 h sample, □ = 6 h sample, and ▨ = 8 h sample.

In no other indicators of inflammation that were used - e.g. nitric oxide production measured as the urinary excretion of cyclic GMP and NO_x , and blood leukocyte count (unpublished data, Table 5.7) - were any differences seen between the lactose and the sucrose intake, with or without ibuprofen.

Table 5.7. Study IV. Blood leukocyte counts ($\times 10^9 / l$) in four lactose maldigesters before and after a lactose tolerance test, with and without a single oral dose of ibuprofen.

Subject n:o	Lactose + Placebo			Lactose + Ibuprofen		
	Baseline	3 h	8 h	Baseline	3 h	8 h
4	3.2	4.5	3.8	3.0	3.6	4.9
5	5.1	4.5	5.2	5.9	5.6	7.7
7	6.0	5.8	6.1	6.4	6.2	7.1
10	9.4	8.2	9.7	10.7	10.1	10.9

5.4. Induction of lactase with lactose

In Study V the dietary modification of lactase enzyme activity was investigated in the rat intestine. Lactase activity was found only in the small intestine, with the exception of those rats that received the largest doses of lactose. In these, it also appeared in the colon. There was no activity present, measured spectrophotometrically, in the oesophagus, the stomach or in the colon of the control animals or those who received the smallest doses of lactose. Water intake and weight gain during the study period can be seen in Table 5.8.

Table 5.8. Study V. The effect of a seven-day supplementation of lactose on weight gain and daily water intake. Mean \pm SEM, $n=5-6$.

	Lactose g/day			
	0	0.7	2.1	2.6
Weight gain (g/week)	44.3 \pm 5.4	20.2 \pm 5.4*	18.2 \pm 7.3*	23.8 \pm 7.9*
Water intake (ml/rat/day)	32.4 \pm 0.7	23.3 \pm 2.1	20.6 \pm 2.6*	12.7 \pm 1.5**

* $p < 0.05$, ** $p < 0.001$ ANOVA with Dunnett's multiple comparison test (control vs test group)

The optical density of lactase immunoreactivity was measured from the proximal part of the jejunum. In the control animals this was 0.19 ± 0.01 arbitrary units. It increased 25-40% in the lactose supplemented groups. Dietary lactose increased the activity of lactase, especially in the proximal ($p=0.01$) and middle parts of the jejunum ($p=0.02$). If the total lactase activity of the control group, measured by combining the activities of five small intestinal biopsies, is 100%, a lactose supplementation of 10-12 g/kg body weight/day increased this activity by about 40% (Figure 5.4).

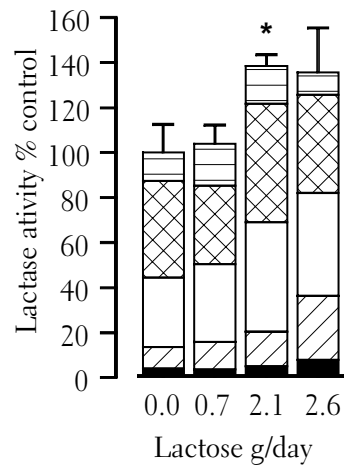


Figure 5.4. Study V. The effect of a seven-day supplementation of lactose on lactase activity in the rat, expressed as a percentage of the control group (control group =100%). ■ = duodenum, ▨ = proximal jejunum, □ = middle jejunum, ▩ = distal jejunum, ▤ = ileum. * $p < 0.05$ ANOVA with Dunnett's multiple comparison test (control vs. test group). $n = 5-6$ animals/group.

6. Discussion

6.1. Evaluation of study designs and methods

6.1.1. Human studies

In this series of studies the purpose was to investigate the possible mechanisms affecting the digestion of lactose, as well as the gastrointestinal symptoms induced by undigested lactose. Side by side with this, the prevalent methods for diagnosing hypolactasia and lactose intolerance came under scrutiny. Lactose maldigestion is usually identified by means of an oral lactose tolerance test, in which, following ingestion of 50 g lactose, maldigestion is detected either by a reduction in the increase of an unabsorbed breakdown monosaccharide component of lactose (glucose or galactose) in either the blood or the urine concentration, or by an increase of hydrogen produced by the colonic fermentation of malabsorbed lactose (see Arola 1994).

Great care was taken to minimise all those factors known to affect test variables for detecting hypolactasia. In this we were enormously supported by the extremely co-operative study subjects, all of whom were healthy volunteers. Pre-test restrictions such as the limited use of medication and a controlled diet before and during the test were imposed in order to improve the comparability of the study periods. It is just possible, however, that the avoidance of coffee among coffee drinkers and smoking among smokers may have influenced the symptoms recorded during the test. Fortunately, rather than affecting gastrointestinal symptoms, coffee withdrawal is probably more likely to result in headache and overall discomfort, and there was only a relatively small number of smokers.

Studies were, whenever possible, randomised (II-IV), double-blinded (II, IV) and cross-overed (II-IV). The evaluation of the usefulness of the Micro H₂ breath hydrogen analyser for detecting hypolactasia (Study I) was not blinded. The analyser proved to be reliable for this purpose compared with the stable hydrogen measurement unit Quintron MicroLyzer or with the Golden Standard. To compare its ability to measure breath hydrogen concentration, it might also have been possible to measure exhaled hydrogen after another undigestible carbohydrate such as lactulose, as we did in a previous study (Teuri *et al* 1999). This combination lactose/lactulose breath hydrogen test enables one to calculate lactose malabsorption by comparing areas under the curves of the lactulose breath test (100% malabsorbed)

and the lactose breath test. It also enables the measurement of intestinal transit time (Hammer *et al* 1996).

However, more experiments are needed to standardise the collection of exhaled hydrogen for analysis with the Micro H₂ analyser. In this study the collection of hydrogen was controlled by strictly limiting the collection time to 70 s and inhibiting the passage of hydrogen through the nose by using a nose clip, as advised by the manufacturer of the equipment. However, even though the subjects were allowed to exhale slowly through the mouthpiece of the Micro H₂ analyser, methods of blowing varied. This possible bias in the inter-individual comparison of results was minimised by using cross-over study designs when no comparison between the subjects was needed.

The total number of study periods in each study was kept to the minimum. This was because of the perennial difficulty in getting lactose maldigesters to participate in a study in which they will have to ingest several large doses of lactose, after which they know, or assume, that they will suffer severe gastrointestinal symptoms. Based on experience from earlier studies, women are more willing to take part in studies such as these than men. It was thought that it would be better to investigate single-sex groups in order to make the study group more homogeneous. General conclusions based on very homogeneous groups should, however, be drawn with circumspection.

There are relatively few reports describing the possible differences in the tolerance of lactose between men and women. Hypolactasia is equally prevalent in both sexes (Sahi 1974b), though female lactose maldigesters seem to be more sensitive to lactose than men (Vesa *et al* 1998) despite the fact that the amount of malabsorbed lactose measured by the breath hydrogen test is similar in both sexes (Krause *et al* 1997).

The influence of gender and the menstrual cycle on gastric emptying and gastrointestinal transit has not been clearly demonstrated. Several studies have found gastric emptying to be faster in men than in women (Datz *et al* 1987, Hutson *et al* 1989, Degen and Philips 1996, Hermansson and Sivertsson 1996, Knight *et al* 1997), whereas others found little or no difference (Horowitz *et al* 1985, Saltzberg *et al* 1988, Madsen 1992). In a recent study, evidence of the postprandial reduction of gastric emptying was found in women, but no differences between men and women during fasting were observed (Caballero-Plasencia *et al* 1999). The phases of the menstrual cycle, however, have not been reported as modifying the gastric emptying of solids or liquids (Caballero-Plasencia *et al* 1999), but Wald *et al* (1981) reported that the whole gastrointestinal transit was reduced by 25% during the luteal phase

compared with the follicular phase. The interesting questions of gender or phases of the menstrual cycle on gastrointestinal transit cannot be answered by our present studies: we were restricted to one gender and the phases of menstrual cycle were not recorded.

The symptom questionnaire we used was based on the questionnaires used previously by us in grading gastrointestinal symptoms in lactose intolerance studies (Vesa *et al* 1996, Teuri *et al* 1999). This type of questionnaire has not yet been statistically validated in recording lactose-induced gastrointestinal symptoms but has been successfully used by us with almost two hundred lactose intolerant subjects. We also compared the numerical grading of symptoms with a visual analogue scaling (VAS). Since no differences in symptom scores were found between these two grading methods, the numerical grading was chosen, mainly because of the more straightforward analysis of results.

6.1.2. Experimental studies

Using experimental animals as a model for investigating hypolactasia provides a valuable tool for detecting mechanisms behind the regulation of the expression and activity of lactase. Even though rodents and humans differ in many ways, the basics of the normal physiological pattern of the lactase enzyme are similar. Thus it makes sense to use the rat as an experimental model for studying hypolactasia. Both human beings and the rat have a high expression of lactase in sucklings, after which the expression and activity both decline to the lower level of adulthood. The time period of this decline differs. In rats the decline begins soon after weaning, as shown by us and others (Büller *et al* 1989), and in humans, later in childhood (Sahi *et al* 1972). However, this is a minor difference when adult animals are used, as they were in this study.

Since we wanted to investigate the effect of a lactose supplementation on low adult levels of lactase, the animals we used were 8-9 weeks old. At this age, the level of lactase was already significantly reduced. The lactose content of the milk of nursing rats varies between 1 and 3.5%, depending on the age of the sucklings (Kuhn 1972). The lowest concentration for supplementation, which was 3%, was chosen to imitate this level of lactose in the milk of nursing rats. Two significantly higher concentrations, 10% and 20%, were also used, in order to see clearly the possible dose dependency in the induction of the lactase enzyme.

Water intake and weight gain were reduced by 50-60% in those animals who received large doses of lactose mixed with water. The intake of the lactose-water solution and the overall well-being of the animals were recorded daily. It is possible that the rats disliked the sweet taste of the lactose and thus drank less. Nor can the other possibility of increased gastrointestinal discomfort by maldigested lactose be excluded. No signs of diarrhoea, however, were noticed. It would be interesting, in an experimental hypolactasia model, to have a suitable piece of equipment for recording the gastrointestinal discomfort of the rat, even though experimental models are for investigating the mechanisms, not the symptoms, of lactose maldigestion.

6.2. Possible tools for reducing lactose-induced gastrointestinal symptoms

6.2.1. The hypothesis of the role of gastric emptying and intestinal motility

Reduced gastric emptying and total gastrointestinal transit time are thought to increase mucosal contact time and thus to reduce the quantities of unabsorbed lactose in the colon. In addition to this hypothesis, reduced intestinal motility may also affect the colonic flora and thus modify fermentation products, as suggested by El Oufir *et al* (1996) and Lewis and Heaton (1997). This may also improve tolerance to lactose.

We found that by inhibiting peripheral muscarinic receptors with an oral dose of propantheline, tolerance to lactose, measured as the severity of gastrointestinal symptoms, improved. It is possible that reduced symptoms in our study after propantheline could, at least partly, be due to the non-specific antispasmodic effect of propantheline. However, in addition to reduced gastrointestinal symptoms, the reduced excretion of breath hydrogen and the increased excretion of urine galactose indicate better absorption of lactose. Among other anticholinergic and antispasmodic properties, propantheline has been shown effectively to delay gastric emptying (Hurwitz *et al* 1977).

Apart from the slight tendency of an earlier appearance of gastrointestinal symptoms, we found no differences in tolerance to lactose after pre-treatment with the prokinetic agent metoclopramide as compared with the placebo. A minor indication of lesser absorption of lactose was seen with an increase in the excretion of breath hydrogen and in the reduced excretion of urine galactose. Gastric emptying of a lactose solution after an overnight fast might be fairly rapid even without a small dose of metoclopramide. Perhaps the dose we used, which was chosen on the basis of

previous studies (Massicotte *et al* 1996) and of the average therapeutic single dose recommendations (Dollery 1991), was not sufficient for all our subjects.

Even though we found no correlation between the total gastrointestinal transit time and the development of gastrointestinal symptoms, the possibility cannot be excluded that the reduction of gastric emptying also modified intestinal motility. In another study, the anti-diarrhoeal drug loperamide reduced intestinal transit in lactose maldigesters by about 30 min (Szilagyi *et al* 1996). This prolongation has been found not only to diminish but also to some extent to delay the development of gastrointestinal symptoms (Szilagyi *et al* 1996), but we were not able to confirm this.

Carminic dye and the subjective estimation of its appearance in the faeces may not have been a sufficiently sensitive way to detect gastric emptying reduction. This method for measuring gastrointestinal transit time was chosen because it is considered a fairly simple, cheap and reliable method (Read *et al* 1980, Marlett *et al* 1981) and was readily accepted by the study subjects. Even though there may have been little value in this method of measuring transit time, it may have increased the compliance of the study subjects since it is a good, and at the same time objective, means which can be observed by them personally. There are more exact methods for measuring gastric emptying, such as the radioscintigraphic technique, the ^{14}C octanoic acid breath test, and the use of ultrasonography or radiopaque capsules, but since these methods are unsuitable for out-patients or need special equipment, a greater number of study periods or the collection of faeces over a long period, the estimation of transit time with dye was chosen.

We thus proved with a simple design, without the affecting factors of any other diet or milk components, that by retarding gastric emptying, tolerance to pure lactose was improved. This confirms previous observations of diminished symptoms after retarding gastric emptying by dietary modifications (Welsh and Hall 1977, Nguyen *et al* 1982, Solomons *et al* 1985, Martini and Savaiano 1988, Marteau *et al* 1990, Mahe *et al* 1994, Dehkordi *et al* 1995, Vesa *et al* 1997a, Vesa *et al* 1997b).

6.2.2. The hypothesis of inflammation

In order to investigate the possible role of inflammation in the symptoms of lactose intolerance, we compared the effects of lactose with a digestible disaccharide, sucrose. The synthesis of prostaglandins, as a marker of inflammation, was reduced by administering ibuprofen at the same time as lactose. Prostaglandins are synthesised via the cyclo-oxygenase pathway (COX) in response to stimuli which, in

the gut, include such things as mechanical stimulation, cell trauma and inflammation (see Hawkey and Rampton 1985). Ibuprofen is a nonselective COX inhibitor, affecting both constitutive COX-1 and inducible COX-2.

In the urinary excretion of the prostaglandin metabolites PGE₂-M and 6-keto prostaglandin F_{1 α} , an increase of about one third was noticed between the lactose and the sucrose intakes. By reducing the synthesis of prostaglandins by inhibiting the COX pathway with ibuprofen, this difference between a purely lactose intake and lactose ingested with ibuprofen was not eliminated. Nor were any differences seen in the plasma PGE₂-M excretion. On the other hand, gastrointestinal symptoms and their severity increased after ibuprofen, both with lactose and with sucrose. Thus it seems probable that gastrointestinal symptoms after ibuprofen are not related to lactose ingestion but rather to COX₁ inhibition, and thus they cannot be abolished by reducing the synthesis of prostaglandins, even though slight changes in the excreted concentrations of prostaglandin were noticed.

Hence our findings do not support the hypothesis that lactose-induced symptoms are related to an increased production of prostaglandins, as suggested previously by Buissert *et al* (1978), Lieb (1978) and Lieb (1980). In agreement with Flatz and Lie (1982) we found no relief in gastrointestinal symptoms after the inhibition of prostaglandin synthesis. Thus the role of prostaglandins in mediating the symptoms of lactose intolerance is minor, if it exists at all.

To test the role of another possible marker of intestinal inflammation, the enhanced production of nitric oxide, we measured the concentrations of its excreted metabolites after a lactose challenge. To our knowledge, no previous studies of the role of NO in lactose intolerance exist. In this study the effect of exogenous nitrate was reduced by restricting the dietary intake of nitrate for 48 h preceding the test, as suggested by Wennmalm (1995). In the excretions of the urinary concentrations of nitrate + nitrite (NO_x), which is a stable metabolite of NO, and cyclic GMP, which is a second messenger of NO, no differences related to lactose ingestion were found.

A new method has been recently introduced to measure by chemiluminescence the luminal concentrations of gaseous NO from rectal gas samples, with no need for colonoscopy (Herulf *et al* 1999). In gastro-enteritis patients, rectal NO measurements taken this way were a hundred times higher than in the healthy controls (Herulf *et al* 1999). This method could also perhaps be a new means of investigating the possible role of NO in lactose intolerance. However, when measuring excreted metabolites,

there is always the problem of whether the results refer to the production or to the metabolism of the substance being investigated.

Taken together, even though inflammation-related changes such as hyperemia and edema have been detected in jejunoscopies after a lactose challenge (Banai *et al* 1984), and even though the symptoms of lactose intolerance resemble those seen in inflammatory gastrointestinal diseases, our findings do not support the theory that inflammation is related to lactose-induced symptoms. The method we used to investigate this was not very refined, but if there had been enhanced production of prostaglandins or NO, which are well documented as being associated with chronic inflammation of the intestine (see Stark and Szurszewski 1992, Lefebvre 1995), this would almost certainly have been apparent with the study design and the methods used.

6.2.3. The hypothesis of induction of lactase by lactose

The effect of dietary modification on the expression or activity of the lactase enzyme was tested by supplementing the diet of 8-week-old rats with lactose for seven days. Both the expression and the activity increased compared to the control animals, who received no lactose. The induction of lactase was most obvious in the proximal and the middle parts of the jejunum. This accords with previous experimental studies in which the reduced activity of lactase found in adult animals was at most doubled by dietary modifications (Bolin *et al* 1969, Wen *et al* 1973, Goldstein *et al* 1974, Goda *et al* 1984, Thoreux *et al* 1998).

In human studies, on the other hand, the continuous intake of lactose did not modify the activity found in post-test biopsies (Cuatrecasas *et al* 1965, Newcomer and McGill 1967, Kreusch *et al* 1969, Gilat *et al* 1972). But these biopsies were only taken from the proximal small intestine before and after the study. As shown recently, the activity of lactase varies greatly on the villus enterocytes depending on the biopsy site (Maiuri *et al* 1992, Rossi *et al* 1997). Thus, one biopsy sample is not sufficient to describe the overall ability of the subject, improved or otherwise, to digest lactose.

To reduce the variability of the activity due to the sampling site, and to observe the variability between the different parts of the intestine, several samples were taken longitudinally the length of the small intestine. To describe better the total capacity to digest lactose we produced an indicator simply by adding the activities of single biopsies. When this indicator was compared proportionately to the control group we proved that lactase activity increased overall by about 35% in those animals who

received the largest dose of lactose. This method could be further improved if the effects of age, the total length of the small intestine and the sampling site were noted in this indicator, for example by using suitable coefficients. In this study the effect of these factors was minimised by using age-, weight- and sex-matched animals.

The dose of lactose with which we showed the induction of the enzyme varied between 10-12 g/kg body weight/day. This accords with previous studies, as far as can be concluded from the original articles (Bolin *et al* 1969, Wen *et al* 1973, Goldstein *et al* 1974, Goda *et al* 1984, Thoreux *et al* 1998). In human studies with lactase activity measured from a biopsy, the daily doses have been about one tenth of this quantity (Cuatrecasas *et al* 1965, Newcomer and McGill 1967, Kreuzsch *et al* 1969, Gilat *et al* 1972). But the smaller dose of lactose, about 0.5-1 g/kg body weight/day, reduced the excretion of breath hydrogen and diminished gastrointestinal symptoms in lactose maldigesters after a few weeks of a lactose-containing diet (Johnson *et al* 1993b, Hertzler and Savaiano 1996, Briet *et al* 1997). At the same time faecal β -galactosidase activity doubled (Briet *et al* 1997) or even tripled (Hertzler and Savaiano 1996) compared with the control periods. These authors (Johnson *et al* 1993b, Hertzler and Savaiano 1996, Briet *et al* 1997) suggest that, rather than the induced activity of lactase, colonic bacterial adaptation was responsible. A similar inference was made after a continuous intake of indigestible lactulose (Florent *et al* 1985, Flourie *et al* 1993).

The results of our study thus confirm the hypothesis that, in an experimental model, lactase is induced by the continuous intake of large dose of lactose. This can also be seen in the Western Blot analysis of intestinal homogenates from rats which received milk for several weeks (unpublished data). Probably because of the far smaller doses used, increased activity has not been demonstrated with humans. Continuous, more normal doses of lactose, less than 1 g/kg body weight, have, however, reduced gastrointestinal symptoms and the excretion of breath hydrogen and increased faecal β -galactosidase activity (Johnson *et al* 1993b, Hertzler and Savaiano 1996, Briet *et al* 1997), all of which are indicators of a better tolerance to lactose.

Taking conjointly our three hypotheses of the possible tools for reducing lactose-induced symptoms, it seems that, rather than being of inflammatory origin, lactose intolerance is, at least partly, the result of motility disorders. This has also been suggested by Hammer *et al* (1996), who emphasised the importance of transit time in the occurrence of the symptoms of lactose intolerance, rather than the importance of the amount of malabsorbed lactose. If either gastric emptying or gastrointestinal

transit time or both can be retarded, the reduced level of the lactase enzyme in the intestinal epithelium of a lactose maldigester may be in better state of balance with the normal dietary intake of lactose. This reduction of motility could be achieved by any factors, dietary or otherwise, which may retard gastric emptying and/or reduce intestinal motility. Continuous daily intakes of lactose may further improve tolerance to lactose by inducing the lactase enzyme, or at least by adapting the colonic bacteria so that they ferment undigested lactose in the colon more effectively and thus reduce the development of gastrointestinal symptoms.

6.3. How should lactose intolerance be diagnosed?

Interestingly, the self-diagnosis of lactose intolerance gave poor results. Only one third of the self-diagnosed subjects proved to be lactose maldigesters, and some of them, indeed, proved to be asymptomatic. Our findings accord closely with previous studies in which lower doses of lactose (15-25 g) were used (Rosado *et al* 1987, Johnson *et al* 1993a, Suarez *et al* 1995, Carraccio *et al* 1998, Saltzman *et al* 1999). In these earlier studies the actual number of symptomatic lactose maldigesters among self-reported maldigesters varied between 10% and 70%. Since the incidence of true maldigesters in our studies and studies by other authors varies greatly, probably depending mainly on the inclusion and exclusion criteria of the study subjects, this phenomenon indicates the very real need for carefully controlled clinical lactose tolerance tests.

We also found that about one third of previously tested lactose intolerant subjects were actually lactose digesters, according to our Golden Standard. This large ratio of changed status in tolerance to lactose could be explained either by a truly changed status due to the high incidence of secondary hypolactasia, or by an incorrectly made diagnosis, either an earlier one or the new one.

The incidence of secondary hypolactasia, as well as many probable factors resulting in it by damage to the epithelial mucosa or by the modification of gastrointestinal motility, is not known. As the severity of inherited hypolactasia may vary, so the severity of secondary hypolactasia may also vary according to the power and the length of the challenge that caused it. Lactase is sensitive to factors harmful to the mucosa, because it is located on the brush border of the mature enterocytes on the top of the villi, and is therefore more exposed than other brush-border enzymes to various factors affecting the jejunal mucosa (see Villako and Maaros 1994).

There are several factors that can lead to an incorrect diagnosis. We tried to minimise these by using the combination of three independent test variables, and by controlling those factors which may affect gastrointestinal motility, or may affect the symptoms, such as dietary intake and the prior use of medication. We also used the standard dose of lactose (50.0 g) dissolved in the standard volume of boiling water.

In Finland the lactose tolerance test is usually performed with 50 g lactose dissolved in 200-400 ml water after an overnight fast (Peuhkuri *et al* 2000a). This dose equals the lactose of a whole litre of milk and cannot be assumed to imitate the normal symptoms produced by dietary lactose. The dose of 50 g is suitable for detecting lactose maldigestion, but too abnormal for detecting lactose intolerance. There are studies that describe the use a dose of 25 g with breath hydrogen analysers for detecting lactose maldigesters (see Hamilton 1998), but more detailed studies of an appropriate dose are needed in order to find more effectively those subjects who really benefit from the restriction of lactose in their diets.

An exact recommendation for the volume of water in which lactose should be dissolved is also needed. As the volume ingested affects gastric emptying (see Cooke 1975, Malagelada 1990), it can be assumed that tolerance to lactose tested with a volume of 400 ml differs from a test performed with a smaller volume. The optimum volume depends on the dose of lactose used. In principle, 1 g lactose can be dissolved in 5 ml room-temperature water or in 2.6 ml boiling water. If the lactose is dissolved in boiling water, a proper cooling to room temperature may improve the reliability of the test, since gastrointestinal symptoms produced by the room-temperature solutions are minor compared with those produced by cold or hot solutions, as we have shown. The volume used may be taken from everyday drinking patterns. 'A glass' (200 ml) seems to be the natural volume that can be swallowed without distaste and without the symptoms induced by the larger volume of liquid after an overnight fast.

We found that, compared to the Golden Standard, on its own none of the test variables used in this study was particularly good for recognising both the lactose maldigesters and the digesters, though on average, the sensitivity and the specificity of the variables used were good. The urine galactose test showed the best sensitivity and the breath hydrogen test the best specificity. The total number of misdiagnosed subjects was at most 9% if the blood glucose test was used on its own. However, it should be borne in mind that the predictive value of each test depends on the prevalence of lactose maldigestion in the study group. In our study the incidence of

lactose intolerance was much higher than in a normal cross-section of the Finnish population.

If these two variables are taken in conjunction, the reliability of the lactose tolerance test may improve. The best combination may be to consider the measurements both of excreted breath hydrogen and of urinary galactose. The urinary galactose test showed the highest sensitivity, recognising 100% of the lactose maldigesters; the breath hydrogen test was best for specificity, detecting 94% of the lactose digesters. It would seem possible to look at the results of these two tests together, since a three-hour urine collection is recommended for excreted galactose measurement (Arola 1988b; Grant *et al* 1989, Buttery and Ratnaike 1995, Alvarez-Coca *et al* 1996) and it is recommended that breath hydrogen measurement should be continued until the baseline value is exceeded by at least 20 ppm or for up to three hours (see Hamilton 1998). The combined $^{13}\text{CO}_2/\text{H}_2$ breath test is in its present form far too cumbersome. If, however, this could be developed further, improving both sensitivity and specificity, simplifying the equipment needed, and reducing costs, this could offer a suitable method in the future.

When diagnosing lactose intolerance (symptomatic lactose maldigestion), the recording of gastrointestinal symptoms should not be disparaged. Both the severity and the intensity of symptoms increase for several hours from the ingestion of the lactose solution, the maximum symptom scores predicted being achieved some hours after the beginning of the test. The most common symptoms, both those that were expected and those actually experienced, seem to be flatulence, borborygmi and abdominal bloating. The recording of symptoms during a lactose tolerance test should be based on the true incidence of single symptoms and should last several hours, preferably of minimum of three.

As was seen in our study, both those who are and those who suppose themselves to be lactose maldigesters expect to experience severe symptoms if they drink a glass of milk. A lactose tolerance test as such may thus, in some subjects, increase the severity and the number of gastrointestinal symptoms. One possible mechanism for this may be via increased intestinal motor activity induced by the stress factor, which in this case is the test situation itself. The modifying effect of emotional factors on intestinal motility may also explain the variability of the symptoms of lactose intolerance, as has also been shown with gastrointestinal symptoms in IBS patients (see Stam *et al* 1997). This may be avoided or reduced by using a blinded test, as we did whenever possible.

This blinding could be achieved by using lactose-free milk or another indigestible disaccharide such as lactulose.

To conclude, in the diagnosing of lactose intolerance, based on our results and those of other investigators, any of the laboratory test variables used for detecting lactose intolerance is superior to self-diagnosing. A breath hydrogen measurement together with a urine galactose test after ingestion of a room-temperature test solution in a natural volume of water is to be recommended. The possibility of reducing the dose of lactose from the prevalent 50 g to a more normal dietary level should be studied further. The recording of gastrointestinal symptoms is an essential part of the tolerance test, even if on its own it has little value in diagnosing lactose intolerance.

7. Conclusions

- The Micro H₂ breath hydrogen analyser is reliable and useful for detecting lactose maldigestion.
- Retarding gastric emptying improves tolerance to lactose.
- With the doses used in this study, ingestion of lactose does not affect the production of prostaglandins or nitric oxide, and thus these indicators of inflammation seem not to be connected with the gastrointestinal symptoms of lactose intolerance.
- Continuous ingestion of a large dose of lactose increases the expression and activity of intestinal lactase, at least in rats.
- A need was observed to re-estimate the practical details, such as the temperature of the test solution, in testing tolerance to lactose. The self-diagnosing of lactose intolerance gives unreliable results.

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Katri Peuhkuri

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