Lactose digestion and maldigestion
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Chapter 1

General Introduction and outline of this Thesis
Chapter 1

1.1 INTRODUCTION
Milk and other dairy products are prominent components of the Western diet. They provide high quality proteins as well as vitamins and are the most significant nutritional source of calcium. The ability to digest milk products is an important factor in the prevention of rickets and osteoporosis, especially in climates with a relatively low amount of sunshine, necessary to activate vitamin D, which regulates calcium uptake from the diet. Until weaning breast milk or humanised bottle-feeds are the only food source for newborns. After weaning the consumption of milk from other mammals such as cow’s, goats, sheep or camels remains as very integral ingredient in the diet in many regions all over the world, especially in the Western world. Besides these easily recognisable dairy products, the diet of most people in the Western world contains a lot of milk ingredients added to a variety of food products. Often consumers are not aware that food products contain components of milk. Milk contains a mixture of important nutrients. Macronutrients are carbohydrate, protein and fat. Micronutrients are calcium and other minerals, vitamins and trace elements. Lactose is nearly the sole carbohydrate in milk.

Maldigestion of lactose is the major cause of gastrointestinal symptoms such as bloating, abdominal pain, flatulence or diarrhoea after consumption of dairy products. These symptoms are not very specific and may therefore be caused by other mechanisms than lactose maldigestion. The small intestinal brush border enzyme lactase is the only enzyme responsible for the digestion of lactose. The occurrence of gastrointestinal symptoms after consumption of animal milk has already been recognized by Hippocrates (4th century B.C) as described in his Aphorisms: "Milk is not recommended for those who suffer from headaches. It is bad too, for patients with fever, those whose bellies are distended and full of rumbling and those who are thirsty." (Fragment)\(^1\). Intolerance symptoms may also be caused by allergic reactions to proteins in mother’s milk or animal derived milk products (for instance Cow’s milk protein allergy or CMPA), but this will not be further discussed in this thesis.

Since elimination of dairy products from the Western diet has major nutritional consequences for calcium, vitamin and protein intake adequate diagnosis of adverse reactions is necessary. The studies described in this thesis focus on the relationship between lactose (mal) digestion and the occurrence of clinical symptoms. A better understanding of this (possible) cause-effect relation may prevent unnecessarily elimination of milk and milk products from the diet. To study this relation we evaluated the available diagnostic methods of lactose maldigestion and developed new techniques to improve the measurement of human lactose digestion.
1.2 Lactose

1.2.1 Chemical structure
Lactose (Galactose β1,4 Glucose) is a disaccharide consisting of equimolar quantities of two monosaccharides, glucose and galactose.

1.2.2 Biological synthesis
Lactose is synthesised by lactose synthethase in the mammary gland during late pregnancy and lactation and excreted in mammalian milk. Human milk contains 7% lactose. The lactose content of milk is not notably altered by a change in the maternal diet or in the level of blood glucose. The most frequently used animal derived milk in human diets, cow’s milk, contains 5% lactose.

1.2.3 Biological functions
Breast milk, which provides approximately 10% of the calories as protein and 45% as fat contains about 45% of the calories as carbohydrate, almost solely lactose. Therefore lactose is an important energy source for newborns. In adults the nutritional contribution of lactose in the diet is less important, since other carbohydrates can also be utilized. For neonates lactose may also have an important function in the bacterial colonisation of the large intestine, since even in neonates not all consumed lactose will be digested under physiological conditions and the spill over from the small intestine will reach the colon.

1.3 Physiology of lactose digestion
Lactose cannot be absorbed by the intact intestinal mucosa. It has to be degraded into its absorbable monosaccharides glucose and galactose.

1.3.1 Lactase, the enzyme
Lactase-phlorizine hydrolase (EC 3.2.1.23, EC 3.2.1.62) is the beta-galactosidase enzyme responsible for the hydrolysis of lactose into glucose and galactose. It is synthesised in the small intestinal microvillus membrane.

1.3.2 Physiological regulation of lactase enzyme expression
From the proximal to the distal small intestine mucosa lactase activity is present in a characteristic gradient. Maximal activity occurs in the proximal to mid jejunum and lower activity in the duodenum and ileum. The expression of enzyme activity is high from the 35th gestational week until the weaning period. In the majority of human subjects lactase enzyme activity decreases after weaning to low levels as found in adults, coded for by an autosomal recessive gene. The gene is located on chromosome 2. However, in a minority of the world population, people originating from the Western world, by genetical determination the capacity to digest lactose after weaning is maintained. Most probably, this is caused by selection of a spontaneous mutation in the lactase-phlorizin hydrolase gene. This mutation is inherited in a dominant autosomal way. With the domestication of cows, goats, camels and sheep, on estimation about 10000 years ago, this mutation was especially of benefit for individuals from populations living in area’s with a marginal availability of calcium and vitamin D.
circumstances prevailed in the northern parts of Europe. It was hypothesised by Sahi et al\textsuperscript{10} that the ability to digest lactose after the weaning period caused a nutritional benefit for individuals with the “lactase persistence mutation”. They were able to digest lactose from non-human mammal species, which allowed them to use milk derived from animals in their diet as an easily available source of protein, vitamins, trace elements, calcium and energy. This nutritional advantage caused a longer life expectancy and a higher reproduction rate compared to the individuals without the mutation. Apart from the described protein and energy nutritional benefit of milk consumption, it is presumed that the ability to digest milk could prevent rickets with less pelvic deformations. Therefore women with lactase persistence had a privileged position in the population because of a higher birth rate compared to women without a persistent presence of lactase\textsuperscript{11}.

1.4 Pathology of lactose digestion
The inability to digest lactose after weaning is the normal condition for most of non-Western individuals in the world population. However before this phenomenon was recognised, these individuals were diagnosed to suffer from “lactase deficiency”, which was considered a pathological condition. It is now generally accepted that high lactase activity beyond early childhood is the exception and that low lactase activity after that age is “normal”. The prevalence of lactase non-persistency in different populations in the world varies from 4\% in Denmark to more than 90\% in Asian regions\textsuperscript{10}.

In infants, children and adults with lactase persistence two other conditions may also cause insufficient lactose digestion.

1.4.1 Mucosal damage of the small intestine
This is the most frequent cause of insufficient lactose hydrolysis in individuals with genetic lactase persistence. Several pathological processes and diseases can cause small intestinal mucosal damage, like celiac disease\textsuperscript{12}, radiation enteritis\textsuperscript{13}, cytostatic treatment\textsuperscript{14}, tropical sprue\textsuperscript{15} or Crohn’s disease\textsuperscript{21,22}. Depending on the localisation and the severity of the mucosal damage expression of all disaccharidases including lactase is impaired.

1.4.2 Congenital absence of lactase enzyme synthesis
Congenital lactase deficiency (CLD) is a very rare autosomal recessive gastrointestinal disorder. In Finland however it is one of the approximately 30 rare recessive disorders that are relatively common. Until 1995 42 Finnish patients have been described, while less than 50 patients have been reported from elsewhere\textsuperscript{23}. Affected patients present with watery diarrhoea starting during the first 1-10 days of life when fed lactose-containing milk. The mutation is located on chromosome 2q21 outside the region of the Lactase Phlorizine Hydrolase gene. There is an almost total lack of lactase activity in jejunal biopsies of these patients\textsuperscript{23}.
1.5 Diagnostic tests used to determine lactose digestion or maldigestion in humans

There are many methods to measure lactose digestion in humans. These methods are based on different principles causing variable accuracy and diagnostic reliability. The possibilities and limitations of the available test methods are discussed below.

1.5.1 Measurement of lactase enzyme activity in a Small Bowel Biopsy Specimen

Lactase activity in a small sample of small bowel mucosa is measured by biochemical methods. Specimens are obtained through endoscopic biopsy and subsequently homogenised and incubated with lactose. Lactase hydrolyses the substrate into glucose and galactose. The glucose concentration of the supernatant presents the capacity of the enzyme to hydrolyse the substrate. Dahlqvist first described this method in 1968\(^\text{24}\). To correct for confounding influences of water content variations in the specimen, results are expressed as activity units per gram protein. Since only a very small part of the mucosa can be tested, the relation between the measured lactase activity with the overall physiological lactase activity of the small intestine has not been well established. Under circumstances of mucosal damage especially, local variation in degrees of damage can be large (so called “patchy lesions”). Apart from this, the lactose hydrolysis capacity \(\text{(in vivo)}\) is not only dependent on lactase activity but also on the contact time with the substrate.

1.5.2 Lactose Tolerance Test (LTT)

After consumption of lactose the substrate will be hydrolysed into glucose and galactose. After intestinal absorption of both monosaccharides the galactose is converted into glucose in the liver. Depending on the feeding state of the individual, glucose is either stored in the liver or released to the blood. In the fed state most glucose will be stored, while in the fasting state the substrate derived glucose will mainly be released to the blood. The rise in serum concentration of glucose is related to the amount of lactose that is hydrolysed. However, the total glucose concentration in blood partly consists of glucose derived from body stores, which makes the test unreliable. Therefore this test has been abandoned in clinical practice.

1.5.3 Lactose Tolerance Test with addition of ethanol

In this test the same substrate and principle as for the LTT is used. The addition of ethanol inhibits the hepatic conversion from galactose into glucose. Therefore galactose will be released by the liver into the blood compartment. Galactose (which has toxic effects) is cleared from the blood by renal excretion. The concentration of galactose in the urine thus represents the hydrolysis of lactose in the intestine and the subsequent uptake of galactose by the intestinal mucosa. Because of the toxic effects of the obligatory ethanol suppletion and the toxic
effects of galactose this test cannot be used in infants and children.

1.5.4. Small intestinal intubation

In the proximal part of the small intestine a catheter with a balloon at the end is placed to occlude the gut lumen. Fluids from the part of the gut proximal of the occlusion can be sampled via side holes in the tube. After addition of substrate, consumed or supplied by nasogastric tube, digestion products can be sampled and further analysed. Using this procedure the enzyme activity in the occluded proximal part of the intestine can be measured. The relation to the over all enzyme activity of the gut has not been evaluated. Due to the invasiveness of the method it is only used in research situations.

1.5.5. \(H_2\) breath test

When lactose is not hydrolysed in the small intestine, undigested lactose will reach the colon. There the bacterial flora can ferment it. This fermentation process leads to the production of gasses including Hydrogen (\(H_2\)), methane (\(CH_4\)) and carbon dioxide (\(CO_2\)), and of lactate and short chain fatty acids, that can all be absorbed by the colonocytes. Subsequently the gasses are transported via the blood and exhaled. Since other biological processes in the human body do not produce hydrogen, the exhaled breath concentration of \(H_2\) represents the fermentation of carbohydrate in the colon. Unfortunately the carbohydrate fermentation process in the colon is not restricted to lactose as substrate. Other carbohydrates such as fibre or undigested starch can also be subject to the similar bacterial fermentation process and lead to the same products in breath. Apart from this, many factors can influence the composition of the colonic flora, such as medication, colonic acidity and thereby the capacity to form \(H_2\). The result of the test is expressed as positive or negative (i.e. maldigestion yes or no) with a cut off point of 10 or 20 parts per million \(H_2\) concentration rise above base line levels. This technique was introduced in the 1970’s \cite{25,26,27}. In understanding the mechanisms behind this test its reliability with respect to the quantity of fermented lactose can be doubted. Despite such uncertainty about results it is until today the most frequently used test to study lactose digestion.

1.5.6 \(^{14}\text{CO}_2\) lactose breath test

The carbon molecules in lactose can be labelled with \(^{14}\text{C}\). After consumption and hydrolysis of such labelled lactose, the resulting \(^{14}\text{C}\)-glucose and \(^{14}\text{C}\)-galactose are metabolised and exhaled as \(^{14}\text{CO}_2\). The cumulative amount of exhaled \(^{14}\text{CO}_2\) is related to the hydrolysis of the substrate. However, the oxidation of the absorbed labelled monosaccharides can vary under different test conditions. Furthermore, radioactivity of \(^{14}\text{C}\) however limits its applicability in medial research, especially in infants, children and pregnant women.
1.6 The scope of this thesis

The important nutritional contribution of dairy products to the Western diet makes that elimination of these products will have negative effects on health, unless compensatory supplements are taken. Supplementation of vitamins and calcium will be necessary and alternative protein sources have to be used. Many individuals eliminate all dairy products from their diet when they experience discomfort after milk use or when tests to measure lactose digestion have indicated lactose malabsorption. As stated before none of the available test methods has been proved to be reliable enough in accurately determining the amount of lactose that can be digested. Most test results only indicate an "all or nothing" phenomenon, while functional lactase capacity should be quantified. The availability of lactose labelled with the stable, non-radioactive isotope $^{13}$C made it possible to study the digestion, absorption and metabolism of consumed lactose without the disadvantages of radioactivity. Using the stable isotope technique we were able to study the digestion of lactose in patients with reported milk intolerance symptoms. Measured digestion could be related to the occurrence of symptoms or the degree of mucosal damage in biopsy specimens.

The specific aims of the study are:

1. To develop a method to reliably measure lactose hydrolysis in humans, which can be applied in clinical practice.
2. To study the interfering factors on the $^{13}$CO$_2$ breath test results:
   a. The influence of variations in substrate oxidation due to different levels of exercise.
   b. The effect of variations in colonic carbohydrate fermentation on the results of the $^{13}$CO$_2$ breath test.
3. To evaluate glucose uptake after hydrolysis of lactose
   a. To improve the diagnostic quality of the lactose tolerance test by use of stable isotope labelled $^{13}$C-lactose and measurement of the $^{13}$C-glucose concentration in blood ($^{13}$C-lactose/$^{13}$C-glucose test)
   b. To develop a method that can correct for individual variations in gastric emptying rate, ileo-coecal transit time and glucose metabolism in the $^{13}$C-lactose/$^{13}$C-glucose test
4. Clinical applications:
   a. To study the relation between intestinal lactose digestion capacity and the occurrence of clinical symptoms.
   b. To study the effect of small intestinal mucosa damage on lactose digestion.
References


