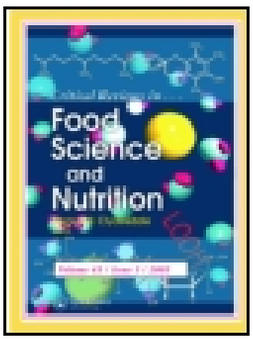

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Recent Advances on Lactose Intolerance: Tolerance Thresholds and Currently Available Solutions

M. Corgneau, J. Scher, L. Ritié-Pertusa, D.T.L. Le, J. Petit, Y. Nikolova, S. Banon & C. Gaiani

To cite this article: M. Corgneau, J. Scher, L. Ritié-Pertusa, D.T.L. Le, J. Petit, Y. Nikolova, S. Banon & C. Gaiani (2015): Recent Advances on Lactose Intolerance: Tolerance Thresholds and Currently Available Solutions, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1123671

To link to this article: http://dx.doi.org/10.1080/10408398.2015.1123671

Accepted author version posted online: 29 Dec 2015.

Recent advances on lactose intolerance: tolerance thresholds and currently available answers

Corgneau, M.[a], Scher, J.[a], Ritié-Pertusa, L.[b], Le, D.T.L.[b], Petit, J.[a], Nikolova, Y.[a], Banon, S.[a], Gaiani, C.[a,*]

[a]Université de Lorraine, Laboratoire d'Ingénierie des Biomolécules, 2, avenue de la Forêt de Haye, TSA 40602, 54518 Vandœuvre-lès-Nancy, France.

[b]Laboratoires SVM, 67130 Muhlbach-sur-Bruche, France.

[*]Corresponding author. Tel.: +33-3-83-59-58-78; Fax: +33-3-83-59-58-04, E-mail address: claire.gaiani@univ-lorraine.fr

Abstract

The genetically-programmed reduction in lactase activity during adulthood affects 70% of the world adult population and can cause severe digestive disorders, which are the sign of lactose intolerance. Lactose intolerance symptoms vary depending on the residual lactase activity, the small bowel transit time, and especially the amount of ingested lactose. To formulate dairy products suitable for the vast majority of lactose intolerants, it is essential to define lactose intolerance threshold. A recent meta-analysis permitted to show that almost all lactose intolerants tolerate 12 g of lactose in one intake and approximately 18 g of lactose spread over the day. The prevalence and severity of lactose intolerance are probably overestimated by the general public. This misconception usually leads to an unnecessary reduction of dairy foodstuff consumption. Nevertheless, dairy products are essential for health mainly due to their calcium content and the positive influence of probiotic bacteria. The formulation of dairy products suitable for most intolerant and suspicious subjects seems necessary. The use of exogenous enzyme preparations,

as well as the consumption of lactose-free products or products rich in probiotic bacteria are proposed as symptom-reducing strategies.

Keywords

dairy, prevalence, lactase, β-galactosidase, low-lactose products, probiotics

Abbreviations

LPH lactase-phlorizin hydrolase

CLD congenital lactase deficiency

LNP lactase non-persistence

LM lactose malabsorption

LI lactose intolerance

SCFAsshort chain fatty acids

IBS irritable bowel syndrome

CMPA cow's milk protein allergy

GOS galacto-oligosaccharides

EU European Union

1. Introduction

Milk, the primary source of nutrients for young mammalians, and dairy products remain beneficial food products even in adulthood. They constitute a high source of proteins, fatty acids, minerals, and vitamins. Milk actually contains high quality proteins including all the essential amino acids (FAO, 2013). Milk proteins are also a good source of bioactive peptides that positively affect various health biomarkers *in vitro*. These include bioactive peptides with antihypertensive, antidiabetic, antiobesity, antioxidant, immunomodulatory, and mineral-binding properties (Nongonierma and FitzGerald, 2015). Milk fat contains approximately 400 different fatty acids (Månsson, 2008), derived from the feed and microbial activity in the rumen of the cow (FAO, 2013). Some of them have demonstrated anticancer activity in animal models: for

instance, rumenic acid is a potent inhibitor of mammary tumorigenesis, sphingolipids seem to prevent the development of intestinal tumours, and butyric acid has showed effect on colon and mammary tumour development (Parodi, 2004). Dairy products have also high contents in minerals (i.e. calcium, magnesium, selenium, zinc, etc.) and vitamins (i.e. B2, B12, D, etc.). Compared to other food sources, milk does not contain any inhibitor of mineral bioavailability; an improvement of mineral assimilation has even been evidenced when dairy products are frequently consumed (Weaver *et al.*, 1999). Calcium and vitamin D are the main dietary factors modulating bone mass. When ingested in sufficient quantities, they reduce the risk of osteoporotic fracture in old people. Diets with low levels of dairy product intake have been associated with increased risk of osteoporosis. Milk is actually a source of bioavailable calcium and just a few other foods naturally contain as much calcium as in milk (i.e. sesame, amaranth, hazelnut, almond) (Souci *et al.*, 2008), hardly consumable in the same proportion as milk. The "calcium paradox", corresponding to higher fractures rates in countries where calcium intake is more important, can be explained by differences in vitamin D, protein, and sodium intake (FAO, 2013). National policies on dietary recommendations vary by country, depending on local food availability, cost, nutritional status, consumption patterns, and food habits. Most of them recommend the consumption of about 500 ml of milk per day. They also advise the consumption of other dairy foods such as cheese, yoghurt, custard, or fermented milks (FAO, 2013). But cow's milk protein allergy and lactose intolerance, corresponding to milk hypersensitivity, affect a significant proportion of the world population. Cow's milk protein allergy is a reproducible clinically abnormal reaction to one or more cow's milk proteins that could involve different immune mechanisms (Vandenplas *et al.*, 2012). It causes frequent regurgitation, vomiting,

swelling of lips or eyes (angioedema), wheezing, rhinitis, and diarrhoea (Solinas *et al.*, 2010). This allergy also causes an alteration of the intestinal mucosa. In contrast, lactose intolerance is a non-immunological reaction, which leads to transient symptoms generally harmless for the gastrointestinal tract (Heyman, 2006). Lactose is the major carbohydrate found in milk and dairy products and it is widely used in processed food (Zheng *et al.*, 2015). It is a disaccharide composed of glucose and galactose. In new-borns, ingested lactose is hydrolysed in the small intestine into glucose and galactose by endogenous lactase-phlorizin hydrolase (LPH) enzyme. The obtained monosaccharides are well absorbed by the intestine. But after weaning, a genetically-programmed reduction in lactase activity can occur and affects 70% of the world adult population (Fox *et al.*, 2015). In case of lactose intolerance, the non-hydrolysed lactose is metabolised by the anaerobic flora of the colon. Metabolites and non-hydrolysed lactose may cause an increase in osmotic load, which can itself lead to digestive disorders. The intolerance degree is highly variable among individuals due to physiological diversity (small bowel transit, colonic water absorption capacity, effect of colonic bacterial fermentation, visceral sensitivity, etc.). The establishment of a lactose tolerance threshold is crucial to develop adapted solutions. The purpose of this review article is to provide an overview of lactose tolerance and to highlight means to formulate functional products suitable for lactose-intolerant consumers.

2. Lactose intolerance

2.1. Lactase deficiency, lactose malabsorption and intolerance

Lactase-phlorizin hydrolase (E.C. 3.2.1.108) is a disaccharidase produced in the intestine of mammalian animals (Plimmer, 1906) and more especially located in the microvillar membrane of epithelial cells in the small intestine. LPH is anchored into the membrane by its C-terminal

end, with the bulk of the molecule turned toward the lumen of the gut. It is a large glycoprotein with two active sites that can catalyse the hydrolysis of a variety of β-glucosides (i.e. phlorizin) and β-galactosides including lactose. Lactase is encoded by a single gene (LCT) of approximatively 50 kb located on chromosome 2 (Shatin, 1968). In humans, this LPH enzyme is synthesised as prepro-LPH, a 1927 amino acids precursor. The first 19 amino acids of this precursor form the signal sequence that is cleaved off in the endoplasmic reticulum, resulting in a 1908 amino acids pro-LPH. This pro-LPH is then glycosylated on the Golgi apparatus (Ouwendijk, 1998). This glycosylation is crucial for the correct folding, proper protein trafficking and subsequent enzymatic activity (Jacob *et al.*, 2000). A subsequent cleavage leads to the formation of a 160 kDa LPHβ and a profragment called LPHα. LPHβ is expressed in the apical membrane and is cleaved by luminal trypsin to obtain the intestinal form. LPHα seems to be enzymatically inactive toward lactose (Ouwendijk, 1998). The mature protein possesses two active sites: Glu1273, in domain III, is responsible for the hydrolysis of glucosides such as phlorizin and Glu1749 catalyses the hydrolysis of galactosides such as lactose. In case of lactase deficiency, a dysfunction of the synthesis is therefore observed. Lactase deficiency can be genetic (primary lactase deficiency) or disease-related (secondary lactase deficiency) (Figure 1). Secondary lactase deficiency is caused by diseases or treatments injuring the intestinal mucosa (Crohn's disease, chronic intestinal inflammation, cancer chemotherapy, etc.). This form is only temporary as lactase activity reappears once the epithelium is healed (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010). Even if lactase seems to be the most vulnerable enzyme, the activities of other disaccharidases and brush border enzymes are also reduced (Flatz, 1987).

For the primary lactase deficiency, two forms are encountered. The most severe one, called congenital lactase deficiency (CLD), is a rare and severe autosomal recessive disorder affecting new-borns (Berg *et al.*, 1969). The most common explanation for CLD is the appearance of a premature stop codon, which is due to frame shifts, missense mutations in the coding region of LPH or exon duplication. The treatment strategy is a rapid removal of lactose from diet to limit life-threatening dehydration and electrolyte loss (Diekmann *et al.*, 2015).

Lactase non-persistence (LNP), the least severe and most encountered form, consists in a down-regulation of lactase activity in intestinal cells after weaning. Details about prevalence and genetic are given in the following subsection.

Lactose malabsorption (LM) indicates that a part of ingested lactose is not hydrolysed in the small bowel and reaches the colon. Lactase deficiency is virtually always responsible for lactose malabsorption (Wilt *et al.*, 2010). Lactose intolerance (LI) is the clinical expression and symptomatic response to lactose malabsorption (i.e. diarrhoea, abdominal discomfort, flatulence, and bloating) (Lomer *et al.*, 2008). Clinical expression of LM depends on the malabsorbed lactose quantity. If small amounts of lactose are ingested, clinical symptoms are limited or absent. Thus, a number of subjects ignore suffering from LM and LNP (Flatz, 1987; Wilt *et al.*, 2010).

2.2. Lactase non-persistence: prevalence and genetics

Lactase synthesis is substantial during the first year of life. After, lactase non-persistence subjects undergo a genetic reduction of lactase synthesis. The onset of LI usually begins between 2 and 3 years and is complete by the age of 5 to 10 years. However, exceptions to this timing have been reported; for example, it mainly occurs during adolescence in Finland (Swallow,

2003). Only 5 to 10% of the initial lactase activity is kept, even in spite of continued intakes of milk or lactose (Paige, 2005).

The prevalence of lactose non-persistence is strongly linked to ethnicity (Welsh *et al.*, 1978). In general, the vast majority of individuals originating from the traditional non-milking zones are maldigesters (70 - 100%). Conversely, individuals coming from a zone with an ancient milk consumption tradition present low prevalence for lactose maldigestion. Thus, the prevalence is low in individuals of northern European descent (15%), medium for African, Latinos, eastern European, and south American (70 - 80 %) and high in many Asian populations (near 100%) (Paige, 2005).

Family studies showed that LP / LNP were due to a genetic polymorphism: lactase non-persistent adults are homozygous for an autosomal recessive allele that cause the post-weaning decline of lactase activity, whereas lactase persistent subjects are either hetero- or homozygous for a dominant allele that allows lactase to persist (Swallow, 2003). Lactase persistence behaves as a dominant trait because half lactase activity is sufficient to show significant lactose digestion capacity. However, as the level of intestinal β-galactosidase is not present in vast excess over requirement, heterozygotes may be more prone to become lactose intolerants in case of stress or mild pathology (Swallow, 2003). Lactase persistence is due to a genetic variation of the LCT promoter located at about 14 kb upstream of the gene coding sequence (Swallow, 2003). The wild-type promoter, which induces a reduction in lactose synthesis after weaning, contains cytosine/cytosine base pair in position 13910. In some cases of lactase-persistence (especially for northern Europeans), cytosine has been replaced by thymine in one or both alleles (Wang *et al.*, 1995), eliminating the programmed reduction in lactase activity (Levitt *et al.*, 2013). Lactase non

persisters have a C/C genotype whereas lactase persisters have C/T (mutation is a dominant trait as indicated above) or T/T genotypes.

This is a way to highlight lactase deficiency with a blood sample only. The only limit of this method is the absence of information about intolerance symptoms (Usai-Satta *et al.*, 2012). Measuring the intestinal lactase activity on jejunum biopsy is another method to directly evaluate lactose deficiency. However, this test seems too invasive for the diagnosis of such a mild condition as LI and the result may be influenced by the heterogeneous distribution of lactase activity throughout the small intestinal mucosa (Usai-Satta *et al.*, 2012). As explained below, information about the persistence/non-persistence of subjects has been largely inferred from measurements of lactose absorption (Wilt *et al.*, 2010).

2.3. Lactose malabsorption: mechanisms, clinical symptoms and measurement

Healthy people cannot absorb lactose as such, it must be hydrolysed into glucose and galactose first. Conversion of lactose into glucose and galactose requires lactase activity. Intestinal LPH has two active sites: one catalyses the hydrolysis of lactose, while the other catalyses the hydrolysis of other carbohydrates (including phlorizin). LPH is located in the small intestine and is most highly expressed in the jejunum. The surface of the small intestinal is composed of hundreds of villi, tiny finger-like structures that protrude from the intestinal wall. Additional extensions called microvilli cover the villi and form the apical brush border of the absorptive epithelium cells. This arrangement maximises the surface area for nutrient absorption. Lactase, as other enzymes involved in the digestion and absorption of carbohydrates, is anchored to the surface of the brush border (Ingram and Swallow, 2009).

After the hydrolysis of lactose by LPH, glucose and galactose are transported across the epithelial cell membranes into the enterocytes (Figure 2) and then into the bloodstream via active transport by a sodium-dependent galactose transporter (Wright *et al.*, 2007). They finally reach the liver, when they enter the pathways of intermediary metabolism and serve as an energy source.

In case of lactase deficiency, ingested lactose is not degraded in the small intestinal and passes into the colon, where it serves as a source of energy for the abundant microflora.

Among the hundreds of colonic bacteria, some have the ability to metabolise lactose, in particular bifidobacteria, lactobacilli and *E. coli*. Bacterial β-galactosidases catalyse the same chemical reactions as lactase but differ from lactase in structure, enzymatic properties, and regulation. When bacterial β-galactosidases release glucose and galactose, intestinal bacteria convert them in a variety of products, including short chain fatty acids (SCFAs), and hydrogen gas (Perlman, 2013) (Figure 3). This fermentation generally causes abdominal pain and bloating.

The fact that lactose is not hydrolysed into glucose and galactose prevents the increase in blood glucose concentration usually observed after lactose intake. Lactose malabsorption can actually be highlighted by a simple blood sample. A blood glucose concentration increasing less than 20 mg/100 mL after ingestion of a large (from 50 to 100 g) dose of lactose is indicative of lactase deficiency (Levitt *et al.*, 2013).

The presence of fermentation products in colon increases the osmotic pressure in lumen. Because of the high hydraulic permeability of colonic mucosa, the gut cannot maintain a good osmotic gradient between blood and lumen so that water moves from blood to lumen to render luminal contents isotonic. Depending on the amount of lactose in colon, water inflow can cause loose

stool and notable diarrhoea (Levitt *et al.*, 2013). The SCFA do not seem to induce trouble as they are absorbed into blood and serve as a source of energy. But besides that, the gas quantity produced by bacterial fermentation reactions can be huge. Degradation of 12.5 g of lactose can release up to 2600 mL CO_2 and 4000 mL H_2 (Wolin, 1981), while the usual excretion rate is below 1000 mL gas per day (Tomlin *et al.*, 1991). A large part of these gases are excreted, causing flatulence, while the rest is absorbed from the intestine and expired via the lungs with breath (Levitt, 1968). Exhaled H_2 is nowadays used as a non-invasive diagnostic for lactose maldigestion. It is commonly considered as the most reliable, non-invasive, and inexpensive technique (Lomer *et al.*, 2008). According to a consensus conference on breath tests (Gasbarrini *et al.*, 2009), it consists in absorb 25 g lactose orally and measuring breath hydrogen levels each 30 min during 4 h (3 h for paediatric use) after oral administration of 25 g lactose. A restricted diet, free of non-absorbable carbohydrates, is advised the evening before the test.

A breath concentration upper than 20 ppm H_2 above baseline indicates lactose intolerance (Järvelä *et al.*, 2009). Breath-test actually shows a good sensitivity and excellent specificity (Usai-Satta *et al.*, 2012). But false negative test occurs in up to 20% of cases (Vernia *et al.*, 2003).

As detailed above, LI triggers abdominal pain, bloating, diarrhoea, and flatus. But LI can also cause nausea, vomiting, and constipation, as well as several systemic symptoms such as headaches, loss of concentration, various allergies, muscle and joint pain (Matthews *et al.*, 2005). In subjects with lactose intolerance, clinical symptoms appear between 1 and 3 h after consumption and is intimately dependent on the amount of ingested lactose, with wide variations among individuals (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)., 2010).

Unlike lactase deficiency and lactose malabsorption, lactose intolerance is based on self-reported symptoms and cannot be objectively demonstrated.

3. Lactose intolerance: tolerance threshold

3.1. Lactose tolerance threshold

The will to highlight the lactose tolerance threshold for intolerance population is not emerging: numerous scientific studies have been carried out for over 40 years (Stephenson and Latham, 1974; Paige *et al.*, 1975; Jones *et al.*, 1976; Lisker and Aguilar, 1978; Newcomer *et al.*, 1978; Cheng *et al.*, 1979; Rorick and Scrimshaw, 1979; Haverberg *et al.*, 1980; Kwon *et al.*, 1980; Rask Pedersen *et al.*, 1982; Lybeck Sørensen *et al.*, 1983; Cavalli-Sforza and Strata, 1987; Johnson *et al.*, 1993b; Suarez *et al.*, 1995a, 1997; Hertzler and Savaiano, 1996; Hertzler *et al.*, 1996; Vesa *et al.*, 1996a, 1997; Xenos *et al.*, 1998; Montalto *et al.*, 2005). Yet, the first meta-analyses showing this threshold value are recent. This can be explained by the fact that some studies do not follow the right methodology, and many are not comparable due to a lack of similar protocols e.g., enrolment criteria, global methodology methods to assess symptoms severity, patient populations. The physiological diversity of subjects also complicates the identification of the threshold. Indeed, symptomatic perceptions of lactose intolerance depend on lactose load, amount of unabsorbed lactose, type of administration (milk is different than a lactose aqueous solution; consumption of lactose during a meal can change its bioavailability), gastric emptying, small bowel transit, colonic water absorption capacity, effect of colonic bacterial fermentation, or visceral sensitivity (Casellas *et al.*, 2010).

Ideally, a large group of subjects in a wide range of age and ethnicity should be randomly recruited and tested for lactose malabsorption (subjects with no lactose malabsorption cannot be

intolerant). Then, lactose malabsorbers would undergo double-blind testing with a maximal physiological dose of lactose (the most standard quantity being 50 g) or an identical placebo to identify lactose intolerants. Lactose intolerant subjects would then be tested in a double-blind crossover study with a dose range of lactose or identical placebo to determine the lactose tolerance threshold. Although the simultaneous consumption of lactose and meal can introduce bias (certain foods such as beans can cause similar symptoms to those induce by LI), this method will be preferred to a single intake. In practice, the vast majority of studies initially administered volunteers with a high dose of lactose (30 to 50 g) in order to classify them as absorbers or malabsorbers based on the BT or blood glucose rise test. Then, no blind control was employed to assess the lactose intolerance of subjects: the classification is only based on reported symptoms during testing. Some of the subjects categorised as lactose intolerants might have had similar symptoms following ingestion of a lactose-free control solution. Lactose-depleted and lactose-free milk, often used as control solutions, are sweeter than conventional milk (glucose and galactose released from lactose have higher sweetening powers than lactose). Some studies did not blind for this taste difference, while other studies employed a variety of methods to mask this taste difference (i.e., the addition of an artificial sweetener to milk or chocolate). Some studies have administered lactose (or low-lactose controls) with meals, while most of them have employed a single dose of milk or control without food ingestion (usually in the morning just after getting up). The heterogeneity of these studies makes them very difficult to summarise.

Another challenge is that physiological diversity of individuals leads to a large variability in tolerated lactose amounts. Lactase non-persistent subjects actually retain a low lactase activity in the brush border of their small bowel. This residual activity remains highly variable across

subjects. The hydrolysed lactose amount also largely depends on the contact time between lactase and lactose, hence to the small bowel transit time. Then, the vast majority of unabsorbed lactose reaches the colon, where it is fermented by colonic bacteria. When low amounts of lactose reach the colon, metabolites are rapidly absorbed by the colonic mucosa and the osmotic load is not sufficient to generate lactose-induced diarrhoea. Conversely, when higher amounts of lactose reach the colon, the production of metabolites by colonic flora may exceed the ability of colonic mucosa to remove them. The osmotic load increases and induces diarrhoea. But differences in faecal bacterial metabolism, colonic mucosal function, and transit time influence the probability of non-persistent lactase subjects to develop diarrhoea as a result of lactose ingestion. Individuals vary also in their response to colonic distention. Thus, for the same degree of distension, hypersensitive subjects perceive discomfort, while less sensitive subjects do not feel any discomfort (Wilt *et al.*, 2010).

Despite these challenges, two major meta-analyses have been conducted on lactose tolerance threshold. The first one (Savaiano *et al.*, 2006) aimed to determine the severity of lactose intolerance among lactose maldigesters after a consumption of between 7 and 25 g lactose in water, milk or commercial food products. This meta-analysis included 21 randomised, crossover, blind studies, with subjects without gastro-intestinal problems. Authors concluded to insignificant differences to distinguish a dose-response relationship. However, the severity of gastro-intestinal symptoms reported by lactose maldigesters was not different after ingestion of 12 g lactose or under placebo-masked conditions. It was concluded that an intake of about 12 g lactose is well tolerated by most people. Conversely, the consumption of 25 g lactose involved symptoms in most of the maldigester population. The second major meta-analysis (Wilt *et al.*,

2010) studied the tolerable doses of lactose in subjects clinically diagnosed as lactose intolerants. To this aim, 38 randomised crossover studies performed between 1968 and 2009 were selected. Studies were heterogeneous in terms of population, interventions, and assessment methods, which complicated the drawing of a general trend. Nevertheless, results indicated that most of individuals diagnosed with lactose intolerance can tolerate 12 g lactose in a single dose with no or minor symptoms. For higher doses, intolerance symptoms became more predominant. However, when lactose was distributed throughout the day and consumed with other nutrients, many maldigesters tolerated doses between 20 and 24 g (Figure 4).

3.2. Lactose intolerance severity

To assess the actual severity of LI, lactose tolerance threshold should be compared to the lactose content of food products. Naturally, lactose contributed to the diet only as mammalian milk and dairy products. Due to its interesting technological properties, lactose is also more and more used in food industry. Indeed, lactose has excellent rheological properties, low sweetness (interesting properties for salted products) and cannot be easily fermented by yeast, preventing unwanted carbon dioxide or ethanol production. In food, lactose may be used as browning agent (mostly in bread and cakes), to add texture or bind water (especially for processed meats such as sausages or hamburgers). It may also be used in the production of soft drinks, breakfast beverages and lagers. Lactose is also injected in some chicken meat and often used in sauces supplied as powders to butchers and restaurants (Matthews *et al.*, 2005). The lactose content of certain food products is presented in Table 1.

Lactose levels vary considerably from trace concentrations in cheddar, mozzarella, fish sticks, or cheeseburger, to more than 2 g / 100 g in yoghurt, milk, sour cream, or chocolate bar (Table 1).

Most studies agreed that the consumption up to 12 g of lactose by intolerants did not cause specific symptoms. On the basis of product composition, a single consumption of 250 mL milk, or three Greek yoghurts is tolerable for most lactose intolerants. Higher doses can be tolerated, especially if lactose is distributed throughout the day and consumed with other food products. The majority of lactose intolerants for example can tolerate 1 serving size of whole milk plus 1 cheeseburger plus 1 Greek yoghurt plus 1 chocolate bar and two cheddar portions in a day. In view of the quantity of lactose-containing products that can be consumed by most lactose intolerants, it appears that the frequency and severity of lactose intolerance is probably overestimated by the general public. Two major factors may explain this overestimation namely the confusion with irritable bowel syndrome (IBS) and the "nocebo effect". IBS is a clinical syndrome affecting 9 to 12% of the population, which is characterised by chronic abdominal pain or discomfort associated with a change in bowel habit that cannot be explained by any organic or biochemical abnormality (Drossman *et al.*, 2002). Meal patterns, caffeine, the type and amount of dietary fibers, fluid intake, gut sensitivity, and food intolerance are the main factors causing the symptoms (Burden, 2001). Lactose intolerance does not lead to IBS (Turnbull, 2000) but subjects with IBS often experience enhanced visceral sensitivity to the luminal effects of lactose (Sciarretta *et al.*, 1984). In addition, lactose intolerance and IBS symptoms are often very similar (Vesa *et al.*, 1998), which makes diagnosis difficult. A large number of subjects with IBS are also diagnosed with lactose intolerance (Alpers, 2006). But some studies have shown that lactose-free milk causes the same symptoms as lactose in subjects, who have been diagnosed with lactose intolerance. In this case, IBS is often the real cause of symptoms (Suarez and Levitt, 1996; Vesa *et al.*, 1998). Lactose intolerance is also confused with

cow's milk protein allergy (CMPA) and 20% of patients with symptoms suggesting lactose intolerance actually suffer from CMPA (Lomer *et al.*, 2008). The "nocebo effect" is a phenomenon that is opposite to the placebo effect, whereby expectation of a negative outcome may lead to the worsening of a symptom (Benedetti *et al.*, 2007). More practically, patients who expect unfavourable side effects before taking a medication are more likely to develop them. These negative expectations tend also to make the subject more attentive to possible side effects and interpret pre-existing ailments as symptoms associated with drug/food intake (Morselli and Garattini, 1970). In the general population, lactose is widely considered as potentially noxious (Vernia *et al.*, 2010). This idea is also communicated by some media dealing with symptoms potentially triggered by lactose (Suarez *et al.*, 1995b). Thus, among the subjects attributing a variety of abdominal complaints to lactose intake, irrespective of the dose, many do not actually have any real lactose intolerance. These misconceptions lead many people to unnecessarily stop their consumption of dairy products. And a major problem arises when self-diagnosed lactose intolerant parents place their children on lactose-restricted diets (even in the absence of symptoms) in the belief that the condition is hereditary. These children and adults tend to be below the recommended doses of calcium and vitamin D, which could decrease bone mineral density (Solomons *et al.*, 1985; Di Stefano *et al.*, 2002). For people with negative expectations on lactose as well as for subjects actually lactose intolerants to low doses, there are some solutions.

4. Solutions to lactose intolerance

4.1. Lactose-depleted and lactose-free dairy products

Some dairy products are naturally low in lactose. In cheese, most of the lactose is lost in the whey during the manufacturing process and in yoghurt, some of the lactose is converted to lactic acid by bacterial action. However, the prevalence of lactose intolerant subjects, or those mistakenly believing it, resulted in the development of industrial lactose-reduced and lactose-free dairy products. For the dairy industry, it allows acquiring new consumers, who otherwise would avoid milk. Enzymatic hydrolysis and membrane processing are the two commercially-used approaches to manufacture lactose-free and lactose-reduced dairy products. The residual lactose content corresponding to these two appellations is not clearly defined. No regulation defining these categories exists in the USA and in the EU. But the EFSA gave a positive opinion on the health claim "Consumption of foods with reduced amount of lactose helps to decrease gastro-intestinal discomfort caused by lactose intake in lactose intolerant individuals." (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011). This is due to the large number of studies convincingly demonstrating that breath-H_2 production and discomfort symptoms are reduced after consuming lactose-free rather than standard milk (Kies, 2014).

Enzymatic hydrolysis of cow's milk began with the commercial availability of β-galactosidase from microbial sources in the early 1970s (Harju *et al.*, 2012). Nowadays, β-galactosidase is one of the most important enzymes used in the food industry (Panesar *et al.*, 2006). Three techniques are industrially used to perform lactose hydrolysis: single use batch (soluble enzyme), recovery system (re-used enzyme), and immobilised system. The choice of process technology depends on substrate nature, enzyme characteristics, and costs of production/storage/marketing of the product (Mahoney, 1997). Indeed, the costs of lactose-hydrolysis process is substantially reduced by using immobilised enzymes (Sprossler and Plainer, 1983). A neutral β-galactosidase is

usually added to pasteurised milk at 6 - 8 °C and left to incubate for 20 - 30 h. The degree of hydrolysis can be controlled by varying the incubation time and temperature, as well as the enzyme dose, and depends on the desired product (lactose-free or depleted-lactose products) (Mahoney, 2002). When this degree is reached, milk is reheated (ultra-pasteurised) for enzyme inactivation and packed. To improve the process, research has been conducted to select a β-galactosidase with an optimum temperature close to the refrigeration temperature (Stougaard and Schmidt, 2012). In a second method, a small quantity of the enzyme (*K. lactis*) can also be sterilised by ultrafiltration and mixed with UHT milk prior to aseptic packaging. During the storage (at least 7 – 10 days) at room temperature, complete hydrolysis takes place. This process is a low-cost solution but the enzyme must be very pure and free from protease activity (Mahoney, 2002).

The products obtained by enzymatic methods are very prone to protein deterioration (including Maillard browning), as lactose is partially replaced by glucose and galactose, which react with lysine at a higher rate than the disaccharide (Naranjo *et al.*, 2013). The browning level depends on the hydrolysis percent (Rehman, 2009). In addition, when lactose is hydrolysed, the number of free molecules actually increases, thus raising the amount of soluble substances in the milk, decreasing its freezing point (Antunes *et al.*, 2014). Lactose-hydrolysed milk also presents a higher sweetness due to the lower sweetness of lactose compared with its monosaccharide constituents, glucose and galactose. This is less appreciated by some consumers (Shakeel-Ur-Rehman, 2009) but, in general, lactose-hydrolysed milk is equally liked as standard milk (Paige *et al.*, 1975; Brand and Holt, 1991). This property could also reduce the amount of added sugar to certain products such as chocolate milk (Li *et al.*, 2015). Treatment of regular milk with yeast

β-galactosidase by the consumer before consumption is also possible. Small packages of neutral β-galactosidase can be bought in order to add few drops to milk one day prior to consumption. Depending on the dosage, lactose can be hydrolysed within 12 – 24 h (Dekker and Daamen, 2011). To produce depleted-lactose milk powders, the latter method can be applied. Two other traditional methods are also available. Lactose in milk can be partially or completely hydrolysed by β-galactosidase treatment before drying. This process usually lead to a strong increase in browning reactions (e.g., Maillard reactions) upon storage, which cause flavour change, reduction of nutritional value, and caking issues. The third method process consists in the addition of active β-galactosidase to milk powder at dry state. But this method causes de-mixing problems during manufacturing and storage, resulting in heterogeneous distributions of lactase activity in milk powders and consequently variations in lactose hydrolysis efficiency upon reconstitution. Another problem is the reduction of lactase activity during storage, especially at elevated temperatures. A brain new method was recently developed by a leader in the food industry. The method proposed to mix β-galactosidase and milk, and perform drying quickly in order to avoid enzyme action. Thus, the enzyme is still active when consumers rehydrate the powder and there is no browning reactions (Braun and Niederreiter, 2012).

Membrane techniques such as microfiltration and ultrafiltration are also commercially used to produce low lactose and lactose-free milks, without taste change. Ultrafiltration membranes retain all the fat and practically all the proteins in milk, but only 30% of the lactose content. When ultrafiltration concentrate is diluted with water to reach the level of proteins and fat in the original milk, the lactose content is less than 1.6 %. The residual lactose is then hydrolysed by a β-galactosidase. Other filtration methods have been patented by different companies to obtain

quasi lactose-free milks. However, urea, amino acids, and minerals unbound to proteins are not retained (Grandison and Glover, 1994). Moreover, milk in which lactose content is reduced by filtration to less than 2% presents a lower cryoscopic value, usually taken as evidence of milk adulteration. This causes labelling issues with regulatory authorities (Shakeel-Ur-Rehman, 2009).

In 2011, the lactose-free market in the USA and Europe grew by 100% compared with 2007 levels. This growth is mainly due to the European market, which tripled in volume over this period. A huge opportunity for lactose-free market lies actually in South Africa or Asia (Mellentin, 2012). The variation of local availability of such products is actually a potential problem (Kies, 2014).

4.2. Exogenous β-galactosidase

As previously noted, the dairy industry uses exogenous β-galactosidase to produce lactose-free or low lactose products. β-galactosidase can also be added in liquid form to milk by the consumer before intake, or be taken in solid form together with milk and dairy products (Levitt *et al.*, 2013). β-galactosidase can be isolated from several sources such as plants, animal organs, yeasts, bacteria, and fungi (Richmond *et al.*, 1981), but common soluble lactases are generally of microbial origin (Greenberg and Mahoney, 1981). Fungal enzymes, from *Aspergillus Niger* or *Aspergillus Oryzae*, have their optimum pH from 3 to 6 and a relatively high optimum temperature (50 - 60 °C). Its major drawback is the strong inhibition by galactose (Mahoney, 1985). Industrially, it is used to hydrolyse lactose in acid wheys and their permeates. Thanks to their activity in the acidic environment of the stomach, fungal enzymes are well suited as dietary supplement. Yeast enzymes, from *Kluyveromyces Lactis* and *Kluyveromyces Fragilis*, have a

neutral optimum pH and an optimum temperature between 30 and 40 °C. Owing to their optimal pH, they are well suited to hydrolyse lactose in milk. However, milk calcium and sodium exert a strong inhibition of these yeast enzymes (Mahoney, 1985) (Table 2).

Neutral and acid β-galactosidase preparations are commercially available. To hydrolyse lactose, preparations can be added to milk in solid or liquid forms prior to consumption. Several studies have evaluated the efficacy of this type of preparations (Payne *et al.*, 1981; Rosado *et al.*, 1984, 1986; Solomons *et al.*, 1985; Barillas and Solomons, 1987; Corazza *et al.*, 1992; Montalto *et al.*, 2005). While the efficacy of exogenous lactase in both reducing exhaled H_2 and overall lactose intolerance symptoms has largely been demonstrated, the exact efficiency rate is somehow discordant, certainly owing to protocol discrepancies.

Studied enzymes were not produced by the same strains, were not added to milk in the same proportions and were conducted on a small number of subjects (Table 3). It is therefore difficult to outline a clear conclusion.

Solid fungal β-galactosidase preparations (in tablets or capsules) are also commercially available. These preparations are consumed before the meal and would allow lactose hydrolysis during digestion. Unfortunately, although some of these studies underlined their interest, only few have examined their efficacy (Biller *et al.*, 1987; Moskovitz *et al.*, 1987; DiPalma and Collins, 1989; Sanders *et al.*, 1992; Lin *et al.*, 1993; Ramirez *et al.*, 1994; Gao *et al.*, 2002; Portincasa *et al.*, 2008; Ojetti *et al.*, 2010). However, the usefulness of solid β-galactosidase preparations is still discussed. Most studies are actually very hard to compare due to a lack of homogeneity in β-galactosidase dose, timing of supplementation, lactose load, and methods to evaluate lactose maldigestion or to measure gastro-intestinal symptoms (Table 4). Many studies were also

conducted at limited scale, and the doses applied are sometimes far above the recommendations recently issued by the EFSA (EFSA, 2010). However, the EFSA Panel concludes that a cause and effect relationship has been established between the consumption of lactase enzyme and breaking down lactose in individuals with symptomatic lactose maldigestion (EFSA, 2009).

Studies on efficiency of different β-galactosidase digestive supplements have shown low *in vitro* enzyme stability under simulated gastrointestinal conditions and more particularly, due to the low pH and the presence of pepsin, in simulated gastric environment. However, β-galactosidase can be protected from gastric degradation by encapsulation in an enteric coating (O'Connell and Walsh, 2006). Enteric coatings are generally composed of pH-sensitive materials that remain stable at the low pH of the stomach and that release the molecule of interest at the higher pH of intestine (Carino and Mathiowitz, 1999).

Research has therefore been carried out to improve β-galactosidase stability. Several previously uncharacterised β-galactosidases have been studied and exhibit more suitable clinical applications than current commercial ones. But these enzymes are still susceptible to digestive proteases and their commercialization would depend on further significant technical improvements and politics of regulation (Turner *et al.*, 2011). To overcome these limitations, a commercial β-galactosidase from *Aspergillus Oryzae* was chemically modified with branched 40-kDA polyethylene glycol (a hydrophilic polymer) to create a zone of steric hindrance around the enzyme. Steric hindrance should be controlled to prevent the action of endogenous digestive enzymes, while allowing substrate to reach catalytic sites (Liu *et al.*, 2013). The chemically-modified enzyme exhibits better stability at acidic pH and in simulated gastric fluids containing pepsin (Turner *et al.*, 2011).

4.3. Fermented milks and yoghurt consumption

The beneficial effect of fermented dairy products has been highlighted since the 1970s (Gallagher *et al.*, 1974). Many studies have subsequently been carried out to check this postulate and understand the involved mechanisms. Most of them have focused on the beneficial effect of yoghurt, a fermented milk product obtain by the action of symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. b*ulgaricus* (Food and Agriculture Organization of the United Nations, 2007).

Most studies compared the effect of fresh yoghurt (with viable bacteria) to pasteurised yoghurt (with reduced or no living cultures), and/or milk in order to investigate the effect on lactose digestion. In most of the studies, the BT was used to evaluate lactose digestion. In nearly all studies, breath-hydrogen production was significantly lower with fresh yoghurt compared to milk and pasteurised yoghurt (Kolars *et al.*, 1984; Savaiano *et al.*, 1984; Dewit *et al.*, 1988; Lerebours *et al.*, 1989; Onwulata *et al.*, 1989; Pochart *et al.*, 1989; Marteau *et al.*, 1990; Martini *et al.*, 1991; Rosado *et al.*, 1992; Varela-Moreiras *et al.*, 1992; Rizkalla *et al.*, 2000; Labayen *et al.*, 2001; Pelletier *et al.*, 2001). Some studies also scored the intolerance symptoms: fewer were noted after consumption of fresh yoghurt than milk, but no difference was observed between fresh and pasteurised yoghurts (Kolars *et al.*, 1984; Onwulata *et al.*, 1989; Rosado *et al.*, 1992; Shermak *et al.*, 1995; Pelletier *et al.*, 2001). Thus, viable yoghurt cultures decrease LM compared to milk and pasteurised yoghurt, and improve symptoms of LI in comparison to milk.

The first explanation was that yoghurt lactose content is inferior in yoghurt than in milk (Gallagher *et al.*, 1974). In fact, although bacteria tend to metabolise lactose, the yoghurt lactose content remained very close to that of milk. This is partly due to the limited lactose consumption

capacity of bacteria (Adolfsson *et al.*, 2004). When yoghurt is enriched with milk powder to raise the levels of solids, the lactose content may even be higher than in milk (Kies, 2014). Consequently, the lactose content can just partially explain the better tolerance of yoghurt than milk in case of LI.

The positive effect of β-galactosidase from yoghurt bacteria on lactose digestion was highlighted in the 1980s (Kolars *et al.*, 1984). In fact, as long as the integrity of the bacteria is retained, lactose can enter the cell via a permease where it is hydrolysed by endogenous β-galactosidase (Adolfsson *et al.*, 2004). The preservation of microbial β-galactosidase during gastric passage can be explained by the huge buffering capacity of yoghurt (Savaiano, 2014). The presence of bile also improves β-galactosidase activity (Gilliland and Kim, 1984), potentially by increasing cellular permeability and allowing more substrate entering the bacterial cell (Noh and Gilliland, 1994), or by degrading the cell bacteria, freeing exogenous β-galactosidase that becomes more accessible (Hove *et al.*, 1999). Thus, the cell wall membrane structure of bacteria plays a key role in β-galactosidase availability (Mustapha *et al.*, 1997).

Finally, most studies show that breath- H_2 concentration peak after consuming fresh yoghurt occurs later than for milk, indicating a slower digestion time for yogurt. It was suggested that this prolonged gastro-intestinal transit time explains part of improved lactose digestion after a yoghurt consumption (Vesa *et al.*, 1996b). This would increase the contact time between lactase (both bacteria β-galactosidase and LPH) and lactose, and thus improve lactose hydrolysis rate (Kies, 2014).

4.4. Colonic adaptation

In case of lactose maldigestion, the colonic bacteria ferment lactose and produce SCFAs and gases. Historically, this fermentation was perceived as a cause of LI symptoms. However, it has been largely highlighted that the fermentation of lactose and other nonabsorbed carbohydrates play an important role for human colonic health and nutritional status (Hertzler *et al.*, 2013). In case of LNP, the loss of lactase activity is definitive: a continuing lactose consumption has no effect on jejunal LPH (Keusch *et al.*, 1969; Reddy and Pershad, 1972; Sahi, 1994). However, several studies have highlighted an improvement of LI symptoms after a gradual introduction of lactose in the diet (Reddy and Pershad, 1972; Habte *et al.*, 1973; Johnson *et al.*, 1993a). Thus, a beneficial role of colonic bacterial adaptation has been proposed. After prolonged nonabsorbed disaccharide feeding, several studies have demonstrated fewer breath hydrogen response and symptoms, and an increasing activity of fecal β-galactosidase (Perman *et al.*, 1981; Florent *et al.*, 1985; Hertzler and Savaiano, 1996, Briet *et al.*, 1997). In fact, the prolonged lactose, lactulose, and other nonabsorbable oligosaccharide feedings stimulate the growth or metabolic activity of bacteria (e.g., bifidobacteria and lactic acid bacteria) that can ferment lactose without hydrogen production. In addition, the growth of some hydrogen-producing bacteria (i.e. clostridia, *Escherichia Coli*, etc.) is inhibited by high Bifidobacteria populations (Hertzler *et al.*, 2013). However, it seems that the symptoms improvement observed in these studies is not necessarily linked to colonic adaptation, but rather a placebo effect. Indeed, acclimatisation to the test procedures (between the initial test and the final one) would reduce the severity of the subjective symptoms by affecting visceral sensitivity or the subject would become more adapted to the scoring system. In conclusion, the role of colonic adaptation in improving symptoms is not clearly established.

4.5. New developments on functional products

Many products for lactose intolerants are currently commercially available, i.e. lactose-depleted and lactose-free products, dietary β-galactosidase supplements. The next step consist to develop products having a second functionality. Examples of such products are given in this subsection.

Between 5 % and 15 % of infants show symptoms suggesting adverse reactions to cow's milk protein, resulting from an immunological reaction to one or more milk proteins. Symptoms include urticarial and angioedema, gastrointestinal symptoms (vomiting and diarrhoea), atopic dermatitis, and respiratory issues (Vandenplas *et al.*, 2007). In order to avoid both CMPA and LI symptoms, a low-lactose milk product where the milk proteins have been hydrolysed by a protease has been developed (Sibakov and Tossavainen, 2012). The developed product presents the same appearance and organoleptic properties than an ordinary product.

The food industry has also an increasingly high demand for low-calorie sweet-tasting food products in order to help overcome the overweight and obesity problems that have become so prevalent in the last 20 years. Sweetness, usually regarded as a pleasurable sensation, greatly varies according to the type of saccharide present in the product. Glucose is thus perceived more than 4 times sweeter than lactose while still having approximately the same level of calories. To enhance fermented dairy products sweetness without adding artificial sweeteners, mutant *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* strains, which excrete high levels of glucose by degrading lactose, have been selected (Johansen *et al.*, 2013). The obtained fermented dairy products are also suitable for lactose intolerants.

The enzyme β-galactosidase usually hydrolyses lactose into D-glucose and D-galactose. But at high lactose concentration, some β-galactosidases are able to transfer galactose to the hydroxyl

groups of D-galactose or D-glucose to form galacto-oligosaccharides (GOS). GOS are carbohydrates, not digestible in humans, comprising up to nine galactose molecules linked by glycosidic bonds. GOS may also include one or more glucose molecules. They present health benefits including low cariogenicity, low caloric content, insulin-independent metabolism, and stimulation of growth and metabolism of specific colonic bacteria (Gänzle, 2012). Thus, a method for obtaining both lactose-depleted and rich GOS products was recently developed (Larsen *et al.*, 2015). The challenge was to obtain GOS even in naturally low lactose products like milk and to stabilise them. Indeed, a small residual β-galactosidase activity after a high temperature pasteurization step is sufficient to degrade formed GOS. The inventors proposed the use of a β-galactosidase derived from a strain of *Bifidobacterium bifidum*, and a specific thermal treatment to avoid GOS degradation.

References

Adolfsson, O., Meydani, S.N., and Russell, R.M. (2004). Yogurt and gut function. *Am. J. Clin. Nutr.* **80**: 245–256.

Alpers, D.H. (2006). Diet and irritable bowel syndrome. *Curr. Opin. Gastroenterol.* **22**: 136–139.

Antunes, A.E.C., Silva e. Alves, A.T., Gallina, D.A., Trento, F.K.H.S., Zacarchenco, P.B., Van Dender, A.G.F., et al. (2014). Development and shelf-life determination of pasteurized, microfiltered, lactose hydrolyzed skim milk. *J. Dairy Sci.* **97**: 5337–5344.

Barillas, C. and Solomons, N.W. (1987). Effective Reduction of Lactose Maldigestion in Preschool Children by Direct Addition of β-Galactosidases to Milk at Mealtime. *Pediatrics.* **79**: 766–772.

Benedetti, F., Lanotte, M., Lopiano, L., and Colloca, L. (2007). When words are painful: unraveling the mechanisms of the nocebo effect. *Neuroscience.* **147**: 260–271.

Berg, N.O., Dahlqvist, A., Lindberg, T., and Studnitz, W.V. (1969). Severe Familial Lactose Intolerance–a Gastrogen Disorder? *Acta Pædiatrica.* **58**: 525–527.

Biller, J.A., King, S., Rosenthal, A., and Grand, R.J. (1987). Efficacy of lactase-treated milk for lactose-intolerant pediatric patients. *J. Pediatr.* **111**: 91–94.

Brand, J.C. and Holt, S. (1991). Relative effectiveness of milks with reduced amounts of lactose in alleviating milk intolerance. *Am. J. Clin. Nutr.* **54**: 148–151.

Braun, M. and Niederreiter, C. (2012) Lactase containing milk powder.

Briet, F., Pochart, P., Marteau, P., Flourie, B., Arrigoni, E., and Rambaud, J.C. (1997). Improved clinical tolerance to chronic lactose ingestion in subjects with lactose intolerance: a placebo effect? *Gut* **41**: 632–635.

Burden, S. (2001). Dietary treatment of irritable bowel syndrome: current evidence and guidelines for future practice. *J. Hum. Nutr. Diet. Off. J. Br. Diet. Assoc.* **14**: 231–241.

Carino, G.P., Mathiowitz, E. (1999). Oral insulin delivery. *Adv. Drug Deliv. Rev.* **35**: 249–257.

Casellas, F., Aparici, A., Casaus, M., Rodríguez, P., and Malagelada, J.R. (2010). Subjective perception of lactose intolerance does not always indicate lactose malabsorption. *Clin. Gastroenterol. Hepatol. Off. Clin. Pract. J. Am. Gastroenterol. Assoc.* **8**: 581–586.

Cavalli-Sforza, L.T. and Strata, A. (1987). Double-blind study on the tolerance of four types of milk in lactose malabsorbers and absorbers. *Hum. Nutr. Clin. Nutr.* **41**: 19–30.

Cheng, A.H., Brunser, O., Espinoza, J., Fones, H.L., Monckeberg, F., Chichester, C.O., et al. (1979). Long-term acceptance of low-lactose milk. *Am. J. Clin. Nutr.* **32**: 1989–1993.

Corazza, G.R., Benati, G., Sorge, M., Strocchi, A., Calza, G., and Gasbarrini, G. (1992). β-Galactosidase from Aspergillus niger in adult lactose malabsorption: a double-blind crossover study. *Aliment. Pharmacol. Ther.* **6**: 61–66.

Dekker, P.J.T. and Daamen, C.B.G. (2011). Enzymes exogenous to milk in dairy technology | β-d-Galactosidase. **In**: Encyclopedia of Dairy Sciences, pp. 276–283. Fox, P.F., McSweetney, P.L.H., Eds., Elsevier.

Dewit, O., Pochart, P., and Desjeux, J.. (1988). Breath hydrogen concentration and plasma glucose, insulin and free fatty acid levels after lactose, milk, fresh or heated yogurt

ingestion by healthy young adults with or without lactose malabsorption. *Nutrition.* **4**: 131–135.

Diekmann, L., Pfeiffer, K., and Naim, H.Y. (2015). Congenital lactose intolerance is triggered by severe mutations on both alleles of the lactase gene. *BMC Gastroenterol.* **15.**:

DiPalma, J.A. and Collins, M.S. (1989). Enzyme replacement for lactose malabsorption using a beta-D-galactosidase. *J. Clin. Gastroenterol.* **11**: 290–293.

Di Stefano, M., Veneto, G., Malservisi, S., Cecchetti, L., Minguzzi, L., Strocchi, A., and Corazza, G.R. (2002). Lactose malabsorption and intolerance and peak bone mass. *Gastroenterology.* **122**: 1793–1799.

Drossman, D.A., Camilleri, M., Mayer, E.A., and Whitehead, W.E. (2002). AGA technical review on irritable bowel syndrome. *Gastroenterology* **123**: 2108–2131.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2009). Scientific Opinion on the substantiation of health claims related to lactase enzyme and breaking down lactose. *EFSA J*, **7**: 1236-1249.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2010). Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia. *EFSA J.* **8**: 1777–1806.

EFSA Panel on Dietetic Products, Nutrition and Allergies. (2011). Scientific Opinion on the substantiation of health claims related to foods with reduced lactose content and decreasing gastro-intestinal discomfort caused by lactose intake in lactose intolerant individuals (ID 646, 1224, 1238, 1339) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. **9**: 2236–2250.

FAO. (2013). Milk and dairy products in human nutrition, Rome.

Flatz, G. (1987). Genetics of lactose digestion in humans. *Adv. Hum. Genet.* **16**: 1–77.

Florent, C., Flourie, B., Leblond, A., Rautureau, M., Bernier, J.J., and Rambaud, J.C. (1985). Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study). *J. Clin. Invest.* **75**: 608–613.

Food and Agriculture Organization of the United Nations ed. (2007). Milk and milk products. Food and Agriculture Organization of the United Nations, Rome.

Fox, P.F., Uniacke-Lowe, T., McSweeney, P.L.H., and O'Mahony, J.A. (2015). Lactose. **In**: Dairy Chemistry and Biochemistry, pp. 21–68. Springer International Publishing, Cham,.

Gallagher, C.R., Molleson, A.L., and Caldwell, J.H. (1974). Lactose intolerance and fermented dairy products. *J. Am. Diet. Assoc.* **65**: 418–419.

Gänzle, M.G. (2012). Enzymatic synthesis of galacto-oligosaccharides and other lactose derivatives (hetero-oligosaccharides) from lactose. *Int. Dairy J.* **22**: 116–122.

Gao, K.-P., Mitsui, T., Fujiki, K., Ishiguro, H., and Kondo, T. (2002). Effect of lactase preparations in asymptomatic individuals with lactase deficiency--gastric digestion of lactose and breath hydrogen analysis. *Nagoya J. Med. Sci.* **65**: 21–28.

Gasbarrini, A., Corazza, G.R., Gasbarrini, G., Montalto, M., Di Stefano, M., Basilisco, G., et al. (2009). Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. *Aliment. Pharmacol. Ther.* **29 Suppl 1**: 1–49.

Gilliland, S.E. and Kim, H.S. (1984). Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. *J. Dairy Sci.* **67**: 1–6.

Grandison, A.S. and Glover, F.A. (1994). Membrane processing of milk. **In**, Modern Dairy Technology, pp: 273-311. Robinson, R.K., Eds., Springer, Boston.

Greenberg, N.A. and Mahoney, R.R. (1981). Immobilisation of lactase (beta-galactosidase) for use in dairy processing: a review. *Process Biochem.* **16**: 2–8.

Habte, D., Sterky, G., and Hjalmarsson, B. (1973). Lactose malabsorption in Ethiopian children. *Acta Paediatr. Scand.* **62**: 649–654.

Harju, M., Kallioinen, H., and Tossavainen, O. (2012). Lactose hydrolysis and other conversions in dairy products: Technological aspects. *Int. Dairy J.* **22**: 104–109.

Haverberg, L., Kwon, P.H., and Scrimshaw, N.S. (1980). Comparative tolerance of adolescents of differing ethic backgrounds to lactose-containing and lactose-free dairy drinks. I. Initial experience with a double-blind procedure. *Am. J. Clin. Nutr.* **33**: 17–21.

Hertzler, S.R., Huynh, B.C., and Savaiano, D.A. (1996). How much lactose is low lactose? *J. Am. Diet. Assoc.* **96**: 243–246.

Hertzler, S.R. and Savaiano, D.A. (1996). Colonic adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. *Am. J. Clin. Nutr.* **64**: 232–236.

Hertzler, S., Savaiano, D.A., Jackson, K.A., Bhriain, S.N., and Suarez, F.L. (2013). Nutrient Considerations in Lactose Intolerance. **In:** Nutrition in the Prevention and Treatment of Disease, pp. 757–772. Coulston, A.M., Boushey, C.J., and Ferruzzi, M.G., Eds., Elsevier.

Heyman, M.B. (2006). Lactose Intolerance in Infants, Children, and Adolescents. *Pediatrics.* **118**: 1279–1286.

Hove, H., Nørgaard, H., and Mortensen, P.B. (1999). Lactic acid bacteria and the human gastrointestinal tract. *Eur. J. Clin. Nutr.* **53**: 339–350.

Ingram, C.J.E. and Swallow, D.M. (2009) Lactose Malabsorption. **In**, Advanced Dairy Chemistry pp. 203–229. McSweeney, P.L.H. and Fox, P.F., Eds., Springer.

Jacob, R., Weiner, J.R., Stadge, S., and Naim, H.Y. (2000). Additional N-glycosylation and its impact on the folding of intestinal lactase-phlorizin hydrolase. *J. Biol. Chem.* **275**: 10630–10637.

Järvelä, I., Torniainen, S., and Kolho, K.-L. (2009). Molecular genetics of human lactase deficiencies. *Ann. Med.* **41**: 568–575.

Johansen, E., Soerensen, K.I., Curic-Bawden, M., and Junge, M.P. (2013). Use of lactic acid bacteria for preparing fermented food products with increased natural sweetness.

Johnson, A.O., Semenya, J.G., Buchowski, M.S., Enwonwu, C.O., and Scrimshaw, N.S. (1993a)/ Adaptation of lactose maldigesters to continued milk intakes. *Am. J. Clin. Nutr.* **58**: 879–881.

Johnson, A.O., Semenya, J.G., Buchowski, M.S., Enwonwu, C.O., and Scrimshaw, N.S. (1993b)/ Correlation of lactose maldigestion, lactose intolerance, and milk intolerance. *Am. J. Clin. Nutr.* **57**: 399–401.

Jones, D.V., Latham, M.C., Kosikowski, F.V., and Woodward, G. (1976). Symptom response to lactose-reduced milk in lactose-intolerant adults. *Am. J. Clin. Nutr.* **29**: 633–638.

Keusch, G.T., Troncale, F.J., Thavaramara, B., Prinyanont, P., Anderson, P.R., and Bhamarapravathi, N. (1969) Lactase deficiency in Thailand: effect of prolonged lactose feeding. *Am. J. Clin. Nutr.* **22**: 638–641.

Kies, A.K. (2014.) 9 - Authorised EU health claims related to the management of lactose intolerance: reduced lactose content, dietary lactase supplements and live yoghurt cultures. **In:** Foods, Nutrients and Food Ingredients with Authorised Eu Health Claims, pp. 177–211. Sadler, M.J., Eds., Woodhead Publishing.

Kolars, J.C., Levitt, M.D., Aouji, M., and Savaiano, D.A. (1984). Yogurt--an autodigesting source of lactose. *N. Engl. J. Med.* **310**: 1–3.

Kwon, P.H., Rorick, M.H., and Scrimshaw, N.S. (1980). Comparative tolerance of adolescents of differing ethnic backgrounds to lactose-containing and lactose-free dairy drinks. II. Improvement of a double-blind test. *Am. J. Clin. Nutr.* **33**: 22–26.

Labayen, I., Forga, L., González, A., Lenoir-Wijnkoop, I., Nutr, R., and Martínez, J.A. (2001). Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. *Aliment. Pharmacol. Ther.* **15**: 543–549.

Larsen, M.K., Cramer, J.F., and Eisele, T. (2015). A method for preparing a dairy product having a stable content of galacto-oligosaccharide(s).

Lerebours, E., N'Djitoyap Ndam, C., Lavoine, A., Hellot, M.F., Antoine, J.M., and Colin, R. (1989). Yogurt and fermented-then-pasteurized milk: effects of short-term and long-term ingestion on lactose absorption and mucosal lactase activity in lactase-deficient subjects. *Am. J. Clin. Nutr.* **49**: 823–827.

Levitt, M.D. (1968). Production and Excretion of Hydrogen Gas in Man. *N. Engl. J. Med.* **281**: 122–127.

Levitt, M.D, Wilt, T., and Shaukat, A. (2013). Clinical implications of lactose malabsorption versus lactose intolerance. *J. Clin. Gastroenterol.* **47**: 471–480.

Lin, M.Y., Dipalma, J.A., Martini, M.C., Gross, C.J., Harlander, S.K., and Savaiano, D.A. (1993). Comparative effects of exogenous lactase (beta-galactosidase) preparations on in vivo lactose digestion. *Dig. Dis. Sci.* **38**: 2022–2027.

Lisker, R. and Aguilar, L. (1978). Double blind study of milk lactose intolerance. *Gastroenterology* **74**: 1283–1285.

Liu, M., Tirino, P., Radivojevic, M., Phillips, D.J., Gibson, M.I., Leroux, J.-C., and Gauthier, M.A. (2013). Molecular Sieving on the Surface of a Protein Provides Protection Without Loss of Activity. *Adv. Funct. Mater.* **23**: 2007–2015.

Li, X.E., Lopetcharat, K., Qiu, Y., and Drake, M.A. (2015). Sugar reduction of skim chocolate milk and viability of alternative sweetening through lactose hydrolysis. *J. Dairy Sci.* **98**: 1455–1466.

Lomer, M.C.E., Parkes, G.C., and Sanderson, J.D. (2008). Review article: lactose intolerance in clinical practice--myths and realities. *Aliment. Pharmacol. Ther.* **27**: 93–103.

Lybeck Sørensen, K., Vergara Meersohn, M., Sonne, J., Larsen, L., Edelsten, D., and Gudmand-Høyer, E. (1983). A new type of low-lactose milk. Tolerance by lactose malabsorbers and evaluation of protein nutritional value. *Scand. J. Gastroenterol.* **18**: 1063–1068.

Mahoney, R. R. (1985). Modification of Lactose and Lactose-Containing Dairy Products with β-Galactosidase. **In:** Developments in Dairy Chemistry—3 Lactose and Minor Constituents, pp. 69-109. Fox, P.F., Eds., Springer.

Mahoney, R.R. (1997). Lactose: enzymatic modification. **In**: Advanced dairy chemistry (2nd ed.). Lactose, water, salts and vitamins, Vol. 3, pp. 77–125. Fox, P.F., Eds Chapman & Hall, London.

Mahoney, R.R. (2002) Enzymes exogenous to milk in dairy technology | Beta-D-Galactosidase. **In:** Encyclopedia of Dairy Sciences, pp. 907–914. Roginski, H., Eds., Elsevier, Oxford.

Månsson, H.L. (2008). Fatty acids in bovine milk fat. *Food Nutr. Res.* **52.**:

Marteau, P., Flourie, B., Pochart, P., Chastang, C., Desjeux, J.F., and Rambaud, J.C. (1990). Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans. *Br. J. Nutr.* **64**: 71–79.

Martini, M.C., Lerebours, E.C., Lin, W.J., Harlander, S.K., Berrada, N.M., Antoine, J.M., and Savaiano, D.A. (1991). Strains and species of lactic acid bacteria in fermented milks (yogurts): effect on in vivo lactose digestion. *Am. J. Clin. Nutr.* **54**: 1041–1046.

Matthews, S.B., Waud, J.P., Roberts, A.G., and Campbell, A.K. (2005). Systemic lactose intolerance: a new perspective on an old problem. *Postgrad. Med. J.* **81**: 167–173.

Mellentin, J. (2012) Lactose-free dairy: Opportunities, strategies and key case studies.

Montalto, M., Nucera, G., Santoro, L., Curigliano, V., Vastola, M., Covino, M., et al. (2005). Effect of exogenous beta-galactosidase in patients with lactose malabsorption and intolerance: a crossover double-blind placebo-controlled study. *Eur. J. Clin. Nutr.* **59**: 489–493.

Morselli, P.L. and Garattini, S. (1970). Monosodium Glutamate and the Chinese Restaurant Syndrome. *Nature.* **227**: 611–612.

Moskovitz, M., C, C., and J, G. (1987). Does oral enzyme replacement therapy reverse intestinal lactose malabsorption? *Am. J. Gastroenterol.* **82**: 632–635.

Mustapha, A., Jiang, T., and Savaiano, D.A. (1997). Improvement of lactose digestion by humans following ingestion of unfermented acidophilus milk: influence of bile sensitivity, lactose transport, and acid tolerance of Lactobacillus acidophilus. *J. Dairy Sci.* **80**: 1537–1545.

Naranjo, G.B., Pereyra Gonzales, A.S., Leiva, G.E., and Malec, L.S. (2013). The kinetics of Maillard reaction in lactose-hydrolysed milk powder and related systems containing carbohydrate mixtures. *Food Chem.* **141**: 3790–3795.

Newcomer, A.D., McGill, D.B., Thomas, P.J., and Hofmann, A.F. (1978). Tolerance to lactose among lactase-deficient American Indians. *Gastroenterology.* **74**: 44–46.

Noh, D.O. and Gilliland, S.E. (1994). Influence of Bile on β-Galactosidase Activity of Component Species of Yogurt Starter Cultures. *J. Dairy Sci.* **77**: 3532–3537.

Nongonierma, A.B. and FitzGerald, R.J. (2015). The scientific evidence for the role of milk protein-derived bioactive peptides in humans: A Review. *J. Funct. Foods.* **17**: 640–656.

O'Connell, S. and Walsh, G. (2006). Physicochemical characteristics of commercial lactases relevant to their application in the alleviation of lactose intolerance. *Appl. Biochem. Biotechnol.* **134**: 179–191.

Ojetti, V., Gigante, G., Gabrielli, M., Ainora, M.E., Mannocci, A., Lauritano, E.C., et al. (2010). The effect of oral supplementation with Lactobacillus reuteri or tilactase in lactose intolerant patients: randomized trial. *Eur. Rev. Med. Pharmacol. Sci.* **14**: 163–170.

Onwulata, C.I., Rao, D.R., and Vankineni, P. (1989). Relative efficiency of yogurt, sweet acidophilus milk, hydrolyzed-lactose milk, and a commercial lactase tablet in alleviating lactose maldigestion. *Am. J. Clin. Nutr.* **49**: 1233–1237.

Ouwendijk, J. (1998). Routing and Processing of Lactase-Phlorizin Hydrolase in Transfected Caco-2 Cells. *J. Biol. Chem.* **273**: 6650–6655.

Paige, D.M. (2005) Lactose intolerance. In, Encyclopedia of Human Nutrition (Second Edition), pp. 113–120. Caballero, B., Eds., Elsevier, Oxford.

Paige, D.M., Bayless, T.M., Huang, S.S., and Wexler, R. (1975). Lactose hydrolyzed milk. *Am. J. Clin. Nutr.* **28**: 818–822.

Panesar, P.S., Panesar, R., Singh, R.S., Kennedy, J.F., and Kumar, H. (2006). Microbial production, immobilization and applications of β-D-galactosidase. *J. Chem. Technol. Biotechnol.* **81**: 530–543.

Parodi, P.W. (2004). Milk fat in human nutrition. *Aust. J. Dairy Technol.* **59**: 3–59.

Payne, D.L., Welsh, J.D., Manion, C.V., Tsegaye, A., and Herd, L.D. (1981). Effectiveness of milk products in dietary management of lactose malabsorption. *Am. J. Clin. Nutr.* **34**: 2711–2715.

Pelletier, X., Laure-Boussuge, S., and Donazzolo, Y. (2001). Hydrogen excretion upon ingestion of dairy products in lactose-intolerant male subjects: importance of the live flora. *Eur. J. Clin. Nutr.* **55**: 509–512.

Perlman, R. (2013). Evolution and Medicine OUP Oxford.

Perman, J.A., Modler, S., and Olson, A.C. (1981). Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. Studies in vivo and in vitro. *J. Clin. Invest.* **67**: 643–650.

Plimmer, R.H.A. (1906). On the presence of lactase in the intestines of animals and on the adaptation of the intestine to lactose. *J. Physiol.* **35**: 20–31.

Pochart, P., Bisetti, N., Bourlioux, P., and Desjeux, J.. (1989). Effect of daily consumption of fresh or pasteurized yogurt on intestinal lactose utilisation in lactose malabsorbers. *Microecol. Ther.* **18**: 105–110.

Portincasa, P., Di Ciaula, A., Vacca, M., Montelli, R., Wang, D.Q.-H., and Palasciano, G. (2008). Beneficial effects of oral tilactase on patients with hypolactasia. *Eur. J. Clin. Invest.* **38**: 835–844.

Ramirez, F.C., Lee, K., and Graham, D.Y. (1994). All lactase preparations are not the same: results of a prospective, randomized, placebo-controlled trial. *Am. J. Gastroenterol.* **89**: 566–570.

Rask Pedersen, E., Jensen, B.H., Jensen, H.J., Keldsbo, I.L., Hylander Møller, E., and Nørby Rasmussen, S. (1982). Lactose malabsorption and tolerance of lactose-hydrolyzed milk. A double-blind controlled crossover study. *Scand. J. Gastroenterol.* **17**: 861–864.

Reddy, V. and Pershad, J. (1972). Lactase deficiency in Indians. *Am. J. Clin. Nutr.* **25**: 114–119.

Rehman, S.U. (2009). Reduced lactose and lactose-free dairy products. **In**, Advanced Dairy Chemistry, pp. 98–104. McSweeney, P.L.H. and Fox, P.F., Eds., Springer.

Richmond, M.L., Gray, J.I., and Stine, C.M. (1981). Beta-Galactosidase: Review of Recent Research Related to Technological Application, Nutritional Concerns, and Immobilization. *J. Dairy Sci.* **64**: 1759–1771.

Rizkalla, S.W., Luo, J., Kabir, M., Chevalier, A., Pacher, N., and Slama, G. (2000). Chronic consumption of fresh but not heated yogurt improves breath-hydrogen status and short-chain fatty acid profiles: a controlled study in healthy men with or without lactose maldigestion. *Am. J. Clin. Nutr.* **72**: 1474–1479.

Rorick, M.H. and Scrimshaw, N.S. (1979). Comparative tolerance of elderly from differing ethnic backgrounds to lactose-containing and lactose-free dairy drinks: a double-blind study. *J. Gerontol.* **34**: 191–196.

Rosado, J.L., Deodhar, A.D., Bourges, H., and Solomons, N.W. (1986). The effect of the digestion products of lactose (glucose and galactose) on its intraintestinal, in vivo hydrolysis by exogenous microbial beta-D-galactosidase. *J. Am. Coll. Nutr.* **5**: 281–290.

Rosado, J.L., Solomons, N.W., and Allen, L.H. (1992). Lactose digestion from unmodified, low-fat and lactose-hydrolyzed yogurt in adult lactose-maldigesters. *Eur. J. Clin. Nutr.* **46**: 61–67.

Rosado, J.L., Solomons, N.W., Lisker, R., and Bourges, H. (1984). Enzyme replacement therapy for primary adult lactase deficiency. Effective reduction of lactose malabsorption and milk intolerance by direct addition of beta-galactosidase to milk at mealtime. *Gastroenterology*. **87**: 1072–1082.

Sahi, T. (1994). Genetics and epidemiology of adult-type hypolactasia. *Scand. J. Gastroenterol. Suppl.* **202**: 7–20.

Sanders, S.W., Tolman, K.G., and Reitberg, D.P. (1992). Effect of a single dose of lactase on symptoms and expired hydrogen after lactose challenge in lactose-intolerant subjects. *Clin. Pharm.* **11**: 533–538.

Savaiano, D.A. (2014). Lactose digestion from yogurt: mechanism and relevance. *Am. J. Clin. Nutr.* **99**: 1251S–1255S.

Savaiano, D.A., AbouElAnouar, A., Smith, D.E., and Levitt, M.D. (1984). Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. *Am. J. Clin. Nutr.* **40**: 1219–1223.

Savaiano, D.A., Boushey, C.J., and McCabe, G.P. (2006). Lactose intolerance symptoms assessed by meta-analysis: a grain of truth that leads to exaggeration. *J. Nutr.* **136**: 1107–1113.

Savaiano, D.A., Ritter, A.J., Klaenhammer, T.R., James, G.M., Longcore, A.T., Chandler, J.R., et al. (2013). Improving lactose digestion and symptoms of lactose intolerance with a novel galacto-oligosaccharide (RP-G28): a randomized, double-blind clinical trial. *Nutr. J.* **12**: 160.

Sciarretta, G., Giacobazzi, G., Verri, A., Zanirato, P., Garuti, G., and Malaguti, P. (1984). Hydrogen breath test quantification and clinical correlation of lactose malabsorption in adult irritable bowel syndrome and ulcerative colitis. *Dig. Dis. Sci.* **29**: 1098–1104.

Shatin, R. (1968). Evolution and lactase deficiency. *Gastroenterology* **54**: 992.

Shermak, M.A., Saavedra, J.M., Jackson, T.L., Huang, S.S., Bayless, T.M., and Perman, J.A. (1995). Effect of yogurt on symptoms and kinetics of hydrogen production in lactose-malabsorbing children. *Am. J. Clin. Nutr.* **62**: 1003–1006.

Sibakov, T. and Tossavainen, O. (2012). Milk product and preparation method.

Solinas, C., Corpino, M., Maccioni, R., and Pelosi, U. (2010). Cow's milk protein allergy. *J. Matern. Fetal Neonatal Med.* **23**: 76–79.

Solomons, N.W., Guerrero, A.M., and Torun, B. (1985). Dietary manipulation of postprandial colonic lactose fermentation: II. Addition of exogenous, microbial beta-galactosidases at mealtime. *Am. J. Clin. Nutr.* **41**: 209–221.

Souci, S.W., Fachmann, W., Kraut, H. (2008). Food composition and nutrition tables. MedPharm Scientific Publishers, CRC Press, Stuttgart.

Sprossler, B. and Plainer, H. (1983). Immobilized lactase for processing whey. *Food Technol.* **37**: 93–95.

Stephenson, L.S. and Latham, M.C. (1974). Lactose intolerance and milk consumption: the relation of tolerance to symptoms. *Am. J. Clin. Nutr.* **27**: 296–303.

Stougaard, P. and Schmidt, M. (2012). Cold-active beta-galactosidase, a method of producing same and use of such enzyme.

Suarez, F. and Levitt, M.D. (1996). Abdominal symptoms and lactose: the discrepancy between patients' claims and the results of blinded trials. *Am. J. Clin. Nutr.* **64**: 251–252.

Suarez, F.L., Savaiano, D.A., and Levitt, M.D. (1995a). A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance. *N. Engl. J. Med.* **333**: 1–4.

Suarez, F.L., Savaiano, D.A., and Levitt, M.D. (1995b). Review article: the treatment of lactose intolerance. *Aliment. Pharmacol. Ther.* **9**: 589–597.

Suarez, F.L., Savaiano, D., Arbisi, P., and Levitt, M.D. (1997). Tolerance to the daily ingestion of two cups of milk by individuals claiming lactose intolerance. *Am. J. Clin. Nutr.* **65**: 1502–1506.

Swallow, D.M. (2003). Genetics of lactase persistence and lactose intolerance. *Annu. Rev. Genet.* **37**: 197–219.

Tomlin, J., Lowis, C., and Read, N.W. (1991). Investigation of normal flatus production in healthy volunteers. *Gut* **32**: 665–669.

Turnbull, G.K. (2000). Lactose intolerance and irritable bowel syndrome. *Nutr. Burbank Los Angel. Cty. Calif* **16**: 665–666.

Turner, K.M., Pasut, G., Veronese, F.M., Boyce, A., and Walsh, G. (2011). Stabilization of a supplemental digestive enzyme by post-translational engineering using chemically-activated polyethylene glycol. *Biotechnol. Lett.* **33**: 617–621.

Usai-Satta, P., Scarpa, M., Oppia, F., and Cabras, F. (2012). Lactose malabsorption and intolerance: What should be the best clinical management? *World J. Gastrointest. Pharmacol. Ther.* **3**: 29–33.

Vandenplas, Y., Brueton, M., Dupont, C., Hill, D., Isolauri, E., Koletzko, S., et al. (2007). Guidelines for the diagnosis and management of cow's milk protein allergy in infants. *Arch. Dis. Child.* **92**: 902–908.

Vandenplas, Y., Gottrand, F., Veereman-Wauters, G., De Greef, E., Devreker, T., Hauser, B., et al. (2012). Gastrointestinal manifestations of cow's milk protein allergy and gastrointestinal motility. *Acta Paediatr.* **101**: 1105–1109.

Varela-Moreiras, G., Antoine, J.., Ruiz-Roso, B., and Varela, G. (1992). Effects of yogurt and fermented-then-pasteurized milk on lactose absorption in an institutionalized elderly group. *J. Am. Coll. Nutr.* **11**: 168–171.

Vernia, P., Di Camillo, M., Foglietta, T., Avallone, V.E., and De Carolis, A. (2010). Diagnosis of lactose intolerance and the "nocebo" effect: The role of negative expectations. *Dig. Liver Dis.* **42**: 616–619.

Vernia, P., Di Camillo, M., Marinaro, V., and Caprilli, R. (2003). Effect of predominant methanogenic flora on the outcome of lactose breath test in irritable bowel syndrome patients. *Eur. J. Clin. Nutr.* **57**: 1116–1119.

Vesa, T.H., Korpela, R.A., and Sahi, T. (1996a). Tolerance to small amounts of lactose in lactose maldigesters. *Am. J. Clin. Nutr.* **64**: 197–201.

Vesa, T.H., Marteau, P., Zidi, S., Briet, F., Pochart, P., and Rambaud, J.C. (1996b). Digestion and tolerance of lactose from yoghurt and different semi-solid fermented dairy products containing Lactobacillus acidophilus and bifidobacteria in lactose maldigesters--is bacterial lactase important? *Eur. J. Clin. Nutr.* **50**: 730–733.

Vesa, T.H., Lember, M., and Korpela, R. (1997). Milk fat does not affect the symptoms of lactose intolerance. *Eur. J. Clin. Nutr.* **51**: 633–636.

Vesa, T.H., Seppo, L.M., Marteau, P.R., Sahi, T., and Korpela, R. (1998). Role of irritable bowel syndrome in subjective lactose intolerance. *Am. J. Clin. Nutr.* **67**: 710–715.

Wang, Y., Harvey, C.B., Pratt, W.S., Sams, V.R., Sarner, M., Rossi, M., et al. (1995). The lactase persistence/non-persistence polymorphism is controlled by a cis-acting element. *Hum. Mol. Genet.* **4**: 657–662.

Weaver, C.M., Proulx, W.R., and Heaney, R. (1999). Choices for achieving adequate dietary calcium with a vegetarian diet. *Am. J. Clin. Nutr.* **70**: 543s–548s.

Welsh, J.D., Poley, J.R., Bhatia, M., and Stevenson, D.E. (1978). Intestinal disaccharidase activities in relation to age, race, and mucosal damage. *Gastroenterology* **75**: 847–855.

Wilt, T.J., Shaukat, A., Shamliyan, T., Taylor, B.C., MacDonald, R., Tacklind, J., et al. (2010). Lactose Intolerance and Health. Evidence report/Technology Assessment No. 192. Agency for Healthcare Research and Quality (US), Rockville (MD).

Wolin, M.J. (1981). Fermentation in the rumen and human large intestine. *Science* **213**: 1463–1468.

Wright, E.M., Hirayama, B.A., and Loo, D.F. (2007). Active sugar transport in health and disease. *J. Intern. Med.* **261**: 32–43.

Xenos, K., Kyroudis, S., Anagnostidis, A., and Papastathopoulos, P. (1998). Treatment of lactose intolerance with exogenous beta-D-galactosidase in pellet form. *Eur. J. Drug Metab. Pharmacokinet.* **23**: 350–355.

Zheng, X., Chu, H., Cong, Y., Deng, Y., Long, Y., Zhu, Y., et al. (2015). Self-reported lactose intolerance in clinic patients with functional gastrointestinal symptoms: prevalence, risk factors, and impact on food choices. *Neurogastroenterol. Motil.* **27**: 1138–1146.

Table 1: Lactose content of some food products.

	Lactose (g/100 g)	Serving size (g)	Lactose per serving size (g)
Whole milk	5.04	244	12.32
Greek yoghurt	2.54	170	4.32
Cheddar	0.18	132	0.24
Mozzarella	0.07	112	0.08
Sour cream	2.91	12	0.35
Multi-grain bread	0.56	28.35	0.16
Chocolate cake	0.46	138	0.63
Energy drink (flavoured)	0.20	30.5	0.06
Cheeseburger (double)	0.39	280	1.09
Salad dressing	1.40	15	0.21
Cheese lasagna	1.00	225	2.27
Vanilla pudding	1.80	28.35	0.51
Chocolate bar	8.21	42	3.45
Fish sticks	0.12	57	0.07

Table 2: Characteristics of some microbial β-galactosidases authorized for food applications.

Source	pH optimum	Temperature optimum (°C)	Ionic activators	Ionic inhibitors
A. niger	3.0 - 4.0	55 - 60	No need	None
A. oryzae	5.0 - 6.2	50 - 55	No need	None
K. lactis	6.5 - 7.3	35	K, Mg, Mn	Ca, Na
K. fragilis	6.6	37	K, Mg, Mn	Ca, Na

Table 3: Efficacy of exogenous enzymes used in milk at mealtime

Study	Subjects number	Enzymatic source	Enzyme activity (FCC)	Lactose load (g)	Delay before consumption (min)
(Barillas and Solomons, 1987)	27	*K. LactisA. Oryzae*	2345 – 3320 – 4355 – 6700 – 9925 – 13270	12	0
(Corazza *et al.*, 1992)	16	*A. niger*	3000	19.2	5
(Montalto *et al.*, 2005)	30	*K. lactis*	3900 - 7800	20	5
(Rosado *et al.*, 1986)	12	*A. niger*	-	18	5
(Rosado *et al.*, 1984)	38	*K. lactisA. niger*	-	18	0
(Solomons *et al.*, 1985)	15	*K. lactisA. niger*	-	18	5

Table 4: Efficacy of exogenous enzymes used as dietary supplements in various studies.

Study	Subjects number	Enzymatic source	Enzyme activity (FCC)	Lactose load (g)	Delay before consumption (min)
(Biller *et al.*, 1987)	16	*A. oryzae*	-	21.7 maximum	5
(DiPalma and Collins, 1989)	10	*A. oryzae*	4000	50	0
(Gao *et al.*, 2002)	10	*A. oryzaeP. multicolor*	10000	18.3	0 - 30
(Lin *et al.*, 1993)	31	*K. LactisA. OryzaeA. niger*	3000 – 6000	20 – 50	0
(Ojetti *et al.*, 2010)	60	*A. oryzae*	9000	25	15
(Portincasa *et al.*, 2008)	134	*A. oryzae*	4623 – 7705	25	0
(Ramirez *et al.*, 1994)	10	*A. oryzae*	6600 – 9900	18	0
(Sanders *et al.*, 1992)	24	*A. oryzae*	9900	50	0

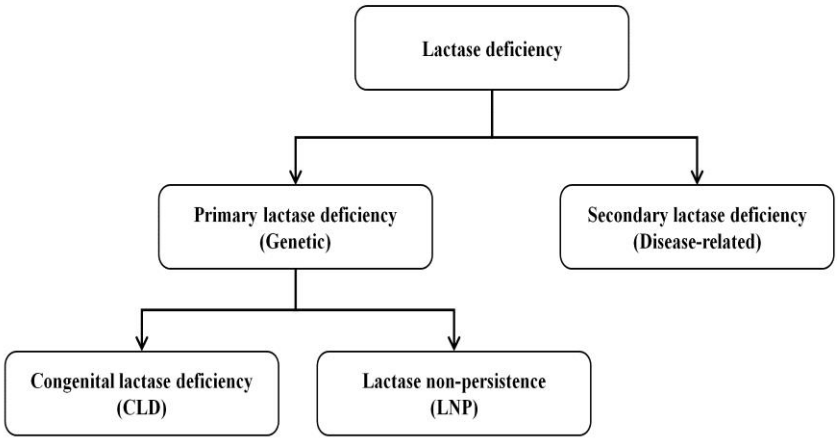

Figure 1: Different types of lactase deficiency.

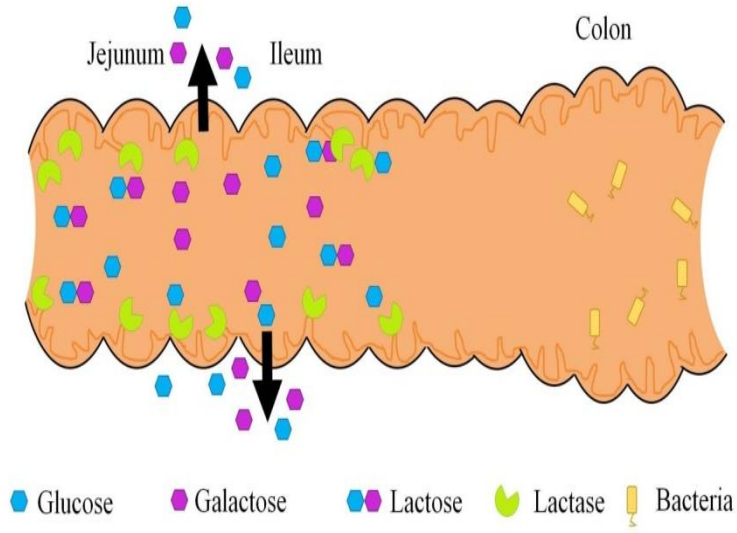

Figure 2: Normal lactose digestion.

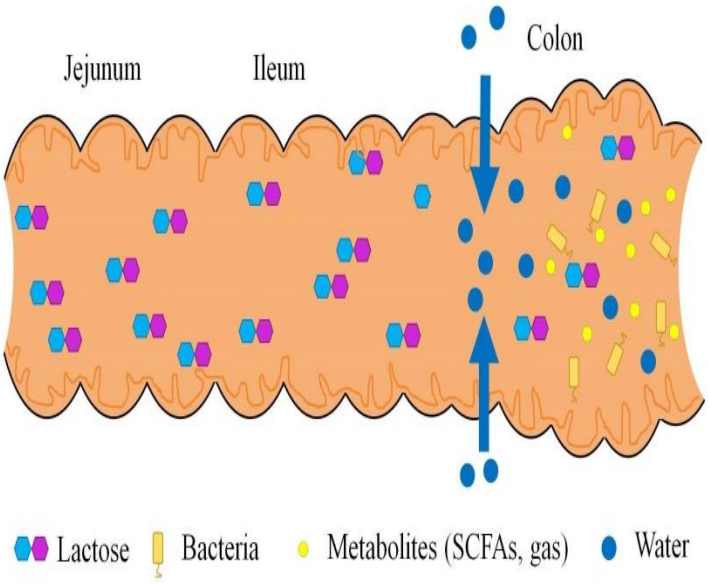

Figure 3: Lactose digestion in case of lactase deficiency

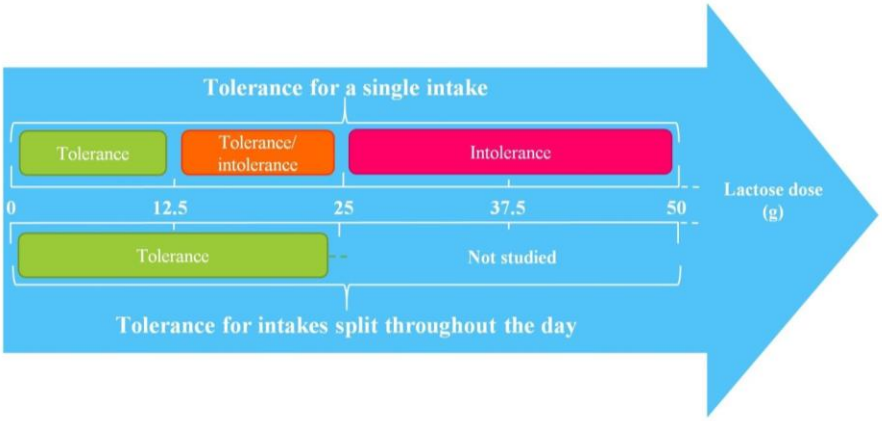

Figure 4: Tolerance of lactose intolerant patients, depending on dose and number of daily intakes.