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Original Research Paper:

Lactose malabsorption and intolerance: a review

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Abstract

Food lactose and lactose intolerance is today a hot topic in food and nutrition knowledge. About 70% of the adult world population is lactose-intolerant, due to low levels of intestinal lactase, also called lactase-phlorizin hydrolase (LPH), a β-D-galactosidase found in the apical surface of the intestinal microvilli. This may be due to the loss of intestinal lactase in adulthood, a condition transmitted by an autosomal recessive gene, which differs in humans according to race. According to the cultural-historical hypothesis, the mutation that allows the metabolization of lactose appeared about 10,000 years ago in the inhabitants of northern Europe where mammalian milk continued in the diet after weaning, and lactase-persistent populations were genetically selected in some areas.

Many intolerant individuals can tolerate low levels of lactose in their daily diet. Probiotics have also been proposed as an alternative that could avoid some symptoms of lactose intolerance. Many products are marketed nowadays as alternatives to dairy products for lactose-intolerant individuals. However, rules for low-lactose foods are currently not harmonised in the European Union.

As scientific knowledge on lactose intolerance has notably advanced in recent decades, the aim of this work was to review the current state of the knowledge on lactose and lactose intolerance, its diagnosis and clinical management, and the various food products that are offered specifically for non-tolerant individuals.

Key words: milk, lactose, lactose malabsorption, lactose intolerance, lactase enzyme, delactosation

1. INTRODUCTION

Milk and dairy products are widely consumed. According to the WHO/FAO, more than six billion people consume milk and its dairy product derivatives, most of these in developed countries. Total consumption has doubled since the 1960s.

These products are in high demand due to their high nutritional value. They contain milk protein with a high biological value, including casein and lactalbumin, and also present varying amounts of fats, most of them constituted of saturated fatty acids. Milk is rich in vitamin A, D and riboflavin, but poor in iron and niacin, and is a source of calcium. The main carbohydrate in milk is lactose, which is not present in other kinds of foods.[1]

It is estimated that lactose represents 6% of the carbohydrates consumed in Western diets. Lactose can serve as a source of energy, but also facilitates the absorption of calcium, phosphate, manganese and magnesium; it is fermented by the gut microbiota and contributes to the development of gram-positive intestinal bacteria (such as Bifidobacterium species), thereby preventing the development of pathogenic microorganisms in the host.[2] Before absorption, disaccharides must first be hydrolyzed in the human digestive tract by the substrate-specific membrane enzymes present in the intestine. The enzyme lactase is responsible for the splitting of lactose into its two components, glucose and galactose.[4]

Lactose intolerance was described by Hippocrates (5^{th} century BC) and Galenus (2^{nd} century AD). Since then, milk is known to be able to produce diarrhoea and other gastrointestinal manifestations in certain people. However it was not until after the Second World War, with the delivery of humanitarian aid (including

milk) to countries in Africa and Asia, that it was noted that many people suffered from flatulence, nausea and diarrhoea shortly after the intake of milk. This was initially attributed to food infections, until researchers at Johns Hopkins Medical School discovered the key to lactose intolerance in 1965.[5] Nowadays it is widely known that some people are lactose-intolerant due to low levels of the enzyme. They reduce or eliminate the consumption of milk and dairy products because they present symptoms when they ingest this type of food, which may lead to less calcium intake in the diet.

The food industry has recently developed a wide range of products for people with this type of intolerance. Lactose-free milk and low-lactose dairy products produced by different methods are available on the market today.

However, the fact that some individuals can digest lactose and others not has lead to widespread misunderstanding in society. Numerous ideas have been raised in various forums, to the point of erroneously considering lactose as a harmful compound in the human diet.

As scientific knowledge of lactose intolerance has notably advanced in recent decades, the aim of this work is to review the present state of knowledge of lactose and lactose intolerance, the clinical management of this situation, and the various products used as food alternatives for non-tolerant individuals.

2. - PROPERTIES AND METABOLISM OF LACTOSE

2.1. - Chemistry and biochemistry of lactose

Lactose is a disaccharide composed of D-galactosyl β (1→4) D-glucose. It is slightly soluble in water (170 g/L at 15º C),[5] exclusively present in the milk of mammals, and six times less sweet than sucrose.[6]

Lactose is obtained from milk serum by ultrafiltration, evaporation and subsequent crystallization, and was first isolated in 1633 by the Italian Fabrizio Bartoletti.[7]

Lactose in milk has two isomers: α-lactose and β-lactose (the C_4 hydroxyl group of galactose in α and β position respectively), which differ in their properties of solubility, crystallization, melting temperature and optical rotation. The α-isomer has a solubility of 70 g/L at 15º C, a melting temperature of 202º C and -89.4º revolving power; while the β-isomer presents values of 500 g/L, 242º and -35º C respectively. The technological treatment of the foods affects the balance of both isomers in milk, depending mainly on the temperatures applied.

Lactose can also be found as anhydrous or hydrated. Hydrated α-lactose is obtained by oversaturated crystallization at a temperature lower than 93.5º C; at higher temperatures, anhydrous β-lactose is obtained.[5]

Lactose is sensitive to heat, which can cause browning of the milk by the lactose joining to amino groups of milk proteins (Maillard reaction), and the caramelization of the lactose molecules.[8] Heat treatments can also cause the isomerization of lactose and produce small amounts of lactulose (galactosyl-fructose). Up to 0.8 g/L of this isomer can be found in sterilized milk.[5]

Lactose is synthesized in the mammary gland from glucose and galactose by the action of the enzyme lactose-synthetase; in the case of ruminants it is also synthesized from volatile acids such as propionic acid, which is produced in the rumen. Lactose synthetase has a subunit with galactosyltransferase activity, and another with regulatory actions (α-lactalbumin). The former catalyzes the transfer of a galactosyl group from UDP-galactose towards the N-acetylglucosamine to form N-acetyllactosamine. Alpha-lactalbumin, in

125 combination with the first subunit, catalyzes the union of UDP-galactose and
126 glucose to form the disaccharide. The concentration of the enzyme in the
127 intestine of the foetus increases during the gestation period.[9]
128 Lactose is the main component of the dry matter in mammals' milk and its
129 content is inversely proportional to fat and proteins. Its average value in human
130 milk is 70 g/L, while cow milk has around 46 g/L, similar to milk from other
131 mammals such as sheep and goats, which have 48 g/L and 41 g/L
132 respectively.[10]
133 Due to its physical-chemical properties such as texture and adhesive qualities,
134 in addition to its hydration properties and flavour, lactose is used as an
135 ingredient in many foods such as processed meats, margarines, breakfast
136 cereals and ready meals, as well as food supplements and as an excipient in
137 medicaments. For use as an ingredient, anhydrous lactose must meet some
138 requirements of purity and identity: richness of no less than 99% m/m, just one
139 molecule of water of crystallization, and humidity no higher than 6%.[11]
140
141 **2.2. - Lactose metabolism**
142 For its physiological use in the human body, lactose must be previously
143 hydrolyzed in the intestine by the lactase enzyme. Once the lactose reaches the
144 proximal intestine, it is broken down into glucose and galactose. Both
145 monosaccharides are absorbed by active transport mediated by membrane
146 proteins: the transporter SGLUT 1 (Sodium-Glucose Linked Transporter 1),
147 which co-transports glucose or galactose accompanied by two ions of Na^+ from
148 the intestinal lumen towards the interior of the enterocyte. The
149 monosaccharides subsequently spread to the blood either passively or by the

facilitating protein GLUT 2 (Glucose Transporter 2). Glucose is used as an energy source, while galactose may be transformed into glucose for its energetic use by the galactokinase and galactose-1Pi-uridyltransferase, or be used as a component of glycolipids and glycoproteins.[12,13]

It is essential to underline the importance of lactose as a precursory molecule of fundamental metabolites in various processes in the human being after its enzymatic metabolism, such as the galactocerebrosides that form part of the plasma membranes of nerve cells, especially in the myelin sheath.[14]

Non-absorbed lactose has osmotic activity and attracts fluid and electrolytes into the intestinal lumen. It is fermented and hydrolyzed by intestinal bacteria producing gases such as H_2 and CO_2. CH_4 can also be obtained after the reutilization of both gases by methanogenic flora. Breathing eliminates these gases after passing into the bloodstream. A certain amount of these gases will lead to flatulence. Short-chain organic acids such as butyric, acetic, propionic, succinic, formic and lactic acid are produced and decrease colonic pH. These products can be refermented by the bacterial flora or absorbed by the large intestine, as acetic, propionic and butyric acids that are absorbed in the caecum and the ascending colon to be used by different tissues.[15]

2.2.1. - The lactase enzyme

Lactase (EC 3.2.1.108; 3.2.1.62), also called lactase-phlorizin hydrolase (LPH), is a β-D-galactosidase found in the apical surface of the intestinal microvilli in the jejunum, and its occurrence gradually decreases towards the ileum. It is the least abundant intestinal disaccharide and does not have a substrate inductor effect through an increase in the ingestion of lactose as it happens to maltase and sucrase.[16] The lactase enzyme is produced as a precursory peptide of 220

KDa, which undergoes a considerable post-translational modification after its initial synthesis during its transport to the cell surface to become a mature yet still inactive protein of 150 KDa. The enzyme is activated due to the action of pancreatic trypsin which produces the excision of two amino acids.[17]

The enzyme has two active sites: the first hydrolyzes lactose, and the other hydrolyzes phloryzin (an aryl alpha-glucoside) as well as a range of dietary glycolipids. Its activity increases progressively in the human foetus, especially from the third trimester of gestation. It reaches its maximum at birth and then begins to decrease from the earliest months of life, and particularly between 3-5 years, by up to 10%, then remains this way in many individuals for the rest of their lives.[18]

This occurs through a decrease in its synthesis and is due to lower gene expression, rather than to low consumption of dairy products as was previously thought.[16] Although this is the normal condition in most mammals; some human beings have kept the lactase activity, and thus the ability to use hydrolize lactose and absorb their components during their whole life, leading to a group of lactase persistent (lactose tolerant individuals), which may be more or less abundant depending on ethnic groups. The exact mechanism involved in the decline of lactase activity at the end of breastfeeding is still unclear; however, part of the enzymatic activity persists after weaning, since the phloryzin site is used, in addition to by glycoside, by a large number of glucolypids in the diet.[12]

Due to the location of the enzyme on the edge of the intestinal villi, its activity is easily affected by aggression and lesions in the intestinal mucosa, and it is more vulnerable to potential damage than other disaccharides.[19]

The optimal activity of lactase occurs in the small intestine at pH 6-8; in the colon, acid lactic bacteria can hydrolyze lactose into glucose and galactose thanks to bacterial lactase; however in this part of the gut, the pH is 4, so bacterial lactase activity is diminished and less lactose fermentation occurs.

Bacterial hyperproliferation in the intestine can decrease the amount of lactase, as the bacterial elastases break down the brush border enzyme site. As a result, more lactose passes into the large intestine and is fermented by bacteria in the colon.[16]

2.2.1.1. - Genetics of lactase

The gene that encodes lactase is located on the long arm of chromosome 2 in position 21 (2q21), and contains 17 exons.

Two possible polymorphisms (single nucleoid polymorphism, SNP), were sequenced by Enhattah *et al* in 2002, associated to the persistence or lack of lactase in adulthood (Table 1). These polymorphisms were found in a gene called MCM6 (minichromosome maintenance complex component 6) near the lactase gene. This gene is not directly involved in lactase synthesis, but overlaps a region of the lactase gene as a key that activates or inhibits the enzyme.[20]

The polymorphism C/T-13910 is the most frequent and is located at approximately 14Kb. It is based on the presence of one cytosine (C) or one thymine (T) in position 13910. The variant C/C is associated with the non-persistence of lactase (intolerant phenotype), while the variants C/T or T/T are related to the persistence of the enzyme's activity.[21]

The second polymorphism (G/A-22108) is located at 22kb; the presence of G/G (guanine/guanine) is associated to non-persistence, while variants G/A (guanine/adenine) and A/A (adenine/adenine) may cause lactase-persistent individuals.[22]

The enzyme is synthesized if at least one of the two variants of the gene associated with the persistence of lactose is present. Only when both expressions are altered is the enzyme's activity and the absorption of lactose reduced. Although these polymorphisms can be used as indicators of the persistence of lactase in the European population, they cannot be applied globally, since other polymorphisms have been identified in the same chromosomal region in the African population.[23] The polymorphisms found in African and Middle Eastern populations are C/G-13915 and G/C-14010, while T/G-14009 is found in Ethiopia.[24]

There are currently eleven gene polymorphisms that are grouped into four haplotypes called A, B, C and U. The first haplotype has a frequency of 86% in the population of northern Europe and only 36% in the south.[16]

The mechanisms responsible for the intolerant phenotype include: decline in mRNA production; or alteration in genetic transcription or translation and even a decline in the number of enterocytes that produce lactase.

The loss of intestinal lactase is transmitted by an autosomal recessive gene while the persistence of enzyme levels (similar to those found in infants) is inherited by an autosomal dominant gene.[25]

Other factors in addition to SNPs may contribute to a decline in mRNA but it is unclear what molecular mechanisms might account for changes in lactase expression. Epigenetic modifications in DNA and histone proteins could

contribute to lactase non-persistence as they effectively regulate gene transcription, differ markedly across tissues and cell types and also change in the same individual over time.[26]

With no symptoms, only 50% of enzymatic activity is necessary for a proper metabolization of lactose.[27] Diets with high starch content have been found to increase levels of mRNA and the amount of lactase, while a high content of long-chain triglycerides in the diet decreases the expression of the gene. Fructose, glucose, galactose and glycerol may also increase the activity of this enzyme.[28]

3. – MANIFESTATIONS AND MAGEMENT OF LACTOSE MALABSORPTION AND INTOLERANCE

According to the definition proposed by the European Academy of Allergology and Clinical Immunology Subcommittee on Adverse Reactions to Food in 1995, food intolerances are those in which there is no immune intervention.[29] Lactose intolerance is an example of an intolerance caused by genome-diet interaction.

Lactose malabsorption occurs when a substantial amount of lactase is not absorbed in the intestine[30-31]. Two types of conditions can be established according to the degree of lactase activity: alactasia (total absence of lactase activity) and hypolactasia (very low lactase activity in the jejunal mucosa, with an imbalance between the amount of lactose ingested and the ability to hydrolyze). Lactose intolerance depends not only on the expression of lactase but also the dose of lactose, intestinal flora, gastrointestinal motility, small intestinal bacterial overgrowth and sensitivity of the gastrointestinal tract to the generation of gas and other fermentations products of lactose digestion.[32]

Lactose intolerance can also be categorized into four types, depending on its origin:

Congenital lactase deficiency

This type of lactose intolerance is a metabolic error in an autosomal recessive trait and is characterized by a total absence or a significant reduction in the enzyme, with a normal histology in the small intestine. It begins at birth and persists throughout the individual's life. It is rare, with very few cases in the world –most of them in Finland– and there is little knowledge of its molecular basis. The first exposure to breast milk or other types of products containing lactose produces a watery diarrhoea. In the past the consequences were fatal, although nowadays with early diagnosis and the provision of an adequate lactose-free diet, the death of the newborn can be prevented.[33]

Developmental lactase deficiency

This is due to low levels of lactase as a result of premature birth (28-32 weeks), since the enzyme's activity in the foetus increases from week 34 and reaches its maximum at birth. However, infants can endure this deficiency thanks to colonic bacterial metabolism: as colonic pH is reduced, colonization by other microbial species as *Bifidobacterium* or *Lactobacillus* is favoured, thus preventing diarrhoea and malnutrition.[34]

Primary lactase deficiency or adult hypolactasia

This is the most frequent form and is the result of the progressive and permanent decrease in enzymatic activity. This is rarely complete, and the level of lactase that persists is an important factor in the development of symptoms.

The process and the time it occurs are variable; the most common average age is between 5 and 7 years and the maximum impact occurs between 30 and 40

298 years. In populations with a high prevalence of hypolactasia, the disorder
299 normally appears around two years of age. In other populations with a lower
300 prevalence, the first symptoms may appear between 11 and 14 years. In
301 Caucasian individuals (prevalence of 25%), lactase activity usually continues
302 until at least age 20.[35]
303 Approximately 70% of the world population presents this type of intolerance
304 although there is great geographical variability, with large differences between
305 geographic areas, ethnic groups and even subpopulations (Table 2). It is
306 estimated that over 90% of the Asian population is lactose intolerant, and 60-
307 70% of African Americans. In Europeans, or populations with European
308 ancestors, the prevalence drops significantly, reaching 5% in countries such as
309 Switzerland and Denmark. For example, in Spain the global percentages are
310 similar to the rest of Europe, although some authors report a 13-15% variation
311 in Barcelona (northeast) and 32.5% in Galicia (northwest). Approximately 50%
312 correspond to elderly people.[33,36,37]
313 Several hypotheses have been suggested to explain this geographical diversity
314 in the response, including the role of lactose in calcium absorption in countries
315 located at high latitudes. According to the cultural-historical hypothesis, the
316 mutation that allows the metabolization of lactose appeared about 10,000 years
317 ago in the inhabitants of Northern Europe, according to DNA evidence from
318 individuals at that time. This coincides with the start of the Neolithic period in
319 that part of the continent and the beginning of livestock breeding and dairy
320 production by North Europeans, and is related to the strong dependence on
321 milk consumption in their diet which would increase the availability of calories
322 and nutrients such as calcium and vitamin D (necessary for calcium

assimilation). The high prevalence of this mutation in this population is the result of selection, acting in favour of those who could consume milk, since they presented lower rates of rickets and osteomalacia. In Southern Europe the main source of vitamin D was solar ultraviolet radiation B (UVB), which is able to synthesize vitamin D_3 in the skin through cutaneous absorption. The inhabitants of Southern Europe were therefore less dependent on diet, and the selective pressure would have been lower.

In Africa, the selection factor favouring the population with the mutation for persistence in the production of lactase would have been the high water content of milk, an important aspect in the arid regions of the African continent. Other authors add that the high riboflavin content in milk could act as protection against malaria caused by *Plasmodium falciparum*, an endemic disease in a large part of the African continent.

These circumstances would not be present in cultures without a relationship with cattle milk production, such as Amerindian or southeast Asian populations, which explains their high prevalence of lactose intolerance.[38]

Secondary lactase deficiency

In this case the affected individuals have normal enzymatic activity. A decrease in lactase occurs for various reasons and through different mechanisms, namely chronic enteropathy (secondary to immunological processes) such as coeliac disease and Chron's disease, atrophy of the villi due to caloric-protein malnutrition, and other gastrointestinal diseases that damage the brush border in the small intestine (such as infections). It is usually reversible when the underlying disease is resolved. The evolution depends on the severity and

duration of the damage caused to the mucosa. It is accompanied by a reduction in the activity of all disaccharidases, although lactase is the most affected.[39]

3.1. - Clinical manifestations

People with lactose intolerance experience typical symptoms that include abdominal pain, swelling, flatulence, diarrhoea, vomiting and bowel (or abdominal) noises, and in some cases also constipation, anorexia and weight loss. Auricchio *et al* was the first that described these symptoms in an article published in 1963.[12]

The gases produced by bacterial fermentation are responsible for the increase in intraluminal pressure and bowel transit time. Flatulence and swelling occur when gas production commences after undigested lactose comes into contact with and is digested by colonic bacteria. Studies show little or no difference in gas production between lactose malabsorbers with and without symptoms. Instead it is the sensitivity to distension that determines the likelihood of symptoms.[40]

Diarrhoea occurs as a result of acidification of the colon due to the production of short-chain fatty acids, which increase the osmotic load that involves the secretion of electrolytes and fluids and rapid transit. The stools are often voluminous, aqueous and foamy.[41]

Symptoms usually begin around one hour after the intake of lactose when its content exceeds the hydrolytic capacity of the lactase. Symptoms cease shortly after the expulsion of flatus and liquid stools. The wide variability in the severity of the symptoms that can be observed in both the same and different individuals depends on the amount and frequency of lactose ingested and the capacity to

digest it. This is due to the wide variability in the ability of the intestinal bacteria to ferment lactose. The symptoms get worse as more milk is consumed.[42]

On many other occasions the colon reabsorbs part of these products and does not produce symptoms, leading to an asymptomatic process. There is no consensus on the minimum dose of lactose leading to symptoms of intolerance. The ingestion of 50 g of lactose produces symptoms in 80-100% of patients with hypolactasia, although those considered extremely intolerant may present symptoms with only 3 g of lactose (equivalent to about 60-70 mL of cow milk). The majority of patients with this intolerance can tolerate 10-12 g of lactose (equivalent to 200-250 mL of milk) without significant symptoms.[43]

Other factors that influence the variability of manifestations include the fat content of foods with lactose, gastric emptying (which slows with higher lipid content), bowel transit time, water absorption capacity, and patients' subjective sensitivity to pain. Various studies have reported that some patients do not relate the symptoms to the intake of dairy products, and so remain undiagnosed and untreated.

Some authors describe non-intestinal symptoms such as headache, memory deterioration, musculoskeletal pain, heart rhythm disorders, dryness in the mucous membranes, depression, ulcers in the oral mucosa and other allergic reactions in 20-80% of patients. The cause could be the toxic effects of compounds such as acetaldehyde, acetone, ethanol, peptides and others that alter cell signals. In this case it is important to assess whether this is due to intolerance or to other disorders; up to 20% of patients with lactose intolerance may also show an allergy to milk proteins.[16]

Sometimes, food intolerance is associated to anxiety, stress, since patients may be hypervigilants to dietary factors that cause them discomfort; there are some studies about psychosocial effects of lactose intolerance in Asian and European patients showing different results. Furthermore, many self-reported lactose intolerants may suffer a “nocebo effect”, probably due to the influence of environmental factors, or the coincidence of other digestive disorders with similar symptoms.[44]

3.2. - Diagnosis

With proper diagnosis the degree of lactose intolerance can be assessed, and treatment can be prescribed.

The clinical history and physical examination of patients suspected of lactose intolerance are also important. The presence of symptoms such as abdominal pain, diarrhoea, nausea, flatulence and bloating are signs of intolerance to carbohydrates even though they have a nonspecific character. Other factors to be taken into account include the personal and family history, the relationship between the symptoms and the ingestion of this carbohydrate, the time elapsed between ingestion and the appearance of symptoms, the matrix in which lactose has been ingested (milk, fermented dairy products…), whether there are any conditions that speed up or slow down intestinal transit, and the age of onset. Abdominal distension and the presence of bowel noises are significant in the physical exploration, as well as perianal erythema caused by acid and explosive stools.

The selection of the diagnostic method will depend on the characteristics of the patient, the therapeutic objective and the available resources. These methods

420 can be classified into invasive or non-invasive and direct or indirect depending
421 on whether they involve intestinal biopsy or not.[6,30]

422 **3.2.1. - Non-invasive or indirect methods**

423 Non-invasive or indirect methods do not involve intestinal biopsy.

424 **- Suppression and provocation test with milk**

425 Lactose is excluded from the diet for two weeks, then reintroduced. If the
426 symptomatology disappears and reappears once it is reintroduced at the end of
427 that period (challenge test), it suggests the person is intolerant. The
428 disadvantage is that it does not allow the diagnosis of asymptomatic individuals
429 suffering from malabsorption.[45]

430 The study by Castiglione and collaborators in 2008 suggests that evaluating
431 different variables in the anamnesis with a simple annotation system is almost
432 as effective an evaluation method as the hydrogen test, and reduces costs and
433 diagnosis times.[46]

434 - **Hydrogen breath test**

435 This is the most common test, since it is a simple, practical and rapid method of
436 determining lactose intolerance. It has a specificity of between 89% and 100%
437 and sensitivity of between 69% and 100%. It is indicated for patients who do not
438 have a conclusive clinical response after the suppression-provocation test. It is
439 the test of choice in clinical practice thanks to its simplicity and safety.[30]

440 The test is based on the fact that intestinal bacteria degrade non-absorbed
441 lactose and produce methane, CO_2 and H_2. This last is disseminated through
442 the intestinal wall and passes into the blood. It reaches the pulmonary alveoli,
443 and is expelled in the breath. Hydrogen can be detected and quantified by gas
444 chromatography.

The test consists of administering lactose (10, 20 and 40 g dissolved in 250 ml of water). Once the intake occurs, hydrogen, methane and carbon dioxide are quantified at time 0, and every 25-30 minutes during 3 hours, and the values obtained are compared. An increase of over 20 ppm in H2 is considered positive; 10-20 ppm is considered an inconclusive value.[47] These reference values may be modified depending on the CO_2 and methane values measured. Ingested lactose can be marked with ^{13}C to improve sensitivity. This process is currently limited to research and is not considered appropriate for clinical use.

False negative results can occur if patients have taken antibiotics for at least one month before the test, or if they have consumed laxatives, probiotics, prokinetics or electrolyte solutions for the preparation of colonoscopies, and due to hyperventilation (crying) or physical exercise. False positives can appear with the intake of non-absorbable carbohydrates the previous day, tobacco (before and during the test), hypoventilation (sleep) or bacterial overgrowth.[48]

- **LacTEST**

Oral 4-galactosil xylose is administered. The amount of xylose is determined in an accumulated 24-hour urine sample. This test can only be applied to the adult population. The LacTEST has proved to be very cost-effective, with a higher sensitivity and specificity than the H_2 test and the intestinal biopsy, and a lower economic cost.[49]

- **Stool acidity test**

The measurement of the pH of stools and reducing substances is only useful if the individual is taking lactose. It is nonspecific and has low sensitivity. In intolerant people, stools are acidic after the intake of the disaccharide. This reduction in pH is due to the presence of volatile fatty acids as a result of the

bacterial digestion of non-absorbed carbohydrates. If the pH is below 5.5, then it is a highly suggestive indicator of lactose malabsorption. It should be noted that the normal faecal pH of infants who are fed breast milk is lower than in the rest of the population due to the relative inadequacy of the enzyme in relation to the high amount of lactose present in breast milk, so this test loses diagnostic value in this group of individuals.

In addition to this method, reducing substances can be searched in faeces to detect the presence of sugars such as lactose in the stool. This test is less sensitive than the previous one because the intestinal bacteria can completely digest non-absorbed carbohydrates.[50]

- Lactose tolerance test

Glucose is determined in the blood plasma before and after ingesting 50 grams of lactose at different time intervals. It is monitored at 0, 60 and 120 minutes. Glucose levels of over 20 mg/dL indicate lactose tolerance. False positives can occur in up to 30% of patients due to a rapid insulin response. False negative results can occur in diabetic patients or with bacterial overgrowth. Because of its low sensitivity and specificity, it should be done only if the exhaled air test is not possible.

Ethanol can be given orally before the ingestion of lactose to inhibit the metabolism of galactose, quantify the levels of glucose and galactose (at least 20 mg/dL and 10 mg/dL respectively) and check for lactose intolerance. It has a specificity of 77-96% and a sensitivity of 76-94%, but is not sensitive to the definitive study of this intolerance.

It is used mainly with adult patients, and only exceptionally in children, given the fact that it has a low sensitivity and requires blood extractions every 30 minutes

over two hours. Although it is a simple and inexpensive test, it tends to produce confusing results and is uncomfortable for the patient, so the exhaled air test is preferred today.[16,30]

3.2.2. - Invasive or direct methods

Invasive or direct methods are based on biopsies of the small intestine.

- **Study of enzymatic activity**

A biopsy must be done on the mucosa of the jejunum, followed by an enzymatic study. It is considered positive when the activity of the lactase is less than 10 U/g with a normal intestinal mucosa. This test can give false negative results because lactase has a very irregular distribution and does not necessarily represent what happens in the entire intestine. Because of its low sensitivity, if compared with other tests such as expired hydrogen, a biopsy procedure and a specialized laboratory test are not required in the diagnosis of hypolactasia.[30]

- **Rapid duodenal biopsy test (Lactose Intolerance Quick Test)**

This is based on a colorimetric reaction in a sample of tissue removed from the duodenum and incubated in lactose for 20 minutes. In people with normal enzyme activity the sample will change colour. The reaction was carried out into two steps: a 15-minutes lactase reaction, followed immediately by a 5-minutes signal reaction in which liberated glucose is measured by glucose oxidase/peroxidise reaction. In patients with severe duodenal hypolactasia, there is a sensitivity of 95% and a specificity of 100%. There are studies showing a high correlation between this test and the genetic study.[30]

3.2.3. - Other methods

- Genotypical study

519 The genetic test can be useful for differentiating primary from secondary
520 hypolactasia and for diagnosing the predisposition of the patients (who are not
521 necessarily ill) to develop the primary intolerance. This is useful in subjects
522 aged over eight years who present clinical signs and have a negative hydrogen
523 test.
524 It identifies the different polymorphisms of the MCM6 gene and marks these
525 phenotypes as intolerant. The genetic study can be done using the specific
526 amplification of DNA extracted from a saliva sample, where the polymorphism
527 of propensity to lactose intolerance by PCR in real time is located and marked
528 with fluorescent probes. The analysis with PCR is a test with a specificity of
529 100% and a sensitivity of 93%. This type of test allows for a quick, definitive and
530 non-invasive diagnosis.
531 The disadvantages of the genetic method are the need for specialized
532 laboratories and its high cost, and it is therefore not widespread.[51]

533 **3.3.- Treatment**

534 The main strategy for treating lactose intolerance is to eliminate or at least
535 reduce the intensity of the symptoms in people with this type of intolerance,
536 while ensuring an adequate intake of nutrients.[30,52]
537 The treatment will depend on the severity of the intolerance and the age of the
538 patient. In children aged under five, this intolerance is almost always secondary
539 and only a few require a low-lactose diet due to the high renewal of the
540 intestinal epithelium. In the case of persistent diarrhoea, it is recommended to
541 mix cereals with milk or to ingest fermented milk.[53]
542 There are four general principles for treating lactose intolerance: reducing or
543 eliminating the intake of lactose; replacing lactose with alternative nutrients;

administering enzymatic substitutes or lactase supplements; and maintaining the intake of calcium and vitamin D.[30]

The other important point of treatment is patient education. The person must learn how to balance and modify their diet until the discomfort disappears. One very important aspect is how to interpret food labels correctly in order to avoid the accidental ingestion of lactose. Not only dairy products must be considered, since lactose is also used in other types of foodstuffs and in medicines.[54]

The best way to reduce lactose in the diet and meet the need for products such as calcium is to use lactose-free milk products. Calcium deficiency can produce osteoporosis, so it is important to maintain an appropriate intake of this element. There are studies showing that hypolactasia and maldigestion of lactose do not alter the absorption of calcium.[55,56] In contrast, other studies argue that calcium absorption is significantly reduced in intolerant individuals when they eliminate lactose from their diet, since the disaccharide stimulates its absorption in the intestine.[55,56]

In recent years the food industry has developed a wide range of high-quality, low-lactose and even lactose-free products to replace normal milk, while preserving the rest of the nutrients in the food. The aim is for an intolerant person not to have to give up consuming dairy products. Plant-based drinks (soybean, oat, rice, almonds...) are also available and are marketed as milk substitutes with added sugars and other substances, together with vitamin D and calcium to enrich their nutritional value.[57]

People who do not consume milk and milk products need dietary alternatives for certain nutrients such as calcium and vitamin D. Other sources of calcium, apart from dairy products, include legumes (beans, chickpeas etc.) or vegetables

569 such as cabbage. Spinach and chard can be excluded since they contain
570 oxalates and block the absorption of the ion. Animal-based foods are also
571 important, including fish (sardines, salmon etc.) and shellfish (prawns, shrimps,
572 clams etc.). Vitamin D can be obtained from oily fish like salmon, from eggs,
573 liver and other foods, and mainly from exposure to sun.
574 Seventy to eighty percent of patients have a positive response to a lactose-free
575 diet. In other cases, the persistence of symptoms may be caused by another
576 underlying cause such as irritable bowel syndrome.[54]
577 As previously mentioned, the vast majority of intolerant individuals can tolerate
578 up to 100-200 ml of milk, which corresponds to about 5-10 g of lactose,
579 distributed throughout the day. It is worth noting that whole milk is better
580 tolerated than skimmed or semi-skimmed dairy products, as fats can delay
581 gastric emptying. The intake of dairy products and other foods such as biscuits,
582 bread, etc. also improves tolerance. Butter can contain traces of lactose, and
583 the disaccharide content can be increased if milk solids are added to the
584 product.[58]
585 After an initial period of lactose exclusion (at least four weeks), it is sometimes
586 recommended to introduce small quantities in the diet to check the tolerance
587 and the adaptation of the colonic flora. Dietary control depends on the affected
588 population learning by trial and error how much lactose they can tolerate.[59]
589 For secondary lactase deficiency the most important strategy is to eliminate the
590 origin of the pathology. The lactase activity will gradually recover in weeks or
591 months once the damage is removed from the intestinal mucosa.

592 **4. - FOOD PRODUCTS FOR LACTOSE INTOLERANT INDIVIDUALS**

593 **4.1. - Delactosation methods**

Delactosation is a technological process that consists of eliminating lactose from milk, or reducing its concentration below a certain threshold. Regulation (EU) No 1169/2011 sets out rules for labelling substances with a scientifically proven allergenic or intolerant effect. These indications are important to enable lactose-intolerant individuals to make safe choices. However, as recognized in Regulation (EU) No 609/2013, labelling rules for reference levels to indicate the absence or reduced presence of lactose in food are currently not harmonized in the European Union, and must be included in Regulation (EU) 1169/2011. These rules should take into account the scientific opinion of authorities on lactose thresholds for lactose intolerance and galactosaemia, which recommends that until rules can be established for claims concerning the absence or reduced presence of lactose in food, a maximum level of 0.01% for lactose-free products and 1% for low-lactose products could be adopted as suitable thresholds for lactose-intolerant individuals. Products in which the lactose is enzymatically hydrolyzed to glucose and galactose and from which the galactose has not been removed are not suitable for patients with galactosaemia, regardless of the residual lactose content, and require different thresholds.[60,61,62]

To achieve these levels, lactose can be removed from dairy products in two ways:

The first consists of hydrolyzing the lactose in milk by means of the β-galactosidase enzyme which converts lactose into glucose and galactose. This is done by adding the enzyme to milk in storage tanks. The process is conditioned by the lactose concentration in the milk, the dose of the enzyme, the temperature of the milk and the time of the process.[16]

619 The enzyme is obtained from different strains of microorganisms such as the
620 yeasts *Kluyveromyces lactis*, *Aspergillus oryzae* and *A. niger*. The first is used
621 primarily for the delactosation of milk while the others are often used to obtain
622 lactose-free cheese whey. Different methods are used to solubilize the β-
623 galactosidase depending on its location within the cell and its stability.
624 Mechanical methods are preferred to autolytic methods, although the former are
625 more expensive and involve separating the enzyme from the cell remains.
626 However autolysis requires a temperature range that could alter the enzyme,
627 and the removal of the detergents used.[63]
628 The delactosation process takes place at a temperature of 6-10º C for 15 to 20
629 hours. This temperature is below the optimum efficiency temperature, which is
630 35-40º C, and the process therefore takes longer. This temperature control is
631 important for preventing the growth of psychrophilic and psychotropic residual
632 bacterial flora which, although the milk undergoes heat pretreatment, can affect
633 the process through the action of thermo-resistant enzymes (lipases and
634 proteases). This method is the most highly rated by the industry despite its
635 disadvantages, such as the time required and the high cost of producing the
636 enzymes. The effectiveness of the process will depend on the rate of hydrolysis
637 of the lactose. In the conditions described above, this percentage is about 85%.
638 Delactosed milks produced using this method have a more intense cooked
639 flavour and sweetness, since glucose and galactose have a greater sweetening
640 power than lactose, which may sometimes pose an obstacle for its
641 consumption.[64]
642 The second method consists of a variation of the former, with the prior
643 ultrafiltration of milk followed by the action of the lactase enzyme to reduce the

lactose to the desired levels. This method eliminates salts, which must be compensated after delactosation. It also involves more manipulation of the product than the previous methods, so the dairy products obtained in this case are known as lacteal products.[16]

4.2. - Lactase food supplements

When lactose cannot be avoided or when a person with symptoms decides to consume foods that contain the disaccharide, lactase supplements can be used. They are administered in capsules, chewable tablets or in liquid preparations. One option is to add commercial lactase to milk followed by incubation for several hours (it is advisable to do this approximately 10 hours before consumption as this has revealed greater tolerance).[65]

The characteristics and properties of these preparations vary depending on the source of the enzyme. Those of fungal origin have a higher thermal stability, with the maximum range of activity varying between 35 and 55º C, and the optimal pH between 4.5 and 6.5. The enzymes obtained from bacteria and yeasts have an optimal activity at 37º C and close to neutral pH. Their activity decreases at a temperature of 55º C and a pH of 5.3, and ceases totally at pH 4.5.[66]

Other preparations include lactase, which is directly ingested and has enough stability to overcome heartburn (inside coated oral dosage forms). These preparations are unable to hydrolyze all dietary lactose, and the results vary depending on each patient so the dosage must be set individually. Intolerant patients can use these supplements to continue to consume dairy products, which are the main sources of calcium, vitamin D, riboflavin, and proteins. They

668 are less commercially successful than lactose-free or low-lactose milk and dairy
669 products.[67]
670 The preparations on the market contain lactase produced by the yeast
671 *Kluyveromyces lactis* or *Aspergillus oryzae*. They are odourless, tasteless liquid
672 products that can be added to milk and dairy products such as creams and
673 chocolate, and transform 70-90% of lactose into glucose and galactose. They
674 can also be found in tablets and chewable tablets for immediate consumption
675 before a meal containing food with lactose. It may be necessary to repeat the
676 administration if the individual continues to consume dairy products.
677 Microencapsulation of lactase (with agarose and a coating of chocolate) is a
678 technological process that has been introduced as an option to meet the
679 growing demand for alternatives for people who are intolerant to lactose.
680 Microencapsulation is a technology used by the food and pharmaceutical
681 industry for encapsulating solid, liquid or gaseous materials. The advantage is
682 that it releases active ingredients at controlled speeds under specific conditions,
683 and can also protect them from reactions with other compounds present in food,
684 and prevent oxidation. Microcapsules help fragile materials withstand
685 processing and packaging and improve the flavour, aroma, stability, nutritional
686 value and appearance of the products. In recent years the microencapsulation
687 of lactase has proven to be a technologically viable alternative for lactose
688 intolerant patients.[68]

689 **4.3. - Probiotics**

690 The term probiotic was first proposed by Lilly and Stillwell to describe
691 substances produced by a microorganism that stimulates the growth of another
692 microorganism.[69] Nowadays, probiotics are defined by the WHO as "live

693 microorganisms that, when administered in adequate amounts, confer a health
694 benefit on the host".[70]
695 Within the framework of the production of functional foods, the use of
696 microorganisms can be very useful in the dietary treatment of various
697 pathologies and disorders such as lactose intolerance, to reduce various types
698 of diarrhoea, and they also have an inmunopromotory and preventive effect on
699 the development of colon cancer.[71] The most frequent starter cultures belong to
700 the lactic acid bacteria group.
701 Yogurt is obtained from the fermentation of *Streptoccocus thermophilus* and
702 various species of *Lactobacillus*, usually *L. bulgaricus.* Intestinal bacteria
703 *Bifidobacterium* is included in some of their starter cultures. Yogurt contains
704 significant amounts of calcium that is bioavailable because it is present in ionic
705 form. The acidity of the yogurt facilitates its intestinal absorption.[72] The
706 fermentation of some other products such as kefir also involves yeast, creating
707 intense interdependent relationships between the organisms considered as
708 probiotic.
709 Various properties related to the improvement and maintenance of health have
710 been attributed to fermented milks since their origins, probably in the Middle
711 East or the Balkans.[73] In the particular case of lactose intolerance, several
712 studies show that the continuous consumption of yogurt for six months
713 improves the digestion and absorption of lactose, although the fermentation
714 process usually reduces the amount of disaccharide by 20%.
715 The increase in lactose absorption appears to be at least partially due to the
716 microbial β-galactosidase, which is still active when it reaches the intestine. This
717 may be caused by the excellent buffer capacity of the yogurt during gastric

718 transit (favoured by casein micelles, calcium phosphate and lactic acid)[74], and
719 the protection of the enzyme by the microbial wall and membrane. It depends
720 on the individual capacity of acid secretion and gastric emptying time, the
721 amount of yogurt ingested and the interaction with other foods ingested at the
722 same time.[75] Other factors include the actions of the secretions and enzymes
723 from the pancreas and intestine, the longer bowel transit time of the yogurt and
724 the lipid emulsion formed by the bile salts. The function of bile acids in this
725 regard is not known for certain, since yogurt bacteria are not resistant to these
726 salts. This could be due to the increased permeability of the bacterial cells,
727 which allows the hydrolysis of the disaccharide in the cells; or because they
728 enable the release of the enzyme from the intracellular space to the lumen.[76]
729 Some literature reviews conclude that certain strains of probiotics can have
730 beneficial effects on lactose intolerance[77], while other reviews contradict this by
731 stating that they are only useful in the treatment of flatulence in adult
732 hypolactasia.[78] More research is therefore necessary in clinical studies that
733 include specific strains and objective methodologies.[79]

734 **4. CONCLUSIONS**

735 Scientific knowledge of lactose intolerance has advanced significantly in recent
736 decades, especially in terms of the genetic basis and diagnosis of this condition.
737 Although the exact mechanism involved in the decline of lactase activity at the
738 end of breastfeeding is still unclear, it has been suggested that it may include a
739 drop in mRNA production, an alteration in genetic transcription or translation
740 and even a decline in the number of enterocytes that produce lactase. However,
741 some enzymatic activity may persist after weaning; the cultural-historical
742 hypothesis postulates that the mutation that gives rise to lactase persistence in

743 adulthood appeared about 10,000 years ago in the inhabitants of Northern
744 Europe. Gene polymorphisms linked to this condition have been sequenced for
745 different human races.

746 As about 70% of the whole world population evolves to reduce lactase activity
747 during their life, the industry has developed many products as alternatives to
748 milk products. Many intolerant individuals can tolerate about 5-10 g of lactose
749 distributed throughout the day. Probiotics have been proposed as an alternative
750 that could avoid some symptoms of lactose intolerance thanks to the microbial
751 β-galactosidase, although the results are still contradictory and further research
752 is needed. Many products are also marketed today as alternatives to dairy
753 products for lactose intolerants, such as plant-based drinks and lactose-free
754 dairy products obtained from the enzymatic hydrolysis of lactose using lactase
755 from different strains of microorganisms such as the yeast *Kluyveromyces*
756 *lactis*. A recommendation by the EFSA for labelling these products specifies a
757 maximum level of 0.01% for lactose-free products, and 1% for low-lactose
758 products. As these indications are important for lactose-intolerant people,
759 labelling rules for low-lactose foods need harmonization in the European Union
760 to enable lactose-intolerant people to make safe choices.

761

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764

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Table 1: Frequency distribution of lactose intolerant people among various ethnic groups[33].

Populations subgroups	Frequency of Lactose intolerance (%)
Northern-Central Europe	5
Southern Europe	15-20
African-Americans	60-70
Asia (SE)	90

Table 2: Genetics polymorphisms of lactase persistence / non-persistance [21,22].

	Lactase persistent	Non lactase persistent
LCT-13910	C/T and T/T	C/C
LCT-22018	G/A and A/A	G/G

C: Cytosine, T: Thymine, G: Guanine, A: Adenine.