Nutrition Research 89 (2021) 23–34

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/NTR

Narrative Review

Lactose intolerance: An update on its pathogenesis, diagnosis, and treatment

Roberto Catanzaro[a,*], *Morena Sciuto*[a], *Francesco Marotta*[b]

[a] *Department of Clinical and Experimental Medicine, Gastroenterology Section, "Gaspare Rodolico" Policlinico Hospital, University of Catania, Catania, Italy*

[b] *ReGenera R&D International for Aging Intervention & San Babila Clinic, Milano, Italy*

ARTICLE INFO

Article history:
Received 27 November 2020
Revised 15 February 2021
Accepted 27 February 2021

Keywords:
Lactose intolerance
Lactase–phlorizin hydrolase
Milk
Lactase non-persistence

ABSTRACT

Lactose intolerance has a high prevalence worldwide, ranging between 57% and 65%. It is caused by a reduction or loss of the activity of the intestinal enzyme lactase–phlorizin hydrolase, responsible for the digestion of lactose. This alteration determines an increased osmotic load in the small intestine and the fermentation of lactose by the bacterial flora, which leads to a high production of short-chain fatty acids and gas. This is followed by the onset of abdominal pain, diarrhea, and flatulence. In addition to these problems, it was found that subjects with lactose intolerance have an increased risk of developing various extra-intestinal diseases, including cancers. The diagnosis is essential to undertake an adequate treatment and, for this purpose, different methods have been tested. These include genetic test, hydrogen breath test (HBT), quick lactase test, and lactose tolerance test. HBT is the most used method because it is non-invasive, inexpensive, and highly sensitive and specific, as well as easy to perform. In clinical practice, the other methods are mainly used as HBT integration tests. There are also many therapeutic options. An appropriate intervention concerns the dietetic style, such as the consumption of lactose–free foods, but with nutritional characteristics comparable to dairy products. Other valid choices are represented by the use of exogenous enzymes, probiotics, prebiotics, the selection of milk containing specific types of beta–caseins. This review is intended to illustrate the diagnostic methods currently available and the possible therapeutic options for lactose intolerance.

1. Introduction

Lactose intolerance is a clinical condition characterized by symptoms attributable to lactose malabsorption. This can be caused by a reduced or absent activity of lactase or by a reduced or absent synthesis of the same enzyme [1]. The severity of the symptoms is subjective and it depends on several factors. These include the concentration of lactase present in the intestinal mucosa, the intestinal flora, the amount of in-

Abbreviations: LCT, lactase gene; RNA, ribonucleic acid; SGLUT-1, sodium-glucose linked transporter-1; Na^+, sodium ion; GLUT-2, glucose transporter-2; H_2, hydrogen; CO_2, carbon dioxide; CH_4, methane; T2DM, type 2 diabetes mellitus; DNA, deoxyribonucleic acid; HBT, hydrogen breath test; ppm, parts per million; 4SLHBT, four-sample lactose hydrogen breath test; SIBO, small intestinal bacterial overgrowth; LTT, lactose tolerance test; BCM-7, beta-casomorphin-7; GOS, galacto-oligosaccharides.

* Corresponding author at. Roberto Catanzaro, Via Santa Sofia, 78 – Catania, 95123, Italy. Tel.: +39 095 3782902
E-mail addresses: rcatanza@unict.it (R. Catanzaro), morena.sciuto@virgilio.it (M. Sciuto), fmarchimede@libero.it (F. Marotta).

https://doi.org/10.1016/j.nutres.2021.02.003
0271-5317/

gested lactose, the gastrointestinal motility, and the individual sensitivity in the perception of symptoms [2].

Currently the prevalence of confirmed cases of lactose intolerance worldwide is about 57% [3]. However, the real prevalence is estimated to exceed 65% and the distribution of cases worldwide is very uneven [4]. It is about 50% in America, 70% in Asia, almost 100% in Africa. In the United States it is 15% among whites, 53% among Mexican-Americans, and 80% among Afro-Americans. In Europe the prevalence is about 28%, but also in this continent it is very variable. In fact, it fluctuates between 2% in Scandinavia and 70% in Southern Italy [5]. Several hypotheses have been put forward to explain these differences. One of these theories is based on the fact that dairy products played an important role in the diet in Northern Europe. This helped to cause a natural selection of subjects capable of digesting lactose. Furthermore, the coexistence, in the same territory, of populations made up of lactose intolerant and non-intolerant subjects is due to the migrations that have occurred over time [4,5].

2. Lactose features

Lactose is a disaccharide composed of D-galactose bound to D-glucose (β-1,4 glycosidic bond) and it is contained in dairy products [6]. The concentration of this carbohydrate is different in the various types of milk of animal origin. In human breast milk it is 7.0 mg/100 ml, in cow milk it is 4.7-5.0 mg/100 ml, in sheep milk it is 4.4-4.8 mg/100 ml, in goat milk it is 4.2-4.8 mg/100 ml, in buffalo milk it is 4.8-5.0 mg/100 ml [7]. In milk of vegetable origin (nut, rice, oat, soy) lactose is absent [8].

Lactose is synthesized from glucose and galactose by the action of lactose synthetase. This is an enzyme composed of two subunits: one possesses galactosyltransferase activity and the second, on the other hand, has regulatory functions. These subunits catalyze the bond of galactose and glucose resulting in the formation of the disaccharide [9].

In order to be exploited by the human body, lactose must be hydrolyzed into glucose and galactose. This process is catalyzed by lactase–phlorizin hydrolase, an enzyme that is present on the surface of the intestinal mucosa. Lactase is encoded by a gene, LCT, of about 50 kb and localized on chromosome 2 (*locus 2q21*) [10,11]. The gene has 17 exons and it encodes a messenger RNA (mRNA) from which is obtained a pre-protein that is processed to a smaller protein, which has one active site [11]. Lactase is expressed by enterocytes of the small intestine, especially the mid-jejunum. This gene can undergo mutations, some of which have been associated with lactose intolerance [10]. To date, several polymorphisms have been sequenced. Those most frequently found in the Western population are two. One is C/T-13910 and it consists in the presence of one cytosine (C) or one thymine (T) in position 13910. The C/C form is related to the non-persistence of lactase, while the variants C/T or T/T are expression of lactase persistence. The second main polymorphism is G/A-22108, characterized by the presence of one guanine (G) or one adenine (A) in position 22108. The variant G/G is related to the non persistence of lactase, while the variants G/A or A/A are expression of lactase persistence. Other polymorphisms are C/G-13907, T/G-13915, T/G-14009, and G/C-14010. These have been found in the populations of the Middle East and Africa [10,12]. In a recent study conducted by *Buzás* [13], the C/C pholymorphism was identified more frequently in female subjects and the incidence was higher in subjects born after 1995 than in those born around 1936. This effect is referred to as a birth cohort phenomenon.

From the eighth week of gestation, the activity of lactase begins on the mucosal surface of our intestine. This activity progressively increases until it reaches its maximum peak at the time of birth [14]. The ability to digest lactose during the breast–feeding is essential for the growth and life of the newborn. In fact, a congenital deficiency in lactase activity has been found to be fatal if not diagnosed shortly after birth. The hydrolysis of lactose determines the formation of the two monosaccharides, glucose and galactose [9]. They are absorbed by active transport mediated by sodium-glucose linked transporter-1 (SGLUT-1), a protein localized in the enterocyte membrane. It co-transports glucose or galactose and two sodium ions (Na^+) from the intestinal lumen to cytoplasm of these cells. Subsequently, the monosaccharides are transferred from the cytosol to the blood by glucose transporter-2 (GLUT-2), present on the other side of the enterocyte membrane [14]. Glucose is used as an energy source, while galactose is transformed into glucose or it is used as a component of glycolipids and glycoproteins [9].

A deficiency of lactase leads to a reduced absorption of lactose present in the intestinal tract and this can cause the onset of the symptoms (Fig. 1). First, the excessive osmotic load increases the intestinal water content. Second, lactose is readily fermented by the colonic microbiome leading to production of short chain fatty acids and gas (mainly hydrogen - H_2 -, carbon dioxide - CO_2 -, and methane - CH_4 -) [15]. Not in all subjects the same amount of lactose ingested causes the onset of symptoms. Symptoms appear in some people after ingesting large amounts of lactose. In others, even a minimal percentage of lactose contained in foods can lead to the onset of related disorders [16]. This depends on several factors: the residual lactase expression, the ingestion of lactose at the same time as other foods, gut–transit time, small intestine bacterial overgrowth, and also composition of the enteric microbiome [15,16].

Symptoms of lactose intolerance generally appear when the percentage of lactase activity is less than 50%. In some cases, tolerance could be induced by adaptation of the intestinal flora. In fact, most people with lactase non-persistence can tolerate small amounts of lactose, especially when it is combined with other foods [17].

3. Clinical features and complications

Typical symptoms of lactose intolerance are abdominal pain, bloating, flatulence, diarrhea (in some cases constipation), borborygmi, nausea, and vomiting. On average, their onset occurs about one hour after ingestion of foods containing lactose, but they can also appear earlier or later [6].

The production of gas causes borborygmi, flatulence, abdominal distension and pain. In these subjects, a lot of gas is produced because lactose is not digested and absorbed in the small bowel, but it is fermented by intestinal bacterial flora

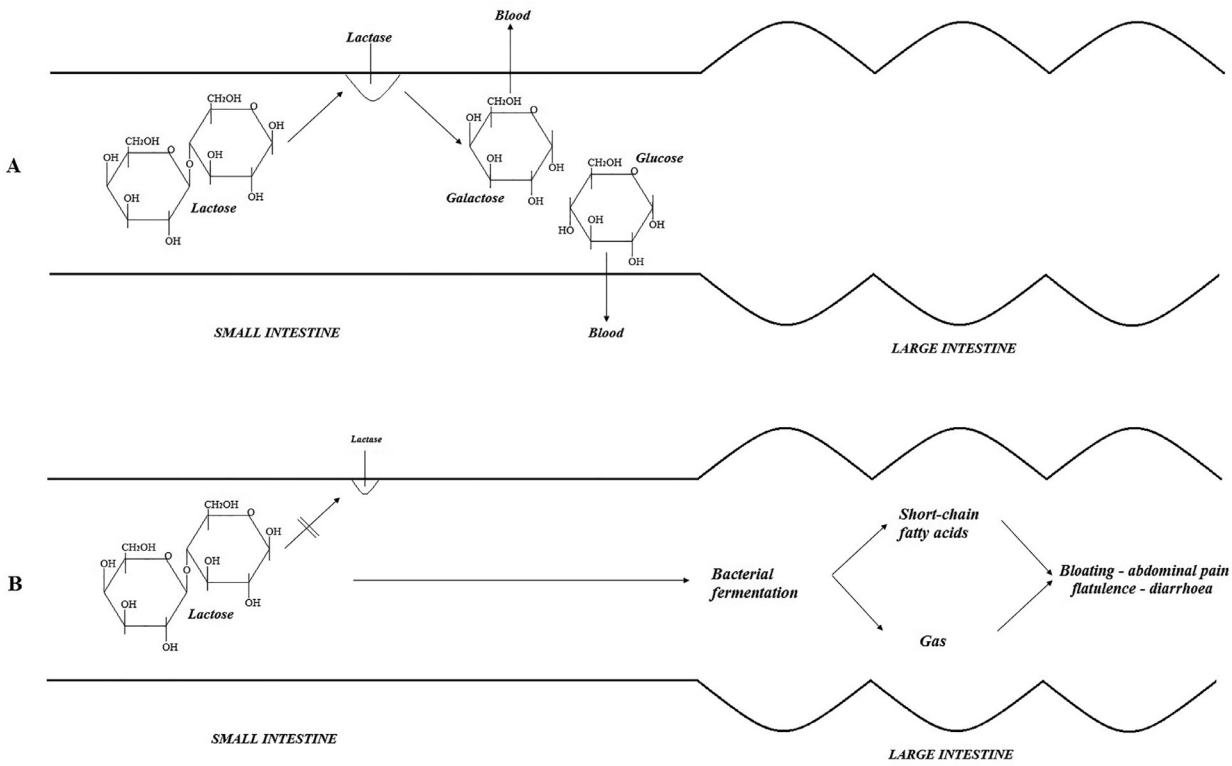

Fig. 1 – A: Normal digestion of lactose in presence of lactase; B: Lactose intolerance due to lactase deficiency or absence and subsequent onset of symptoms.

[18]. However, not all intolerant people develop symptoms of the same intensity. This is due not only to the amount of gas that is produced, which obviously varies from person to another, but also depends on the sensitivity to intestinal distension. For example, people affected by irritable bowel syndrome tend to develop more severe symptoms than people not lactose intolerant [3]. Also, there are other factors that influence the bloating. Among these, a very important role is played by the bacterial flora of the colon, which, if altered, can contribute to the increase in gas production, as well as bacterial proliferation [3,18].

Diarrhea is caused by acidification of intestinal contents that occurs as a result of the high production of short–chain fatty acids, which increase the osmotic load. This is followed by an increase in electrolytes and fluid secretion and a rapid intestinal transit time [19].

This symptom feature can affect the patients' quality of life. In fact, anxiety, depression and fatigue are found in a high percentage of intolerant subjects [20]. In other cases, a real eating disorder develops, which can be classified according to the criteria of the DSM V. This happens because patients tend to significantly reduce their food intake to avoid suffering from abdominal pain, diarrhea, and all the other symptoms related to lactose intolerance [8].

In addition to these, extra–intestinal symptoms may also occur in subjects with lactose intolerance: memory deterioration, headache, musculoskeletal pains, heart rhythm disorders, depression, anxiety, ulcers in the oral mucosa, dyspareunia, disturbances of menstrual cycle, eczema and other allergic–based pathologies (sinusitis, rhinitis, asthma), acne, increased frequency of urination [21,22]. These disorders could be caused by the excessive production of chemical substances such as acetone, acetaldehyde, ethanol, peptides, and others which are formed in the course of maldigestion and malabsorption of lactose and which have toxic effects on the body [9,23].

In addition to the disorders directly related to the altered metabolism of lactose, over time it has emerged that intolerant subjects have an increased risk of developing certain diseases. This may be due both to the alteration of the intestinal microbiome and to the excessive production of the toxic substances already mentioned. Furthermore, it seems that the substances contained in dairy products carry out various beneficial actions, even if not yet been well defined. Failure to assimilate them could expose the intolerant subject to a greater risk of developing various diseases, including some types of cancer [24].

Among the cancers whose incidence appears to be influenced by dairy foods is colorectal cancer. This tumor has a high incidence worldwide. In fact, there are about 250,000 new cases per year, and in industrialized countries the risk of developing this cancer is about 5% for healty subjects who are not genetically predisposed. There are several risk factors and, among these, a primary role is played by the diet [25]. In particular, it has been shown that ingesting milk reduces the risk of developing colorectal cancer. This could be due to its components, including calcium and vitamin D [26].

Calcium is considered an anti–carcinogenic factor as it appears to bind secondary bile acids and ionized fatty acids, which act on the colic mucosa by stimulating its proliferation. Calcium is also involved in the mechanisms of cell differentiation and apoptosis, as well as in the reduction of the risk of mutations in the K-RAS gene (involved in the genesis of colorectal cancer). Its dose considered anti–neoplastic is higher than 1200 mg/day and the main source of calcium is represented by dairy products [27].

The protective effects of dairy products also appear to be there with regard to bladder cancer, although the results are not entirely certain. Since a beneficial effect of lactobacilli on the bladder surface was observed, it was hypothesized that lactose from dairy foods could exert an probiotic–like effect. The meta-analysis conducted by *Bermejo et al.* [28] showed a reduction of this type of cancer with the consumption of medium quantities of dairy products and with medium–high quantities of not–whole milk. Conversely, the risk increases if large quantities of whole milk are consumed.

A meta-analysis realized by *Dong et al.* [29] indicated that increased consumption of total dairy food, excluding milk, may be associated with a reduced risk of breast cancer. In another meta–analysis by *Zang et al.* [30] it was found that low fat dairy and yogurt reduced breast cancer risk. In this type of cancer, it seems that the protective anti–carcinogenetic effect is due to calcium, vitamin D, butyrate, and lactoferrin, and conjugated linoleic acid, that are found, in fact, in dairy products.

A meta–analysis of studies on the association between dairy consumption with gastric cancer risk suggested that total dairy foods might be related to the reduction of gastric cancer risk in Europe and United States of America, while milk consumption was not associated with gastric cancer risk [31].

On the other hand, in some types of cancer, consuming large quantities of dairy products is not beneficial. However, on this point, the data available today are often conflicting. This is the case of ovarian cancer. Some studies tend to support the association between the high consumption of dairy products and the increased risk of ovarian cancer. In fact, an excessive amount of galactose seems to be toxic for the germ cells of the ovary [26]. In other cases, this association has not emerged, while in some cases an opposite result has even emerged, ie, a reduction in the risk of cancer associated to a high consumption of dairy foods and cow's milk [32,33].

The risk of developing prostate cancer also appears to be related to increased consumption of dairy products. This is what has emerged in several studies. *Agarwal et al.* [34] found a lower incidence of lactose intolerance among prostate cancer patients than in the general population. In other cases milk consumption has been shown to be a possible risk factor for more severe and aggressive forms of prostate cancer [33].

These results suggest that it would be convenient not to exceed the dietary intake of milk, but to ingest moderate quantities daily. In this way it is possible to obtain the benefits that have been proven while avoiding the increased risk of certain diseases.

In addition to cancer, consumption of dairy products is also a protective factor against other diseases, including chronic inflammatory bowel diseases. Three studies have shown that the pre-disease intake of dairy products can reduce risk of Crohn's disease and idiopathic ulcerative colitis [35-37]. In particular, there was a statistically significant protective effect of milk consumption compared to non–consumers.

In a meta–analysis by Drouin-Chartier *et al.* [38] it also emerged that several studies support favorable associations (ie, decreased risk) between dairy foods intake, in particular of low–fat–dairy, and the risk of hypertension, stroke, and metabolic syndrome. Lactose also determines a reduction in the risk of developing type 2 diabetes mellitus (T2DM). In fact, this disaccharide has a low glycemic index, and several scientific evidences show that the ingestion of carbohydrates with a low glycemic index reduces the risk of developing T2DM [39].

Finally, one of the best known associations is that between the intake of dairy products and bone remodelling. Calcium, vitamin D, and protein are needed for the maintenance of bone mass and its architecture [40]. In addition, vitamin A, potassium, zinc, and magnesium present in dairy products are also important nutrients in bone formation. In lactose intolerant subjects there is also a reduced intestinal absorption of calcium [41]. This malabsorption causes, together with the reduced intake of dairy products, an increased risk of osteoporosis and fractures [42]. In fact, reduced bone density is found much more frequently in subjects with the C/C genotype of the LCT gene than in subjects with the T/T genotype [41].

4. Diagnostic methods

Currently, several diagnostic methods are available to identify individuals with lactose intolerance. Some of them analyze the various reactions of the digestive tract following the administration of pure lactose and not foods containing lactose. To settle the question, a dairy food tolerance test [43] was proposed aimed at determining the onset of gastrointestinal symptoms after the ingestion of dairy products. This test would also be tailored to each patient since it would also take into account the individual person's food culture. In this way, it would be possible to identify those who develop lactose intolerance symptoms even after ingesting minimal amounts of food. On the other hand, those who are able to tolerate the ingestion of these foods can simply reduce, rather than completely abolish, the consumption of dairy products [43].

The characteristics of the methods currently used for the diagnosis of lactose intolerance are illustrated in Table 1.

4.1. *Genetic test*

As described above, several studies over the last few decades have revealed a genetic predisposition to lactose intolerance. Polymorphism have been identified in the gene that codes for lactase and which appear to be predisposing to this intolerance. Based on this knowledge, a method that can be used to diagnose lactose intolerance is the genetic test. It consists in the isolation of DNA, obtained by means a blood sample, and the subsequent analysis of polymorphisms LCT-13910C>T and LCT-22018G>A [44]. However, this test has important limitations. The presence of a predisposing polymorphism (C/C or G/G) does not necessarily imply intolerance nor does it allow us to predict if and when this will develop. This method does not allow to detect any secondary

Table 1 – Summary table of main diagnostic methods for lactose intolerance.

Diagnostic method	Sample tested	Substance or molecule measured or researched	Expected changes in intolerant subjects	Advantages	Disadvantages
Genetic test [44,45]	DNA by blood sample	LCT gene	Presence of the polymorphisms 13910 C>T and 22018 G>A	It allows to confirm or exclude a primary form of intolerance; it is minimally invasive	It does not allow to identify a secondary form; the presence of polymorphisms does not necessarily imply that the subject will develop a lactose intolerance in the course of life
HBT [19,46,52,53]	Exhaled air sample	Hydrogen exhaled before and after ingestion of 25 or 50 g of lactose	At least 20 ppm increase in exhaled hydrogen from baseline	Inexpensive; non-invasive; high sensitivity and specificity; easy to perform and to interpret	Long duration (3-6 h); however, false-positives and false-negatives can be obtained
Quick lactase test [56,58,59]	Post-bulbar duodenal mucosa biopsy sample	Lactase activity	Absent or reduced activity	Diagnosis of certainty of hypolactasia	It is invasive; it requires the presence of a laboratory technician in endoscopy room; it is expensive
Lactose tolerance test [60,61]	Blood sample	Glycaemia 30, 60, and 120 min after lactose ingestion	No change in glycaemia	Minimally invasive; low cost	Low sensitivity and specificity; closely related to the patient's characteristics (gastric emptying time, etc.)

DNA: deoxyribonucleic acid; LCT: lactase gene; HBT: hydrogen breath test; ppm: parts per million.

intolerance, but it is important to distinguish primary from secondary hypolactasia in order to undertake appropriate therapy. Therefore the genetic test could be performed after having ascertained the presence of lactose intolerance, with another method, to exclude or confirm a primary form [44].

A case in which the genetic test would be preferred as first choice is the presence of conditions that do not allow to be performed the other methods, for example, in children under 6 years old [45].

4.2. *Hydrogen breath test*

Hydrogen breath test (HBT) is the most frequently used method to diagnose this intolerance. In fact, it has several advantages: it is inexpensive, non−invasive, with high sensitivity and specificity, and easy to perform and to interpret [46].

The test exploits the phenomenon whereby the fermentation of lactose by the microbial flora, which occurs when it is not digested and it accumulates in the gut, determines the production of gas, including hydrogen. The test involves the measurement of exhaled hydrogen fasting and the subsequent ingestion of 25-50 g of lactose. Then the hydrogen measurement is performed every 15 minutes for 3-6 hours. An increase in exhaled hydrogen concentration greater than 20 ppm (parts per million) from baseline is suggestive of hypolactasia [19].

A disadvantage of HBT is its long duration (3-6 hours). During this time span there are from 12 to 24 measurements, one every 15 min. To overcome this relative discomfort, Yang *et al.* [47] have proposed a new protocol (four-sample lactose hydrogen breath test, 4SLHBT) which provides in total only 4 measurements distributed over a period of 3 hours. They found a high concordance between classical HBT and 4SLHBT. This improves patient compliance. In addition, it allows to reduce waiting lists in public hospitals as more people can perform the breath test on the same day.

HBT can also detect false-negatives. One reason is that the intestine can also be colonized by methane-producing bacteria (CH_4). In a study by Houben *et al.* [48], the measurement of CH_4, together with that of hydrogen, would allow an increase in the diagnostic accuracy of the breath test. This would allow the diagnosis of lactose intolerance even in patients with a bacterial flora that does not produce hydrogen. Also according to Rojo *et al.* [49] this method could be exploited to identify intolerant subjects with normal H_2 excretion.

False-negative can also caused by physical exercise, slow oro−cecal transit, and all those conditions that alter the normal bacterial flora, such as recent use of oral antibiotics, abuse of laxatives, or invasive procedures that require preparatory bowel cleansing with purgatives and/or enemas. The use of probiotics can also affect the validity of the HBT because they also cause an alteration of the intestinal bacterial flora [50].

According to a recent study by Gallagher *et al.* [51], false-negatives can also be obtained if the test is stopped at the third hour. In fact, they showed that 18.5% of the patients they recruited would have tested falsely negative for HBT if had been stopped at the third hour.

False-positives results can also be obtained in addition to false-negatives and several condition can be the causes.

An excessive physical activity close to performing the breath test can also result in a false positive [52]. Another condition is the recent use of drugs such as aspirin or proton pump inhibitors. Smoking can also cause false-positives as the combustion of tobacco causes an increase in gases, including hydrogen. Some foods such as beans or corn should also be mentioned, which can cause an increase in the production of hydrogen whose concentration increases, consequently, in the exhaled air [53].

Also, one condition that can cause a false positive result is the small intestinal bacterial overgrowth (SIBO). In fact, an observational study conducted by *Varjù et al.* [54] compared the results obtained in a group of patients undergoing simultaneous HBT and lactose tolerance test (illustrated below). From this comparison it emerged that 9.1% of HBT positives was indeed affected by SIBO and not by lactose intolerance, as in these subjects the lactose tolerance test was negative.

A further explanation of the discrepancy in the results observed in the various studies is to be found in the lack of standardization criteria both for the indications for the breath-test and for the methods of execution and interpretation of the results [55].

4.3. *Quick lactase test*

Quick lactase test consists in performing biopsies of the postbulbar duodenal mucosa. These are subsequently incubated with lactose on test plate. This process aims to check the presence or absence of lactase activity. If lactase activity is present, a dark blue reaction occurs; if partial enzyme activity is present, a light blue color will appear; if no staining develops, this result will indicate a total absence of lactase activity and, therefore, severe hypolactasia [56].

Ojetti *et al.* [57] proposed that this method could be used when the common HBT turns out to be negative, despite the symptoms, instead of the genetic test. In fact they have found an agreement between the quick test and the HBT of 81%.

Also Tsadok Perets *et al.* [58] found a high agreement between HBT and the quick test for intolerant subjects with positive HBT results. In these patients a null or reduced lactase activity was detected in duodenal biopsies. On the other hand, in the case of subjects who tested negative for HBT, the agreement between the two tests was lower. This is because many patients with negative HBT results had indeed a bioptic picture of hypolactasia. From this study it can be deduced that the execution of the quick test is useful only in the case of a negative result of HBT, since this is an invasive test that can be avoided in the case of a positive HBT.

In any case, the quick test has limitations. Among these is the size of biopsies which, if larger or shorter than 2 mm, may give false negatives or false positive hypolactasia, respectively, due to the irregular expression of lactase. Another limitation is represented by the invasiveness, as well as the high cost of the endoscopic method. One more problem, being a bioptic examination, is that this is conditioned by the coagulation and the patient's clinical conditions. Furthermore, the incubation of the samples must be performed quickly and this requires the presence of a laboratory technician in the endoscopy room [59].

4.4. *Lactose tolerance test*

Lactose tolerance test (LTT) involves the administration of 50 g of lactose and the measurement of blood glucose before lactose intake and after 30 min, 60 min, and 120 min. The digestion of lactose causes the elevation of glycaemia and the absence of this increase indicates a lack of lactose absorption [19]. This test is rarely performed due to low sensitivity and specificity. False-positive and false-negative test results occur in 20% of normal subjects. These altered results are attributable to the gastric emptying time, which varies from patient to patient, and to glucose metabolism, which is also subjective [60].

According to Goshal *et al.* [61], lactose tolerance test could be used alongside HBT or even alone in centers where the latter is not available. In fact they have demonstrated a validity of this test comparable to the breath test. Among the advantages of the LTT there is the possibility of diagnosing lactose intolerance even in subjects who have a bacterial flora that does not produce hydrogen and who, therefore, are negative for HBT. However, an important limitation is represented by diabetes mellitus. In diabetic patients there can be an increase in blood glucose levels after ingesting lactose, even in the presence of intolerance.

Varjù et al. [54] have come to similar conclusions. Since in their study this test determined false positives in 9.8% of cases, the authors believe that it is more appropriate to perform the lactose tolerance test in combination with the HBT.

5. Clinical management and therapeutic options

Several approaches are currently possible for managing lactose intolerance. The first intervention concerns nutrition. Although in the past the focus was mainly on the exclusion of milk and dairy products from the diet, today we try to maintain a minimum daily income of these foods. As previously mentioned, dairy products are an important source of calcium, proteins, minerals, polyunsaturated fatty acids, and other substances which help prevent certain diseases and perform various physiological functions, such as bone remodelling [6]. It is no longer recommended to adopt a dairy-free diet in patients with lactose intolerance and many intolerant individuals can tolerate up to 12-15 grams of lactose per day [62,63]. So a valid strategy would be to increase lactose tolerance in these patients.

To ensure the intake of the substances contained in dairy products, without causing abdominal discomfort caused by lactose, the production of lactose-free foods was started. From a nutritional point of view, they are comparable to classic dairy products, with the difference that they do not contain lactose [64]. It would be useful to encourage the consumption of aged cheeses which, unlike fresh ones, contain little or no amount of lactose. In fact, during the ripening process, the bacteria consume all the lactose present [65].

A valid option is represented by enzymatic integration with exogenous lactase obtained from non-human sources. It can be obtained from yeast (*Kluyveromyces lactis*) or fungi

(*Aspergillus oryzae*, *Aspergillus niger*).The role of exogenous lactase is to replace the functions performed by the native enzyme, that is the splitting of lactose into glucose and galactose [66]. Its intake, in the form of pills, capsules or liquids, is expected every time foods containing lactose are ingested. Its use has allowed to obtain an improvement in the clinical features in children, adolescents and adults with lactose intolerance [67].

Ibba *et al*. [68] conducted a study to evaluate the efficacy of exogenous lactase in lactose intolerant subjects. The enzymatic compound exploited by these authors was Beta-Galactosidase, obtained from the fermentation of *Aspergillus oryzae*. A reduction in hydrogen excretion, as measured by HBT, was achieved in 40% of patients. On the other hand, in the remaining 60% of them the amount of hydrogen excreted did not change, but symptoms improvement was seen in a very high percentage of patients.

Ojetti *et al*. [69] conducted a randomized clinical trial to compare the effects of exogenous lactase and those of *Lactobacillus reuteri*. The effects of the administration of one or the other substance were monitored by HBT, performed before and after administration. The result was a reduction in the amount of hydrogen excreted in both patients who took the probiotic and those who received exogenous lactase. This reduction was more marked in the case of exogenous lactase. Symptoms associated with intolerance (bloating, abdominal pain, flatulence, diarrhoea) also improved in both groups. But even in this case the improvement was greater for patients who had taken lactase. According to Ojetti *et al*. [69], the use of exogenous lactase is recommended for the treatment of lactose intolerance, so as to be able to ingest milk and dairy products.

In addition to lactose, other substances contained in milk that could cause gastrointestinal disorders were also considered. Among these are the beta−caseins. Two isoforms have been identified, A1 and A2, which may be present in the milk individually or together. Type A2 is considered the original variant, as the gene encoding A1 is the result of a point mutation in the A2 gene, with proline substitution with histidine at position 67. In the gut, beta−caseins are lysed with the formation of various peptides, including beta-casomorphin-7 (BCM-7). Larger amounts of BCM-7 are obtained from A1 type proteolysis compared to A2 [70]. BCM-7 exerts an opioid activity because it binds to μ-opioid receptors, involved in the pathogenesis of pain and which are also present in the human gastrointestinal tract [71].

BCM-7 influences gastrointestinal motility and its overproduction causes a slowdown in intestinal transit, which leads to a greater consistency of the stools and a tendency towards the constipation [72]. The efficacy of casein-free diets has been investigated on the basis of the “opioid excess theory”: a high intake of casein can lead to an excessive production of opioid compounds. This results in an increase in the effects caused by these substances, such as constipation. However, this type of diet can lead to nutritional deficiencies, including vitamin D deficiency [73].

A possible alternative can be represented by the consumption of products containing only one isoform of casein. Several epidemiological studies conducted so far show a relationship between the consumption of milk containing A1 beta−casein and some gastrointestinal diseases. Conversely, it is hypothesized that milk containing the A2 isoform may have positive effects on digestive health [74].

In two study conducted by Jianqin et al. [75] and He et al. [76] it was found that the consumption of milk containing both isoforms caused the worsening of gastrointestinal symptoms in lactose intolerant patients, an increase in intestinal transit time, an increase in serum inflammation markers, a slowing of cognitive abilities, an increase in elimination with the faeces of short-chain fatty acids. Instead, these events did not occur during the administration of milk containing only A2 beta−casein. In both studies, it was concluded that type A1 was harmful to the digestive system, in particular for subjects with lactose intolerance.

Another valid therapeutic option is represented by probiotics. These are microorganisms which are capable of determining beneficial effects for the host [77]. These benefits include the improvement of gastrointestinal microflora, the reinforcement of immune system, the reduction of total serum cholesterol, the resolution of chronic diarrhoea and constipation, the improvement of the clinical features that characterizes chronic inflammatory bowel diseases, the reduction of food intolerances, including lactose intolerance [78]. The mechanisms by which probiotics are able to determine these effects are manifold. They act by increasing the hydrolytic capacity of the intestinal flora, they behave like antagonists towards gas-producing bacteria, they regulate the permeability of the intestinal mucosa, and they maintain constant and low levels of short-chain fatty acids in the bowel, which are implicated in the genesis of abdominal pain and diarrhoea [79,80]. Bacteria most frequently used for the production of probiotics are *Lactobacillus* spp. (*L. acidophilus*, *L. rhamnosus*, *L. casei*, etc.), *Bifidobacterium longum* spp., *Streptococcus thermophilus*, and *Saccharomyces boulardii* [79]. The latter has proved capable of counteracting intestinal hyperpermeability induced by stressful events, such as the aforementioned hyperproduction of short-chain fatty acids [81]. Therefore, the administration of *Saccharomyces boulardii* can also determine a further positive effect, which is represented by the reduction of the translocation of potential antigens into the blood [82].

Several studies have been conducted to evaluate the efficacy of probiotics in patients with lactose intolerance. In some cases both a reduction in the amount of exhaled hydrogen (measured with HBT) and an improvement in symptoms was obtained. However, in most cases only improvement in symptoms was achieved, with no change in the amount of exhaled hydrogen [83]. In the study conducted by Cano−Contreras et al. [84], patients who received the probiotic obtained a regression of symptoms related to lactose intolerance, in particular abdominal pain and flatulence.

In other cases, adding vitamins to probiotics has also proved beneficial. In the clinical trial of *Vitellio et al*. [85], a combination of *Bifidobacterium longum, Lactobacillus rhamnosus* and vitamin B_6 was used. From this study it emerged that this association resulted in the improvement of the clinical picture of lactose intolerant. This is due to the fact that the composition and the activity of the intestinal bacterial flora are modulated not only by the probiotic, but also by the vitamin B_6. Therefore, the addition of vitamin compounds, in particular vitamin B_6,

Table 2 – Summary table of clinical trials with preparation used, number of subjects, age, and effect of preparation.

Authors	Preparation used	Subjects (number)	Age (yrs)	Effect of preparation on symptoms
Gingold-Belfer et al. 2020 [83]	BIO-25	8	> 18	Reduction of intolerance symptoms (in particular bloating and flatulence); no modification of the amount of excreted hydrogen
Cano-Contreras et al. 2020 [84]	i3.1 probiotic	48	n/a	Reduction of the area under the curve of expired hydrogen; significant reduction in symptoms (especially abdominal pain and flatulence)
Vitellio et al. 2019 [85]	*Bifidobacterium longum* BB536, *Lactobacillus rhamnosus* HN001 and Vitamin B6	23	20-67	Improvement of some GI symptoms and metabolism of intestinal microbiota
He M et al. 2017 [76]	Milk containing only A2 β-casein vs milk containing A1 and A2 β-casein	600	20-50	Milk with A2 β-casein attenuates symptoms; milk with A1/A2 β-casein reduces lactase activity and it worsens symptoms
Jianqin S et al. 2016 [75]	Milk containing only A2 β-casein vs milk containing A1 and A2 β-casein	45	26-68	Milk with A1/A2 β-casein increases GI inflammation, worsens symptoms, delayes intestinal transit time, deteriorates cognitive processes; milk with only A2 does not worsen the symptoms
Ibba et al. 2014 [68]	Beta-Galactosidase obtained from *Aspergillus oryzae*	96	18-65	Reduction of hydrogen excreted in 40% of cases; symptom improvement in most patients (although there was no correlation between excreted hydrogen levels and symptoms)
De Vrese et al. 2014 [88]	Combination of acid lactase from *Aspergillus oryzae* and yogurt bacteria	24	> 18	Reduction of hydrogen excretion peak at HBT; improvement of abdominal pain and flatulence
Ojetti V et al. 2010 [69]	Supplementation with *Lactobacillus reuteri* or exogenous lactase	60	18-65	Reduction of hydrogen excretion at HBT (more with lactase than *L. reuteri*); improvement of abdominal pain, flatulence, bloating, and diarrhoea (more with lactase than *L. reuteri*)
He T et al. 2008 [87]	Capsules of *Bifidobacterium longum* and a yogurt with a specific probiotic strain (*Bifidobacterium animalis* DN173010)	11	23-54	Changes in the metabolism of intestinal bacterial flora; improvement of symptoms

n/a: data is no provided; GI: gastrointestinal; HBT: hydrogen breath test.

could also be considered in the case of patients with lactose intolerance.

Probiotics are often added to dairy products, both as fermenting agents and as food additives. An example is yogurt, a food obtained from the fermentation of milk and it is rich in calcium, vitamins, and proteins. It is an excellent source of these substances for patients with lactose intolerance [80]. Yogurt, once ingested by an intolerant subject, does not cause the onset of typical symptoms. It would seem that the lactose contained in yogurt is more easily digested [86].

In a study by He et al. [87] it was found an increase in fecal β-galactosidase activity in lactose intolerant subjects after consumption of yogurt containing probiotics. In particular, the yogurt used by these authors was a derivative of fermented milk containing both traditional yogurt strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and a specific probiotic strain (*Bifidobacterium animalis*). This combination led to an improvement in symptoms related to lactose intolerance. Similar results were also obtained in the study by de Vrese et al. [88], who administered yogurt containing *Streptococcus thermophilus* and *Lactobacillus delbrückii* ssp. *bulgaricus*.

In addition to probiotics, prebiotics have also been considered, which are non−digestible oligosaccharides fructans and galactans [89]. They stimulate the growth and proliferation of bacterial flora capable of fermenting lactose. In an indirect way, they also determine positive effects on the metabolism of lactose and on the symptoms caused by its maldigestion [79]. Pure prebiotic galacto−oligosaccharides (GOS) have been shown to be capable of causing a reduction in symptoms of lactose intolerance. In fact, GOS induce changes in the composition of the intestinal bacterial flora, increasing the concentration of *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium*, and *Roseburia* spp., which ferment lactose. These changes suggest that somehow prebiotics may help regain the ability to digest lactose although these effects do not occur in all individuals [90]. Table 2 schematically shows the studies cited in this section.

6. Conclusions

Ultimately, as regards the diagnostic methods of lactose intolerance available today, HBT represents the most valid choice

for diagnostic accuracy, non-invasiveness, ease of execution, low cost. Since this method is also not free from false positive or negative results, it would be desirable to associate it with another diagnostic technique and it would be useful to associate the methane measurement with the exhaled hydrogen dosage. This would make the method extremely efficient.

As regards the treatment of these patients, it is not possible to define a standardized therapy. In fact, the response of the bowel to the various substances that can be used to increase or restore the ability to digest lactose is not the same in all individuals. We should define a treatment tailored to each patient, evaluating which therapeutic options are most effective for the person in question.

We believe, however, to highlight that the current diagnostic and therapeutic approach to lactose intolerance will quickly require a thorough critical review that takes into account not so much the molecule alone as the causative agent of gastrointestinal disorders, but rather the various food components. In this way it would also be possible to take into account the individual person's food culture.

Acknowledgment

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.The authors declare no conflict of interest.

REFERENCES

[1] Vandenplas Y. Lactose intolerance. Asia Pac J Clin Nutr 2015;24:S9–13. doi:10.6133/apjcn.2015.24.s1.02.
[2] Martínez Vázquez SE, Nogueira de Rojas JR, Remes Troche JM, Coss Adame E, Rivas Ruíz R, Uscanga Domínguez LF. The importance of lactose intolerance in individuals with gastrointestinal symptoms. Rev Gastroenterol Mex 2020;85:321–31. doi:10.1016/j.rgmx.2020.03.002.
[3] Catanzaro R, Sciuto M, Singh B, Pathak S, Marotta F. Irritable Bowel Syndrome and lactose intolerance: the importance of differential diagnosis. A monocentric study. Minerva Gastroenterol (Torino) 2021;67(1):72–8. doi:10.23736/S1121-421X.20.02734-8.
[4] Storhaug CL, Fosse SK, Fadnes LT. Country, regional, and global estimates for lactose malabsorption in adults: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2017;2:738–46. doi:10.1016/S2468-1253(17)30154-1.
[5] Lember M. Hypolactasia: a common enzyme deficiency leading to lactose malabsorption and intolerance. Pol Arch Med Wewn 2012;122:60–4.
[6] Szilagyi A, Ishayek N. Lactose intolerance, dairy avoidance, and treatment options. Nutrients 2018;10. doi:10.3390/nu10121994.
[7] Silanikove N, Leitner G, Merin U. The interrelationships between lactose intolerance and the modern dairy industry: global perspectives in evolutional and historical backgrounds. Nutrients 2015;7:7312–31. doi:10.3390/nu7095340.
[8] Misselwitz B, Butter M, Verbeke K, Fox MR. Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management. Gut 2019;68:2080–91. doi:10.1136/gutjnl-2019-318404.
[9] Ugidos-Rodríguez S, Matallana-González MC, Sánchez-Mata MC. Lactose malabsorption and intolerance: a review. Food Funct 2018;9:4056–68. doi:10.1039/c8fo00555a.
[10] Kuokkanen M, Enattah NS, Oksanen A, Savilahti E, Orpana A, Järvelä I. Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. Gut 2003;52:647–52. doi:10.1136/gut.52.5.647.
[11] Anguita-Ruiz A, Aguilera CM, Gil Á. Genetics of lactose intolerance: an updated review and online interactive world maps of phenotype and genotype frequencies. Nutrients 2020;12:2689. doi:10.3390/nu12092689.
[12] Swallow DM. Genetics of lactase persistence and lactose intolerance. Annu Rev Genet 2003;37:197–219. doi:10.1146/annurev.genet.37.110801.143820.
[13] Buzás GM. Birth-cohort analysis in adult-type hypolactasia. Orv Hetil 2019;160:549–54. doi:10.1556/650.2019.31332.
[14] Lomer MCE, Parkes GC, Sanderson JD. Review article: lactose intolerance in clinical practice - myths and realities. Aliment Pharmacol Ther 2008;27:94–6. doi:10.1111/j.1365-2036.2007.03557.x.
[15] Shepherd SJ, Lomer MC, Gibson PR. Short-chain carbohydrates and functional gastrointestinal disorders. Am J Gastroenterol 2013;108:707–17. doi:10.1038/ajg.2013.96.
[16] Misselwitz B, Pohl D, Frühauf H, Fried M, Vavricka SR, Fox M. Lactose malabsorption and intolerance: pathogenesis, diagnosis and treatment. United. European Gastroenterol J 2013;1:151–9. doi:10.1177/2050640613484463.
[17] Shaukat A, Levitt MD, Taylor BC, MacDonald R, Shamliyan TA, Kane RL, et al. Systematic review: effective management strategies for lactose intolerance. Ann Intern Med 2010;152:797–803. doi:10.7326/0003-4819-152-12-201006150-00241.
[18] Zhu Y, Zheng X, Cong Y, Chu H, Fried M, Dai N, et al. Bloating and distention in irritable bowel syndrome: the role of gas production and visceral sensation after lactose ingestion in a population with lactase deficiency. Am J Gastroenterol 2013;108:1516–25. doi:10.1038/ajg.2013.198.
[19] Swagerty DL Jr, Walling AD, Klein RM. Lactose intolerance. Am Fam Physician 2002;65:1845–50.
[20] Casellas F, Aparici A, Pérez MJ, Rodríguez P. Perception of lactose intolerance impairs health-related quality of life. Eur J Clin Nutr 2016;70:1068–72. doi:10.1038/ejcn.2016.80.
[21] Matthews SB, Waud JP, Roberts AG, Campbell AK. Systemic lactose intolerance: a new perspective on an old problem. Postgrad Med J 2005;81:167–73. doi:10.1136/pgmj.2004.025551.
[22] Aghasi M, Golzarand M, Shab-Bidar S, Aminianfar A, Omidian M, Taheri F. Dairy intake and acne development: a meta-analysis of observational studies. Clin Nutr 2019;38:1067–75. doi:10.1016/j.clnu.2018.04.015.
[23] Schiffner R, Kostev K, Gothe H. Do patients with lactose intolerance exhibit more frequent comorbidities than patients without lactose intolerance? An analysis of routine data from German medical practices. Ann Gastroenterol 2016;29:174–9. doi:10.20524/aog.2016.0009.
[24] Shrier I, Szilagyi A, Correa JA. Impact of lactose containing foods and the genetics of lactase on diseases: an analytical review of population data. Cancer Nutr 2008;60:292–300. doi:10.1080/01635580701745301.
[25] Hull R, Francies FZ, Oyomno M, Dlamini Z. Colorectal cancer genetics, incidence and risk factors: in search for targeted therapies. Cancer Manag Res 2020;12:9869–82. doi:10.2147/CMAR.S251223.
[26] Amiri M, Diekmann L, von Köckritz-Blickwede M, Naim HY. The diverse forms of lactose intolerance and the putative linkage to several cancers. Nutrients 2015;7:7209–30. doi:10.3390/nu7095332.
[27] Aune D, Lau R, Chan DS, Vieira R, Greenwood DC, Kampman E, et al. Dairy products and colorectal cancer risk:

a systematic review and meta-analysis of cohort studies. Ann Oncol 2012;23:37–45. doi:10.1093/annonc/mdr269.
[28] Bermejo LM, López-Plaza B, Santurino C, Cavero-Redondo I, Gómez-Candela C. Milk and dairy product consumption and bladder cancer risk: a systematic review and meta-analysis of observational studies. Adv Nutr 2019;10:S224–38. doi:10.1093/advances/nmy119.
[29] Dong JY, Zhang L, He K, Qin LQ. Dairy consumption and risk of breast cancer: a meta-analysis of prospective cohort studies. Breast Cancer Res Treat 2011;127:23–31. doi:10.1007/s10549-011-1467-5.
[30] Zang J, Shen M, Du S, Chen T, Zou S. The association between dairy intake and breast cancer in Western and Asian Populations: a systematic review and meta-analysis. J Breast Cancer 2015;18:313–22. doi:10.4048/jbc.2015.18.4.313.
[31] Guo Y, Shan Z, Ren H, Chen W. Dairy consumption and gastric cancer risk: a meta-analysis of epidemiological studies. Nutr Cancer 2015;67:555–68. doi:10.1080/01635581.2015.1019634.
[32] Koralek DO, Bertone-Johnson ER, Leitzmann MF, Sturgeon SR, Lacey JV Jr, Schairer C, et al. Relationship between calcium, lactose, vitamin D, and dairy products and ovarian cancer. Nutr Cancer 2006;56:22–30. doi:10.1207/s15327914nc5601_4.
[33] Willett WC, Ludwig DS. Milk and health. N Engl J Med 2020;382:644–54. doi:10.1056/NEJMra1903547.
[34] Agarwal MM, Rana SV, Mandal AK, Malhotra S, Khandelwal N, Kumar S, et al. Lactose intolerance in prostate cancer patients: incidence and associated factors. Scand J Gastroenterol 2008;43:270–6. doi:10.1080/00365520701676831.
[35] Octoratou M, Merikas E, Malgarinos G, Stanciu C, Triantafillidis JKA. Prospective study of pre-illness diet in newly diagnosed patients with Crohn's disease. Rev Med Chir Soc Med Nat Iasi 2012;116:40–9.
[36] Abubakar I, Myhill DJ, Hart AR, Lake IR, Harvey I, Rhodes JM, et al. A case-control study of drinking water and dairy products in Crohn's Disease - further investigation of the possible role of Mycobacterium avium paratuberculosis. Am J Epidemiol 2007;165:776–83. doi:10.1093/aje/kwk067.
[37] Opstelten JL, Leenders M, Dik VK, Chan SS, van Schaik FD, Khaw KT, et al. Dairy products, dietary calcium, and risk of Inflammatory Bowel Disease: results from a European prospective cohort investigation. Inflamm Bowel Dis 2016;22:1403–11. doi:10.1097/MIB.0000000000000798.
[38] Drouin-Chartier JP, Brassard D, Tessier-Grenier M, Côté JA, Labonté ME, Desroches S, et al. Systematic review of the association between dairy product consumption and risk of cardiovascular-related clinical outcomes. Adv Nutr 2016;7:1026–40. doi:10.3945/an.115.011403.
[39] Romero-Velarde E, Delgado-Franco D, García-Gutiérrez M, Gurrola-Díaz C, Larrosa-Haro A, Montijo-Barrios E, et al. The importance of lactose in the human diet: outcomes of a Mexican consensus meeting. Nutrients 2019;11:2737. doi:10.3390/nu11112737.
[40] Martin-Bautista E, Muñoz-Torres M, Fonolla J, Quesada M, Poyatos A, Lopez-Huertas E. Improvement of bone formation biomarkers after 1-year consumption with milk fortified with eicosapentaenoic acid, docosahexaenoic acid, oleic acid, and selected vitamins. Nutr Res 2010;30:320–6. doi:10.1016/j.nutres.2010.05.007.
[41] Obermayer-Pietsch BM, Bonelli CM, Walter DE, Kuhn RJ, Fahrleitner-Pammer A, Berghold A, et al. Genetic predisposition for adult lactose intolerance and relation to diet, bone density, and bone fractures. J Bone Miner Res 2004;19:42–7. doi:10.1359/JBMR.0301207.
[42] Fedota O, Babalian V, Ryndenko V, Belyaev S, Belozorov I. Lactose tolerance and risk of multifactorial diseases on the example of gastrointestinal tract and bone tissue pathologies. Georgian Med News 2020(303):109–13.
[43] Lukito W, Malik SG, Surono IS, Wahlqvist ML. From 'lactose intolerance' to 'lactose nutrition'. Asia Pac J Clin Nutr 2015;2:S1–8. doi:10.6133/apjcn.2015.24.s1.01.
[44] Santonocito C, Scapaticci M, Guarino D, Annicchiarico EB, Lisci R, Penitente R, et al. Lactose intolerance genetic testing: Is it useful as routine screening? Results on 1426 south-central Italy patients. Clin Chim Acta 2015;439:14–17. doi:10.1016/j.cca.2014.09.026.
[45] Tomczonek-Moruś J, Wojtasik A, Zeman K, Smolarz B. Bąk-Romaniszyn L. 13910C>T and 22018G>A LCT gene polymorphisms in diagnosing hypolactasia in children. United European Gastroenterol J 2019;7:210–16. doi:10.1177/2050640618814136.
[46] Robles L, Priefer R. Lactose intolerance: what your breath can tell you. Diagnostics (Basel) 2020;10:412. doi:10.3390/diagnostics10060412.
[47] Yang JF, Fox M, Chu H, Zheng X, Long YQ, Pohl D, et al. Four-sample lactose hydrogen breath test for diagnosis of lactose malabsorption in irritable bowel syndrome patients with diarrhea. Worl J Gastroenterol 2015;21:7563–70. doi:10.3748/wjg.v21.i24.7563.
[48] Houben E, De Preter V, Billen J, Van Ranst M, Verbeke K. Additional value of CH_4 measurement in a combined $^{13}C/H_2$ lactose malabsorption breath test: a retrospective analysis. Nutrients 2015;7:7469–85. doi:10.3390/nu7095348.
[49] Rojo C, Jaime F, Azócar L, Hernández C, Villagrán A, Miquel JF, et al. Concordance between lactose quick test, hydrogen-methane breath test and genotyping for the diagnosis of lactose malabsorption in children. Neurogastroenterol Motil 2018;30:e13271. doi:10.1111/nmo.13271.
[50] Braden B. Methods and functions: breath tests. Best Pract Res Clin Gastroenterol 2009;23:337–52. doi:10.1016/j.bpg.2009.02.014.
[51] Gallagher J, Kirton E, Anderson-Leary L, Hewitt S, Burke J, Jackson W. PWE-079 Lactose intolerance hydrogen breath testing: extending the sample duration time reduces a false negative diagnosis. Gut 2019;68:A209. doi:10.1136/gutjnl-2019-BSGAbstracts.399.
[52] Robles L, Priefer R. Lactose intolerance: what your breath can tell you. Diagnostics (Basel) 2020;10:412. doi:10.3390/diagnostics10060412.
[53] Levitt MD, Hirsh P, Fetzer CA, Sheahan M, Levine AS. H_2 excretion after ingestion of complex carbohydrates. Gastroenterology 1987;92:383–9. doi:10.1016/0016-5085(87)90132-6.
[54] Varjú P, Ystad B, Gede N, Hegyi P, Pécsi D, Czimmer J. The role of small intestinal bacterial overgrowth and false positive diagnosis of lactose intolerance in southwest Hungary - A retrospective observational study. PLoS One 2020;15:e0230784. doi:10.1371/journal.pone.0230784.
[55] Rezaie A, Buresi M, Lembo A, Lin H, McCallum R, Rao S, et al. Hydrogen and methane-based breath testing in gastrointestinal disorders: the North American Consensus. Am J Gastroenterol 2017;112:775–84. doi:10.1038/ajg.2017.46.
[56] Kuokkanen M, Myllyniemi M, Vauhkonen M, Helske T, Kääriäinen I, Karesvuori S, et al. A biopsy-based quick test in the diagnosis of duodenal hypolactasia in upper gastrointestinal endoscopy. Endoscopy 2006;38:708–12. doi:10.1055/s-2006-925354.
[57] Ojetti V, La Mura R, Zocco MA, Cesaro P, De Masi E, La Mazza A, et al. Quick test: a new test for the diagnosis of duodenal hypolactasia. Dig Dis Sci 2008;53:1589–92. doi:10.1007/s10620-007-0027-7.
[58] Perets TT, Shporn E, Aizic S, Kelner E, Levy S, Bareli Y, et al. A diagnostic approach to patients with suspected lactose malabsorption. Dig Dis Sci 2014;59:1012–16. doi:10.1007/s10620-013-2980-7.

[59] Mattar R, Basile-Filho A, Kemp R, dos Santos JS. Comparison of quick lactose intolerance test in duodenal biopsies of dyspeptic patients with single nucleotide polymorphism LCT-13910C>T associated with primary hypolactasia/lactase-persistence. Acta Cir Bras 2013;28:77–82.
[60] Domínguez-Jiménez JL, Fernández-Suárez A, Ruiz-Tajuelos S, Puente-Gutiérrez JJ, Cerezo-Ruiz A. Lactose tolerance test shortened to 30 minutes: an exploratory study of its feasibility and impact. Rev Esp Enferm Dig 2014;106:381–5.
[61] Ghoshal UC, Kumar S, Chourasia D, Misra A. Lactose hydrogen breath test versus lactose tolerance test in the tropics: does positive lactose tolerance test reflect more severe lactose malabsorption? Trop Gastroenterol 2009;30:86–90.
[62] Rozenberg S, Body JJ, Bruyère O, Bergmann P, Brandi ML, Cooper C, et al. Effects of dairy products consumption on health: benefits and beliefs - A commentary from the Belgian Bone Club and the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases. Calcif Tissue Int 2016;98:1–17. doi:10.1007/s00223-015-0062-x.
[63] Boukria O, El Hadrami EM, Boudalia S, Safarov J, Leriche F. Aït-Kaddour A. The effect of mixing milk of different species on chemical, physicochemical, and sensory features of cheeses: a review. Foods 2020;9:1309. doi:10.3390/foods9091309.
[64] McCain HR, Kaliappan S, Drake MA. Invited review: sugar reduction in dairy products. J Dairy Sci 2018;101:8619–40. doi:10.3168/jds.2017-14347.
[65] Facioni MS, Raspini B, Pivari F, Dogliotti E, Cena H. Nutritional management of lactose intolerance: the importance of diet and food labelling. J Transl Med 2020;18:260. doi:10.1186/s12967-020-02429-2.
[66] Corgneau M, Scher J, Ritie-Pertusa L, Le DTL, Petit J, Nikolova Y, et al. Recent advances on lactose intolerance: tolerance thresholds and currently available answers. Crit Rev Food Sci Nutr 2017;57:3344–56. doi:10.1080/10408398.2015.1123671.
[67] Santos GJ, Rocha R, Santana GO. Lactose intolerance: what is a correct management? Rev Assoc Med Bras 2019;65:270–5. doi:10.1590/1806-9282.65.2.270.
[68] Ibba I, Gilli A, Boi MF, Usai P. Effects of exogenous lactase administration on hydrogen breath excretion and intestinal symptoms in patients presenting lactose malabsorption and intolerance. Biomed Res Int 2014;2014:680196. doi:10.1155/2014/680196.
[69] Ojetti V, Gigante G, Gabrielli M, Ainora ME, Mannocci A, Lauritano EC, et al. The effect of oral supplementation with Lactobacillus reuteri or tilactase in lactose intolerant patients: randomized trial. Eur Rev Med Pharmacol Sci 2010;14:163–70.
[70] Pal S, Woodford K, Kukuljan S, Ho S. Milk intolerance, beta-casein and lactose. Nutrients 2015;7:7285–97. doi:10.3390/nu7095339.
[71] Thiruvengadama M, Venkidasamyb B, Thirupathic P, Chunga IM, Subramanian U. β-Casomorphin: a complete health perspective. Food Chem 2021;337:127765. doi:10.1016/j.foodchem.2020.127765.
[72] Ho S, Woodford K, Kukuljan S, Pal S. Comparative effects of A1 versus A2 beta-casein on gastrointestinal measures: a blinded randomised cross-over pilot study. Eur J Clin Nutr 2014;68:994–1000. doi:10.1038/ejcn.2014.127.
[73] Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejía JL, Hansen LH, et al. A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome. 2018;6:133. doi:10.1186/s40168-018-0523-3.
[74] Küllenberg de Gaudry D, Lohner S, Schmucker C, Kapp P, Motschall E, Hörrlein S, et al. Milk A1 β-casein and health-related outcomes in humans: a systematic review. Nutr Rev 2019;77:278–306. doi:10.1093/nutrit/nuy063.
[75] Jianqin S, Leiming X, Lu X, Yelland GW, Ni J, Clarke AJ. Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk. Nutr J 2016;15:35. doi:10.1186/s12937-016-0147-z.
[76] He M, Sun J, Jiang ZQ, Yang YX. Effects of cow's milk beta-casein variants on symptoms of milk intolerance in Chinese adults: a multicentre, randomised controlled study. Nutr J 2017;16:72. doi:10.1186/s12937-017-0275-0.
[77] Shi LH, Balakrishnan K, Thiagarajah K, Mohd Ismail NI, Yin OS. Beneficial properties of probiotics. Trop Life Sci Res 2016;27:73–90. doi:10.21315/tlsr2016.27.2.6.
[78] Jarocki P, Komoń-Janczara E, Glibowska A, Dworniczak M, Pytka M, Korzeniowska-Kowal A, et al. Molecular routes to specific identification of the Lactobacillus casei group at the species, subspecies and strain level. Int J Mol Sci 2020;21:2694. doi:10.3390/ijms21082694.
[79] Leis R, de Castro MJ, de Lamas C, Picáns R, Couce ML. Effects of prebiotic and probiotic supplementation on lactase deficiency and lactose intolerance: a systematic review of controlled trials. Nutrients 2020;12:1487. doi:10.3390/nu12051487.
[80] Trush EA, Poluektova EA, Beniashvilli AG, Shifrin OS, Poluektov YM, Ivashkin VT. The evolution of human probiotics: challenges and prospects. Probiotics Antimicrob Proteins 2020;12:1291–9. doi:10.1007/s12602-019-09628-4.
[81] Takadanohara H, Catanzaro R, Chui DH, He F, Yadav H, Ganguli A, et al. Beneficial effect of a symbiotic preparation with S. boulardii lysate in mild stress-induced gut hyper-permeability. Acta Biomed 2012;83:208–16.
[82] Naito Y, Marotta F, Kantah MK, Zerbinati N, Kushugulova A, Zhumadilov Z, et al. Gut-targeted immunonutrition boosting natural killer cell activity using Saccharomyces boulardii lysates in immuno-compromised healthy elderly subjects. Rejuvenation Res 2014;17:184–7. doi:10.1089/rej.2013.1500.
[83] Gingold-Belfer R, Levy S, Layfer O, Pakanaev L, Niv Y, Dickman R, et al. Use of a novel probiotic formulation to alleviate lactose intolerance symptoms - a pilot study. Probiotics Antimicrob Proteins 2020;12:112–18. doi:10.1007/s12602-018-9507-7.
[84] Cano-Contreras AD, Minero Alfaro IJ, Medina López VM, Amieva Balmori M, Remes Troche JM, Espadaler Mazo J, et al. Efficacy of i3.1 Probiotic on improvement of lactose intolerance symptoms: a randomized, placebo-controlled clinical trial. J Clin Gastroenterol 2020 Online ahead of print. doi:10.1097/MCG.0000000000001456.
[85] Vitellio P, Celano G, Bonfrate L, Gobbetti M, Portincasa P, De Angelis M. Effects of Bifidobacterium longum and Lactobacillus rhamnosus on gut microbiota in patients with lactose intolerance and persisting functional gastrointestinal symptoms: a randomised, double-blind, cross-over study. Nutrients 2019;11. doi:10.3390/nu11040886.
[86] Aljutaily T, Huarte E, Martinez-Monteagudo S, Gonzalez-Hernandez JL, Rovai M, Sergeev IN. Probiotic-enriched milk and dairy products increase gut microbiota diversity: a comparative study. Nutr Res 2020;82:25–33. doi:10.1016/j.nutres.2020.06.017.
[87] He T, Priebe MG, Zhong Y, Huang C, Harmsen HJ, Raangs GC, et al. Effects of yogurt and bifidobacteria supplementation on the colonic microbiota in lactose-intolerant subjects. J

Appl Microbiol 2008;104:595–604. doi:10.1111/j.1365-2672.2007.03579.x.
[88] de Vrese M, Laue C, Offick B, Soeth E, Repenning F, Thoß A, et al. A combination of acid lactase from Aspergillus oryzae and yogurt bacteria improves lactose digestion in lactose maldigesters synergistically: a randomized, controlled, double-blind cross-over trial. Clin Nutr 2015;34:394–9. doi:10.1016/j.clnu.2014.06.012.
[89] Rastall RA, Gibson GR. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. Curr Opin Biotechnol 2015;32:42–6. doi:10.1016/j.copbio.2014.11.002.
[90] Arnold JW, Simpson JB, Roach J, Bruno-Barcena JM, Azcarate-Peril MA. Prebiotics for lactose intolerance: variability in galacto-oligosaccharide utilization by intestinal Lactobacillus rhamnosus. Nutrients 2018;10:1517. doi:10.3390/nu10101517.