

Lactose intolerance: from diagnosis to correct management

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Abstract. This review discusses one of the most relevant problems in gastrointestinal clinical practice: lactose intolerance.

The role of lactase-persistence alleles the diagnosis of lactose malabsorption the development of lactose intolerance symptoms and its management.

Most people are born with the ability to digest lactose, the major carbohydrate in milk and the main source of nutrition until weaning. Approximately, 75% of the world's population loses this ability at some point, while others can digest lactose into adulthood. Symptoms of lactose intolerance include abdominal pain, bloating, flatulence and diarrhea with a considerable intraindividual and interindividual variability in the severity.

Diagnosis is most commonly performed by the non invasive lactose hydrogen breath test.

Management of lactose intolerance consists of two possible clinical choice not mutually exclusive: alimentary restriction and drug therapy.

Keywords:

Lactose breath test, Diagnosis, Therapy.

Introduction

Lactose is a disaccharide sugar found in mammalian milk; it makes up around 2-8% of milk (by weight), although the amount varies among species and individuals: 7.2 g/100 mL in mature human milk, 4.7 g/100 mL in cow's milk but is negligible in the milk of some marine mammals. Lactose is a large sugar molecule that is made up of two smaller sugar molecules, glucose and galactose. Lactose is first broke down into D(+)glucose and D(+)galactose by lactase enzyme, then absorbed by intestinal enterocytes into the bloodstream.

Lactose and lactase

Lactose digestion takes place in the small intestine by the work of lactase-phlorizin hydrolase, a protein expressed on the brush border of intestinal villi (with the highest expression in the mid-jejunum), fingerlike protrusions, which increase the total surface area and, therefore, the ability to absorb different substrates. The enzyme lactase is encoded by LCT gene that maps on chromosome 2 (2q21). Lactase has two active sites: the first hydrolyzes lactose in the two monosaccharides glucose and galactose, making them absorbable by the intestinal mucosa, the second hydrolyzes phlorizin¹.

Hydrolysis typically occurs in the first part of the small intestine (jejunum), which has low concentrations of bacteria; so just little portion of lactose is fermented.

Glucose and galactose are absorbed by intestinal enterocytes into the blood-stream; glucose is ultimately utilized as a source of energy and galactose, into the liver, becomes a component of glycolipids and glycoproteins².

If the lactase enzyme is absent (alactasia) or deficient (hypolactasia), unabsorbed lactose molecules osmotically attract fluid into the bowel lumen, leading to an increased volume and fluidity of the intestinal content. In addition, the unabsorbed lactose passes into the colon, where it is fermented by bacteria producing short-chain fatty acids and gases (CO₂, CH₄, H₂) possibly leading to various gastrointestinal symptoms³.

Human lactase

Since 8 weeks of gestation, lactase activity can be detected on mucosal surface in the human gut. The activity increases up to 34 weeks from birth, were lactase expression reaches its peak of expres-

sion. However, since the first month of life, lactase activity starts to decrease (*lactase non persistence*) and its decline is highly variable from weaning to undetectable levels as a consequence of the normal maturational down-regulation of lactase activity^{4,6}. In humans, about 30% of the population has continuous activity of lactase after weaning and in adulthood (*lactase non persistence*)^{2,7}.

Hypolactasia

Hypolactasia, or lactase deficiency, a condition that determines the malabsorption of lactose, occurs generally no earlier than 6-7 year age, but is also sometimes much later⁸, showing a steady increase in prevalence even in the age groups above 65 years⁹. The kinetics of the reduction and the amount of residual lactase have considerable variability between different ethnic groups and even between individuals. However, the reduction of up to 50% of lactase is sufficient to ensure effective digestion of lactose¹⁰.

Hypolactasia, exists in three distinct forms: congenital, primary and secondary.

The congenital is an autosomal recessive (AR) condition characterized by severe diarrhea with event of watery stools since the first intake of milk by the infant; it persists for all the life and requires the complete exclusion of the sources of lactose. This extremely rare condition has been described in about 40 cases, in which the non-lactase activity was caused by a non-sense mRNA. The deficiency of nutrients causes a delay in growth, with the rapid onset of dehydration and alkalosis.

The primary lactase deficiency (*lactase non persistence*) is an autosomal recessive (AR) condition resulting from the physiological decline of lactase activity of intestinal cells and features a large proportion of individuals. It is a well-known cause of abdominal disorders such as diarrhea, bloating and flatulence.

The primary hypolactasia is a condition extremely widespread in the world population, but with substantial variations between different ethnic groups, the prevalence of disease is minimal in Northern European populations (Finland, Sweden, Germany, Austria, Switzerland, France, Great Britain, Holland, Ireland) and groups descended from them, and is particularly high in Asia, Africa and Australia. In Italy, the *lactase non persistence* effects average 40-50% of the population, particularly in south (Campania and

Sicily) were could reach 70% of populations^{11,12}. The “normal” condition is represented by the loss of lactase expression, defined as “non-persistent”. In fact, in human life, the power supply is based exclusively on breast milk for the first few months of life. It is, therefore, understandable how the adjustment expression of the lactase gene may predict its progressive decline in later stages of life. However, the genotype that determines the persistence of lactase, is found only in Northern Europe populations, in some African and Arab nomadic tribes. In Europe, the persistence or not persistence of the expression of lactase is associated with the so-called point polymorphism C/T 13910. This consists in the substitution of a single nucleotide base in a sequence of DNA, that carries regulatory on the lactase gene: genotype CC is associated with hypolactasia (lactase residual is approximately 10% compared to the levels of birth), while the TT genotype with lactase persistence of activity. The presence of a CT genotype, instead, predisposes to the presence of levels of intermediate expression¹³.

The secondary hypolactasia occurs, instead, when a mucosal damage of bowel causes a temporary lactase deficiency. Typically, all diseases of the small intestine, such as the Celiac or Crohn's disease, ulcerative colitis, irritable bowel syndrome, radiation, immunological deficiencies, are capable of causing a secondary deficiency of lactase, but more commonly bacterial or viral infections, parasitic infections (such as a giardiasis) or drug treatment⁶ induce a transient loss of enzyme in the mucosa affected by the process inflammatory or infectious. It is obviously a reversible condition requiring a lactose-free diet until it is restored to a normal intestinal mucosa¹⁴.

Lactose malabsorption in coeliac patients

A recent study by our group demonstrated that a large proportion of patients with lactose malabsorption is affected by an otherwise silent coeliac disease, a gluten-sensitive enteropathy caused by a permanent intolerance to gliadin and its related proteins in individuals genetically susceptible. Celiac disease damaging the brush border causes a partial deficiency of lactase.

Moreover, other data indicate that a large proportion of patients with coeliac disease experienced a regression of lactose malabsorption after being on a gluten-free diet^{15,16}. In some patient's

intraluminal deficit, which brings to the damage of the brush border, could be associated with many diseases, as chronic pancreatitis, chronic hepatitis, irritable bowel syndrome (IBS) and small intestinal bacterial overgrowth (SIBO).

IBS is a common chronic disorder of unknown origin, characterized by abdominal pain, bloating and constipation alternating with diarrhoea¹⁷⁻¹⁹. Abnormal visceral sensation, altered motility and psychosocial factors may play a role in the occurrence and severity of IBS^{17,18}.

SIBO and lactose intolerance

Small intestinal bacterial overgrowth (SIBO) is a condition characterized by abnormally high bacterial population level in the small intestine, exceeding 10^6 organisms/mL²⁰. SIBO is clinically characterized by symptoms such as abdominal pain, flatulence, diarrhea and/or signs of malabsorption, comparable to those observed in IBS pts²¹. Recent findings suggested that SIBO may play a role in IBS: in particular, some trials reported a high prevalence of SIBO in IBS (78-84%) and a significant improvement in IBS symptoms after eradication^{22,23}. Lactose²⁴⁻²⁷, fructose and sorbitol malabsorption^{28,29}, have also been blamed for IBS symptoms. Sugar malabsorption could be primary (congenital enzymatic/carrier deficiency) or acquired (developing after intestinal damage: acute gastroenteritis, medications, celiac disease, Crohn's disease, others)³⁰. When carbohydrates are not properly hydrolyzed and absorbed, they cause in the bowel a high bacterial production of short-chain fatty acids and gas, with the onset of a syndrome characterized by meteorism, abdominal pain and diarrhea, thus, mimicking IBS symptoms. Hydrogen lactose, fructose and sorbitol BTs are commonly used to detect specific sugar's malabsorption. There is a recent interest in fructose intolerance as a possible explication for unexplained gastrointestinal symptoms³¹. In fact, IBS patients showed a similar pattern of malabsorption in various tested fermentable substrates²⁹. For many patients, bacterial overgrowth could be responsible for the association between sugar intolerance and IBS rather than true sugar intolerance. Indeed, Nucera et al³² showed a regression of lactose (86.6%), fructose (97.5%), and sorbitol (90.9%) malabsorption after SIBO eradication.

The normalization of Lactulose breath test one month after antibiotic treatment was associ-

ated with a normalization of the majority of previously positive lactose, fructose and sorbitol breath test. These findings suggest that, in presence of SIBO, the large amount of intestinal bacteria may unspecifically ferment sugars, causing an abnormal H₂ production and consequently, a misleading diagnosis of lactose, fructose or sorbitol malabsorption. An alternative hypothesis could be that the bacterial overgrowth leads to a damage of the small bowel mucosa, thus, inducing a transient enzymatic or carrier protein deficiency and, then, multiple sugar's malabsorption. After SIBO eradication the intestinal mucosa comes back to play its normal functions, and the sugar's malabsorption disappear. Similarly, Pimentel et al reported that while the number of IBS patients with true lactose intolerance was low (16%), a much higher number (58%) had an abnormal lactose breath test result and there was a significant correlation between lactulose (SIBO) and lactose breath test result¹⁹. Normalization of lactulose breath test after neomycin treatment was associated with a significant reduction in IBS symptoms. These studies showed that the prevalence of true lactose malabsorption was lower than the prevalence of abnormal LBT in SIBO thus suggesting that the expansion of gut bacterial flora proximally results in abnormal interaction of substrate and gut bacteria leading to a positive lactose BT³³.

In conclusion, LBT can be useful in the management of patients presenting with IBS symptoms, in order to detect and treat a possible SIBO. The presence of SIBO should be always assessed at first, before searching sugar malabsorption and specific sugars-free diets. Lactose, fructose and sorbitol BT could become a useful diagnostic approach in SIBO-negative or eradicated patients with refractory symptoms^{19,34}.

Symptoms of lactose intolerance

Malabsorption of sugar does not necessarily mean lactose intolerance; in fact, the development of gastrointestinal symptoms such as abdominal pain, flatulence, nausea, bloating, and diarrhea, only occur in about one-third of "malabsorbers"³⁵. However, the undigested lactose causes a rise in the osmotic load in the intestinal lumen, leading to an increased excretion of electrolytes and fluids. Lactose intolerance is clinically characterized by abdominal pain and bloat-

ing. The unabsorbed lactose is fermented by gut microflora with a production of short chain fatty acids (SCFA), and gases (H₂, CH₄), thus increasing colonic distention and accelerating the oro-cecal transit time. Patients also complain flatulence, diarrhea, borborygmi, and sometimes, nausea and vomiting. More rarely there is increased production of methane with a delayed transit time and a consequent constipation. Some authors have reported that the clinical presentation of lactose intolerance is not restricted to gut symptoms. Neurological symptoms such as headache, vertigo, memory impairment, lethargy or cardiac arrhythmia can develop even in less than 20%^{5,36}. One of the possible factors responsible for these systemic symptoms can be the production of toxic metabolites, generated by lactose fermentation in colonic bacteria^{5,37}, that can alter cell signaling mechanism. When systemic complaints are present, it is important to exclude allergy to cow's milk protein, which is attendant in up to 20% of patients with symptoms of lactose intolerance. There is considerable intraindividual and interindividual variability in the severity of gastrointestinal symptoms, according to the amount of lactose ingested and the patient's ability to digest it. Fat content of lactose-containing food oro-cecal transit time, visceral sensitivity contributes to this variability³⁸. Valid evidence is missing for a relationship between symptoms and amount of lactose ingested^{2,38}.

Lactose doses of 15-18 g are well tolerated when offered together with other nutrients. With higher doses than 18 g, intolerance becomes progressively more frequent, and quantities over 50 g elicit symptoms in most individuals^{39,40}.

Diagnosis of lactose malabsorption

Different methods have been used for the diagnosis of lactose malabsorption. The gold standard for the diagnosis of adults-type hypolactasia is the measurement of lactose, sucrase and maltase activities and the determination of the lactose to sucrase ratio (L:S) in intestinal biopsies^{41,42}.

However, it seems too invasive for the diagnosis of such a mild condition and its results may be influenced by the irregular dissemination of lactase activity throughout the small intestine mucosa^{14,43,44}.

Determination of genotypes

Genomic DNA was extracted from peripheral venous blood. A positive genetic test for lactase non-persistence indicates a decline in lactase activity but does not give information on actual patient symptoms. Meanwhile, a lactose breath test with intolerance symptoms gave clear information about the clinical aspect. Genotyping of lactose tolerance tests has limitations. In children, genotyping could only be used as a rule-out test since it does not tell anything about the age at which a child with the CC genotype begins to decrease lactase expression. In patients where secondary hypolactasia is suspected, e.g. coeliac disease, genotyping should be done together with tolerance tests. Although, more studies are needed to prove that the C-13910T SNP is a causative DNA variant, and not merely a highly associated marker, the test is still useful for clinical purposes⁴⁵. The present real-time PCR method offers several advantages over the RFLP method. It is less laborious and the risks for PCR contaminations are fewer. Furthermore, several steps in the method could be automated. The method is suited for a clinical laboratory, and in this sense comparable to pyrosequencing or minisequencing^{13,46}. The genetic analysis identified a single nucleotide polymorphism that shows complete association with the lactase non-persistence/persistence³⁹ and is characterized by a C to T change in the position 13910 of the lactase phlorizin hydrolase (LPH) gene. In particular, it has been shown that the genotype C/C -13910 is associated with adult-type hypolactasia, whereas genotypes C/T -13910 and T/T -13910 are associated with lactase persistence. Moreover, these three genotypes perfectly correlate with the level of the lactase activity in intestinal biopsy samples, and their L:S ratio^{13,47}.

Other diagnostic tests available are:

- **Lactose tolerance test (LTT):** In this test patient's drinks 50 g of lactose dissolved in water. Samples of capillary blood to test the plasma glucose concentration were taken at -5, 0, 15, 30, 45 and 60 min. The average of the -5 and 0 min determinations was used as the pre-challenge glucose concentration. Glucose was measured in whole blood on a plasma-calibrated Hemocue 201 (Hemocue AB, Angelholm, Sweden). A maximal plasma-glucose increase of 1.4 mmol/l or higher indicate lactose tolerance. The digestion of lactose determines the elevation of blood glucose: the absence of such increase indicates failure ab-

sorption of lactose. This test is burdened by the onset of severe gastrointestinal symptoms in patients with lactose intolerance to high dose of lactose administered⁴⁸.

- **Quick lactose test (QLT):** a new method for the endoscopic diagnosis of adult-type hypolactasia, has been developed over the last few years. This test is based on a colorimetric reaction that develops when the endoscopic biopsy from the post-bulbar duodenum is incubated with lactose on a test plate. The color reaction develops within 20 min after hydrolysis of lactose in patients with normolactasia (positive result), while no reaction develops in patients with severe hypolactasia (negative result)⁴⁹.

QLT comparison of genetics and exhibition good correlation between the different techniques (sensitivity 95-100%, specificity 100%)⁴⁹. Our group compared the efficacy of the QLT with the H₂ lactose BT. Our results showed a valid correlation between the two techniques, with a concordance rate of 81%. This percentage became higher and reached 96% when we considered CH₄ and H₂ production. In our study, the QLT seems to be able to identify the subgroup of patients with adult-type hypolactasia and low H₂ production that is not identified by the commonly used H₂ BT. The former is also simpler and less expensive than the genetic tests. Based on these observations QLT could be a reliable test for the diagnosis of adult-type hypolactasia, with a sensitivity higher than that of the BT and comparable to that of the more difficult to perform genetic tests⁵⁰.

Lactose breath test

Lactose BT represents an indirect test for lactose malabsorption, and it is commonly considered the most reliable, non-invasive and inexpensive technique. Based on several different studies, lactose BT shows good sensitivity (mean value of 77.5%) and excellent specificity (mean value of 97.6%)^{43,44}.

How the test look like?

To minimize the basal hydrogen excretion, patients were asked to have a carbohydrate-restricted dinner on the day before the test and to be fasting for at least 12 h on the testing day. Before starting the test patients did a mouth wash with 20 ml of chlorhexidine 0.05%. Smoking and physical exercise were not allowed for 30 min before and during the test. End-alveolar breath samples were collected before lactose ingestion. Dose of

lactose administered was 25 g for adults and 1 mg/kg in children. End-alveolar breath samples were collected immediately before lactose ingestion and every 30 min for 4 h using a two-pack system. Samples were analyzed immediately for H₂ using a model of solid sensor gas-chromatograph. Results were expressed as parts per million (p.p.m.). H₂-LBT was considered positive for lactose malabsorption when an increase in H₂ value more than 20 parts per million (p.p.m.) over the baseline value was registered⁵¹. Some researcher or clinician suggested to perform the test directly with milk. However the use of galenic preparations is required for standardized test according to available guidelines.

Problems

It is possible to find false-negative breath tests, due to the inability of colonic flora to produce H₂ after ingestion of non-absorbable carbohydrates, or after a recent use of antibiotics. False-positive breath tests are less frequent and are mainly due to small bowel bacterial overgrowth or abnormal oral microflora⁵².

In both adults and children, we propose to record and score the most common GI symptoms (abdominal pain, bloating, flatulence and diarrhea) during and 8 h after the test, by a visual-analogue scale (VAS).

It is important to underline the fact that not all patients with lactose malabsorption present intolerance symptoms during the test.

Management of lactose intolerance

Management of lactose intolerance consists of two possible clinical choice not mutually exclusive: alimentary restriction and drug therapy.

The usual behavior for this condition is the avoidance of milk and dairy products from the diet. However, this restriction leads to a reduction of intake of substances such as calcium, phosphorus and vitamins and may be associated with decreased bone mineral density⁵³. This diet should be given only in patients with gastrointestinal symptoms of intolerance (diarrhea, bloating, abdominal pain, flatulence), so defined "lactose intolerants" not also in "lactose malabsorbers".

In primary hypolactasia milk and dairy products are forbidden for 2-4 weeks, time required for remission of symptoms. Then, should recommend a gradual reintroduction of dairy products low in lactose up to a threshold dose of individual toler-

ance. In secondary hypolactasia, associated with various intestinal disorders, diet is necessary only until the regression of these acquired disorders⁵⁴.

In the case of unearned baby, milk without lactose completely solves the problem.

What is the dose tolerated by lactose intolerant daily?

Available data suggest that adults and adolescents with diagnosis of lactose intolerance could ingest at least 12 g of lactose in a single dose (equivalent to the lactose content in 1 cup of milk) without or with minor symptoms⁵⁴. Other strategies that should be suggested to patients are: to consume milk with other foods; to use fermented dairy products and cheeses; to distribute the amount of milk during the day to increase lactose dose assumption making colon able to an adaptation.

Montalto et al⁵⁵ shows that low-dose lactose, such as in drugs, neither increase breath hydrogen excretion nor causes gastrointestinal symptoms.

Drug therapy

Enzyme supplementation therapy with lactase from nonhuman sources to hydrolyze lactose is another important approach. Exogen lactase is obtained from *Aspergillus oryzae* (Lacdigest, Italchimici, Pomezia, Rome, Italy) or from *Kluyveromyces lactis* (Silact, Sofar, Trezzano, Milano, Italy) are able to break down lactose into glucose and galactose to allow a better absorption⁵⁶.

The use of exogenous β -galactosidase in lactose malabsorbers, also when added at mealtime, is efficacious and free of side effects. More recently, the administration of probiotics endowed with a β -galactosidase activity was useful to treat patients with lactose intolerance. Probiotics are live microorganisms; some of them have a beta-galactosidase that may aid in the digestion of lactose ingested. These microorganisms can be added to food products, such as milk and yogurt, or used as supplements. Many bacterial families with over 500 species house our gastrointestinal tract with the highest concentration in the colon. It has been demonstrated that malabsorbed lactose is salvaged by the distal ileal and colonic lactic acid bacteria. *Lactobacillus*, *Bifidobacterium*, *Staphylococcus*, *Enterococcus*, *Streptococcus* are defined as "Lactic acid bacteria", they ferment lactose to produce lactate, hydrogen, methane, and short-chain fatty acids. In the process of fermentation, microbial lactase, present in lactic acid

bacteria, initially breaks down unabsorbed lactose by hydrolysis to its monosaccharides, glucose and galactose, that may be absorbed. However, there is a huge variability in the amount of lactase activity in different probiotics. Ojetti et al⁵⁶ shows in a placebo-controlled trial that the addition of tilactase to a lactose load improves gastrointestinal symptoms, and reduced hydrogen production during the LBT. *Lactobacillus reuteri* also is effective but lesser than tilactase. This probiotic may represent an interesting treatment option for lactose intolerance since its use is simple, and its effect may last in the time after stopping administration. On the other hand, probiotic is administered at a standard dosage, regardless of the dosage of lactose the patients are going to ingest.

Other strategies for management of Lactose Intolerance may include gut decontaminating agents and anti-microbials agent, such as rifaximin.

Conflict of interest

The Authors declare that they have no conflict of interests.

References

- 1) CAMPBELL AK, WAUD JP, MATTHEWS SB. The molecular basis of lactose intolerance. *Sci Prog* 2005; 88(Pt 3): 157-202.
- 2) LOMER MC, PARKES GC, SANDERSON JD. Lactose intolerance in clinical practice-myths and realities. *Aliment Pharmacol Ther* 2008; 27: 93-103.
- 3) SPOHR A, GUILFORD WG, HASLETT SJ, VIBE-PETERSEN G. Use of breath hydrogen testing to detect experimentally induced disaccharide malabsorption in healthy adult dogs. *Am J Vet Res* 1999; 60: 836-840.
- 4) KRETCHMER N. Lactose and lactase-a historical perspective. *Gastroenterology* 1971; 61: 805-813.
- 5) MATTHEWS SB, WAUD JP, ROBERTS AG, CAMPBELL AK. Systemic lactose intolerance: a new perspective on an old problem. *Postgrad Med J* 2005; 81: 167-173.
- 6) VESA TH, MARTEAU P, KORPELA R. Lactose intolerance. *J Am Coll Nutr* 2000; 19(2 Suppl): 165S-175S.
- 7) SAVAIANO DA, LEVITT MD. Milk intolerance and microbe-containing dairy foods. *J Dairy Sci* 1987; 70: 397-406.
- 8) SEppo L, TUURE T, KORPELA R, JÄRVELÄ I, RASINPERÄ H, SAHI T. Can primary hypolactasia manifest itself after the age of 20 years? A two-decade follow-up study. *Scand J Gastroenterol* 2008; 43: 1082-1087.

- 9) DI STEFANO M, VENETO G, MALSERSIVI S, STROCCHI A, CORAZZA GR. Lactose malabsorption and intolerance in the elderly. *Scand J Gastroenterol* 2001; 36: 1274-1278.
- 10) SWALLOW DM. Genetics of lactase persistence and lactose intolerance. *Annu Rev Genet* 2003; 37: 197-219.
- 11) BURGIO GR, FLATZ G, BARBERA C, PATANÉ R, BONER A, CAJOZZO C, FLATZ SD. Prevalence of primary adult lactose malabsorption and awareness of milk intolerance in Italy. *Am J Clin Nutr* 1984; 39: 100-104.
- 12) DE RITIS F, BALESTRIERI GG, RUGGIERO G, FILOSA E, AURICCHIO S. High frequency of lactase activity deficiency in small bowel of adults in the Neapolitan area. *Enzymol Biol Clin (Basel)* 1970; 11: 263-267.
- 13) ENATTAH NS, SAHI T, SAVILAHTI E, TERWILLIGER JD, PELTONEN L, JÄRVELÄ I. Identification of a variant associated with adult-type hypolactasia. *Nat Genet* 2002; 30: 233-237.
- 14) USAI-SATTA U, SCARPA M, OPPIA F, CABRAS F. Lactose malabsorption and intolerance: What should be the best clinical management? *World J Gastrointest Pharmacol Ther* 2012; 3: 29-33.
- 15) OJETTI V, NUCERA G, MIGNECO A, GABRIELLI M, LAURITANO C, DANESE S, ZOCCO MA, NISTA EC, CAMMAROTA G, DE LORENZO A, GASBARRINI G, GASBARRINI A. High prevalence of celiac disease in patients with lactose intolerance. *Digestion* 2005; 71: 106-110.
- 16) OJETTI V, GABRIELLI M, MIGNECO A, LAURITANO C, ZOCCO MA, SCARPELLINI E, NISTA EC, GASBARRINI G, GASBARRINI A. Regression of lactose malabsorption in coeliac patients after receiving a gluten-free diet. *Scand J Gastroenterol* 2008; 43: 174-177.
- 17) SANDLER RS. Epidemiology of irritable bowel syndrome in the United States. *Gastroenterology* 1990; 99: 409-415.
- 18) TALLEY NJ, GABRIEL SE, HARMSSEN WS, ZINSMEISTER AR, EVANS RW. Medical costs in community subjects with irritable bowel syndrome. *Gastroenterology* 1995; 109: 1736-1741.
- 19) LIN HC. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. *JAMA* 2004; 292: 852-858.
- 20) DONALDSON RM JR. Normal bacterial populations of the intestine and their relation to intestinal function. *N Engl J Med* 1964; 270: 1050-1056.
- 21) SINGH VV, TOSKES PP. Small bowel bacterial overgrowth: presentation, diagnosis, and treatment. *Curr Treat Options Gastroenterol* 2004; 7: 19-28.
- 22) PIMENTEL M, CHOW EJ, LIN HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol* 2000; 95: 3503-3506.
- 23) PIMENTEL M, CHOW EJ, LIN HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003; 98: 412-419.
- 24) BOHMER CJ, TUYNMAN HA. The clinical relevance of lactose malabsorption in irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 1996; 8: 1013-1016.
- 25) SCIARRETTA G, GIACOBazzi G, VERRI A, ZANIRATO P, GARUTI G, MALAGUTI P. Hydrogen breath test quantification and clinical correlation of lactose malabsorption in adult irritable bowel syndrome and ulcerative colitis. *Dig Dis Sci* 1984; 29: 1098-1104.
- 26) VERNIA P, DI CAMILLO M, MARINARO V. Lactose malabsorption, irritable bowel syndrome and self-reported milk intolerance. *Dig Liver Dis* 2001; 33: 234-239.
- 27) VERNIA P, RICCIARDI MR, FRANDINA C, BILOTTA T, FRIERI G. Lactose malabsorption and irritable bowel syndrome. Effect of a long-term lactose-free diet. *Ital J Gastroenterol* 1995; 27: 117-121.
- 28) LEDOCHOWSKI M, WIDNER B, BAIR H, PROBST T, FUCHS D. Fructose- and sorbitol-reduced diet improves mood and gastrointestinal disturbances in fructose malabsorbers. *Scand J Gastroenterol* 2000; 35: 1048-1052.
- 29) GOLDSTEIN R, BRAVERMAN D, STANKIEWICZ H. Carbohydrate malabsorption and the effect of dietary restriction on symptoms of irritable bowel syndrome and functional bowel complaints. *Isr Med Assoc J* 2000; 2: 583-587.
- 30) SWAGERTY DL JR, WALLING AD, KLEIN RM. Lactose intolerance. *Am Fam Physician* 2002; 65: 1845-1850.
- 31) CHOI YK, JOHLIN FC JR, SUMMERS RW, JACKSON M, RAO SS. Fructose intolerance: an under-recognized problem. *Am J Gastroenterol* 2003; 98: 1348-1353.
- 32) NUCERA G, LUPASCU A, GABRIELLI M. Sugar intolerance in irritable bowel syndrome: The role of small bowel bacterial overgrowth. *Gastroenterology* 2004; 126: A511-A511.
- 33) PIMENTEL M, KONG Y, PARK S. Breath testing to evaluate lactose intolerance in irritable bowel syndrome correlates with lactulose testing and may not reflect true lactose malabsorption. *Am J Gastroenterol* 2003; 98: 2700-2704.
- 34) NUCERA G, GABRIELLI M, LUPASCU A, LAURITANO EC, SANTOLUQUIDO A, CREMONINI F, CAMMAROTA G, TONDI P, POLA P, GASBARRINI G, GASBARRINI A. Abnormal breath tests to lactose, fructose and sorbitol in irritable bowel syndrome may be explained by small intestinal bacterial overgrowth. *Aliment Pharmacol Ther* 2005; 21: 1391-1395.
- 35) CORAZZA G.R, STROCCHI A, GASBARRINI G. Fasting breath hydrogen in celiac disease. *Gastroenterology* 1987; 93: 53-58.
- 36) HARRINGTON LK, MAYBERRY JF. A re-appraisal of lactose intolerance. *Int J Clin Pract* 2008; 62: 1541-1546.
- 37) CAMPBELL AK. Bacterial metabolic 'toxins': a new mechanism for lactose and food intolerance, and irritable bowel syndrome. *Toxicology* 2010; 278: 268-276.
- 38) SUCHY FJ, MATTHEWS SB, VASSEL N, COX CD, NASEEM R, CHAICHI J, HOLLAND IB, GREEN J, WANN KT. National Institutes of Health Consensus Development Conference: lactose intolerance and health. *Ann Intern Med* 2010; 152: 792-796.
- 39) RASINPERA H, SAVILAHTI E, ENATTAH N.S, KUOKKANEN M, TÖTTERMAN N, LINDAHL H, JÄRVELÄ I, KOLHO KL. A genetic test which can be used to diagnose adult-type hypolactasia in children. *Gut* 2004; 53: 1571-1576.

- 40) SHAUKAT A, LEVITT M.D, TAYLOR B.C, MACDONALD R, SHAMLIYAN T.A, KANE R.L, WILT T.J. Systematic review: effective management strategies for lactose intolerance. *Ann Intern Med* 2010; 152): 797-803.
- 41) MESSER M, DAHLOVIST A. A one-step ultramicro method for the assay of intestinal disaccharidases. *Anal Biochem* 1966; 14: 376-392.
- 42) SHAW AD, DAVIES GJ. Lactose intolerance: problems in diagnosis and treatment. *J Clin Gastroenterol* 1999; 28: 208-216.
- 43) NEWCOMER AD, MCGILL DB, THOMAS PJ, HOFMANN AF. Prospective comparison of indirect methods for detecting lactase deficiency. *N Engl J Med* 1975; 293: 1232-1236.
- 44) METZ G, JENKINS DJ, PETERS TJ, NEWMAN A, BLENDIS LM. Breath hydrogen as a diagnostic method for hypolactasia. *Lancet* 1975; 1(7917): 1155-1157.
- 45) RIDEFELT P, HAKANSSON LD. Lactose intolerance: lactose tolerance test versus genotyping. *Scand J Gastroenterol* 2005; 40: 822-826.
- 46) NILSSON TK, JOHANSSON CA. A novel method for diagnosis of adult hypolactasia by genotyping of the -13910 C/T polymorphism with Pyrosequencing technology. *Scand J Gastroenterol* 2004; 39: 287-290.
- 47) 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: 647-652.
- 48) HERMANS MMH, BRUMMER RJ, RUIJGERS AM, STOCKBRÜGGER RW. The relationship between lactose tolerance test results and symptoms of lactose intolerance. *Am J Gastroenterol* 1997; 92: 981-984.
- 49) KUOKKANEN M, MYLLYNIEMI M, VAUHKONEN M, HELSKE T, KÄÄRIÄINEN I, KARESVUORI S, LINNALA A, HÄRKÖNEN M, JÄRVELÄ I, SIPPONEN P. A biopsy-based quick test in the diagnosis of duodenal hypolactasia in upper gastrointestinal endoscopy. *Endoscopy* 2006; 38: 708-712.
- 50) OJETTI V, LA MURA R, ZOCCO MA, CESARO P, DE MASI E, LA MAZZA A, CAMMAROTA G, GASBARRINI G, GASBARRINI A. Quick test: a new test for the diagnosis of duodenal hypolactasia. *Dig Dis Sci* 2008; 53: 1589-1592.
- 51) USAI-SATTA P. Methodology and indications of H₂-breath testing in gastrointestinal diseases: the Rome Consensus Conference. *Aliment Pharmacol Ther* 2009; 31: 166-166.
- 52) DI STEFANO M, VENETO G, MALSERVISI S, CECCHETTI L, MINGUZZI L, STROCCHI A, CORAZZA G.R. Hydrogen breath test in the diagnosis of lactose malabsorption: Accuracy of new versus conventional criteria. *J Lab Clin Med* 2004; 144: 313-318.
- 53) DI STEFANO M, VENETO G, MALSERVISI S, CECCHETTI L, MINGUZZI L, STROCCHI A, CORAZZA G.R. Lactose malabsorption and intolerance and peak bone mass. *Gastroenterology* 2002; 122: 1793-1799.
- 54) USAI-SATTA P, SCARPA M, OPPIA F, CABRAS F. Lactose malabsorption and intolerance: what should be the best clinical management? *World journal of gastrointestinal pharmacology and therapeutics* 2012; 3: 29-33.
- 55) MONTALTO M, GALLO A, SANTORO L, D'ONOFRIO F, CURIGLIANO V, COVINO M, CAMMAROTA G, GRIECO A, GASBARRINI A, GASBARRINI G. Low-dose lactose in drugs neither increases breath hydrogen excretion nor causes gastrointestinal symptoms. *Aliment Pharmacol Ther* 2008; 28: 1003-1012.
- 56) OJETTI V, GIGANTE G, GABRIELLI M, AINORA ME, MANNOCCI A, LAURITANO EC, GASBARRINI G, GASBARRINI A. 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.