LACTOSE INTOLERANCE – CURRENT STATE OF KNOWLEDGE*

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Abstract. Lactase-phlorizin hydrolase (LCT), more commonly known as lactase, is an enzyme responsible for cleaving lactose into absorbable monosaccharides, glucose and galactose. LCT deficiency (hypolactasia – HL) is caused by a decreased activity of LCT in the small intestinal villi and potentially results in lactose malabsorption what may lead to the development of clinical symptoms (diarrhea, bloating, flatulence and cramps) and avoiding milk products in the diet. HL is the world’s most common enzyme deficiency in humans. HL exists in three distinct forms – congenital, primary and secondary. Adult type hypolactasia (ATH) is the most common phenotype found in human. It is a genetically predetermined physiological condition inherited through an autosomal recessive mode which results in a decline of lactase activity after weaning. ATH is associated with the LCT -13910 C>T polymorphism worldwide, except in Africa. Lactase non-persistence has been observed in individuals with the C/C-13910 genotype, whereas lactase persistence in subjects with remaining allelic variants. Small intestine biopsy is the only diagnostic procedure allowing for the direct measurement of LCT activity, however due to its invasive nature it is hardly accepted by patients. Therefore, LCT status is often inferred simply by assessing the patient’s lactose digestion. A lactose tolerance test can be performed after lactose load and then measuring blood glucose concentration or breath hydrogen (preferably hydrogen and methane) expiration. A genetic test of the C/T-13910 polymorphism is also available at present. It is a reliable method in excluding/confirming ATH predisposition. However, it definitely does not assess lactose tolerance or malabsorption.

Key words: lactase, hypolactasia, lactose malabsorption, lactose intolerance, lactase gene polymorphism, hydrogen breath test

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BACKGROUND

Lactose intolerance is a common, described throughout the world, gastrointestinal disorder. Its prevalence is highly variable, depending on ethnicity, ranging from 5% in north-western Europe to almost 100% in some Asian populations [Solomons 2002, Swallow 2003, Itan et al. 2010]. Hypolactasia or lactase deficiency may result in lactose malabsorption, which combined with clinical symptoms consists in the image of lactose intolerance. Management of lactose intolerance requires a total or partial exclusion of dairy products from one's diet [Martini and Savaiano 1988].

LACTOSE

Lactose (milk sugar) is a key nutrient in mammalian milk and the major carbohydrate source during the neonatal period. From an evolutionary and biological viewpoint, lactose is a unique sugar, as it exists as a free molecule only in milk. It is synthesized by lactose synthetase exclusively in the mammary gland of virtually all placental mammals (except the sea lion) during late pregnancy and lactation. Lactose concentration in milk is inversely related to the content of fat and protein. Interestingly, human milk contains the highest concentration (7%) of lactose in mammals [Solomons 2002].

Lactase-phlorizin hydrolase (LCT), more commonly known as lactase, a small intestine beta-galactosidase is responsible for cleaving lactose into absorbable monosaccharides, glucose and galactose [Ingram et al. 2009]. This enzyme is critical in the nourishment of newborn mammals, whose sole source of nutrients is milk and lactose is the major carbohydrate component. LCT has two active sites, one which splits lactose and another hydrolyzing phlorizin (an aryl a-glucoside) as well as a range of dietary glycolipids [Campbell et al. 2005]. A number of actions of the phlorizin site are useful in humans and this may explain why some enzyme activity is retained after weaning. Lactase is present on the apical surface of enterocytes in the small intestine brush border with its highest expression found in the mid-jejunum. The enzyme is produced as a 220 kDa precursor peptide, which undergoes considerable post-transcriptional modifications during transport to the cell surface where it appears as the mature 150 kDa protein. Luminal factors also contribute to a final modification of the protein – cleavage of two further amino acids by pancreatic trypsin produces the active form of the enzyme [Zecca et al. 1998].

Lactase deficiency is the major cause of milk intolerance in children and adolescents worldwide [Semenza et al. 2001]. Although several authors have suggested that consuming small amounts of milk does not exert any adverse effects in subjects with hypolactasia [Martini and Savaiano 1988], this condition is apparently the most common reason for avoiding milk products [Di Stefano et al. 2002]. Since milk is usually one of the most important sources of calcium in one's diet, people with lactose intolerance who consume less dairy may have reduced calcium intake. On the other hand, lactase-deficient subjects with normal dairy product consumption may experience malabsorption and therefore all its related negative consequences.
MECHANISM OF LACTOSE INTOLERANCE

Lactase deficiency (hypolactasia) is caused by a decreased activity of LCT in the small intestinal villi. It is the world’s most common enzyme deficiency in humans. Lactase-deficient individuals are not able to hydrolyze lactose into glucose and galactose after consuming products containing lactose. When undigested lactose reaches the large intestine it is metabolised by colonic microflora. The non-hydrolysed lactose in the small intestine causes an increase in osmotic load and along with the bacterial metabolites is considered to play an important role in the genesis of the classic symptoms of intolerance. The exact mechanism involves the osmotic load acting to increase the osmotic gradient across the intestinal wall [Christopher and Bayless 1971, Vernia et al. 2003, Pimentel et al. 2006]. This gradient then pulls water into the lumen and thus results in symptomatic diarrhea. Secondly, when the lactose is fermented by bacteria present in the colon, fatty acids and gaseous by-products such as hydrogen and methane are produced and these can potentially cause a patient’s discomfort, bloating and flatulence [Matthews et al. 2005, Vesa et al. 2000]. However, despite this mechanism the majority of lactose intolerant individuals can still tolerate small amounts of lactose (as in tea or coffee), and some can even consume greater amounts without experiencing any negative clinical symptoms [Martian and Savaiano 1988]. Only about 50% of lactase activity is necessary for effective lactose metabolism and utilization [Swallow 2003]. For smaller amounts even quite low enzyme activity could be sufficient. As is the case of other intestinal disaccharides the enzyme is only translated in amounts that are required based on the individual’s diet [Semenza et al. 2001].

TYPES OF LACTOSE INTOLERANCE

Hypolactasia exists in three distinct forms – congenital, primary and secondary [Saavedra and Perman 1989]. Congenital lactase deficiency (CLD) is associated with the least lactase activity of the three known forms. It is believed to be an autosomal recessive trait, although very little is still known about its molecular basis. The hallmark symptom of CLD is watery diarrhea that the newborn develops soon after the first doses of breast milk (or any other – beyond lactose-free formula), in a short time causing its failure to thrive. CLD is extremely rare, with only about 40 cases ever reported in the literature. The only treatment is complete avoidance of lactose from birth throughout life [Swallow 2003]. Secondary. In a minority of individuals, the gastrointestinal symptoms after lactose consumption are due to a secondary lactase deficiency that is caused by distinct pathologies of the small intestine. Secondary hypolactasia occurs as a result of gastrointestinal disease that is related to damage of the brush border of the small intestine, for example as in giardiasis, celiac disease, viral gastroenteritis, Crohn’s disease, radiotherapy, chemotherapy or even from the use of some medications. With adequate treatment of the underlying disease this condition is usually reversible [Saavedra and Perman 1989, Gudmand-Hoyer and Skovbjerg 1996].
ADULT TYPE HYPOLACTASIA

Adult type hypolactasia (ATH), also known as primary lactose malabsorption or lactase non-persistence, is the most common phenotype found in humans. It is a genetically predetermined physiological condition inherited through an autosomal recessive mode which results in a decline of lactase activity after weaning. Adult type hypolactasia was previously associated with the LCT -13910C>T polymorphism worldwide, except in Africa [Enattah et al. 2002]. All healthy newborns normally display an adequate expression of LCT, which decreases after the infant is weaned off breast milk. In most adults worldwide, milk is no longer the main dietary product, and the decline of lactase activity in adulthood simply represents an evolutionary adaptation [Rasinpera et al. 2006].

The prevalence of adult type hypolactasia in Europe increases towards the South and East and reaches 70% in southern Italy and Turkey. A majority of Europeans are able to digest large quantities of lactose throughout their life and are known to be lactase persistent. The down-regulation of intestinal lactase activity varies according to ethnicity, and most commonly start to appear around 5-6 years of age [Matthews et al. 2005]. The differences in the timing of the down-regulation may be considerable. The majority of Thai children were shown to become hypolactasic by the age of two years. In black populations adult-type hypolactasia was documented to manifest between one to eight years, whereas in white populations low lactase levels are rarely seen in children under five years of age [Sahi 1994].

LACTASE GENE AND ITS REGULATION

The lactase gene is approximately 20 kb in size [Boll et al. 1991] and is located on chromosome 2 [Harvey et al. 1993, Enattah et al. 2002]. Enattah et al. (2002) identified a variant allele polymorphism LCT -13910C>T upstream from the lactase gene locus, associated with hypolactasia/lactase persistence in Finland and elsewhere. An exception is noted in Africa, where three identified single nucleotide polymorphisms have been found: LCT -14010G>C, LCT -13915T>G, and LCT -13907C>G [Tishkoff 2007]. Sequence analysis and association analyses revealed a link between lactose intolerance and a polymorphism in a position 13910 C/T, about 14 kb upstream from the LCT locus. This association can be partially explained by the cis-acting element properties of the polymorphism and its influences on the LCT gene promoter site. Lactase non-persistence has been observed in individuals with the C/C-13910 genotype, whereas lactase persistence in T/T-13910 genotypic people [Boll 1991, Enattah et al. 2002, Swal- low 2003]. Over 600 intestinal biopsy specimens have been used to identify the association of the C/T-13910 variant with the disaccharidase activities and the lactase/sucrase ratio (L/S) [Enattah et al. 2007].

The in vitro studies have demonstrated that the lactase persistence trait-related T-13910 allele binds Oct-1 transcription factor with a higher affinity than does the C-13910 allele. Additionally, a wider DNA region encompassing the C/T-13910 variant has been found to contain an enhancer element which has binding sites for several transcription factors. These factors most probably conduce the regulation of the lactase gene in intestinal cells and include: Oct-1 and GATA-6 (region from _13909 to _13934), HNF4a and Fox/HNF3a (region _13857 to _13817), and Cdx-2 (region _14022 to
Importantly, the expression of Oct-1 has also been shown to drive the reporter gene expression from both T-13910 and C-13910 variant/LCT promoter constructs only when it is coexpressed with HNF1a, thus indicating that the 13910 enhancer effect is most likely mediated through HNF1a binding to the proximal promoter of the LCT gene [Olds and Sibley 2003].

**Lactase persistence hypothesis.** The dairy culture was initiated some 10,000 years ago in the Middle East with the domestication of sheep, goat, and cattle. Simoons and McCracken proposed a cultural historical hypothesis and by referencing the origins of the dairy culture they tried to explain the differences in the prevalence patterns of hypolactasia worldwide. As previously mentioned people in the Middle East have a lower prevalence of lactose intolerance. Therefore, it was suggested that people with lactase persistence could survive better than those without because they could absorb all nutrients adequately from milk without having diarrhea. It is therefore then possible that they were healthier and had more children than subjects who presented with hypolactasia [Simoons 1970, Beja-Pereira et al. 2003]. A different idea known as the calcium absorption hypothesis was proposed by Flatz and Rotthauwe [1973]. This hypothesis was put forward to explain the prevalence of lactase persistence in Northern Europe. The nutritional supply of vitamin D in this region was low and it was proposed that lactose could enhance absorption of calcium and thus individuals with lactase persistence had less rickets and pelvic deformities therefore having more children. The natural selection was acting in both instances in order to benefit lactase-persistent individuals.

**DIAGNOSTIC PROCEDURES**

Small intestine biopsy is the only diagnostic procedure allowing for the direct measurement of lactase activity. However, due to invasive nature it is hardly accepted by patients unless they have to undergo gastrointestinal endoscopy for other reasons. Therefore, lactase persistence/nonpersistence status is often inferred simply by assessing the patient’s lactose digestion [Swallow 2003, Gugatschka et al. 2005]. A lactose tolerance test can be performed and usually involves consuming a lactose load after an overnight fast and then measuring blood glucose concentration or breath hydrogen/(preferably hydrogen and methane) expiration. A baseline measurement of blood glucose or breath gases is taken before ingestion of the lactose, and then the measurements are repeated at 30 min intervals up to 2 and 3 h, respectively [Matthews et al. 2005]. An increase in blood glucose indicates lactose digestion (glucose produced from the lactose hydrolysis is absorbed into the bloodstream), and lack of proper increase (<20 mg%) is indicative of a lactose maldigestion (probable lactase nonpersistent) phenotype. An increase in breath hydrogen, methane or hydrogen/methane indicates maldigestion (20, 12 and 15 ppm, respectively) and reflects colonic fermentation of the non-absorbed lactose [Matthews et al. 2005]. In both cases, somewhat arbitrary cut-off points have to be set for distinguishing the two phenotypes and both methods inform upon the person’s ability to digest lactose rather than the given individual’s lactase expression.

The hydrogen breath test with lactose loading has been used for more than 30 years to diagnose lactase deficiency in clinical practice [Shaw and Davies 1999]. However, the procedure that is based upon a combined measurement of hydrogen and methane is considered the ‘gold standard’ of the non-invasive tests to diagnose lactose intolerance.
[Lisowska et al. 2009]. Although hydrogen production appears more ubiquitous, methane production is seen in up to 10-15% of healthy subjects. Available evidence suggests some clinical implications of gas profiles in different entities [Gilat et al. 1978]. The sensitivity and specificity of breath tests to diagnose lactose intolerance is high and appears to have better results for the combined measurement of hydrogen and methane [Pohl et al. 2010]. Although the lactose breath hydrogen test actually measures lactose malabsorption rather than lactose hydrolysis and monosaccharide uptake, its sensitivity and specificity are higher than those of the lactose absorption test. Moreover, the hydrogen breath test is simple, cheap and noninvasive [Shaw and Davies 1999].

A genetic test of the C/T-13910 polymorphism is also available at present. It is a reliable method in excluding/confirming adult-type hypolactasia in subjects with milk-related symptoms. However, it definitely does not assess lactose tolerance or malabsorption [Rasinperä et al. 2004].

REFERENCES


NIETOLERANCJA LAKTOZY – WSPÓŁCZESNY STAN WIEDZY


**Słowa kluczowe:** laktaza, hipolaktazja, zaburzenia trawienia i wchłaniania laktozy, nietolerancja laktozy, polimorfizm genu laktazy, wodorowy test oddechowy

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