SCIENTIFIC OPINION

Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

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ABSTRACT

This Opinion of the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) deals with lactose thresholds in lactose intolerance and galactosaemia. LACTASE DEFICIENCY AND LACTOSE INTOLERANCE: Primary lactase deficiency, also referred to as lactase-nonpersistence (LNP), is genetically determined and a normal, developmental phenomenon characterised by the down-regulation of lactase activity. In adults with LNP, undigested lactose reaches the colon where it can elicit symptoms of lactose intolerance. Lactose tolerance varies widely among individuals with lactose maldigestion. A single threshold of lactose for all lactose intolerant subjects cannot be determined owing to the great variation in individual tolerances. Symptoms of lactose intolerance have been described after intake of less than 6 g of lactose in some subjects. The vast majority of subjects with lactose maldigestion will tolerate up to 12 g of lactose as a single dose with no or minor symptoms. Higher doses may be tolerated if distributed throughout the day. GALACTOSAEMIA: Galactosaemia is caused by three different genetic enzyme defects in the metabolism of galactose. Severe galactosaemia, if untreated, is accompanied by a potentially fatal impairment of hepatic and renal function and with cataracts in the newborn and the young infant. The dietetic principle in the management of all types of galactosaemia is the elimination of all sources of galactose, including human milk, as far as possible. Dietetic management is started with lactose free infant and later follow-on formulae with a lactose content ≤10 mg/100 kcal. In older infants, children and adults, foods containing milk or milk products or lactose as an ingredient must be avoided, as far as possible, so that the overall daily lactose intake will be about 25 mg/100 kcal. A precise threshold for galactose/lactose intake below which adverse effects are not elicited cannot be given.

KEY WORDS

Lactose, lactase, lactase-nonpersistence, galactosaemia, intolerance, threshold.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia. Lactose is a disaccharide of glucose and galactose and the primary sugar of mammalian milk. Ingested lactose is hydrolysed by lactase, an enzyme of the microvillus membrane of the enterocytes, into its components, glucose and galactose, which are absorbed. If lactase activity is low or absent, undigested lactose may induce the symptoms of lactose intolerance.

Subjects with galactosaemia, an inherited disorder of galactose metabolism, do not "tolerate" lactose either, but their symptoms are more severe and differ widely from those of subjects with lactose intolerance.

Lactase deficiency and lactose intolerance

Primary lactase deficiency, also referred to as lactase-nonpersistence (LNP) is genetically determined and a normal, developmental phenomenon characterised by the down-regulation of lactase activity, which occurs soon after weaning in most ethnic groups. LNP prevalence and the age of manifestation vary considerably amongst different ethnic populations.

In adults with LNP, undigested lactose reaches the colon where it is degraded to lactic acid, acetic acid, hydrogen and carbon dioxide by intestinal bacteria. Lactose maldigestion can elicit symptoms of lactose intolerance, such as abdominal pain, bloating, flatulence and diarrhoea. However, lactose maldigestion will not lead to symptoms of lactose intolerance in all LNP subjects.

The most common tests used to measure the digestion of lactose are the hydrogen breath test and the lactose tolerance test. An analysis of polymorphisms of the lactase gene can add useful information. LNP can be confirmed by determination of the lactase activity in a small bowel biopsy. The diagnosis of lactose intolerance, however, is more difficult because it depends on self-reported symptoms (diarrhoea, abdominal cramping, audible bowel, flatulence, vomiting) not all of which can be assessed objectively.

The only satisfactory treatment of lactose intolerance is a diet with reduced lactose content.

Lactose tolerance varies widely among individuals with lactose maldigestion.

The Panel notes that according to a recent systematic review most individuals diagnosed with lactose intolerance or lactose maldigestion can tolerate 12 g of lactose as a single dose (particularly if taken with food) with no or minor symptoms. Single doses of 24 g usually lead to appreciable symptoms. There is some evidence that many lactose maldigesters tolerate daily doses of 20 to 24 g of lactose if distributed throughout the day and consumed together with other nutrients. Consuming 50 g of lactose per day induces symptoms in the vast majority of lactose maldigesters and in many of these symptoms will be severe. There are a few studies with a small number of subjects with lactose maldigestion who self-reported abdominal symptoms and diarrhoea with lactose intakes below 12 g, in some cases with 3 to 5 g of lactose. The Panel notes that the testing procedure with daily increases of the lactose dose and the insufficient masking of the test solutions needs to be taken into account when interpreting the results.

Lactose intolerance prevalence is generally very low in young children and remains low into early adulthood among individuals of Northern European descent. The Panel notes that there are not enough data on children with lactose intolerance, but it appears that similar thresholds may exist as observed in adults with a similar variability in individual sensitivity.

The Panel concludes that a single threshold of lactose for all lactose intolerant subjects cannot be determined owing to the great variation in individual tolerances. Symptoms of lactose intolerance have been described after intake of less than 6 g of lactose in some subjects.

The Panel concludes that the vast majority of subjects with lactose maldigestion will tolerate up to 12 g of lactose as a single dose with no or minor symptoms. Higher doses may be tolerated if distributed throughout the day.

Galactosaemia

Galactosaemia is caused by three different genetic enzyme defects in the metabolism of galactose. Severe galactosaemia, if untreated, is accompanied by a potentially fatal impairment of hepatic and renal function and with cataracts in the newborn and the young infant which is reversed by elimination of dietary galactose. Despite lifelong dietetic management there is retarded development and growth deficiency in most patients and ovarian insufficiency in most female patients.

Galactosaemia can be suspected on the basis of clinical symptoms in a newborn or on the basis of newborn screening programmes which exist in many European countries.

The dietetic principle in the management of all types of galactosaemia is the elimination of all sources of galactose, including human milk, as far as possible, particularly in infants and young children. Dietetic management is started with lactose-free infant and later follow-on formulae with a lactose content ≤10 mg/100 kcal. In older infants, children and adults, foods containing milk or milk products or lactose as an ingredient must be avoided as far as possible so that the overall daily lactose intake will be about 25 mg/100 kcal.

The existing criterion of ≤10 mg lactose/100 kcal for labelling infant and follow-on formulae as "lactose-free" permits that these formulae can be safely used in the dietetic management of patients with galactosaemia.

A precise threshold for galactose/lactose intake below which adverse effects are not elicited cannot be given.

Milk (beverages) in which lactose is (partially) enzymatically hydrolysed to glucose and galactose and from which the latter is not removed are not suitable for patients with galactosaemia regardless of the residual lactose content.

Consequences of technology of lactose reduction in foods

Information on compositional changes resulting from the technological processes applied to remove lactose from products is limited. These changes might result in lower carbohydrate content and, in cases of ultrafiltration or chromatographic separation, also in small decreases in mineral content which are unlikely to be significant. The available evidence does not allow a scientific conclusion to be drawn on a possible effect of lactose on calcium absorption. No negative nutritional consequences can be expected from the consumption of lactose hydrolysed dairy products in either LNP or healthy people, if the only difference between conventional and lactose hydrolysed dairy products is the lactose content. The avoidance of conventional dairy products without supplementation or appropriate adaptation of dietary habits may result in low intakes of calcium, vitamin D and riboflavin.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Foods for particular nutritional uses or dietetic foods as described in Article 1 of Directive 2009/39/EC are foodstuffs which, owing to their special composition or manufacturing process are clearly distinguishable from foodstuffs for normal consumption and fulfil the particular nutritional requirements of certain categories of persons whose digestive processes or metabolism are disturbed.

Recital 22 of Regulation (EC) N°1924/20062 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods mentions that the conditions for claims such as 'lactose-free' or 'gluten free' addressed to a group of consumers with specific disorders should be dealt under European Parliament and Council Directive 2009/39/EC.

Lactose intolerance refers to the inability to metabolise lactose, a sugar found in milk and other dairy products, which is due to the absence or reduced production of the required enzyme lactase. If degradation of lactose does not occur or occurs only partially, the lactose acts as a laxative: increasing water content in lumen, flatulence and abdominal pain. Since lactose intolerance poses no further threat to a person's health, managing the condition consists in minimising the occurrence and severity of symptoms by avoiding lactose-containing products.

Currently there are dairy products on the market that have been specially manufactured to reduce their lactose content and are designated as 'low in lactose' or 'lactose-free'.

As there are currently no harmonised rules at EU level for the use of terms such as 'lactose-free', Member States may maintain or adopt relevant national measures, which may cause confusion for lactose intolerant people and not ensure the same level of consumer protection within the EU. In particular, the level of lactose set as threshold for claims related to the presence of lactose.

It should be noted that conditions for making a nutrition claim such as 'lactose free' are already laid down for infant formulae in Commission Directive 2006/141/EC of 22 December on infant formulae and follow-on formulae and prescribe that the lactose content should not be greater than 2.5 mg/100 kJ (10 mg/100 kcal). See Report of the Scientific Committee on Food on the Revision of Essential requirements of Infant Formulae and Follow-on Formulae: http://ec.europa.eu/food/fs/sc/scf/out199_en.pdf.

The determination of the lactose threshold(s) for foods or food ingredients that no longer induce adverse reactions in lactose intolerant people is important as the basis for setting common rules for the use of terms indicating the reduction and/or absence of lactose.

Sometimes galactosaemia is confused with lactose intolerance, but galactosaemia may have more serious consequences. Galactosaemia is a rare genetic metabolic disorder which affects an individual's ability to properly metabolise the sugar galactose. In individuals with galactosaemia, adverse effects can result in hepatomegaly (an enlarged liver), cirrhosis, renal failure, cataract, and brain damage. The main source of galactose in the diet is the milk sugar lactose which is broken down by the body into glucose and galactose.

Therefore, the Commission would ask EFSA in addition to advise on the potential tolerable threshold of lactose for galactosaemic individuals in the context of the opinion it will issue under the terms of reference given below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to determine the threshold(s) for lactose in a food or a food ingredient which may be tolerated by lactose intolerant people, taking into account the variability in sensitivity between individuals with respect to the dose of lactose required to trigger an adverse effect.

ASSESSMENT

1. Introduction

Lactose is a disaccharide of glucose and galactose and the primary sugar of mammalian milk.

Ingested lactose is hydrolysed by lactase, an enzyme of the microvillus membrane of the enterocytes, into its components, glucose and galactose, which are absorbed. If lactase activity is low or absent, undigested lactose may induce the symptoms of lactose intolerance. Intolerance to lactose is normally dependent on the activity of lactase and, therefore, also dependent on the acute dose and the frequency of consumption as well as the total daily amount of lactose ingested.

The descriptive term "lactose intolerance" includes all causes of symptomatic intolerance to lactose, which is the consequence of undigested lactose remaining in the distal ileum and colon.

Lactose intolerance is not to be confused with intolerance or allergy to (cow's) milk proteins.

Subjects with galactosaemia, an inherited disorder of galactose metabolism, do not "tolerate" lactose either, but their symptoms are more severe and differ widely from those of subjects with lactose intolerance. These patients do not only have to avoid lactose but also galactose.

In Community legislation, the term "lactose-free" has only been defined for infant and follow-on formula as ≤10 mg/100 kcal. This value is based on the empirical guidance values for a galactose (both free and β-glycosidic) intake of 50 (to 200) mg per day for infants with galactosaemia (SCF, 2003).

Some EU Member States have set threshold levels at national level for the use of the terms "lactose-free", "very low lactose" and "low lactose" for foodstuffs other than foodstuffs intended for infants. These threshold levels are given in Table 1.

Table 1: Threshold levels in some EU Member States for the use of the terms "lactose-free" and "low-lactose" in foods other than foods for particular nutritional uses.

Country	"Lactose-free"	"Low lactose"
Denmark	10 mg/100 g*	1 g/100 g*
Estonia	10 mg/100 g*	1 g/100 g*
Finland	10 mg/100 g*	1 g/100 g*
Norway	10 mg/100 g*	1 g/100 g*
Sweden	10 mg/100 g*	1 g/100 g*
Germany	100 mg/100 g*	NA
Slovenia	100 mg/100 g*	NA
Hungary	100 mg/100g or mL*	NA
Ireland	No lactose present No galactose present	1 g/100 g*

* final product

Different types of "lactose-free" or "low-lactose" products are currently on the market in the EU Member States ranging from only a few available products in some Member States to almost the whole range of dairy products in others.

2. Lactase deficiency and lactose intolerance

2.1. Definitions

A stable, low or absent lactase activity results from either an alteration (in infants) or a reduction (in adults) in the expression of the lactase gene (primary lactase deficiency). Intestinal disease processes which damage the epithelium of the small intestine may cause secondary lactase deficiency, which is reversible with the correction of the underlying disease.

2.1.1. Primary lactase deficiency

Primary lactase deficiency is genetically determined. Congenital lactase deficiency (CLD) (OMIM, #223000) is a severe form of lactase deficiency in which lactase activity is very low or absent in the intestinal epithelium from birth.

In contrast, lactase deficiency in the adult, also referred to as lactase-nonpersistence (LNP) (OMIM, #223100) is a normal, developmental phenomenon characterised by the down-regulation of lactase activity, which occurs soon after weaning in most ethinic groups. The activity of the enzyme drops to around one tenth or less of the infant level. In populations where the prevalence of LNP is high the decline in lactase activity begins at the age of two to three years, while in populations with low LNP prevalence this most commonly occurs in adolescence. Based on family studies, both CLD and LNP are inherited as autosomal recessive traits (Vesa et al., 2000).

Lactase activity is expressed in units per 1 g of wet mucosa or 1 g of protein (Dahlqvist, 1970; Lojda et al., 1972). Values above 50 U/g protein are usually considered to be associated with lactase persistence.

2.1.2. Secondary lactase deficiency

Secondary lactase deficiency results from diseases of the small intestine that damage the intestinal epithelium leading to subsequent lactose maldigestion of different degrees. Acute gastroenteritis, untreated coeliac disease, chronic intestinal inflammation or cancer chemotherapy may be associated with hypolactasia. However, when the epithelium heals the activity of the lactase returns (Vesa et al., 2000).

Potential lactose maldigestion secondary to diarrhoea in children over three months of age is clinically not important and does not normally require lactose-free foods (Heyman, 2006; Sandhu et al., 1997).

2.2. Mechanisms and prevalence

Ingested lactose is hydrolysed by lactase (lactase-phlorizin hydrolase or LPH; EC 3.2.1.108), an enzyme of the microvillus membrane of the enterocytes. It is split into the monosaccharides, glucose and galactose, which are rapidly and completely absorbed within the small intestine (Paige, 2005). Lactase activity appears by the 9th week of gestation, increases by the 14th week and is very high at term. The high lactase activity accounts for the efficient use of lactose, the major carbohydrate in human milk, by the neonate. Contrary to other intestinal enzymes, lactase activity is not inducible by its substrate (Gilat et al., 1972).

CLD is an extremely rare disease with only a few dozen cases documented in the world, most of them in Finland. It results from mutations in the translated region of the lactase gene (LCT) (Kuokkanen et al., 2006; Torniainen et al., 2009), which impair the trafficking and subcellular localisation of the mutant lactase (Behrendt et al., 2009). As a result, lactase activity assayed in small intestinal biopsies is very low. The osmotic load of undigested lactose causes the secretion of fluid and electrolytes into the duodenum and the jejunum, which in turn accelerates intestinal transit, leading to diarrhoea. CLD will not be addressed further in this opinion.

LNP prevalence and age of manifestation vary considerably amongst different ethnic populations. It affects about 70 % of the world adult population. In fact, LNP is the ancestral condition for humans

and indeed for all mammals (Swallow, 2003). In Europe, LNP frequency varies from 4 to 56 % and the lactase persistence gene "travelled" with the spread of milk farming in Europe as only around 4 to 5% of the population in Northern Europe are affected by LNP (Ingram et al., 2009a). Table 2 summarises LNP frequencies in several European countries.

Table 2: Frequency of LNP in European countries.

Country	LNP frequency (%)
Austria	20
Britain	23
Denmark	4
Estonia	43
Finland	17
France	38
Germany	14
Greece	46
Hungary	40
Ireland	4
Italy	56
Poland	37
Spain	34

(after Ingram et al., 2009a)

2.3. Clinical presentations of lactase deficiencies

2.3.1. Lactase nonpersistence (LNP)

In adults with LNP, undigested lactose reaches the colon where it is degraded to lactic acid, acetic acid, hydrogen and carbon dioxide by intestinal bacteria. Lactose maldigestion can elicit symptoms of lactose intolerance (Jouet et al., 1996). However, lactose maldigestion will not lead to symptoms of lactose intolerance in all LNP subjects. Even after ingestion of large amounts of lactose, a small percentage of LNP subjects remains free of symptoms (Scrimshaw and Murray, 1988).

Adverse reactions, which may develop 1 to 3 hours after a lactase-deficient and lactose intolerant individual ingests lactose in food, include abdominal pain, bloating, flatulence and diarrhoea. The clinical effects of lactose ingestion are closely related to the dose and there is a wide variation among individuals regarding the dose–response.

The symptoms attributed to lactose intolerance are also common in the absence of lactose ingestion and are highly susceptible to a placebo effect (Shaukat et al., 2010). Self-reported symptoms that patients associate with lactose intolerance are often unrelated to lactose maldigestion (Casellas et al., 2010; Jellema et al., 2010). Overall, however, their presence was more often associated with lactose maldigestion than digestion in a setting of specialised medical care. No data are available on the prevalence of lactose intolerance as assessed by lactose-related symptoms and breath hydrogen measurement in the general population (Jellema et al., 2010).

The Panel notes that lactose intolerance is normally not the primary explanation of severe gastrointestinal symptoms in infancy, since primary congenital lactase deficiency (CLD) is extremely rare. Lactose intolerance in infants often indicates an underlying (mucosal) abnormality.

Table 3 gives an overview about the symptoms of lactose intolerance in individuals with lactose maldigestion and their occurrence rate.

Table 3: Symptoms reported by individuals at the time of diagnosis of lactose intolerance.

Symptoms	Number of people with symptoms (% of total)
Abdominal pain	100
Gut distension	100
Borborygmi	100
Flatulence	100
Nausea	78
Vomiting	78
Diarrhoea	70
Constipation	30

(after Harrington and Mayberry, 2008; Savaiano et al., 2006; Swagerty et al., 2002)

For reasons that are poorly understood, lactose presented in a solid food may be less likely to induce symptoms than an identical load of lactose presented in solution. One relevant factor may be the rate of gastric emptying, so the fat content of the food or drink consumed may slow the entrance of lactose into the small intestine (Savaiano et al., 2006).

Although lactase cannot be induced by its substrate, some studies have indicated that daily lactose consumption may result in metabolic adaptation by the gut microbiota, thus dampening the symptoms of lactose intolerance in subjects with lactose maldigestion (Saavedra and Perman, 1989). Studies investigating colonic adaptation are few, examined different products to prevent lactose intolerance symptoms and used a wide variety of patients, interventions, comparisons, and outcomes. Results either did not show a difference in symptom score or reported clinically insignificant differences, mostly in symptoms of flatulence. Symptoms of abdominal pain, diarrhoea, or overall score were not improved, which may be more clinically relevant to lactose maldigesters (Wilt et al., 2010).

2.3.2. Secondary lactose intolerance

Symptoms of secondary lactose intolerance are often similar to those of lactose maldigestion and are described in detail in section 2.3.1.

Disaccharide intolerance including lactose intolerance may occur as a transient phenomenon associated with a wide variety of diseases of the small intestine in childhood including gastroenteritis, coeliac disease, giardiasis, protein calorie malnutrition, cow's milk protein intolerance, immunodeficiency syndromes and intestinal resections (Heyman, 2006; Vesa et al., 2000).

The Panel agrees with the recommendations of the WHO/UNICEF (WHO/UNICEF, 1985) for the management of children with acute diarrhoea that food, including breast-milk or diluted milk, should not be withheld from infants and children, or, in cases of dehydration, should be offered as soon as initial rehydration therapy has been completed and that the routine use of any special infant formulae (e.g. lactose-free products) for diarrhoea cases is not advised.

Multiple pathology

Since loss of lactase activity may be the norm in some ethnic groups, lactose maldigestion and lactose intolerance will often coexist with other diseases. Since coincident lactose intolerance may modify the pattern of clinical presentation, a period on a lactose-free diet may be of diagnostic value in patients with a puzzling combination of symptoms. For example, lactose intolerance will clearly affect faecal volume and gastrointestinal symptoms in patients with Crohn's disease or ulcerative colitis and it may be sensible to establish the catalytic activity of lactase using hydrogen breath analysis or blood glucose measurements after a lactose load in these patients (Cox, 2003; Harrington and Mayberry, 2008).

Abdominal pain in children

Recurrent abdominal pain in children is almost as common as irritable bowel syndrome. The post-weaning drop in intestinal lactase activity may occur as early as two years in some ethnic groups, or at

five years in most European children, so that schoolchildren occasionally may already be intolerant to lactose. Studies of recurrent abdominal pain in children in the USA have shown clinical lactose intolerance in a substantial proportion, particularly in African-American, Hispanic and Asian children. Lactose intolerance associated with abdominal pain is particularly relevant in children of ethnic groups with a high prevalence of LNP (Heyman, 2006).

Diarrhoea after gastric surgery

Gastric surgery and surgery of the small intestine radically alter the physiology of the upper gastrointestinal tract. As noted above, the rate of gastric emptying may affect the tolerance to lactose in a susceptible individual. With lactose feeding after surgery, a lactase-deficient person may develop bloating, faintness and diarrhoea (Saavedra and Perman, 1989; Tamm, 1994).

2.4. Genetics and diagnosis

2.4.1. Genetics of lactose nonpersistence (LNP)

The LNP/LP phenotype is genetically determined, with lactase persistence (LP) being dominant over LNP. In Europe, LP is strongly associated with a single C to T transition located 13910 kilobases (kb) upstream of the LCT gene (lactase gene). Correlation of lactase activities with LP/LNP genotypes shows a trimodal distribution, with LNP genotype C/C-13910 displaying the lowest lactase activity (about 6 U/g protein; about 10 % of normal specific activity). The polymorphisms C/T-13910 and T/T-13910 are closely associated with lactase persistence (Rasinperä et al., 2005). The decline in lactase activity in LNP subjects is related to a reduction in the transcription of the LNP alleles (Rasinperä et al., 2005). At present, it is unclear whether the polymorphisms play an important role in lactase expression or simply provide markers for an as-yet-unidentified regulatory element (Grand and Montgomery, 2008; Rasinperä et al., 2005) and the molecular mechanism that induces the natural down-regulation of the LNP alleles remains to be clarified (Enattah et al., 2007; Enattah et al., 2008; Ingram et al., 2009b).

2.4.2. Diagnosis

Poor correlation of abdominal symptoms with the level of lactase activity has made the clinical diagnosis of LNP a challenge (Järvelä et al., 2009).

The most common tests used to measure the digestion of lactose are the hydrogen breath test and the lactose tolerance test. An analysis of polymorphisms of the lactase gene can add useful information. LNP can be confirmed by determination of the lactase activity in a small bowel biopsy.

The diagnosis of lactose intolerance, however, is more difficult because it depends on self-reported symptoms (diarrhoea, abdominal cramping, audible bowel, flatulence, vomiting) not all of which can be assessed objectively. When 353 subjects with a self-diagnosis of lactose intolerance were subjected to a 50 g lactose tolerance test, 164 (46.3 %) were classified as lactose maldigesters by breath hydrogen measurement and their symptoms reported at home before the challenge and after the challenge were comparable though somewhat more severe at home. The 189 lactose absorbers on the contrary reported more and more severe symptoms at home than after the challenge. Reported symptoms alone are a highly unreliable tool to establish symptomatic lactose maldigestion (Casellas et al., 2010).

2.4.2.1. Hydrogen breath test

The hydrogen breath test measures the amount of hydrogen in a person's breath. Normally, very little hydrogen is detectable. However, undigested lactose in the colon is fermented by bacteria, and various gases, including hydrogen, are produced. The hydrogen quickly diffuses into the blood and is exhaled. In the test, the patient drinks a lactose-loaded drink, and the breath is analysed at regular intervals. Raised levels of hydrogen in the breath (by more than 20 ppm) indicate impaired digestion of lactose. This test is available for children and adults. About 15 to 20 % of all individuals are hydrogen non-

excreters after a test load (Hammer et al., 1996). The specificity and the sensitivity of the hydrogen breath test vary, leading to both false negative and positive results (Järvelä, 2005). Both the sensitivity and the specificity of the hydrogen breath test are highest (100 % up to 12 hours after the lactose challenge) in subjects with the C/C-13910 genotype (Matthews et al., 2005).

The combination of a lactose tolerance test (see section 2.4.2.2.) and hydrogen breath test improves the detection of lactose maldigestion, but even if a combined diagnostic approach is used, around 5 % of patients presenting with symptoms suggestive of lactose intolerance may not be classified by these diagnostic means. An analysis of polymorphisms of the lactase gene together with a breath hydrogen test can also be used for screening and diagnostic purposes in subjects with lactose intolerance due to LNP (Matthews et al., 2005; Nagy et al., 2009).

2.4.2.2. Lactose tolerance test

Normally, when lactose reaches the digestive system, the lactase breaks it down into glucose and galactose which are absorbed. The liver then converts the galactose into glucose, which enters the bloodstream and raises the blood glucose concentrations. If lactose is not or incompletely broken down, the blood glucose concentrations do not rise (by <1.1 mmol/L) and a diagnosis of lactose maldigestion is confirmed (Heyman, 2006; Matthews et al., 2005). The specificity of the lactose tolerance test has been reported to range from 77 to 96 % and the sensitivity from 76 to 94 %, leading to both false negative and positive results (Järvelä, 2005). The variability of the test makes it less reliable than the breath hydrogen test and the measurement of the mucosal lactase activity (see section 2.4.2.3.).

2.4.2.3. Intestinal small bowel biopsy

The diagnosis of LNP is based on the direct measurement of lactase, sucrase and maltase activities and the lactase:sucrase (L/S) ratio in the samples obtained from an intestinal biopsy (Järvelä, 2005). A lactase activity below 10 U/g protein is considered to be related to LNP (Dahlqvist, 1970; Koetse et al., 1999).

2.4.2.4. Polymorphism analysis (genetic testing)

For the diagnosis of LNP several methods have been developed to detect the C/T-13910 genotype, including minisequencing, enzyme digest, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-genotyping, and pyrosequencing. Sequencing is the most reliable method to detect all variants known so far in patients from multi-ethnic populations. The age should be taken into account in the interpretations of the results in children (Järvelä et al., 2009).

2.5. Dietetic treatment

The Panel notes that the strict diagnosis of lactose intolerance relies on objective measurements of the clinical effects of the withdrawal and reintroduction of lactose. Milk is such an important component of the diet that before recommending a “low-lactose” diet with the avoidance of milk, lactose intolerance should be formally confirmed by one of the techniques described in section 2.4.2. The only satisfactory treatment of lactose intolerance is a diet with reduced lactose content. It is important to avoid calcium, vitamin D and riboflavin deficiency states. Foodstuffs high in lactose, such as fresh milk, powdered milk and milk puddings, should be avoided, but most lactose intolerant subjects can tolerate around 10 g of lactose in milk products per serving (EFSA, 2004). Lactose-reduced and “lactose-free” milk and milk products are commercially available. Individuals need to adapt their lactose consumption to their individual tolerance. Addition of external lactase enzymes to milk products with the aim of reducing the lactose content may be helpful in individual cases (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2009b).

2.6. Thresholds

Controlled dose escalation studies in well characterised subjects with lactose maldigestion or lactose intolerant individuals are very few in the literature. Prospective studies should take the lactase

polymorphism status of the lactose intolerant individual into account. Clinical studies report a sizeable placebo response of individuals under investigation. Even though good studies are blinded, it seems very difficult to mask the appearance and taste of lactose containing products. Most studies do not report whether the masking had been achieved.

Savaiano et al. (2006) conducted a meta-analysis to determine the severity of symptoms of lactose intolerance among lactose maldigesters after consuming between 7 and 25 g of lactose in water, milk or other foods in comparison to placebo (0 to 3.75 g of lactose) under masked conditions. The meta-analysis included 21 articles published between 1966 and January 2002. Eleven of the 21 eligible studies reported the severity of symptoms; seven of these were double-masked. Fifteen studies reported the incidence of symptoms and of these 11 were double-masked. A pooled symptom effect size was calculated for each reported symptom (abdominal bloating, abdominal pain, degree of loose stools or diarrhoea, flatulence) at the lactose dose closest to one cup of milk (~12 g lactose) and given with the lowest amount of fat. Incidence differences were calculated per symptom and pooled after weighting for sample size. The severity of gastrointestinal symptoms reported by maldigesters were not different after they consumed about 12 g of lactose (240 mL of milk) compared to placebo under masked conditions. The authors concluded that there was insufficient variation in the study doses to distinguish a dose-response relationship. They also concluded that the dose of lactose which resulted in symptoms in the majority of maldigesters was probably near 25 g (that found in around 500 mL of milk); whereas symptoms (both incidence and severity) after doses at or below 12 g were not noticeable under masked conditions. The Panel notes that this meta-analysis due to its design is not suitable to determine thresholds of lactose consumption for eliciting symptoms of lactose intolerance in subjects with lactose maldigestion.

In a systematic review by Wilt et al. (2010) tolerable doses of lactose in subjects with clinically diagnosed lactose intolerance (by challenge) were investigated. Multiple electronic databases for original studies published in English from 1967 to November 2009 were searched. Twenty-eight randomised cross-over trials with lactose intolerant subjects were included. Diagnosis of lactose maldigestion was performed by hydrogen breath test in 13 studies, by blood glucose after lactose challenges in 11 studies, by galactose excretion in urine in one study and was not reported in three studies. Most trials were conducted in a “double blind” way, but three studies were single blinded or did not attempt to mask the tastes of the test preparations. The majority of studies were small (<30 subjects) with trial populations ranging from six to 150 subjects and age ranges from 10 to 77 years. Studies often used a single dose of lactose and a “lactose-free” control administered in water or milk without food, frequently in a fashion in which blinding was not totally achieved. Results were heterogeneous in terms of study populations, interventions, assessment methods and outcome definitions, thus precluding pooling. The classification of symptoms, particularly, has not been done in a uniform and comparable way. The authors differentiate “no or minor”, “moderate” and “severe” symptoms. A sizeable variability of the response of maldigesters to the placebo was observed in various studies. Evidence indicated that most individuals diagnosed with lactose intolerance can tolerate 12 g (10 to 15 g) of lactose (~240 mL milk) as a single dose if taken with food with no or minor symptoms, whilst the same single dose in water may be symptomatic. However, as the dose was increased above 12 g (18 to 25 g), intolerance became more prominent, with single doses of 24 g usually leading to appreciable symptoms. There is some evidence that if the intake of 20 to 24 g of lactose was distributed throughout the day and consumption occurred together with other nutrients, many lactose maldigesters tolerated this dose. Lactose in a dose of 50 g per day induced symptoms in the vast majority of lactose maldigesters, in many of them severe.

Table 4 compiles the occurrence of symptoms of different severity following the consumption of different amounts of lactose either together with or without other foods than milk from the systematic review (Wilt et al., 2010). It is apparent that in the latter case symptoms occur at somewhat lower doses and tend to be more severe. It is also apparent that the number of subjects tested is in most instances limited. No clear statement was included in the review what trivial, minor and severe symptoms are.

Table 4: Symptomatic responses (-, ±, + or ++) of adult lactose maldigesters to lactose consumed with or without foods other than milk.

Lactose dose with other foods than milk (g)	**3-6**	**7**			**12**		**15**		**18**		**22**			**30**	**34**	**49**	**50**
Symptoms	-	-			-		-		-		-			++	+	-	++
Number of maldigesters studied	59	16			103		16		89		19			16	31	18	74
Lactose dose without other foods than milk (g)	**2-6**		**8**	**10**	**12**	**13**	**15**	**17**		**20**		**23**	**25**	**30**		**49**	**50**
Symptoms	-		±	-	-	±	-	+		++		+	+	+		++	++
Number of maldigesters studied	96		40	17	35	40	19	45		33		52	17	28		9	71

Symptoms:- no or trivial; ± trivial to minor, + minor; ++ severe
(data from Wilt et al., 2010)

From the same review, the percentage of subjects with lactose maldigestion who reported abdominal pain associated with different amounts of ingested lactose can be calculated (either in dose finding studies or in studies investigating the dietetic management of individuals with diagnosed lactose intolerance) (see Table 5). It appears that subjects with lactose maldigestion or lactose intolerance report abdominal symptoms even with the use of "low-lactose" products in a considerable percentage.

Table 5: Percentage of subjects with lactose maldigestion reporting abdominal pain in relation to the lactose content of conventional milk and lactose-reduced milk, with or without chocolate flavour.

Lactose content	**Percentage with symptoms**	**Number of subjects studied**
0-2 g	4-37 %	121
>2-7 g	20-67 %	56
~12 g	22-37%	51
~20 g	33-71 %	56

(data from Wilt et al., 2010)

The Panel notes the high placebo response and that the results of individual dose-response studies are inconsistent. Two studies showed no differences in symptoms between stepwise increased lactose doses of 0 and 7 g added to 200 mL fat-free lactose-free milk (Vesa et al., 1996), and graded dose of 0 to 18 g of lactose added to a lactose-free preparation (Ensure®) and consumed together with a sweet roll (Newcomer et al., 1978), whilst a significant increase of slight symptoms was observed in 13 adult lactose maldigesters when more than 12 g lactose was consumed in aspartame sweetened water without food (Hertzler et al., 1996). When 40 subjects with lactose maldigestion and 31 subjects who were lactose digesters were tested in a parallel and double blind way with daily increasing amounts of lactose (125 mL, 250 mL, 500 mL and 1000 mL of skim milk, corresponding to about 5 g, 12.7 g, 25 g and 51 g lactose and low-lactose skim milk, corresponding to 0.8 g, 1.6 g, 3.25 g and 6.5 g lactose) while on a diet free of milk and dairy products, lactose maldigesters showed a higher frequency of symptoms than lactose digesters and there was a significant association between dose and frequency of symptoms which was not observed in lactose digesters. 32.5 %, 45 %, 49 % and 68 % of lactose maldigesters reported symptoms with the increased intake of normal-lactose skim milk (the percentages in lactose absorbers were 13 %, 16 %, 29 % and 37 %). The fact that lactose maldigesters also reported an increase in symptoms at a somewhat lower level with the increase of consumption of low-lactose milk (20 %, 28 %, 45 % and 50 %; the corresponding figures in the lactose absorbers were 23 %, 26 %, 29 % and 36 %) suggests, however, that the volume of the test milks has an effect on the frequency of symptoms (Cavalli-Sforza and Strata, 1987). These four studies were included in the systematic review, which excluded the two studies described below (Wilt et al., 2010).

Bedine and Bayless (1973) investigated 20 subjects (18-70 years of age) with low levels of jejunal lactase acivity (<2 U per g wet weight). All subjects received initially 12 g lactose or 12 g sucrose or a mixture of 6 g glucose and 6 g galactose in 200 mL of an electrolyte solution and reported subsequent symptoms (1+ abdominal distention and borborygmy, 2+ severe distention, flatulence and loose bowel movements, 3+ diarrhoea). Thereafter, they received either lower or higher doses on the subsequent days depending on the presence or absence of symptoms. The lowest dose of lactose at which they reported at least symptoms classified as 1+ which differed from their baseline status was 3 g in two subjects, 6 g in three subjects, 12 g in 10 subjects, 24 g in two subjects, 48 g in two subjects and 96 g in one subject. 75 % of the subjects noted abdominal fullness, excessive flatulence or diarrhoea within 3 to 4 hours of ingesting 12 g lactose, whilst 25 % were asymptomatic. However, seven of the 15 subjects sensitive to 12 g lactose or less and two of the five subjects sensitive to higher (>24 g) lactose doses had the irritable bowel syndrome which could interfere with the reported symptoms.

In 13 of 20 subjects with demonstrated lactose maldigestion (no blood glucose rise on lactose challenge; diarrhoea on lactose challenge and/or normal blood glucose rise on challenge with glucose plus galactose; low lactase activity in jejunal biopsy) who had become free of symptoms after three weeks of a lactose-free diet, provocation tests with lactose added in amounts of 0 g, 5 g, 10 g, 15 g, 20 g and 25 g to 200 mL of "lactose-free" (<0.5 g/100 mL) milk consumed before breakfast every other day were performed. Patients reported the occurrence of diarrhoea and any abdominal symptoms, which were classified as "slight" when the symptoms involved discomfort and did not interfere with usual activities including work, and as "severe" when they restricted or prevented usual daily activities. Two subjects reported slight complaints and diarrhoea with 5 g of lactose, seven reported slight or severe complaints with 10 g (with watery diarrhoea in two cases) and 12 subjects reported slight or severe complaints accompanied by diarrhoea in nine cases with 25 g, whilst one subject had no abdominal complaints at all (Gudmand-Høyer and Simony, 1977). The Panel notes the small number of subjects and the fact that the subjects were tested after a considerable period on a lactose-free diet which might enhance the sensitivity for small amounts of lactose.

Lactose intolerance prevalence is generally very low in young children and remains low into early adulthood among individuals of Northern European descent. For African American, Hispanic, Asian, and American Indian populations lactose intolerance rates may be 50 percent higher in late childhood and adulthood (Wilt et al., 2010). Gremse et al. (2003) following a study in 30 American children concluded that children (average age 11 year) with lactose maldigestion diagnosed through the breath hydrogen test had increased abdominal pain scores following daily ingestion of 12 g of lactose from regular milk when compared to hydrolysed milk during two weeks. In a study with nine children in Denmark (average age 10 years) children had significantly less symptoms after lactose free milk (1.25 g of lactose) versus ordinary milk (25 g of lactose) (Nielsen et al., 1984). Ladas et al. (1991) reported that in 150 Greek children 12 g lactose (240 mL milk) caused symptoms in 7.3 % of the digesters and 8.6 % of maldigesters. The Panel notes that there are not enough data on children with lactose intolerance, but it appears that similar thresholds may exist as observed in adults with a similar variability in individual sensitivity.

The Panel notes that according to the systematic review by Wilt et al. (2010) most individuals diagnosed with lactose intolerance or lactose maldigestion can tolerate 12 g of lactose as a single dose (particularly if taken with food) with no or minor symptoms. Single doses of 24 g usually lead to appreciable symptoms. There is some evidence that many lactose maldigesters tolerate daily doses of 20 to 24 g of lactose if distributed throughout the day and consumed together with other nutrients. Consuming 50 g of lactose per day induces symptoms in the vast majority of lactose maldigesters and in many of these symptoms will be severe (Shaukat et al., 2010). There are a few studies with a small number of subjects with lactose maldigestion who self-reported abdominal symptoms and diarrhoea already with lactose intakes below 12 g, in some cases with 3 to 5 g of lactose. The Panel notes that the testing procedure with daily increases of the lactose dose and the insufficient masking of the test solutions needs to be taken into account when interpreting the results.

The Panel concludes that a single threshold of lactose for all lactose intolerant subjects cannot be determined owing to the great variation in individual tolerances. Symptoms of lactose intolerance have been described after intake of less than 6 g of lactose in some subjects.

The Panel concludes that the vast majority of subjects with lactose maldigestion will tolerate up to 12 g of lactose as a single dose with no or minor symptoms. Higher doses may be tolerated if distributed throughout the day.

3. Galactosaemia

3.1. Definitions

"Galactosaemia" describes the unusual presence of galactose in blood following lactose/galactose ingestion. This term is commonly used to signify the severe form of galactosaemia I ("classical galactosaemia") caused by a deficiency in galactose-1-phosphate uridyltransferase (GALT) with a residual enzyme activity <1% of normal (OMIM, #230400). This was first described by Goppert (1917) in an infant with hepatomegaly, icterus, failure to thrive and urinary excretion of sugar who tolerated all kinds of sugar except lactose and galactose. Whilst the elimination of these two sugars reversed all acute symptoms the child remained mentally retarded (Suzuki et al., 2001).

Galactokinase (GALK) deficiency causes "galactosaemia II", an autosomal-recessive disorder first described by Gitzelmann (1967) in two siblings of a Roma family with juvenile cataract and galactosuria (OMIM, #230200).

UDP-galactose-4-epimerase (GALE) deficiency (or galactosaemia III) in circulating red and white blood cells and elevated blood galactose was originally described as a benign condition in a healthy newborn by Gitzelmann (1972). A second "generalised" form of GALE deficiency with symptoms resembling severe galactosaemia I was described by Holton et al. (1981). Both forms represent the extremes of a continuum of symptoms caused by different mutations (Timson, 2006). Generalised GALE deficiency is a rare disorder whilst benign GALE deficiency may be as common as galactosaemia I in some populations (OMIM, #230350).

3.2. Mechanisms and prevalence

Four consecutive enzymes convert most of dietary galactose into glucose in the human body: galactose mutarotase (ß-D-galactose into α-D-galactose), GALK (α-D-galactose into galactose-1-phosphate), GALT (galactose-1-phosphate plus UDP-glucose into glucose-1-phosphate and UDP-galactose) and GALE (UDP-galactose into UDP-glucose). Reduced activity of GALK, GALT and GALE leads to accumulation of galactose which is rapidly excreted in the urine or converted either to galactitol by aldose reductase (Weinstein and Segal, 1968) or by an as-yet-unknown mechanism to galactonate (Cuatrecasas and Segal, 1966; Wehrli et al., 1997). The latter is excreted in the urine or completely oxidised. Small amounts of both galactose and galactitol can be found in the urine of healthy subjects. The sum of both in the urine of healthy controls ranged from 1.4 to 41.2 mg per day. Postabsorptive galactose excretion in treated patients with severe galactosaemia I was similar to healthy subjects, whilst galactitol excretion was about 50 times higher. The total galactose excretion was 150 to 200 mg per day in children and 250 to 300 mg per day in adults with severe galactosaemia I under strict dietary control (Schadewaldt et al., 2003). This amount corresponds to about 30 % of endogenously formed galactose by adults. Endogenous galactose production is age related, highest in infants and young children and decreases thereafter by about 50 % (Berry et al., 1995; Schadewaldt et al., 2004). Endogenous galactose synthesis is not modified by exogenous galactose supplementation (Huidekoper et al., 2005) and is mostly derived from glycoprotein and galactolipid degradation or from UDP-glucose (Berry et al., 1995).

In GALT deficiency, galactose-1-phosphate accumulates in the foetus and after birth in various tissues when lactose or galactose is ingested. Galactose-1-phosphate has been considered to inhibit glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, phosphoglucomutase, UDP-hexose

pyrophosphorylase and phosphorylase and, moreover to cause sequestration of phosphorus by futile galactose phosphorylation and dephosphorylation (Gitzelmann, 1995).

Galactitol is highly increased in plasma and urine in GALT, GALK and generalised GALE deficiency It does not completely normalise with treatment (Jacobs et al., 1995) and it is considered to be responsible for cataract formation and for pseudotumor cerebri symptomatology observed in some newborns (Bosch, 2006).

Possible reasons for the unsatisfactory mental, motor and language development observed despite adequate dietetic control are a chronic intoxication by galactose metabolites or deficiencies and structural and functional abnormalities of galactose-containing glycoproteins or glycolipids critical for normal myelin formation and normal cellular signalling pathways (Coman et al., 2010; Kadhom et al., 1994; Lai et al., 2003; Petry et al., 1991; Segal, 1995). The same mechanisms are also discussed to be at the bottom of the impaired ovarian function with hypergonadotropic hypogonadism in women with severe galactosaemia I (Forges et al., 2006).

The birth incidence of galactosaemia I in the Western European population is between 1:23,000 and 1:44,000 (Suzuki et al., 2001). Severe GALT deficient galactosaemia occurred in 1 in 480 Travellers in Ireland and in 1 in 30,000 in the non-Traveller Irish population (Murphy et al., 1999).

3.3. Symptoms

The type of symptoms and frequency of their occurrence in severe galactosaemia I are compiled in Table 6.

Failure to thrive, vomiting and diarrhoea begin in the newborn with severe galactosaemia I within a few days of lactose ingestion. This is followed by haemolysis, jaundice and coagulopathy accompanying hepatopathy. Incipient cataracts have been seen within a few days after birth. Neonatal death is frequently caused by a fulminant *E. coli* sepsis. Despite early dietetic management with apparently good control of galactose intake the long-term results are generally not satisfactory (Schweitzer et al., 1993; Waggoner et al., 1990). Verbal dyspraxia is found in many (60 %) children with treated severe galactosaemia I and a delayed mental development with slowing of cognitive function is observed in 50 % of children aged over six years. Learning disabilities increase with age whilst IQs decrease, more in females than in males. Motor function and balance is impaired in 13 % of children over three years of age and 20 % show growth retardation. Neurological abnormalities like ataxia, tremor and dysmetria (inability to properly direct or limit motions) may occur. 80 % of females with a residual GALT activity below 1 % of normal have primary gonadal failure (Bosch, 2006; Bosch et al., 2004; Kaufman et al., 1995; Nelson et al., 1991; Waggoner et al., 1990; Widhalm et al., 2002). Pregnancy is, therefore, rare in women with severe galactosaemia I. Pregnant women with galactosaemia show galactosuria as soon as lactose biosynthesis starts in the mammary glands in the second trimester, and a case of self-intoxication presumably due to lactose synthesis has been reported in a breastfeeding woman with GALT deficiency despite a strict galactose-free diet (Brivet et al., 1989).

Most patients with GALK deficiency have cataracts. Other associated clinical symptoms, particularly mental retardation, are probably not causally related to the enzyme defect (Bosch et al., 2002).

Subjects with peripheral or isolated GALE deficiency in blood cells are asymptomatic (Gitzelmann et al., 1977), whilst the symptoms of generalised GALE deficiency are similar to severe galactosaemia I (Walter et al., 1999).

Table 6: Type and frequency of symptoms in severe galactosaemia I.

Symptoms	Number of people with symptoms
Neonatal cases n=270	
Hepatocellular damage	89 %
Food intolerance	76 %
Failure to thrive	29 %
Lethargy	16 %
Seizures	1 %
Sepsis a) suspected b) positive cultures	 a) 30 % b) 10 %
Long-term outcome	
Developmental delay at 6 years of age and age-related decline of IQ, particularly in females	45 % of n=177
Speech disorder at > 3.5 years	56 %
Gonadal function: a) primary amenorrhoea at >17 years b) pregnancies at >17 years c) elevated FSH levels at > 15 years	 a) 24 % of n=34 b) 14 % of n=37 c) 80 % of n=47
Growth: height <3rd %ile at 5-16 years	33 % of n=93 females; 12 % of n=72 males
Disturbed motor function at >3.5 years	18 % of n=206

(after Waggoner et al., 1990)

3.4. Genetics and diagnosis

3.4.1. Genetics of severe galactosaemia I

All types of hereditary galactosaemia are inherited autosomal recessively.

The gene for GALT is located on chromosome 9p13 and more than 180 different mutations at the GALT locus have been identified which largely determine the severity of the disorder. This ranges from the severe galactosaemia I syndrome due to e.g. the p.Q188R mutant which is the most frequent in the white population (65 % in Western Europe) (Elsas et al., 1995; Tyfield et al., 1999), to mild symptoms due to the p.S135L mutant which is the most frequent mutation (50 %) in the African American population with a residual GALT activity of 5-10 % in leukocytes, to apparent clinical normality in the "Duarte" variant (Elsas et al., 2001; Hammersen et al., 1975). Most patients are compound heterozygotes (Murphy et al., 1999).

In recent years epigenetic phenomena due to defective glycosylation of proteins involved in gene expression are considered to partly explain the variable long-term clinical outcome of patients and siblings with identical mutations (Coman et al., 2010; Hughes et al., 2009).

3.4.2. Diagnosis

Galactosaemia can be suspected on the basis of clinical symptoms in a newborn or on the basis of newborn screening programmes which exist in many European countries, although the time for testing may be too late for some infants. Two search strategies are applied, either for increased galactose concentrations in blood, which will detect GALK and GALT deficiency, and with certain modifications, also GALE deficiency, provided the sample was taken after the infant had received lactose-containing human milk or formulae. GALT activity measurement in red blood cells will detect GALT deficiency, provided no blood (exchange) transfusion has been performed earlier (Schweitzer, 1995). Total body galactose oxidation as assessed by $^{13}CO_2$ enrichment in expired air after a bolus dose of 7 mg of ^{13}C-D-galactose/kg body weight has been proposed as an alternative and fast screening test in newborns to predict GALT deficiency after it had been demonstrated to predict both severity of disease and genotype (Barbouth et al., 2007). The diagnosis for GALT deficiency depends

on GALT activity measurements in red blood (or other body) cells. Elevated levels of galactose-1-phosphate in red blood cells (>10 mg/100 mL RBC), of galactose and galactitol in plasma and urine will support the diagnosis.

The diagnosis of GALK deficiency relies on the enzyme activity measured in red blood cells. Elevated levels of galactose and galactitol in plasma and urine may support the diagnosis.

The diagnosis of GALE deficiency is based on enzyme activity in red blood cells and, for generalised GALE deficiency, in other tissues. Both galactose-1-phosphate and UDP-galactose are elevated in red blood cells. Elevated levels of galactose and galactitol in plasma and urine support the diagnosis.

Challenges with galactose or lactose are contraindicated in suspected cases of galactosaemia.

3.5. Dietetic treatment

The dietetic principle in the management of all types of galactosaemia is the elimination of all sources of galactose, including human milk, as far as possible, particularly in infants and young children.

The level of galactose-1-phosphate in red blood cells is barely measurable in healthy subjects (<0.57 µmol/g Hb or <1mg/dL RBC (Ficicioglu et al., 2008; Kalderon et al., 1992)) and highly increased in newborns with severe galactosaemia I (>100 mg/dL RBC). It gradually decreases to 2.5 to 4.5 mg/dL in red blood cells but does not become normal, even when elemental formula devoid of lactose is administered (Ficicioglu et al., 2008; Zlatunich and Packman, 2005). This may be due to an incomplete elimination of galactose from the diet in the case of feeding a formula based on soy protein or to endogenous synthesis of galactose-1-phosphate from glucose via UDP-galactose through a pyrophosphorylase reaction, since GALE is normally active already in the embryo (Gitzelmann, 1995). The endogenous synthesis of galactose is estimated to amount to 1 to 2 g per day in adults (Gitzelmann and Auricchio, 1965).

Infants suspected of galactosaemia as a rule receive an infant formula based on soy protein isolate which is lactose and galactose free according to the criteria laid down in Directive 2006/141/EC[4] or an elemental formula free of both lactose and galactose. Soy based formula contains small amounts of bound galactose (about 1.4 mg/dL). It is not known at present if the more rapid decrease of galactose-1-phosphate levels with an elemental formula has an impact on the long-term outcome.

Complementary feeding is introduced in galactosaemic infants at the appropriate age (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2009a) under careful avoidance of dairy products and of all processed foods with lactose containing ingredients. Fruits and vegetables with small amounts of free and β-glycosidic bound galactose may be consumed without restriction. Alpha-glycosidic bound galactose in e.g. legumes need not be of concern. The galactose intake on a strict lactose-free diet has been estimated to be 10 to 50 mg per day. In recent years, a less severe restriction of dietary galactose has been observed to be without harm in galactosaemic patients from the age of about three years (APS, 1997; Bosch, 2006; Bosch et al., 2004). Dietetic management of older children and adults with galactosaemia is possible without specially manufactured foods, if the lactose or galactose content of foods is taken into account.

3.6. Thresholds

Contrary to subjects with lactose intolerance, patients with disorders of galactose metabolism need to observe both the lactose and galactose content of foods. There is also a quantitative difference in the amounts of lactose tolerated by subjects with lactose intolerance and by patients with inherited disorders of galactose metabolism: lactose reduction may be sufficient in lactose intolerance, while lactose elimination, as far as possible, will be required in foods suitable for individuals with

[4] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p. 1–33.

symptomatic galactosaemia. Milk products in which the lactose content has been reduced by enzymatic hydrolysis contain equivalent amounts of galactose and glucose which correspond in sum to the original amount of lactose and are, therefore, not suitable for patients with galactosaemia.

Sources of galactose are mainly milk and its derivatives which contain lactose (cow's milk contains 4.5 to 5.5 g lactose/100 mL or 2.3 g galactose/100 mL). Many fruits and vegetables and fermented foods contain some amounts of free galactose (yoghurt 900 to 1600 mg, cheddar cheese 236 to 440 mg, blueberries 26 ± 8.0 mg, honeydew melon 27 ± 2.0 mg, pineapple 19 ± 3.0 mg/100 g fresh weight). Galactose intake of healthy people in industrial countries varies between 3 and 14 g per day (Forges et al., 2006; Gropper et al., 2000).

It is not known at which dose of dietary galactose precisely RBC galactose-1-phosphate, starts to rise. In some infants 100 mg of lactose per day have been found to sustain mild jaundice and failure to thrive. For ethical reasons, only few loading studies have been performed (Gitzelmann, 1995; Kalderon et al., 1992).

It has been suggested to allow in the diet of patients with severe galactosaemia I only foods with a galactose content of ≤5 mg/100 g, restrict those with a galactose content of 5 to <20 mg/100 g and to disallow all foods with a content of >20 mg/100 g (Gropper et al., 2000).

An estimate of recommendable galactose intakes for patients with severe galactosaemia I has been based on observed feasible diets in well-controlled patients from a number of European centres for the treatment of inherited disorders of metabolism: infants 50 (to 200) mg, toddlers 150 (to 200) mg, school children 200 (to 300) mg, adolescents 250 (to 400) mg and adults 300 (to 500) mg of both free and β-glycosidic bound galactose per day (APS, 1997). Taking these recommendations as a basis and assuming energy intakes of 600, 1,100, 1,500, 2,000 and 2,500 kcal per day, respectively, this would require a diet with about 8 mg (16 mg lactose), 14 mg (28 mg lactose), 13 mg (26 mg lactose), 13 mg (26 mg lactose) and 12 mg (24 mg lactose) of galactose/100 kcal, respectively. This can be achieved by a careful selection of both natural and processed foods according to their lactose content in addition to substituting foods and beverages made with cow's milk by foods made from soy protein.

The Panel considers that the existing criterion of ≤10 mg lactose/100 kcal for labelling infant and follow-on formulae as "lactose-free" permits that these formulae can be safely used in the dietetic management of patients with galactosaemia I, with galactosaemia II (GALK deficiency) and with generalised galactosaemia III (GALE deficiency). If additional special lactose and galactose free foods for patients with galactosaemia were produced, the same criterion as for infant and follow-on formulae should be applied.

Milk (beverages) in which the lactose is (partially) enzymatically hydrolysed to glucose and galactose and from which the latter is not removed are not suitable for patients with galactosaemia regardless of the residual lactose content.

4. Consequences of technology of lactose reduction in foods

The technological process for removing lactose is based either on the extensive hydrolysis of lactose by microbial β-galactosidase or the removal of lactose by physical means (e.g. ultrafiltration, chromatography) with a subsequent hydrolysis of the residual lactose. As in both processes lactose is hydrolysed to glucose and galactose, these processes are not suitable to produce galactose-free products.

The β-galactosidase most commonly used in the hydrolysis of lactose is derived from *Kluyveromyces lactis* and *Kluyveromyces fragilis*. β-galactosidase derived from *Aspergillus oryzae* is also used in food production but to a lesser extent (Saavedra and Perman, 1989).

Information on compositional changes resulting from the technological processes applied to remove lactose from products is limited. These changes might result in lower carbohydrate content and, in

cases of ultrafiltration or chromatographic separation, also in small decreases in mineral content which are unlikely to be significant.

4.1. Nutritional impact of lactose reduced dairy products

Few studies are available on the nutritional and physiological impact of lactose hydrolysed dairy products. A specific need for lactose has not been proven. The effects of monosaccharide absorption in the small intestine on the absorption of water and sodium and of calcium by passive non-saturable diffusion are not restricted to the monosaccharides resulting from the cleavage of lactose (SCF, 2003).

In 1988, Scrimshaw and Murray reviewed existing studies on the effect of the absence of lactose on calcium absorption and concluded that most of the evidence indicated a favourable or neutral effect of lactose on calcium absorption in both lactose digesters and maldigesters (Scrimshaw and Murray, 1988).

Abrams and coworkers (2002) assessed the absorption of calcium and zinc from lactose-containing and "lactose-free", glucose polymer-containing infant formulas in 18 full-term infants. They reported that fractional and total calcium absorption was significantly greater from the lactose-containing formula than from the "lactose-free" formula. Absorption of calcium from a "lactose-free" infant formula was, however, judged to be adequate to meet the calcium needs of full-term infants when the formula's calcium content is similar to that of lactose containing, cow-milk-based infant formulae (Abrams et al., 2002).

The Panel notes that the available evidence does not allow a scientific conclusion to be drawn on a possible effect of lactose on calcium absorption.

No negative nutritional consequences can be expected from the consumption of lactose hydrolysed dairy products in either LNP or healthy people, if the only difference between conventional and lactose hydrolysed dairy products is the lactose content. The avoidance of conventional dairy products without supplementation or appropriate adaptation of dietary habits may result in low intakes of calcium, vitamin D and riboflavin.

4.2. Analytical methods

A large number of methods for the determination of carbohydrates in milk have been developed, including, spectrophotometric (infrared), polarimetric, gravimetric, enzymatic, and chromatographic methods.

The lower limits of detection and quantification range from 10 µg to 10 mg/100 mL. For details see Appendix 1.

CONCLUSIONS

Lactase deficiency and lactose intolerance

- Lactose intolerance can be due to genetic non-persistence of lactase. In individuals with LNP dietary lactose is not or incompletely split by intestinal lactase and residual lactose is fermented by the colonic microbiota leading to abdominal symptoms.

- Lactose tolerance varies widely among individuals with lactose maldigestion. A single threshold of lactose for all lactose intolerant subjects cannot be determined owing to the great variation in individual tolerances. Symptoms of lactose intolerance have been described after intake of less than 6 g of lactose in some subjects. The vast majority of subjects with lactose maldigestion will tolerate acute doses of up to 12 g lactose as a single dose with no or minor symptoms. Higher doses may be tolerated if distributed throughout the day.

Galactosaemia

- Galactosaemia is caused by three different genetic enzyme defects in the metabolism of galactose. Severe galactosaemia if untreated is accompanied by a potentially fatal impairment of hepatic and renal function and with cataracts in the newborn and the young infant which is reversed by elimination of dietary galactose. Despite lifelong dietetic management there is retarded development and growth deficiency in most patients and ovarian insufficiency in most female patients.

- Dietetic management is started with lactose-free infant and later follow-on formulae with a lactose content ≤10 mg/100 kcal. In older infants, children and adults, foods containing milk or milk products or lactose as an ingredient must be avoided as far as possible, so that the lactose content of the daily diet will correspond to about 25 mg/100 kcal. A precise threshold for galactose/lactose intake below which adverse effects are not elicited cannot be given.

- Milk (beverages) in which the lactose is (partially) enzymatically hydrolysed to glucose and galactose and from which the latter is not removed are not suitable for patients with galactosaemia regardless of the residual lactose content.

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APPENDIX

ANALYTICAL METHODS OF LACTOSE DETERMINATION IN FOODS

Polarimetric methods (e.g. AOAC 896.01 - lactose in milk), gravimetric methods (e.g. AOAC 930.28 - lactose in milk) and mid-infrared spectrophotometric methods (e.g. AOAC 972.16 - fat, lactose, proteins and solids in milk) do not allow the differentiation between carbohydrates and are not suitable for measuring lactose in products where the content of lactose has been reduced by enzymatic hydrolysis.

A considerable number of enzymatic methods to determine lactose have been reported. They have the common reaction of enzymatic hydrolysis of lactose to glucose and galactose, followed by the enzymatic determination of one of the liberated monosaccharides. The difference in the monosaccharide content before and after hydrolysis represents the amount of lactose in the sample. The most common enzymatic method to measure galactose is based on its oxidation by β-galactose dehydrogenase to galacturonic acid in the presence of NAD that is reduced to NADH. The absorbance of NADH at 340 nm is measured before and after the addition of the enzyme and the amount of lactose is calculated based on the differences in readings (Nollet and Toldrá, 2010). Two ISO standards and one AOAC official method (AOAC 984.15 – lactose in milk) are available describing the analysis of lactose by enzymatic methods (AOAC, 2007; ISO and IDF, 2002a, 2002b). The limit of quantification of enzymatic methods can be as low as 0.01 g/100 g, although they are more reliable at high-lactose concentrations (>1 % w/w).

High-Performance Liquid Chromatography (HPLC) allows direct detection of carbohydrates. The most common sugar-detection system after HPLC separation is the refractive index (RI). However, the sensitivity of this detector is quite low with a limit of quantification of 0.05 g/100 mL. Alternative detection systems are light-scattering detectors providing better sensitivity and baseline stability, and electrochemical detectors. Among the various available chromatographic systems, the reverse-phase and cationic exchange resin-based columns are the most used in carbohydrate analysis. High-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) is an alternative analytical technique with very high sensitivity and good resolution for non-derivatised carbohydrates (Nollet and Toldrá, 2010) which allows detection of lactose in amounts below 0.01 g/100 g.

Lactose in milk powder samples were satisfactorily determined also by capillary electrophoresis with electrochemical detection (CE-ED) with a detection limit of 0.01 mg/100 mL (Zhang et al., 2001).

Actually, the reference method for the determination of the lactose content of raw milk, heat-treated milks, dried milk and raw and pasteurised cream is the ISO 22662, IDF 198 (2007) standard. It is not applicable to fermented milks and milks to which oligosaccharides have been added. This chromatographic method uses a cation exchange column in the lead form and detection by a differential refractometer detector or any other suitable detector (ISO and IDF, 2007). Values are expressed as mass fraction % (i.e. in cream 1,461 %).

GLOSSARY AND ABBREVIATIONS

AOAC	Association of Analytical Communities
CLD	Congenital lactase deficiency
FSH	Follicle-stimulating hormone
GALE	UDP-galactose-4-epimerase
GALK	Galactokinase
GALT	Galactose-1-phosphate uridyltransferase
HPAEC	High-performance anion-exchange chromatography
HPLC	High-Performance Liquid Chromatography
ISO	International Organization for Standardization
LCT	Lactase gene
LPH	Lactase-phlorizin hydrolase
NAD/NADH	Nicotinamide adenine dinucleotide
PAD	Pulsed amperometric detection
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
RBC	Red blood cells
UDP	Uridine diphosphate