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ORIGINAL ARTICLE

Lactose malabsorption in children and adolescents: diagnosis through breath hydrogen test using cow milk

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Abstract

Objective: to determine the prevalence of lactose malabsorption and its association with skin color and age in children and teenagers attending public schools in Porto Alegre, Brazil.

Methods: a cross-sectional study was performed with 225 subjects between 8 and 18 years attending two public schools in Porto Alegre, Brazil. Patients were randomly selected. Subjects were classified according to skin color (white and non-white) and age group (8 to 12 and 13 to 18 years). Lactose malabsorption was diagnosed using the breath hydrogen test after ingestion of 250 ml of whole cow milk. The test lasted for 3 hours, with collections after fasting and 60, 120, and 180 minutes after milk ingestion. Malabsorption was determined in the presence of increase of $\geq$20 ppm in hydrogen concentration regarding the basal levels.

Results: two-hundred and twenty-five students were studied, with a mean age ± standard deviation of 12.2 ± 2.0 years. The subjects consisted of 134 females (59.6%); 154 white (68.4%); and 71 nonwhite. Lactose malabsorption was observed in 19/225 cases (8.4%). It was diagnosed in 8/154 white patients (5.2%) and in 11/71 non-white patients (15.5%) (P = 0.02). Regarding the age group, we found 15/143 cases of malabsorption in students between 8 and 12 years (10.5%), and 4/82 cases in students between 13 and 18 years (4.9%) (P = 0.227).

Conclusions: the prevalence of lactose malabsorption in students attending public schools in Porto Alegre is significant, especially if we consider that the physiological doses (250 ml of milk) were used for diagnosis. The malabsorption rate was higher among non-white children, which confirms the influence of race on primary adult type hypolactasia. No association was observed between lactose malabsorption and age group.

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Introduction

During the digestive process, lactose must be hydrolyzed into glucose and galactose to be absorbed by the small intestine mucosa. These monosaccharides are absorbed via sodium-dependent carrier-mediated active transport.[1] Lactose is hydrolyzed by a betagalactosidase, known as lactase.

After weaning, there is a gradual decrease in lactase activity in most of the world population.[1] This phenomenon,

called primary lactase deficiency, is the most common form of genetically determined disaccharidase deficiency.[1] The persistence of the enzyme activity in adult life has an autosomal dominant heritage, while lactase deficiency is an autosomal recessive disorder.[2,3] Lactose malabsorption is the predominant phenotype in native populations of Australia, Oceania, eastern and southeastern Asia, Tropical Africa and Americas.[1]

Lactase levels progressively decline during childhood and adolescence, and malabsorption often increases with age.[4-6] Studies on the prevalence of lactose malabsorption showed lower rates of malabsorption in whites than in non-whites.[7,8] The doses and forms of administration of lactose used as diagnostic tool also influence the prevalence rates. Leis et al.,[6] in a study that comprised 850 individuals aged between three and 85 years, found 32.5% of malabsorption with the administration of 2g/kg of lactose in an aqueous solution at 20%. The individuals who tested positive after the dose were retested with the administration of 250 ml of milk and 250 ml of yoghurt. The malabsorption rates were 13.7 and 3.8%, respectively.

Currently, the hydrogen breath test is one the most widely used techniques in the diagnosis of lactose malabsorption.[9] The fermentation of the lactose that is not absorbed by the intestinal flora produces hydrogen. Part of this gas is eliminated by the lungs and can be detected in breath. The increased hydrogen content in breath samples after lactose administration indicates malabsorption and fermentation of this carbohydrate, since there are no other endogenous sources of hydrogen in mammals.[10] The standard dose used on the breath test is 2g/kg of lactose in an aqueous solution at 20%. However, as the hydrogen breath test can detect up to two grams of nonabsorbed lactose, some authors have proposed the use of lower and more physiological doses, in addition to the use of milk or yoghurt as sources of lactose.[6,11]

There are no published articles on lactose malabsorption in infants in the state of Rio Grande do Sul. The extrapolation of data obtained from other regions of Brazil is hindered by racial diversity, which influences the prevalence rates.

The present study aims at determining the prevalence of malabsorption in physiological doses of lactose using the hydrogen breath test. The association of lactose malabsorption with the complexion and age of children and adolescents from public schools in the city of Porto Alegre is also analyzed.

Methods

A cross-sectional study was carried out between May and August 2000. The study population consisted of children and adolescents aged between eight and 18 years, who were attending public schools in the city of Porto Alegre.

Two schools were selected for the study. These schools have a great number of morning groups. Only students of these morning groups were included in the study because the tests had to be performed during school activities and the selected individuals had to fast for at least eight hours. In addition, we selected schools located in the outskirts of Porto Alegre, where we could find a greater number of non-white individuals. The students were selected by randomly drawing the numbers attributed to each student on the roll call. The selected students received a consent form that had to be signed by parents or guardians and be returned before the test. Individuals with the following conditions were not included in the study: a) previous diagnosis of a disease in which milk was contraindicated; b) fasting less than eight hours; c) use of antibiotics, enemas or laxatives fifteen days before the test; d) diarrhea on the test day or in the previous seven days; e) smoking six hours before the test or during the test; f) falling asleep during the test; g) not able to perform the test properly; h) not wanting to participate in the study or no signature on the consent form by parents.

A form with information about each patient was filled out. This form included full name, age, complexion, and anthropometric data. The students were classified as white or non-white, according to their complexion. They were divided into two age groups: eight to 12 years old, 13 to 18 years old. Two indicators of nutritional status were used for each student: weight/age and height/age, and the comparison of the sample with the reference standard (National Center of Health Statistics curves) was made by means of the z score.[12] Students with a z score less than or equal to –2 were considered malnourished.

By using an expected malabsorption rate of 10% between white children and/or adolescents and 30% between non-white students (OR $\geq$ 3), the sample consisted of approximately 150 individuals for a 0.05 alpha level and 0.20 beta level. With this sample size, we could estimate the prevalence of lactose malabsorption with a 6% error margin.

The hydrogen breath test was performed in two stages: collection and storage of the biological material in schools, and the analysis of hydrogen levels by means of gas chromatography at Hospital de Clínicas de Porto Alegre. The Quintron Microlyzer model 12i gas chromatograph was used to measure hydrogen breath level.

A collecting system and vessels supplied by Quintron® were used for the collection and storage of hydrogen breath samples. The first samples were collected early in the morning after an eight-hour fasting period. After that, each participant was given a 250-ml glass of industrialized cow's milk, corresponding to 12.5g of lactose. The subsequent samples were collected at 60, 120 and 180 minutes after milk intake. Lactose malabsorption was characterized by a maximum increase in hydrogen concentration (H_2 delta) was greater than or equal to 20 parts per million (ppm). H_2 delta was calculated as the difference between the baseline

value and the highest value of hydrogen concentration obtained during the test.

The data were processed and analyzed by Epi-Info version 6.04, PEPI version 3.0 and SPSS version 10.0 programs. The quantitative variables were described by the central tendency measures (mean and median) and dispersion (standard deviation) and the categorical variables were expressed in percentages. The categorical variables were compared by the chi-square test with Yates' correction. Student's t test was used for the comparison of the continuous variables means. A P value less than 0.05 was considered to be significant.

The protocol of the present study and the informed consent form were approved by the Research and Ethics Committee of Hospital de Clínicas de Porto Alegre.

Results

Three hundred eight students were selected and were given the informed consent form to participate in the study. Eighty-three students were not included, among which 66 did not bring the consent form signed by parents or guardians, five did not follow the eight-hour fasting requirement, five were using antibiotics, four presented with diarrhea, and three ate during sample collection. One hundred seventy-eight students aged between eight and 12 years were selected and 32 students were not included in the study. In the 13-18 age group, 130 students were selected and 51 were not included in the study (39%) (P< 0.001).

Among the 225 individuals included in the study, 134 (59.6%) were females. The age ranged from eight to 18 years (mean =12.1 ± 2.0 years and median = 11.8 years). One hundred fifty-four students (68.4%) were white, and 71 (31.6%) were non-white. With respect to the nutritional status, the z score mean was 0.16 ± 1.42 for weight/age and –0.17 ± 0.97 for height/age. Five students (2.2%) were considered malnourished (z score less than or equal to -2 for weight/age and/or height/age). The anthropometric data were not obtained from one student only.

The comparison between white and non-white students in terms of age, sex, and nutritional status means did not show significant differences (Table 1).

No significant difference in terms of sex and weight/age z score was observed in either of the age groups. The height/age z score was significantly lower in older children (Table 2).

The hydrogen breath test was positive in 19 of the 225 analyzed patients, showing an 8.4% (95%CI: 5.2- 12.9) prevalence of lactose malabsorption.

The mean hydrogen levels observed in the fasting period and at 60, 120 and 180 minutes after lactose intake in the 19 students whose test results were positive are shown in Figure 1.

Table 1 - Characteristics of children admitted to the PICU

Variables	White n (%)	Non-white n (%)	P
Gender			
Male	61 (39.6)	30 (42.2)	0.819
Female	93 (60.4)	41 (57.8)	
Age (years)			
Median±standard deviation	12.1±1.9	12.2±2.0	0.559
Index (z score±standard deviation)			
Weight/age	-0.01±1.08	0.25±1.08	0.108
Height/age	-0.23±0.96	-0.03±1.00	0.160

The highest increase in hydrogen levels compared to the baseline values on positive tests ranged from 21 to 44, mean of 26.2 ± 5.7. This H_2 delta peak occurred at 120 minutes in three of 19 individuals (16%), and at 180 minutes in the remaining 16 individuals (84%). The first H_2 delta greater than 20 was observed in one individual at 60 minutes (5%), in three at 120 minutes (16%), and in 15 at 180 minutes (79%).

The difference between the prevalence rates of lactose malabsorption in white and non-white students (5.2% x 15.5%) was statistically significant (OR= 3.0; 95%CI= 1.3-7.1; P= 0.02) (Table 3).

Table 2 - Comparison of general characterisitics of students in both age groups

Variables	8-12 years n (%)	13-18 years n (%)	P
Gender			
Male	57 (39.9)	34 (41.5)	0.924
Female	86 (60.1)	48 (58.5)	
Index (z score±standard deviation)			
Weight/age	0.15±1.11	-0.06±1.03	0.183
Height/age	-0.04±0.98	-0.39±0.93	0.009

No statistically significant difference was found in the prevalence of lactose malabsorption as far as age was concerned. Malabsorption occurred in 4.9% of students aged between 13 and 18 years, and in 10.5% of those between eight and 12 years (OR= 0.47; 95%CI= 0.16-1.35; P= 0.227) (Table 4). With respect to complexion, white students aged between eight and 12 years had a malabsorption

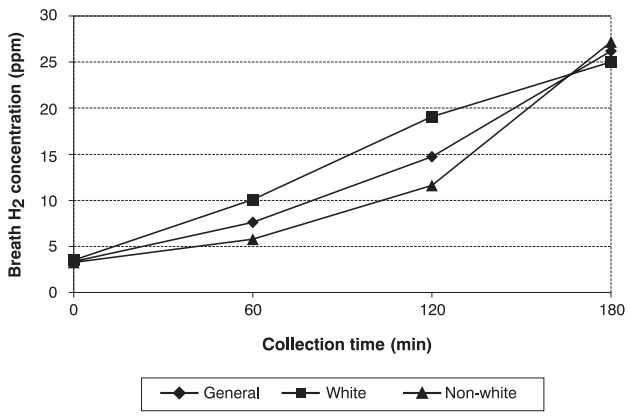

Figure 1 - Mean values of hydrogen concentration in the air expired by students with positive tests

prevalence of 6.9%, and those aged between 13 and 18 showed a prevalence of 1.9%. In non-white students, the prevalence rates were 19% for those aged between eight and 12 years and 10.3% for those between 13 and 18 years (P= 0.506).

Table 3 - Prevalence of lactose malabsorption related to color

Color	n	f	(%)	PR	CI95%	p
White	154	8	5.2			
Non-white	71	11	15.5	3.0	1.3 - 7.1	0.020

n: number of subjects
f: total number of subjects with malabsorption
PR: prevalence ratio
CI: confidence interval

Discussion

Our study assessed the prevalence of lactose malabsorption by means of the hydrogen breath test after the administration of 250ml of whole cow's milk. The test lasted three hours, and the samples were collected at 60, 120 and 180 minutes after milk intake. By using lactose instead of an aqueous solution in the formula, sample collection should extend for three or five hours due to the delayed gastric voiding and excretion of hydrogen.[13,14] In our study, the samples of hydrogen were collected at intervals of 60 minutes. According to Solomons et al.,[15] this interval does not reduce the sensitivity to the test, even if low doses of lactose (12.5 g) are used.

An increase in hydrogen levels to 20 ppm or more in relation to the baseline value was considered as a positivity criterion. In pediatric literature, this is the most frequently adopted criterion, since it has greater specificity.[11,16-19] Some authors, however, use an increase in hydrogen levels greater than 10 ppm.[20,21]

The prevalence of malabsorption of physiological doses of lactose in children and adolescents aged between eight and 18 years was 8.4% in this study. By considering both age groups, the prevalence was 10.5% for students between eight and 12 years, and 4.9% for those between 13 and 18 years (Table 4).

There are few Brazilian publications on lactose malabsorption in children. In addition, lactose is usually given in greater doses and in an aqueous solution, which prevents us from comparing our data with those obtained in other studies. The use of milk would optimize lactose uptake.

Reis et al.[19] carried out a study to assess the prevalence of lactose malabsorption in schoolchildren from the outskirts of Marília, state of São Paulo. Eighty-three students, whose age ranged from seven to 15 years, were included in their study. As diagnostic tool, they used the hydrogen breath test after an 18-gram dose of lactose in an aqueous solution at 10%. With an H_2 delta cutoff point greater than 20 ppm, 19 (22.9%) students were classified as lactose malabsorbers. Although similar doses were used, the prevalence rate found by these authors is higher than the one we obtained in the present study. However, the means for the administration of lactose were different.

Figueiredo[22] conducted a study with Brazilian children in the state of Minas Gerais, in the town of Rio Acima, assessing 435 children between seven and 15 years old by means of the hydrogen breath test after the intake of 25g of lactose in an aqueous solution at 10%. The malabsorption prevalence was 49.2%, while lactose intolerance was 24.3%. The results of that study cannot be compared with ours due to the different doses of lactose and to the means used to administer it.

Table 4 - Prevalence of lactose malabsorption according to age group

Age group	n	f	(%)	PR	CI95%	p
8-12 years	143	15	10.5			
13-18 years	82	4	4.9	0.47	0.16 - 1.35	0.227

n: number of subjects
f: number of subjects with malabsorption
PR: prevalence ratio
CI: confidence interval

Alves et al.[23] assessed 251 children from two indigenous villages in the state of Mato Grosso do Sul and found a lactose malabsorption prevalence rate of 34.5% in children older than four years. No case of malabsorption was observed among the 54 children younger than four years. As diagnostic tool, these authors used the hydrogen breath test. They administered 18g of lactose in an aqueous solution at 10%.

Our results are similar to those obtained by Pitzalis et al.[24] in Rome, Italy. These authors found a lactose malabsorption prevalence rate of 5.7% in 70 children aged between eight and 15 years when using 250 ml of milk.

Lower rates of malabsorption, with the use of similar milk doses, were found in Greece by Ladas et al.,[16] in 150 children between five and 12 years. All the children were submitted to two tests: the first consisted of 2g/kg of lactose, and the second one, carried out four weeks afterwards, consisted of 240 ml of milk. The malabsorption prevalence rates were 46% and 2% for the first and second tests, respectively. If we extrapolate these data to our population, we could infer higher rates of malabsorption with greater doses of lactose.

As far as complexion is concerned, our study revealed malabsorption rates three times higher in non-white than in white students (Table 3). In São Paulo, Troncon et al.[25] also found higher malabsorption rates in non-white patients compared to white individuals (94.45% x 68.75%). These data were obtained from adult patients submitted to the lactose tolerance test. Another study carried out by Seva-Pereira et al.,[8] with adults in São Paulo, showed malabsorption rates of 85% in non-white individuals and 50% in white ones. The study conducted by Figueiredo,[22] in the state of Minas Gerais, with 435 children, also revealed higher rates of malabsorption in black children (60.3%) and in dark-skinned children (50.7%) when compared to white children (37.5%).

Our study did not find any association between lactose malabsorption rates and age of the students. We found a 10.5% malabsorption rate in children aged between eight and 12 years, and 4.9% in children between 13 and 18 years (Table 4). Although the difference showed no statistical difference, there was a tendency towards fewer cases of lactose malabsorption in older children. This paradox between lower rates of malabsorption and aging also occurred in other studies that compared similar age groups.[4,26] In the study conducted by Leis et al.,[6] the malabsorption prevalence rate found among children between six and 13 years (36.4%) was quite similar to that observed in the 14-18 group (36.5%). This tendency observed in our study could be explained if there were more non-white students in the 8-12 age group. However, this tendency persisted even after we established the classification according to complexion. There was a significant difference in one of the nutritional status indicators (height/age z score) between the two age groups analyzed (Table 2). A higher prevalence of severe malnutrition in students aged between eight and 12 years could explain the greater incidence of lactose malabsorption in this age group. Nevertheless, the height/age z score was lower in students between 13 and 18 years old, with lower rates of lactose malabsorption. In addition to random happenstance, another factor could contribute towards these results, since no statistical significance was observed (P= 0.227). It is possible that part of the individuals with lactose intolerance and, consequently, lactose malabsorption, refused to participate in the study. The exclusion rate was significantly higher in the 13-18 age group. This selection bias might have left a great number of students with lactose malabsorption out of this age group and, obviously, out of the whole sample. In this case, the malabsorption rates observed in the present study might have been underestimated.

Our study was somewhat limited in the sense that we could not test the hydrogen-producing capacity of the intestinal flora in the included individuals. However, there are some authors who found a rate of up to 9.2% children whose intestinal flora is unable to produce hydrogen.[27]

Our study assessed the lactose malabsorption prevalence rate and its association with complexion and age of the analyzed students. The major cause of lactose malabsorption in the analyzed age groups was primary lactase deficiency. We should also remember that some of the cases diagnosed in our study might stem from secondary lactase deficiency. However, most children and adolescents did not show malnutrition or chronic diseases. It is also important to remember that cases of diarrhea were ruled out.

The prevalence of lactose malabsorption observed with the administration of 250ml of lactose was 8.4%. The comparison of our results was hampered by the lack of other similar studies carried out in Brazil. This study confirmed the association between lactose malabsorption and complexion. However, we found no association between lactose malabsorption and age. The hydrogen breath test is simple, noninvasive, and ideal for epidemiological studies.

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