

POSSIBLE DISCRIMINATION BETWEEN GENOTYPES OF LACTASE PERSISTENCE PHENOTYPE

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ABSTRACT

Persistence of lactase activity in adult life is accepted to be inherited as an autosomal dominant trait. However, investigation of the frequency of adult-type hypolactasia among Brazilian Caucasoids suggests that homozygous lactose absorbers may be distinguished from heterozygous absorbers. A lactose loading test was applied to 88 healthy adult volunteers, glucose levels being determined in venous blood samples collected before, 20, 40 and 60 minutes after this test. The distribution of the maximum blood glucose rise (MBGR) of the examined individuals, instead of the expected bimodal distribution, suggested trimodality, corresponding to three phenotypes: adult-type hypolactasia (MBGR less than 16 mg%), heterozygous lactose absorbers (MBGR between 16 and 56 mg%) and homozygous lactose absorbers (MBGR of 56 mg% or higher). The observed distribution agrees well with that expected according to the Hardy-Weinberg law.

INTRODUCTION

Lactase (EC 3.2.1.23) activity of the small-intestinal brush border membrane may begin to decrease after weaning, reaching very low levels before six years of age or, at latest, during adolescence (Lisker *et al.*, 1974; Dahlqvist, 1977). The gene for lactase has been cloned and its location on chromosome 2 has been determined recently (Kruse *et al.*, 1988). However, the mechanism leading to the decline of this enzyme is little understood. It may be related to a shorter lifespan of the enterocyte, or to a decreased rate of synthesis (processing or homing) of lactase or to a combination of both causes (Tsuboi *et al.*, 1979; Jonas *et al.*, 1985; Smith and James, 1987).

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This type of lactase deficiency, named *adult-type hypolactasia*, causes lactose malabsorption and is associated with lactose or milk intolerance. This trait is determined by a main autosomal gene in its homozygous state and should not be confused with general disaccharidase deficiency, which is secondary to several small-bowel diseases (Sevá-Pereira *et al.*, 1983).

Adult type hypolactasia is the rule for most human populations, since it is manifested by the majority of adults from the Far East and Middle East Countries, as well as from Africa, being also predominant among Indians from North and South America. The main exceptions thus far are the populations living in or originating from Northern Europe. Among them lactase activity persists among most adults (Sevá-Pereira *et al.*, 1983).

This trait, which enables lactose absorption during adult life, is named *lactase persistence phenotype* and may be associated with idiopathic presenile and senile cataracts (Simoons, 1982; Rinaldi *et al.*, 1984; Spinelli *et al.*, 1987; Bhatnagar *et al.*, 1989). It is determined by an allele of the adult type hypolactasia gene and is considered to be transmitted as a dominant autosomal trait (Sahi *et al.*, 1973; Sahi, 1974; Lisker *et al.*, 1975; Ransome-Kuti *et al.*, 1975; Sahi and Launiala, 1977; Johnson *et al.*, 1977).

The idea that homozygous and heterozygous genotypes of lactase persistence phenotype are indistinguishable comes from the data of Sahi *et al.* (1973) on the distribution of Finnish individuals, according to the maximum blood glucose rise after a lactose tolerance test. Since these authors observed a bimodal distribution, with the antimode falling between 18 and 21 mg% they considered, and it is usually accepted, that after a lactose loading test a maximum blood glucose rise below 20 mg% indicates hypolactasia. The lactase persistence phenotype obviously would be indicated by a maximum blood glucose rise above 20 mg%.

In a previous paper, while investigating the frequency of adult-type hypolactasia in Brazilian populations, we obtained data suggesting that homozygous lactose absorbers may be distinguished from heterozygous individuals (Sevá-Pereira *et al.*, 1983). The present paper adds evidence in support of this suggestion.

SUBJECTS AND METHODS

A lactose loading test was applied to 88 non-consanguineously related Brazilian Caucasoid volunteers (48 males and 40 females) recruited among students and hospital personnel from medical schools of two Brazilian universities (*Universidade Estadual de Campinas* and *Fundação Universidade Federal do Rio Grande*). On average the age of the males (24.04 ± 3.49 years) was not different from that of the females (23.80 ± 4.11 years) ($t = 0.292$; $0.70 < P < 0.80$). All tested individuals were in good health and well nourished. None of them had any history of previous gastro-intestinal diseases, or chronic

ingestion of drugs and/or alcohol. Moreover, three consecutive weekly protoparasitological examinations revealed that none of them had intestinal parasites.

The loading test consisted of 50 g of lactose as a 10% aqueous solution ingested after an overnight fast (Peternel, 1965; McGill and Newcomer, 1967; Sahi, 1974). Glucose levels were determined by the orthotoluidine method (Cooper and McDaniel, 1970) in 3 ml venous blood samples collected before, and then 20, 40 and 60 minutes after the ingestion of lactose, in order to evaluate the maximum blood glucose rise of each individual.

Each subject was questioned about occurrence of cramps, meteorism and diarrhea during the 24 hours following the ingestion of lactose. Lactose intolerance was attributed when any of these symptoms was reported, as recommended by the Protein Advisory Group of the United Nations (1972).

RESULTS

The distribution of the examined individuals by maximum blood glucose rise after the lactose loading test is shown in Figure 1. Since this distribution shows an antimode in the interval between 12 and 16 mg% a maximum blood glucose rise less than 16 mg% after the lactose loading test was considered to indicate adult-type hypolactasia instead of the 20 mg% limit proposed by Sahi *et al.* (1973). On the basis of this criterion adult-type hypolactasia was manifested by 36 out of the 88 Brazilian Caucasoids examined (41%).

Table I shows the frequency of lactose intolerance among the 88 individuals classified according to the presence of adult-type hypolactasia. These two traits are strongly associated ($\chi^2 = 46.357$; 1 d.f.; $P \ll 0.001$) since only four of the 36 persons with adult-type hypolactasia did not complain of clinical symptoms of lactose intolerance, while only eight of 52 subjects with lactase persistence phenotype manifested these symptoms.

DISCUSSION

As is shown in Figure 1 the distribution of Brazilian Caucasoids by maximum blood glucose rise is strikingly different from that observed by Sahi *et al.* (1973) among Finnish individuals. Besides the antimodal value, which discriminates lactose malabsorbers from lactose absorbers (12 to 16 mg%), two other depressions are shown in the distribution: one falling at the 32-36 mg% interval and the other at the 52-56 mg% interval.

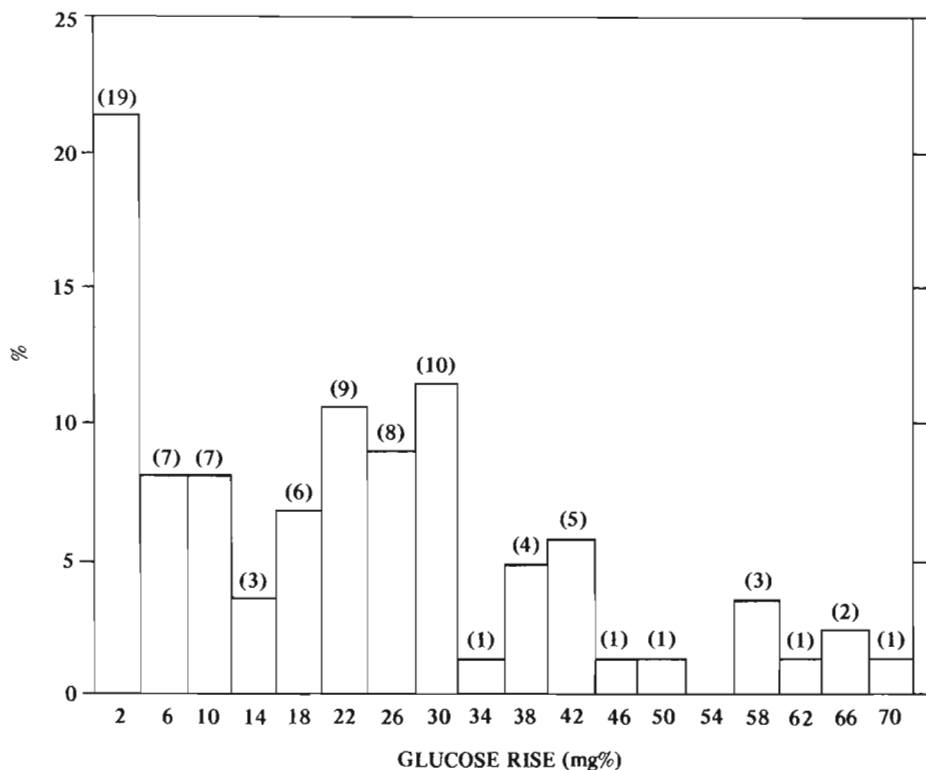


Figure 1 - Distribution of 88 Brazilian Caucasoids by the maximum blood glucose rise after a lactose loading test.

Table I - Frequency of lactose intolerance among 88 Brazilian Caucasoids classified according to the presence of adult-type hypolactasia.

Adult-type hypolactasia	Lactose intolerance		Total
	Yes	No	
Yes	32 (88.9%)*	4	36
No	8 (15.4%)**	44	52
Total	40 (45.5%)	48	88

* 8 without diarrheha

** 3 without diarrheha

A Population Genetics approach favors the hypothesis that 52-56 mg% is a second antimode which discriminates between genotypes of lactase persistence phenotype. If one considers that the group analysed represents a population in Hardy-Weinberg equilibrium, the frequency of the gene for adult-type hypolactasia among Brazilian Caucasoids would be estimated as $q = \sqrt{0.4091} = 0.6396$, while the frequency of its allele, which determines lactase persistence, would have an estimated frequency of $p = 1 - q = 0.3604$. In a random sample of adult individuals we would expect to find $(2nq)/(1+q) = 40.6$ heterozygous and $n - [(2nq)/(1+q)] = 11.4$ homozygous persons among the $n = 52$ lactose absorbers.

These proportions do not differ significantly from the observed ratios, when one takes the interval between 52 and 56 mg% as a true antimode, since 45 individuals are between 16 and 56 mg%, and seven individuals have a maximum blood glucose rise above 56 mg% ($\chi^2 = 2.175$; 1 d.f.; $0.10 < P < 0.20$).

If this hypothesis is true we may have better estimates of the gene frequencies by simple counting, that is

$$q = \frac{36 + 22.5}{88} = 0.6648 \quad \text{and} \quad p = 1 - 0.6648 = 0.3352.$$

Moreover, the observed distribution agrees well with that expected according to Hardy-Weinberg law: $Nq^2 = 38.9$ adult type hypolactasic individuals, $2Npq = 39.2$ heterozygous lactose absorbers, and $Np^2 = 9.9$ homozygous lactose absorbers among the $N = 88$ examined individuals ($\chi^2 = 1.924$; 1 d.f.; $0.10 < P < 0.20$).

If our hypothesis is true, all the eight individuals with lactase persistence phenotype who did complain of clinical symptoms of lactose intolerance would be classified as heterozygous, since their maximum blood glucose rise varied between 16 and 28 mg%.

ACKNOWLEDGMENTS

This work was supported by grants from the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

Publication supported by FAPESP.

RESUMO

A persistência da atividade da lactase na vida adulta tem sido aceita como um fenótipo autossômico dominante. Uma investigação da frequência da hipolactasia do tipo adulto em caucasóides brasileiros sugere, entretanto, que, entre os indivíduos capazes de absorver lactose, os homozigotos podem ser distinguidos dos

heterozigotos. Um teste de tolerância à lactose foi realizado em 88 voluntários adultos, sendo a glicemia determinada em amostras de sangue venoso colhido antes e 20, 40 e 60 minutos depois do teste. Ao invés da esperada distribuição bimodal, a distribuição do aumento máximo da glicemia (MBGR) dos indivíduos examinados sugeriu trimodalidade correspondente a três fenótipos: a hipolactasia do tipo adulto (MBGR menor que 16 mg%), a persistência da atividade da lactase heterozigótica (MBGR entre 16 e 56 mg%) e a persistência da lactase homozigótica (MBGR igual ou superior a 56 mg%). A distribuição observada e a esperada de acordo com a lei de Hardy e Weinberg mostraram bom ajustamento.

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(Received February 14, 1992)