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TREATMENT OF LACTASE DEFICIENCY IN CHILDREN'S OBESITY WITH GENOTYPE C/C 13910 OF LACTASE GENE

LECZENIE NIEDOBORU LAKTOZY U OTYŁYCH DZIECI Z GENOTYPEM C/C 13910 DLA GENU LAKTAZY

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ABSTRACT

Introduction: Excess lactose in the diet of modern man causes the development of not only lactase deficiency, but it can be a factor that contributes to obesity.

The aim: To study associations between obesity and genotype C/C 13910 of lactase gene (*LCT*) in children, to investigate the effectiveness of treatment using drug exogenous lactase and a low-lactose diet.

Materials and methods: genotyping of lactase gene by real-time polymerase chain reaction, determining the level of lactose maldigestion by hydrogen breath test (HBT), estimating the insulin resistance with the HOMA-IR index in 70 obese children and 40 healthy children 6 - 18 years. Obese children with genotype C/C 13910 and lactose maldigestion (n=40) were randomized in two groups: children from group I (n=20) received an exogenous lactase preparation, and children from group II (n=20) - low-lactose diet.

Results: in obese children, the genotype C/C 13910 is 2 times more often than in healthy children. Obese children with genotype C/C 13910 have a significantly higher value of HBT (32.8–39.8 ppm) compared to healthy children ($p<0.05$), and an increased value of the HOMA-IR index. After treatment, there was a significant decrease in HBT and the HOMA-IR index in the two comparison groups.

Conclusions: signs of insulin resistance are observed in children with obesity, genotype C/C 13910 and lactose maldigestion. The use of exogenous lactase in the therapy or the administration of a low-lactose diet cause approximately the same decrease in the HOMA-IR index.

KEY WORDS: hydrogen breath test, genotype C/C 13910, lactose maldigestion, obesity

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INTRODUCTION

The leading cause of the disorder of lactose digestion is the discrepancy between the level of lactose load and the activity of lactase production. Single nucleotide polymorphisms (SNP) of the lactase gene (*LCT*) affect the characteristics of the production of this enzyme during human ontogenesis. According to modern concepts, at position 13910 of the *LCT* gene sequence, the majority of representatives of the human population have cytosine (C). This cytosine base is located approximately 14 kb upstream of the *LCT* transcription site, in the regulatory region of the lactase gene - *MCM6* (minichromosome maintenance complex 6). The gradual age-related decrease in the level of lactase production, most often manifested from the second year of life, is characteristic of the individual's homozygous state (C/C). The homozygous state of C/C 13910 is recorded in, more than 32% of Caucasians, 99% of East Asians, 74% of South Asians and 59% of individuals of other or mixed ethnic groups [1]. The loss of the lactase activity of the lactase and the ability to synthesize lactase at a sufficiently high level throughout life is characteristic of individuals with the SNP of the *LCT* gene, which are characterized by replacement of the cytosine by the thymine base (T) at position 13910. The SNP data of the *LCT* gene lead to the appearance two variants of genotypes: heterozygous (C/T) and homozygous (T/T). Taking into account, that allele T is dominant; both these genotypes are accompanied by lactase persistence (LP) [2; 3; 4; 5].

The maximum level of LCT expression is noted in the duodenum (121.7 RPKM (reads per kilobase per million), and the jejunum (73.8 RPKM) and provides hydrolysis of lactose disaccharide to two monosaccharides - glucose and galactose, capable of being absorbed by intestinal enterocytes [6]. Galactose is metabolized in the liver, where epimerization to glucose occurs upon accession to uridine-5'-diphosphate (UDP), with the formation of UDP-glucose. UDP-glucose is a direct precursor of glycogen synthesis and can not be reversed through UDP-glucose pyrophosphorylase (EC 2.7.7.9) to glucose-1-phosphate in vivo [7]. Due to this pathway, which is perhaps one of the evolutionary advantages of galactose as a lactose, almost 100% of the dietary galactose absorbed from the gastrointestinal tract, is converted into hepatic glycogen. While with lactase deficiency (LD), it becomes impossible.

Most often clinical manifestations of LD begin to be registered from 10 to 16 years of age, which coincides with

the critical period of risk of obesity and the formation of insulin resistance in the pubertal period. It is in adolescents 12-19 years, the incidence of obesity has the highest level - 20.5%, while in the children's population as a whole, this figure is about 17% [8, 9, 10].

It is also interesting that the occurrence of LP in the geographical regions of Europe has a negative feedback from the prevalence of obesity. Thus, the maximum frequency of occurrence of LP, more than 90%, is observed among the population of the Nordic countries of Scandinavia and the Netherlands, and the minimum among the population of southern Europe (Italy and Spain). The lowest prevalence of overweight and obesity in the European Region is registered in Norway and is 15% among children aged 7- 8 years (13.5% for boys and 17.8% for girls), the highest level is in Italy and is 36% (among boys – 37.2% and among girls – 34.7%) [11]. Therefore, we believe that the genotype C/C 13910, associated with adult-type LD, is targeted for the emergence of a systemic inflammatory response and the formation of insulin-resistant obesity [12].

The occurrence of obesity and its complicated course in recent years, especially among children living in large cities, is increasing at an epidemic rate, but the causes of this pathomorphosis remain poorly understood. In particular, the role of lactose in the formation of insulin resistance associated with obesity in children is not reflected.

THE AIM

Objective: to study associations between obesity and genotype C/C 13910 of the lactase gene (*LCT*) in children, to investigate the effectiveness of treatment using drug exogenous lactase and a low-lactose diet.

MATERIALS AND METHODS

We inform that patients gave their informed consent to participate in the study. The study was carried out according to the ethical principles of the Medical Study conducted with the participation of people set forth in the Helsinki Declaration. The study was with the permission of the local bioethics commission in accordance with the requirements of the bioethical committee (protocol No. 2 of the bioethical examination of the State Institution "DMA of Ministry of Health of Ukraine" dated 10.02.2016. Head of the Commission: MD, Professor V.V. Koldunov).

Genotyping was carried out at the polymorphic locus of the *LCT* gene by real-time polymerase chain reaction (RT-PCR) in 70 obese children and 40 healthy children from 6 to 18 years of age. The SNP *LCT* study was carried out in a certified Synevo laboratory, as the analyzer used the detector "CFX96 (BioRad)", USA. The material for the study was venous blood.

To determine insulin resistance, the Homeostasis Model Assessment was calculated, before and after treatment, based on basal insulinemia and blood glucose in venous blood, using the Immunochemical Test Method with Electrochemiluminescent Detection (ECLIA) in the Synevo laboratory. The presence of insulin resistance was recorded with the index HOMA-IR>95th percentile, respectively, with the percentile curves recommended by the IDEFICS consortium for the European population according to the age and sex of the child [13].

A hydrogen breath test with lactose load (at a rate of 1 g/kg but not more than 25 g of lactose in the form of a 10% aqueous solution) was performed at the "Gastro+ Gastrolyser" Gas Analyzer of the British company "Bedfont Scientific Ltd" at the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine. The hydrogen concentration in the patient's exhaled air was determined in parts per million (ppm). The test was considered positive when the level of hydrogen concentration in the exhaled air increased by more than 20 ppm (0.002%) after 60 minutes compared to the basal level and the appearance of clinical symptoms of LD during the next three hours of observation [14]. An increase of H2 in air samples without the appearance of characteristic symptoms indicated the lactose maldigestion, with the appearance of characteristic symptoms - of lactose intolerance [15].

Children with obesity, with genotype C/C 13910 and lactose maldigestion (n=40), to study the effectiveness of various treatments, were randomized in two comparison groups. Patients from group I (n=20) received an exogenous lactase preparation "Mamalac" (National Enzyme Company Inc., USA, Distributor in Ukraine: Pharmunion BSV Development Ltd.), 30 mg (3000 ALU Aspergillus orizae) three times a day for 1 month, and patients in group II (n=20) had a low-lactose diet, according to the computer program „Low- lactose diet" [16]. In addition, all patients and their parents received counseling on lifestyle modification, psychologist's recommendations, in the case of insulin resistance; children over 12 years of age were prescribed metformin at the age-appropriate dosage.

Criteria for the effectiveness of treatment were the normalization of the HOMA-IR index and HBT indices.

Statistical processing of the results of the study used the methods of variation statistics using the STATISTICA software package (version 6.1) StatSoftInc. serial number AGAR 909E415822FA, adapted for biomedical research with the False Discovery Rate control (FDR) to compare the effectiveness of various methods of treatment ($p<0.05$).

RESULTS

Insert the text of the results here. In children with obesity of 6-18 years (n=70), the genotype C/C 13910 was registered in 57.1% (n=40), the genotype of C/T 13910 - in 28.6% (n=20), and the genotype T/T 13910 - in 14.3% of cases (n=10). Of 40 healthy children 6-18 years old, the genotype C/C 13910 was registered in 37.5% (n=15), the genotype of C/T 13910 was 55% (n=22), and the genotype T/T 13910 - in 7.5% of cases (n=3).

The level of hydrogen concentration in the exhaled air, determined using the HBT in obese children and with genotype C/C 13910 was 36.03±3.71 ppm; in children with genotype T/T 13910 – 1.35±0.83 ppm; and in healthy children with genotype C/C 13910 – 14.21±3.54 ppm, $p<0.05$. It is interesting

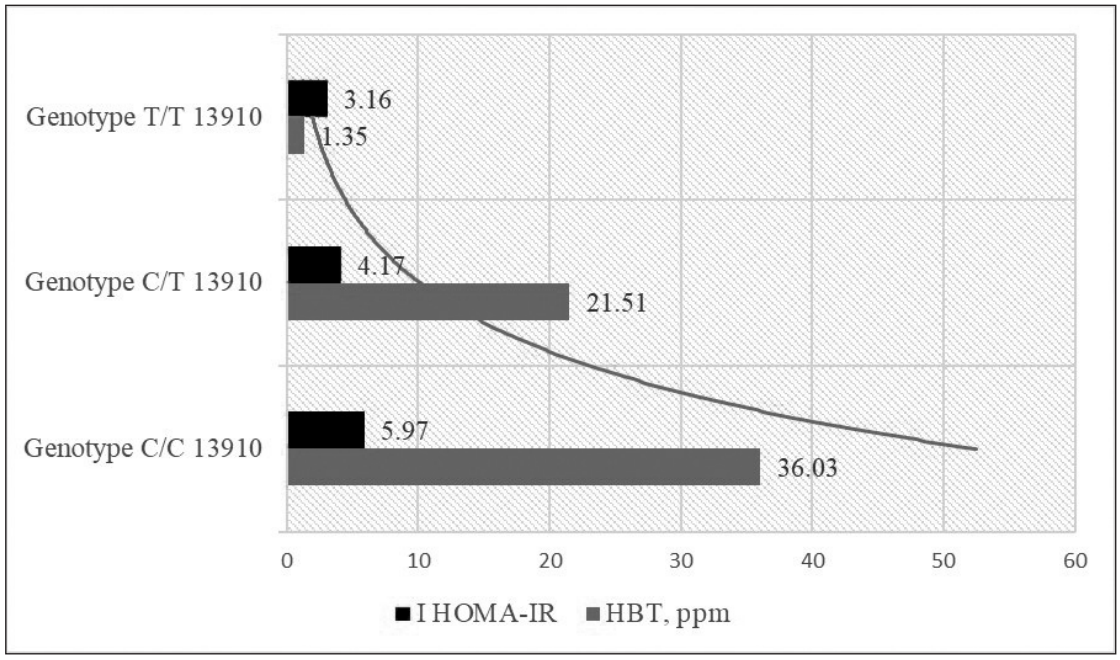

Figure 1. Association figures hydrogen concentration in exhaled air in a HBT and insulin resistance in obese children with genotypes LCT

that the level of hydrogen concentration in the exhaled air with HBT in healthy children with genotype C/C 13910 did not have a statistical difference compared to obese children with the genotype C/T 13910– 21.51±3.1 ppm ($p>0.05$).

It was identified that a high level of HOMA-IR index of 5.97±0.86 was recorded in children with obesity and genotype C/C 13910. With the C/T 13910 genotype, the HOMA-IR index level was 4.17±0.21 and was lower than in genotype C/C 13910 ($p<0.05$). The lowest HOMA-IR index was characteristic of children with obesity and genotype T/T 13910 – 3.16±0.66 ($p<0.05$), figure 1.

The fact that the level of HOMA-IR index in children with obesity and genotype T/T 13910 was comparable to that of healthy children (HOMA-IR index=2.24±0.51, p>0.05) is of great interest. Probably, this circumstance emphasizes the special role of lactase deficiency in the development of insulin resistance in children's obesity.

The study of the effect of diet and pharmacological therapy on insulin resistance in obese children showed that both methods of treatment are quite effective. Thus, in children of group I, before the prescription of the drug for exogenous lactase, the value of the HOMA-IR index was 7.36±0.34, and after one month of treatment they had a significant decrease in 1.85 times, up to 3.97±0.6 ($p<0.05$). In children of group II, the HOMA-IR index before treatment was 5.55±0.68, and after the administration of the low-lactose diet for one month its value decreased by 1.75 times and was 3.16±0.35 ($p<0.05$).

DISCUSSION

Insert the text of the discussion here. The genotype of C/C 13910 in children with obesity aged from 6 to 18 years is 2 times more common than in children with physiological body weight. According to HBT data, the highest frequency of lactose maldigestion is registered among children with genotype C/C 13910, which coincides with the results of other studies [9]. According to the literature, the value of the correlation coefficient between the determination of genotype C/C 13910 by genotyping SNP *LCT* and the probability of a positive HBT is 0.74 [17]. Concetta Santonocito et al. [18] demonstrated that in persons with positive HBT genotype C/C 13910 was determined in 97% of cases. Our study showed that the ratio between the levels of the hydrogen breath test in 70 children with obesity and different genotypes of T/T, C/T, C/C 13910 corresponds to 1:16:27. It follows that in children with obesity and genotype C/C 13910, the risk of lactose maldigestion is 27 times higher than in T/T 13910 genotype. Changes in the results of HBT, which occurred as the consequence of treatment, indicate that the use of exogenous lactase "Mamalac" (National Enzyme Company Inc., USA, Distributor in Ukraine: Pharmunion BSV Development Ltd.) and a low-lactose diet according to our computer program "Low-lactose diet" significantly ($p<0.05$) contribute to lactose digestion in patients with genotype C/C 13910.

Excess lactose in the diet of modern man causes clinical manifestations of lactase deficiency. Food interviewing conducted by Lucyna Ostrowska et al. [19] showed that clinical lactase persistence in adult males correlates with a low HOMA-IR index. Studies Priska Stahel et al. [20] have shown that in male Sprague-Dawley rats, eating a diet that contains 15% galactose increases insulin sensitivity, and the use of higher doses of galactose promotes the formation of type 2 diabetes.

In our study, the level of insulin resistance studied by calculating the HOMA-IR index was directly correlated

with the level of lactose maldigestion in obese children and was the highest in the genotype of C/C 13910, which is identical to the data of other authors [21].

The ratio of HOMA-IR index in children with T/T, C/T, C/C 13910 genotypes was 1:1,3:2. It is likely that the risk of insulin resistance in children with obesity and genotype C/C 13910 is 2 times higher than in genotype T/T 13910. It is possible that the deficiency of lactase is accompanied by the interaction of excess lactose with galectin 9 (Gal-9), which blocks it binding to the Tim-3 receptor and prevents the activation of signaling pathways that activate Treg - cells. Inhibition of the Gal-9/Tim-3 signaling pathway leads to the development of a low-level inflammatory response and, as a consequence, to the formation of insulin resistance [11].

The use of an exogenous lactose drug or a low-lactose diet in treating children with genotype C/C 13910 and obesity, approximately, equally contributes to a decrease in the level of insulin resistance. It should be noted that the administration of exogenous lactase preparations preserves the possibility of taking dairy products, so necessary in the childhood period.

CONCLUSIONS

In this study, we demonstrated for the first time the significance of lactose maldigestion associated with genotype C/C 13910 *LCT*, when insulin resistance is formed in obese children. The genotype of C/C 13910 is 2 times more often detected in obese children than in healthy children and is associated with lactose maldigestion, causing an increase in insulin resistance ($p<0.05$). The use of the drug exogenous lactase or low-lactose diet prevents the formation of insulin resistance in children with obesity and genotype C/C 13910 *LCT*. However, being aware of the fact, that dairy products are the source of other nutrients, we recommend prescribing exogenous lactase preparations especially for young children.

In the future, it is planned to study the effect of lactose maldigestion on the level of expression of the main ligand of the T-cell apoptosis receptor (Tim-3) of galectin 9 in children with obesity and genotype C/C 13910.

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Authors' contributions:
AA analyzed the results of the study, interpreted the research data and drafted the paper. YuS designed the written above study, was in charge of HBT. AN was responsible for data collection in patients from randomized groups. All authors contributed to revisions into the paper. All authors read and approved the final manuscript.

Conflict of interest:
The Authors declare no conflict of interest.

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