Biomarkers of the nutrition quality and environmental impact

Tatyana Mazhaeva[1,2,3], *Yuliya* Chernova[2], *Olga* Chugunova[1], and *Dmitriy* Grashchenkov[1]

[1]Ural State University of Economics, Ekaterinburg, Russian Federation
[2]Ekaterinburg Medical Scientific Center for Prevention and Health Protection of Industrial Workers of Rospotrebnadzor, Ekaterinburg, Russian Federation
[3]Ural State Medical University of the Ministry of Health, Ekaterinburg, Russian Federation

Abstract. Modern "Omic" technologies are increasingly used to isolate biomarkers of the nutrition model. In order to find the impact markers of the food quality and environment on the metabolic profile of preschoolers from two territories with different ecology (main group (Preschool Educational Institution 1) and comparison group (Preschool Educational Institution 2)), a nutrition assessment was carried out (data analysis of the cyclic two-week menu for the Preschool Educational Institution and nutrition outside the Preschool Educational Institution). The results of the food set assessment and the nutritional value of the menu were compared with the norms established by the requirements of legislation and medical recommendations. The content of organic acids in the urine of 60 children was analyzed to identify markers of environmental exposure, metabolic disorders of macro- and micronutrients using gas chromatography and mass spectrometry. It was found that the menu of the Preschool Educational Institution 1 was more rational in terms of the food set in comparison with the menu of the Preschool Educational Institution 2. The menu of the Preschool Educational Institution 2 had insufficient levels of macronutrients necessary for normal metabolism, such as proteins by 1.7%, vegetable fats by 22%, including PUFA by 1.9%, dietary fiber by 10.9%, as well as micronutrients vitamins B1 by 25.9%, PP by 15.2%, and calcium by 18.2%. In the Preschool Educational Institution 1 there was deficiency in the amount of vegetable fats by 8%, vitamin B1 by 13%, and calcium by 19.4%. Analysis of organic acid biomarker data showed that the lowest titer values of markers for branched amino acid metabolism deficiency (valine, leucine, isoleucine) were detected for 3-hydroxy-isovalerian (leucine metabolite) acid in the children of the Preschool Educational Institution 1. However, the average values don't significantly differ (p=0.7). Correlation analysis showed the relationship between the content of nutrients and organic acids in the urine of the children. A high amount of organic acids, markers of vitamin B6, B9, and B12 deficiency were identified in children of both groups. The titers of markers of sulfur-containing amino acids (2-hydroxybutyric and pyroglutamic acids) are higher in children of the Preschool Educational Institution 1, which indicates the need for them in detoxification reactions.

1 Introduction

The environment in which we live is increasingly confirming its negative role in formation of the population health. The child population deserves special attention [1,2], because it is more vulnerable to the influence of destructive environmental factors. Children are more sensitive to the damaging effects of xenobiotics due to the peculiarities of their metabolism and critical stages of the growth and development. Investigation of children's health are widely used in complex scientific researches to study the effects of the environment [1,3,4]. The tense environmental situation carries the risk of developing various diseases in children, especially due to the limited adaptive abilities of the child's body. This is due to the changes in metabolic processes, disorders of the immune and neuroendocrine systems. As a result, the negative trends in the health indicators of the child population continue to persist.

Currently, metabolomics, along with genomics, proteomics, lipidomics, etc., is widely used both in medical practice for personalized effective treatment, and in nutritionology in order to evaluate the nutrition model and prevent metabolic disorders [5,6]. «Omics» technologies are technologies based on the achievements of genomics, transcriptomics, proteomics, and metabolomics. These are sciences that study how the genome is arranged and how the information encoded in it is implemented. How it is transformed into the structure of proteins and, in the future, into some signs of the body that may be important for the diagnosis and treatment of diseases. The results of recent studies identify circulating metabolites and metabolic pathways associated with the use of a high-quality diet. They identify a set of potential biomarkers of nutrition models that can serve to improve the assessment of the quality of food consumed using traditional questionnaires [7]. Based on the identified features, more effective measures to prevent the development of diseases are being developed at the individual level using personalized, systematic, modern research methods [8].

The aim of the study is to search for the markers of the impact of the nutrition quality and the environment on the metabolic profile of children.

2 Research Methods

A comparative assessment of the body's response to the effects of nutrition and the environment impact was carried out in the Preschool Educational Institutions in two territories of the Sverdlovsk region. The study included 98 children (the main group) aged 3 to 7 years, attending the Preschool Educational Institution 1 of the Territory-1 (hereinafter Preschool Educational Institution 1) located in the zone of high aerogenic risk (from 8 to 25). In the control area 2, the Preschool Educational Institution 2 (hereinafter Preschool Educational Institution 2) was selected by random numbers. 99 children aged 3-7 years were examined in it (comparison group). Parents of all children gave voluntary informed consent to the examination. The permission was obtained for the study by the local Ethics Committee of the FBIS «Ekaterinburg Medical Scientific Center for Prevention and Health Protection of Industrial Workers» of Rospotrebnadzor No. 5 dated 12/27/2021.

The nutrition of children outside the Preschool Educational Institution was assessed according to the questionnaire data by a semi-quantitative frequency method. Processing and analysis of the data of the cyclic two-week menu of the Preschool Educational Institution and the nutrition outside the Preschool Educational Institutions was carried out using the computer program «Calculation system for catering» [9]. The results of the assessment of the food set and the nutritional value of the menu were compared with the norms established by the requirements of legislation and recommendations [10,11].

By the method of random numbers, 30 children were selected from two Preschool Educational Institutions, whose nutrition was assessed using the software of the Institute of Nutrition of the Russian Academy of Medical Sciences « «NUTRITEST-IP»®». 60 organic acids in urine were studied to identify markers of environmental exposure, metabolic

disorders of macro and micronutrients. In accordance with the requirements of the standard, the preparation for urine collection was carried out, studies were carried out by gas chromatography and mass-spectrometry [12].

The Microsoft Excel package and the IBM SPSS Statistics 20 program were used for statistical data processing. The analysis of independent and related samples was carried out using the nonparametric Mann-Whitney method. Pearson's $\chi2$ was used to compare differences between categorical variables. The relationship between the parameters was considered significant at a level of $p < 0.05$. The analysis of paired correlations for variables was carried out using the Spearman correlation coefficient.

3 Research Results

The assessment of children's nutrition according to the two-week cyclic menu of the Preschool Educational Institution showed that it is irrational. The results of the analysis of compliance with the norms of fulfillment for the food set showed that in the menu of two Preschool Educational Institutions there were fewer dairy products than the recommended– on average by 11.5%. Meat products were not enough in the Preschool Educational Institution 2, where the norm was not fulfilled almost 2 times. In the same the Preschool Educational Institution the norm for fish was not fulfilled by 29.2%, for vegetables by 25%, similarly for fresh fruits, dried fruits, juices. At the same time for children in the Preschool Educational Institution 2 there was an excess of products containing free sugars by an average of 2 times. That is, the menu of the Preschool Educational Institution 1, compared with the menu of the Preschool Educational Institution 2, was more rational in the food set.

In accordance with the irrational food set of the menu, the Preschool Educational Institution 2 had an insufficient level of the content of macronutrients necessary for normal metabolism, such as proteins by 1.7%, vegetable fats by 22%, including PUFA by 1.9%, dietary fiber by 10.9%, as well as micronutrients of vitamins B1 by 25.9%, PP by 15.2%, and calcium by 18.2%. In the Preschool Educational Institution 1 the amount of vegetable fats was insufficient by 8%, vitamin B1 by 13%, and calcium by 19.4% (Table 1).

Table 1. Comparison of the fulfillment for the food consumption norm in the menu of the Preschool Educational Institution for two comparison groups, %

Nutritional value	Recommended daily rate	Preschool Educational Institution 1 (M±m)	Preschool Educational Institution 2 (M±m)	Reliability of differences in Mann-Whitney groups
Caloric content, kcal	1800,0	1626,6±35,2	1240,4±34,9	p=0,036
Total proteins, g	54	67,5±4,1	43,0±2,3	p=0,003
including proteins of animal origin, g	32,4	45,2±4,7	26,6±2,3	p=0,018
Total fats, g	60	52,3±2,7	38,7±1,6	p=0,028
Including fats of vegetable origin, g	18	14,9±1,3	10,0±1,0	p=0,026
Total carbohydrates, g	7,2	229,2±9,9	187,3±6,7	p=0,049
Mono- and disaccharides, g	261	91,6±5,4	85,2±3,0	
Starch, g	22	118,4±7,9	87,2±5,7	
Dietary fiber, g	0,9	19,2±1,1	14,9±1,0	
Vitamin B1, mg	1	0,7±0,0	0,5±0,0	
Vitamin B2, mg	11	1,5±0,1	0,8±0,1	
Vitamin C, mg	50	63,5±11,2	29,3±4,6	p=0,004

Table 1. Continued

Vitamin A, mcg	500	2637,1±679,4	864,1±154,9	p=0,000
Vitamin E (tocopherol.eq), mg	7	10,4±0,8	7,2±0,5	
Ca, mg	900	653,1±49,5	533,4±56,9	
P, mg	700	1001,9±44,9	728,9±44,4	
Mg, mg	200	232,5±7,2	187,4±7,6	
Fe, mg	10	13,8±1,2	8,0±0,3	p=0,001

The assessment of the daily diet conducted by the semi-quantitative frequency method of the survey showed that the calorie content of the daily diet in children of the Preschool Educational Institution 1 was less by 16.1% of the need, and in children of the Preschool Educational Institution 2 - by 26%. Calorie deficiency in children of the Preschool Educational Institution 1 was detected in 71% of children, and in children of the Preschool Educational Institution 2 – 90.7% (p=0.019). Of the macronutrients, there is a slight deficiency of protein and carbohydrates, as well as vegetable fats. Compared with the Preschool Educational Institution 1, in which the proportion of children with protein deficiency in daily diets is 35.5%, in the Preschool Educational Institution 2 such children are almost 2 times more (68.5%), and the number of children with fat deficiency is 23% (p=0.028).

In the structure of micronutrients of the daily diet of children in the Preschool Educational Institution 2, there is a deficiency of vitamin C by 44.2%, B1 by 15%, calcium by 38.8%, magnesium by 5.1%, as well as violations of the balance of calcium, magnesium and phosphorus (1:0.4:1.6). In children of the Preschool Educational Institution 1, vitamin C deficiency was detected by 21.5%, vitamin B1 – by 36.6%, calcium – by 50.1%, magnesium – by 11.8%. The Ca:Mg:P ratio is 1:0.4:1.7, which does not correspond to the recommended values [20]. The number of children with iron and vitamin A deficiency in the Preschool Educational Institution 2 is 35% higher (p=0.002), and vitamin C is 21% higher (p=0.002).

Analysis of organic acid biomarker data showed that the lowest titer values of markers of branched amino acid metabolism deficiency (valine, leucine, isoleucine) were detected for 3-hydroxy-isovalerian (leucine metabolite) acid in children of the Preschool Educational Institution 1, however, the average values did not significantly differ (p=0.7). The number of children with markers (3-Hydroxyisovaleric acid) of leucine deficiency in the Preschool Educational Institution 2 is less (p=0.02) than in the Preschool Educational Institution 1 and amounts to 14.7% and 44.4%, respectively. As for the markers of the amino acid tryptophan (quinoline, picoline, xanthurenic, kynurenic acids), the titers between the Preschool Educational Institutions also don't significantly differ, the number of children with low titers in children of the Preschool Educational Institution 1 and the Preschool Educational Institution 2 is 9.3% and 7.6%, respectively. As for the markers of deficiency of sulfur-containing amino acids (2-hydroxybutyric and pyroglutamic acids) in the Preschool Educational Institution 2, the number of children with values of below the reference is 15.7%, and the Preschool Educational Institution 1 is 1% (p=0.002). Correlation analysis showed a weak negative relationship between the protein and the titers of glycolic acid (hydroxyacetic acid) (r=-0.32; p=0.04), lactate/pyruvate ratio (r=0.37; p=0.02), as well as positive with tricarballinic acid (r=0.31; p=0.04).

The number of children in the Preschool Educational Institution 2 with heightened titers of markers of energy metabolism disorders and mitochondrial dysfunction [13] identified by intermediate metabolites of the Krebs Cycle (CC): citric acid, cis-aconitic acid, isolic acid, 2-ketoglutaric acid. Significant excess of the average concentration of organic acids in children of Preschool Educational Institution 2 compared with the Preschool Educational Institution 1 were detected by citric acid (p=0.008), cis-aconitic acid (p=0.003), isolic acid

(p=0.024). In the Preschool Educational Institution 1, the number of children with higher reference values of other CC titers, such as succinic acid, fumaric acid, malic acid is 16.7%, 20%, 6.7%, respectively, but no significant differences in the average concentration of these acids were found [13].

According to xanthurenic and kynurenic acids, it is possible to judge vitamin B6 deficiency, which was detected only in 6.7% of children of the Preschool Educational Institution 2 (p=0.04). High titers of formiminoglutamic acid, a marker of folic acid deficiency, were found in 47% in the Preschool Educational Institution 1 and 33% in the Preschool Educational Institution 2, and high titers of methylmalonic acid, a marker of vitamin B12 deficiency, were found in 10% in the Preschool Educational Institution 1 and 20% in the Preschool Educational Institution 2. An excess of metabolites of medium-chain fatty acids (sebacic, adipic, suberic) indicates an excess of saturated fatty acids in the diet, which was detected in 15% of children of the Preschool Educational Institution 1 and 18% of children of the Preschool Educational Institution 2. It was found that the content of glutaric acid is significantly higher (p=0.015) in children in the Preschool Educational Institution 2 (0.83mmol/L) compared with the Preschool Educational Institution 1 (0.66 mmol/L), which may indicate the content of medium-chain fatty acids. In addition, the acid may be a marker of vitamin B1 and B2 deficiency.

The content of hypuric acid (N-benzoyl glycine) in children of the Preschool Educational Institution 1 is 1.75 mmol / l, which is 22.8% higher (p=0.004) compared to the Preschool Educational Institution 2 (1.35 mmol/L), and which indicates that children of the Preschool Educational Institution 1 consume more vegetables and fruits than children of the Preschool Educational Institution 2.

The correlation analysis showed the relationship between the content of nutrients and organic acids in the urine of children. So the content of 3-Methyl-2-oxovaleric acid (3-methyl-2-oxopentane), 3-Hydroxy-3-methylglutaric acid (meglutol), pyroglutamic acid (5-oxoproline) it is negatively correlated with the energy value of the children's diet (r= -0.34, p=,003; r= -0.38, p=0.01; r= -0.36, p=0.02, respectively). The ratio of organic acids lactate \ pyruvate, titers of glycolic acid (hydroxyacetic acid), tricarballylic acid (1,2,3-propantricaboxylic acid) correlate with the protein content in the diet (r= - 0.37, p=,001; r= -0.32, p=0.04; r= - 0.31, p=0.04, respectively).

The content of pyroglutamic acid is negatively correlated with the energy value of the diet (r= -0.36; p=0.02), the content of total fat, polyunsaturated fatty acids PUFA (r= -0.35; p=0.02), omega-6 fatty acids (r= -0.32; p=0.04), mono-disaccharides (r= -0.33; p=0.03), added sugar (r= -0.35; p=0.02), total carbohydrates (r= -0.37; p=0.02), niacin (r= -0.45; p=0.03).

4 Discussion

We used data on the content of organic acids in order to identify markers of the nutrition pattern and metabolism of individual food substances, as well as to identify markers of exposure to toxic substances. The obtained data on the markers of individual amino acids (leucine, valine, isoleucine, phenylalanine, tyrosine, tryptophan, glycine, lysine) showed that, in general, the titers of organic acids indicate a sufficient intake of proteins with the diets of children of two the Preschool Educational Institutions. At the same time, about 30% of children in the Preschool Educational Institution 2 have heightened titers of markers of energy metabolism disorders and mitochondrial dysfunction, determined by intermediate metabolites of the central nervous system (citric, cis-aconite, isolimonic and 2-ketoglutaric acids). They may increase due to high carbohydrate intake in children of the Preschool Educational Institution 2. In the Preschool Educational Institution 1, located in an ecologically disadvantaged area, there are more children with high titers of other markers of

the same cycle (succinic, fumaric and malic acid), due to possible excessive fat consumption. At the same time, it should be noted that an increase in the production of pyruvic acid may contribute to the excessive consumption of free sugars observed in the Preschool Educational Institution 2.

A high amount of organic acids, markers of vitamin sufficiency may indicate a deficiency of vitamins B6, B9, B12. According to literature sources, more than 95% of the tryptophan supplied with food is transformed by the Kynurenic pathway (KP) into kynurenic and xanthurenic acids, followed by conversion into nicotinic acid (NIA+). The latter occurs under the influence of the immune system in case of an urgent need for the production of NIA+ in immune cells. Vitamin B6, more precisely its active metabolite pyridoxal-5-phosphate, is a cofactor of kynureninase, which transforms kynurenine into quinolic acid. In the case of activation of the immune system, its cells require more energy and a cofactor in the form of vitamin B6. At the same time the activity of the Kynurenine pathway becomes higher and the number of its metabolites in the urine increases, which indicates a deficiency of B6 [14-16]. High titers of xanthurenic and quinorenic organic acids (above the reference values or closer to the upper limit) are more common in children of the Preschool Educational Institution 2.

Formiminoglutamic and methylmalonic organic acids, markers of vitamin B9 and B12 sufficiency, which interact closely with each other through the folate cycle and they are interdependent. The scientists knew about the excretion large amounts of formiminoglutamic acid in the urine during a period of B12 deficiency in the body the 1960s [16]. A greater deficiency of vitamin B9 was detected in the Preschool Educational Institution 1 in 47% of children, but in the Preschool Educational Institution 2 it is in 33%. The deficiency of B12 is in the Preschool Educational Institution 1 in 10%, and in the Preschool Educational Institution 2 in 20% of children, i.e. in both Preschool Educational Institutions the risks associated with impaired metabolic processes of the folate cycle are almost the same.

The cofactors of glutathione formation from sulfur-containing amino acids: folic acid (B9), methylcobalamin (B12) and pyrodoxal-5-phosphate (B6) affect the functionality of the folate cycle through S-adenosyl-L-methionine or through the gamma-glutamyl cycle [17-18]. The titers of markers of sulfur-containing amino acids (2-hydroxybutyric and pyroglutamic acid) are deficient in 15.7% of children in the Preschool Educational Institution 2, which may indicate their lack in the diet. On the contrary, in children of Preschool Educational Institution 1, the total number of these organic acids is more by 50.41 mmol/mol of creatinine, which indicates the need in children of Preschool Educational Institution 1 for glutathione and vitamins (B12, B9) as suppliers of methyl groups in detoxification reactions [19-22].

5 Conclusion

The obtained data on the negative correlation between the content of pyroglutamic acid and food substances, with high consumption contributing to the risk of overweight (energy value of the diet, total fat content, mono-disaccharides, added sugar, total carbohydrates) can be used as a marker of a high-calorie nutrition model. In addition, data on the quantitative content of individual organic acids, indicating excessive carbohydrate intake, deficiency of meat, vegetable products and fruits, vitamins B6, B9 and B12 in the Preschool Educational Institution 2 may also indicate an irrational model of nutrition of children in the Preschool Educational Institution 2. It needs more correction than in the Preschool Educational Institution 1. The diet of the Preschool Educational Institution 1 should be adjusted according to the detoxification model, aimed at reducing the negative impact of environmental factors.

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