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Improving milk quality to prevent microelement deficiencies: a socio-hygienic perspective on adding bioavailable trace elements

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ABSTRACT

Based on the study of actual nutrition and the availability of macro- and microelements, it was found that the adult population of the North Caucasus Federal District (NCFD) of Russia belongs to the risk group for the development of micronutrient insufficiency associated with a low content in the diet of several essential elements (copper, zinc, calcium, selenium), which are a priority for correction. This is because 89% of the population in the NCFD has a diet that is significantly out of balance both quantitatively and qualitatively, negatively impacting nutritional status and the dispersion of trace elements. It was found that a significant part of the population of the NCFD is characterized by a lack of dairy products in the diet (59.8%), as well as insufficient intake of vitamins B2, B6, C, PP, folic acid, I, Se, Cu, Zn, Mg, Ca, fiber, polyunsaturated fatty acids, tryptophan. Volunteers were selected for the experiment – adult men living in the NCFD. The volunteers took 200 ml of “Voznesenovskiy Ecoproduct” milk (2.5% fatness) for 60 days, produced by a local enterprise using the technology proposed by the authors. Significant violations of mineral metabolism were found in 68.3% of the population at the start of the trial, according to the findings of screening examinations conducted on the hair of the experiment's volunteer participants. Among the priorities for the correction of essential elements are: Se (deficiency in 88.2% of the examined), J (82.2%), Cu (59.1%), Zn (66.7%), Ca (29.8%). The proportion of people with calcium deficiency decreased from 29.8 to 21.5%, copper from 59.1 to 36.2%, selenium from 88.2 to 72.4%, zinc from 66.7 to 38.4%, and iodine from 82.2 to 68.4% when “Voznesenovskiy Ecoproduct” was added to the milk diet. At the end of the preventive course, an increase in the concentration in the hair was noted: calcium (by 26.6%), zinc (by 11.0%), copper (by 10.1%), iodine (by 32.5%) and selenium (by 38.9%). Regular consumption of “Voznesenovskiy Ecoproduct” milk allowed to increase the consumption of dairy products among the study participants, to receive a rapid physiological response of the body in the form of an increase in the content of the studied micro- and macroelements in the hair, reducing the number of people with calcium, zinc and selenium deficiency.

Keywords: milk, microelementosis, fortification, rational nutrition

INTRODUCTION

To minimize the prevalence of hypovitaminosis and microelementosis, which have become pervasive, it is currently of special importance to research the reasons and devise strategies for improving population nutrition [1]. World experience shows that the most effective and economical way to improve the supply of micronutrients to the population is the regular inclusion of specialized mass-consumption foods enriched with vitamins and trace elements [2], [3].

Studies of the actual nutrition of the population in various countries have shown the presence of both general and specific problems depending on socioeconomic, environmental and industrial factors, as well as on the dietary traditions of a particular population group [4], [5]. This negative process is expressed by the chronization of human diseases, an increase in mortality in the employable population, an increase in mortality in childhood, and a decrease in the birth rate and average life expectancy of a person. The state of health is not least determined by

malnutrition. A special role in the normal functioning of all physiological systems of the body is assigned to trace elements, which are part of at least 2000 enzymes that catalyze a variety of biochemical reactions [6], [7].

Trace elements enter plants from the soil, and animals and humans receive them with food [8]. It has been established that higher mortality from cardiovascular diseases is observed with a general deficiency of trace elements in the soil. Thus, in Europe, the highest mortality from coronary artery disease is observed in the northern regions of Great Britain and the northeastern region of Finland, where podzol soils with a deficiency of trace elements predominate [9]. Stomach and lung cancer is more common among residents of settlements located on soils poor in selenium, gededz, cobalt, and zinc [10], [11], [12]. 80% of the Russian population has an inadequate selenium supply (less than 70 µg/l) [13].

Violation of trace element metabolism and imbalance of trace elements involved in maintaining homeostasis and normal functioning of the human body at the cell level must be considered in treating a variety of diseases [14]. The main micronutrient risks have been identified in the North Caucasus Federal District of Russia, and the leading directions for the prevention of alimentary-dependent diseases, primarily oncological, cardiovascular, and endocrine diseases, have been identified [15]. One of the most effective solutions to the problem of deficiency of essential trace elements can be the development of daily diet foods enriched with essential trace elements and bioavailable form following the established indicators of deficiency of specific trace elements in a particular region. This study aimed to solve the problem in the direction of preventive nutrition on the territory of Russia's North Caucasus Federal District (NCFD).

Scientific Hypothesis

Enriching milk with essential trace elements in bioavailable form is an effective solution in the fight against microelementosis. In particular, daily consumption of milk enriched with calcium, zinc, copper and iodine will correct the deficiency of these elements in the body.

MATERIAL AND METHODOLOGY

Samples

For the experiment, Ecoproduct Voznesenovskiy (Voznesenovskoye, Russia) produced specialized milk (2.5% fatness) enriched with trace elements. To study the effect of milk on the microelement balance of the experimental participants, the hair of adult volunteers living in the NCFD was used as the experimental samples.

Chemicals

We used reagents of recognized analytical purity and distilled water. For atomic emission spectroscopy and mass spectrometry with inductively coupled argon plasma, state standard samples of Calcium, Copper, Iron, Iodine, Magnesium, Selenium, and Zinc, were purchased in LenReactive LLC (Sants Petersburg, Russia).

Animals and Biological Material

The work used biological material - the hair of adult volunteers living in the NCFD.

Instruments

Laboratory Spray Dryer BIORUS BIO-8000 (BIORUS, Moscow, Russia), Muffle furnace UED-7-10D (UED, Saint Petersburg, Russia), atomic emission spectrometer with microwave plasma Agilent 4210 (Agilent, Santa Clara, CA, USA), inductively coupled argon plasma mass spectrometer NexION 350 IPS-MS (PerkinElmer, Akron, Ohio, USA).

Laboratory Methods

The study of the actual nutrition of the adult population of the NCFD (n = 1000) was carried out by the method of 24-hour (daily) reproduction of nutrition recommended for these purposes by the Federal State Budgetary Research Institute of Nutrition (Moscow, Russia) [16]. Additionally, a specially designed questionnaire was used.

The macro-, and microelement composition of "Voznesenovskiy Ecoproduct" milk was studied by atomic emission and mass spectrometry with inductively coupled argon plasma.

To assess the preventive effect of milk enriched with trace elements, a before-after study was conducted on 200 adult volunteers aged 16-59 years living in the territory of the NCFD and selected following the inclusion-exclusion criteria (informed consent to participate in the study, absence of acute, diseases, chronic diseases in the stage of exacerbation or decompensation) who took 200 ml of the product daily for 60 days. The change in the amount of macro- and microelements in the body served as the yardstick for measuring the study's effectiveness (according to the hair mineralograms at the points "before" and "after" the course of taking the product).

In parallel, the actual nutrition was monitored by analyzing the frequency of food consumption.

Determination of the content of mineral elements in the hair was carried out by atomic emission and mass spectrometry with inductively coupled argon plasma. The selection of persons for the study (n = 50) was carried

out among the participants of the previous stage of the study, subject to their informed consent. The sample was representative.

Description of the Experiment

Sample preparation: “Voznesenovskiy Ecoproduct” milk was dried on a BIO RU BIO-8000 laboratory spray dryer (BIO RUS, Moscow, Russia). Milk powder was mineralized in a muffle furnace UID-7-10D (UED, Saint Petersburg, Russia) and sent to atomic emission spectroscopy and mass spectrometry with inductively coupled argon plasma to determine the mineral composition.

The hair was taken from adult volunteers naturally using tweezers (3 hairs per selection). The selected hair was placed in a sterile bag with a tag containing the person's contact details, the date and the time of selection. Before studying the trace element composition, the hair was mineralized in a muffle furnace UID-7-10D (UED, Saint Petersburg, Russia) and sent to atomic emission spectroscopy and mass spectrometry with inductively coupled argon plasma.

Number of samples analyzed: 301

Number of repeated analyses: 903

Number of experiment replication: 1

Design of the experiment: The subject of the study was the adult population of the NCFD, the subject of the study was the structure of nutrition of the population, and the object of the study was the content of trace elements in biosubstrates (hair). In the course of the work, the preventive effectiveness of “Voznesenovskiy Ecoproduct” milk was established. At the first stage of the study, the study of the actual nutrition of the adult population of the NCFD ($n = 1000$) was carried out by the method of 24-hour (daily) reproduction of nutrition recommended for these purposes by the FSBI Research Institute of Nutrition (Moscow, Russia) [16]. Additionally, a specially designed questionnaire was used.

A “before-after” study was conducted on 200 adult volunteers aged 16-59 living in the North Caucasus Federal District to assess the preventive efficacy of milk enriched with microelements. Volunteers were selected using online survey for adults living in the suding region. Volunteers participating in the experiment were daily given “Voznesenovskiy Ecoproduct” milk enriched with macro- and microelements. The milk required for the experiment was provided by Ecoproduct Voznesenovskiy (Voznesenovskoye, Russia). The elemental composition of milk was studied at the beginning of the experiment by atomic emission spectrometry and mass spectrometry with inductively coupled argon plasma. Standardized milk (2.5% fatness) “Molochnaya legenda” (Nalchik Dairy Plant, Nalchik, Russia) was used as a control sample for comparison. Conclusions on the preventive efficacy of milk were made based on the analysis of the elemental composition of hair.

Determination of the content of mineral elements in the hair was carried out using atomic emission and mass spectrometry with inductively coupled argon plasma. The selection of individuals for the study ($n = 50$) was carried out from among the participants of the previous stage of the study, provided that their informed consent was obtained. The sample was representative.

Statistical Analysis

The normality of the distribution of quantitative traits was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and the hypotheses about the equality of general variances were tested using the Levene test. To compare the numerical data of two independent groups, the Mann-Whitney U test was used; to compare the qualitative data of two or more independent groups, the Fisher test was used.

RESULTS AND DISCUSSION

An assessment of the structure of actual nutrition and consumption of basic nutrients by the population of the NCFD showed a relatively low amount of fish products in the diet (377.2 ± 5.3 g/day), as well as dairy products (296.1 ± 7.4 g/day) which are significantly less than the recommended values. Insufficient consumption of dairy products (including cottage cheese, sour cream, cheese, etc.) was observed in 59.8% of the population. The importance of the consumption of milk and dairy products as a part of rational nutrition was mentioned in several works [17], [18], [19]. Moreover, recent studies have found a correlation between insufficient consumption of dairy products and the risks of cardiovascular disease [20], diabetes [21], lymphoma [22] and even bones fracture [23].

When analyzing the balance of the diet, it was found that only 11% of the population had the content of basic nutrients within the recommended values. The optimal ratio between energy consumption and the energy value of the diet was 14% of the population. An unbalanced diet in terms of the ratio of proteins: fats: carbohydrates was observed in 89% of the population.

Dietary protein consumed by the population of the NCFD contained the only limiting amino acid, tryptophan (75.7%). The main sources of protein, providing a third of its amount, were meat and meat products, 21% of the

protein came from bakery products, 14% from dairy products. The data obtained are consistent with those presented by Chmyrev et al. [16].

In general, the daily intake profile of the most important nutrients by the population of the NCFD was characterized by a significant lack of vitamins B2, B6, C, PP, folic acid, a number of macro- and microelements, fiber, polyunsaturated fatty acids (PUFA) with excessive consumption of salt, cholesterol, triglycerides and alcohol. This is in line with the trend declared by Gerasimov et al. [24].

The values of the daily intake of a number of the most important micronutrients are given in Table 1. The largest proportion of people with insufficient consumption was noted among women, mainly in the age groups of 30–39 years old (zinc, copper) and 18–29 years old (calcium, selenium, iodine). The median daily intake of selenium was 38.3 µg for women and 46.0 µg for men, about half of the recommended intake. The diet's low calcium content (655.9 mg in men and 566.1 mg in women) also deserves close attention, consistent with the above data on insufficient amounts of milk and dairy products, vegetables and fruits (Table 1).

Table 1 Values of the average daily intake of the most important macro- and microelements by the population of the NCFD by sex and age.

Index	Age, years	Man		Women	
		median	rate of consumption below RDI*	median	rate of consumption below RDI
Calcium, mg	18-29	566.5	66.7	476.6	99.9
	30-39	549.0	70.1	517.2	81.3
	≥40	652.5	65.4	585.4	78.3
	≥18	655.9	63.9	566.1	79.1
Copper, µg	18-29	1450.1	42.3	1118.9	42.1
	30-39	1473.8	44.5	1181.2	46.5
	≥40	1488.8	41.6	1193.0	42.3
	≥18	1455.3	41.3	1133.7	42.8
Zinc, µg	18-29	8628.1	51.2	7707.2	65.1
	30-39	9129.2	47.8	7816.3	67.6
	≥40	9207.7	46.2	7833.9	64.9
	≥18	9044.2	46.0	7819.1	65.2
Selenium, µg	18-29	55.2	49.7	27.9	77.1
	30-39	47.7	49.1	28.8	76.5
	≥40	46.3	50.2	36.6	65.2
	≥18	46.0	51.8	38.3	68.9
Iodine, µg	18-29	77.3	76.6	58.1	65.3
	30-39	77.8	78.2	48.7	56.6
	≥40	66.5	70.5	45.3	55.1
	≥18	66.9	71.3	49.7	58.6

Note: * RDI – recommended daily intake.

As a result, the NCFD adult population's diet was characterized by a quantitative and qualitative imbalance, which contributed to negative changes in nutritional status and the proliferation of microelementoses [25], [26].

In a study of the provision of the body of residents of the NCFD with chemical bioelements, it was found that significant violations of mineral metabolism (moderate and pronounced) were present in 70.5% of the population [16]. The priority essential elements for correction were: Se (deficiency in 88.2%), I (82.2%), Cu (59.1%), Zn (66.7%) and Ca (29.8%). Thus, based both on the data of the assessment of actual nutrition and on the results of the assessment of the provision of the population with macro- and microelements, micronutrients were identified that are a priority in terms of correction for the adult population of the NCFD: folic acid, vitamins PP, B2, B6 and C, minerals: I, Se, Cu, Zn, Mg, Ca. One of the most effective areas of population prevention of pathology associated with micronutrient deficiencies is the enrichment of essential foods with them [27], [28].

Considering the range of micronutrients identified as priorities for correction, we have developed the composition of new preventive milk, considering the enrichment principles given in several works [29], [30], [31]. As enrichers, it is proposed to use a mineral premix (Ca, Zn, Cu) and an additive containing selenopyran. The content of introduced micronutrients (Ca, Zn, Cu, Se) in one serving ranged from 15 to 25% of the RDI, and from the upper safe intake level – from 7.6% (copper) to 19.3% (zinc). The presence of additives did not change the organoleptic properties of the enriched product, as in other works [32], [33]. Following the developed technology, regulatory and technical documentation was developed, permits were obtained for the production of innovative milk in the conditions of Ecoproduct Voznesenovskoye (Voznesenovskoye, Russia). Milk “Voznesenovskoye Ecoproduct” is a source of the organic form of selenium, which is best absorbed and does not have toxicity even at very high concentrations [13], [34]. The introduction of this form of selenium into a dairy product containing tryptophan contributes to the rapid inclusion of both components in metabolism [35]. To exclude antagonism in the process of assimilation, the compatibility and interaction of the introduced mineral substances were taken into account, based on the works [36], [37].

Before starting the experiment with volunteers, we studied the mineral composition of “Voznesenovskoye Ecoproduct” milk. The research results are presented in Table 2.

Table 2 Mineral composition of “Voznesenovskoye Ecoproduct” milk.

Element	Content in “Molochnaya legenda” milk (2.5% fatness)	Content in “Voznesenovskoye Ecoproduct” milk (2.5% fatness)
Calcium, mg/l	24.7 ±1.2	35.6 ±1.7
Copper, µg/l	14.9 ±0.8	48.8 ±2.3
Iron, µg/l	76.6 ±3.1	214.3 ±8.1
Iodine, µg/l	9.5 ±0.4	20.2 ±0.9
Magnesium, µg/l	22.7 ±0.9	43.8 ±1.6
Selenium, µg/l	2.2 ±0.1	8.1 ±0.6
Zinc, µg/l	0.4 ±0.1	2.3 ±0.2

To assess the preventive efficacy of the bioproduct in relation to the provision of the body with micronutrients, a before-after” study was conducted with the involvement of a group of volunteers (n = 200) [38]. At the first point (starting stage), 88.2% of the hair examined for the content of microelements had an insufficient supply of selenium, 82.2% – iodine, 66.7% – zinc, 59.1% – copper, 29.8% – calcium (Figure 1). The inclusion of “Voznesenovskoye Ecoproduct” milk in the daily diet resulted in a decrease in the proportion of persons with calcium deficiency (from 29.8 to 21.5%, $p = 0.017$), copper (from 59.1 to 36.2%, $p < 0.001$), selenium (from 88.2 to 72.4%, $p = 0.022$), zinc (from 66.7 to 38.4%, $p < 0.001$) and iodine (from 82.2 to 68.4%, $p < 0.001$). As a result of the intervention (inclusion of “Voznesenovskoye Ecoproduct” milk in the daily diet), the concentrations of elements in biosubstrates increased (Table 3). On average, the increase in hair concentrations for calcium was 26.6% ($p = 0.032$), zinc – 11.0% ($p = 0.002$), copper – 10.1% ($p < 0.001$), iodine – 32.5% ($p < 0.001$) and selenium – 38.9% ($p < 0.001$). At the same time, the proportion of individuals with a deficiency of the studied elements decreased [39].

Table 3 The content of elements in the hair of the subjects at the points “before” and “after” a 2-month prophylactic course with “Voznesenovskoye Ecoproduct” milk (in µg/g; n = 50; Wilcoxon paired test).

Element	P25	P50	P75	p (before-after)
Ca before	863.72	1615.49	2692.11	
Ca after	853.51	1998.45	2591.07	0.032
Cu before	7.85	8.54	10.20	
Cu after	8.13	9.68	11.61	<0.001
Se before	0.21	0.51	0.66	
Se after	0.28	0.60	0.68	<0.001
I before	0.16	0.47	0.72	
I after	0.19	0.53	0.71	<0.001
Zn before	122.17	153.08	169.79	
Zn after	140.22	178.15	195.77	0.002

The normal content of selenium in hair is 0.7-1.5 µg/g. The level of 0.3-0.7 µg/g corresponds to suboptimal provision [13].

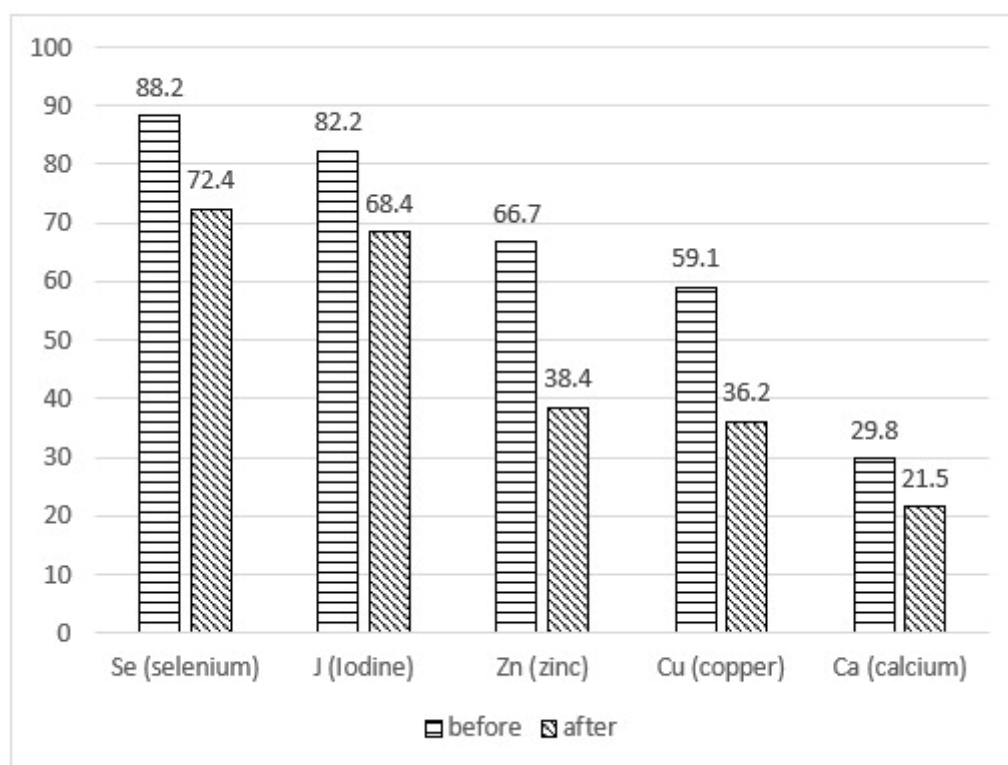


Figure 1 The share of persons with insufficient provision of macro- and microelements at the points "before" and "after" a 2-month prophylactic course with "Voznsenovsky Ecoproduct" milk.

Evaluation of the effectiveness of this intervention showed the possibility of correction and prevention of conditions caused by absolute and relative deficiency of calcium, copper, zinc, iodine and selenium [40], [41]. Given that, according to the analysis of actual nutrition, some vitamins are classified as deficient nutrients, it is important to research to study the provision of the population with water-soluble vitamins and consider the possibility of creating products enriched with a complex of micronutrients.

Several works are worth considering regarding enriching milk and dairy products with essential micronutrients. Pfrimer et al. obtained milk richer in vitamin E, polyunsaturated fatty acids and Se produced by cows fed a diet supplemented with these nutrients. Immunologic analysis revealed a positive effect of the consumption of biofortified milk on inflammation in institutionalized older people [42]. Adegboye et al. in their study [43] prepared Vitamin D and calcium-fortified milk for pregnant women with periodontitis. Clinical trials showed that fortified milk helps women to deal with issues related to metabolic disorders and inflammation associated with periodontitis, which may have important health consequences for the pregnant woman and her offspring. With a similar product, Khadgawat et al. carried out a study on the effect of milk reached with vitamin D on the status of healthy school children aged 10-14 years (300 boys and 413 girls) [44]. The authors found that fortifying milk for 12 weeks is a safe and effective strategy for dealing with widespread vitamin D deficiency in school children. Sharifan et al. found that intake of fortified dairy products containing nano-encapsulated vitamin D₃ was associated with improved anthropometric indices, glucose homeostasis, and lipid profiles, particularly in individuals receiving fortified milk [45]. The authors declared that along with other benefits, fortifying dairy products with vitamin D may be a practical approach to improve some cardiometabolic indicators, such as insulin resistance. All these results prove that milk can be used for biocorrection and balance of the physiological status of people of different genders, ages and activities. However, the most promising direction is the correction of microelementosis by diagnosing the regional characteristics of the microelement status of the population and the development of enriched milk and dairy products of regional significance. In the future, we plan to develop this project in other regions and various population groups.

CONCLUSION

Nutrition of the North Caucasus Federal District population is characterized by a significant imbalance in the diet in quantitative and qualitative terms (89% of the population), contributing to adverse changes in the nutritional status and the spread of microelementoses. A significant part of the population is characterized by a lack of dairy products in the diet (59.8%), as well as insufficient intake of vitamins B2, B6, C, PP, folic acid, I, Se, Cu, Zn, Mg, Ca, fiber, PUFA, tryptophan. According to the results of screening studies of hair, significant disorders of mineral metabolism were present in 68.3% of the population. Among the priorities for the correction of essential elements are: Se (deficiency in 88.2% of the examined), J (82.2%), Cu (59.1%), Zn (66.7%), Ca (29.8%). The inclusion of “Voznsenovskiy Ecoproduct” in the diet of milk resulted in a decrease in the proportion of persons with calcium deficiency (from 29.8 to 21.5%), copper (from 59.1 to 36.2%), selenium (from 88.2 to 72.4%), zinc (from 66.7 to 38.4%) and iodine (from 82.2 to 68.4%). At the end of the preventive course, an increase in the concentration in the hair was noted: calcium (by 26.6%), zinc (by 11.0%), copper (by 10.1%), iodine (by 32.5%) and selenium (by 38.9%). Regular consumption of “Voznesenovskiy Ecoproduct” milk allowed to increase in the consumption of dairy products among the study participants to receive a rapid physiological response of the body in the form of an increase in the content of the studied micro- and macroelements in the hair, reducing the number of people with calcium, zinc and selenium deficiency. Thus, it is necessary to recommend using this enriched product in the nutrition of the North Caucasus Federal District population, as well as other regions and countries, and to continue studying the long-term preventive effects of the systematic use of the product under study.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

To study the effect of milk on the microelement balance of the experimental participants, the hair of adult volunteers living in the NCFD was used as the experimental samples. A corresponding agreement was concluded with all participants of the experiment. The experiment was approved by ethics commission of North Ossetian State Medical Academy (Protocol #SOGMA_1/22). Additional information is available upon request from the author for correspondence.

Limitations:

The study does not consider the reasons for the population's inadequate nutrition, such as cultural or economic factors, which could have an impact on the design of interventions to address the nutritional issues. Additionally, the article does not explore the association between the population's nutritional status and their health outcomes. Further research will explore the underlying causes of the inadequate nutrition and the health consequences of the population's poor nutritional status.

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