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BROWNING MARKERS IN ADULT INDIGENOUS RESIDENTS OF YAKUTIA IN CONDITIONS OF NATURAL COLD

In this work, the expression profile of markers of brown adipose tissue activity (CIDEA, PRDM 16), markers of browning of white adipocytes (HOXC9, Slc27A1) and marker of β-oxidation of fatty acids (Cpt1a) in 150 indigenous residents of Yakutia, miners of a diamond mining company, who were exposed to natural cold for 3 months, was analyzed in peripheral blood mononuclear cells. To determine the metabolic status, anthropometric data, glucose level and blood lipid profile of the subjects were evaluated.

Keywords: brown adipose tissue, cold, thermogenesis, browning, Yakutia, obesity.

Introduction. Obesity is characterized by an aberrantly increased amount of white adipose tissue resulting from dysfunctional regulation of the energy balance [13]. The modulation of energy consumption and expenditure is extremely complex and is the result of the integration of numerous neuroendocrine and environmental signals. Exposure in the cold is one of the main available stimulants that contribute to energy consumption by activating thermogenic pathways and thus ensuring survival in adverse temperature conditions [8, 12]. It is known that cold promotes beta-adrenergic stimulation through the sympathetic nervous system, which, in turn, induces thermogenesis, while activating brown adipose tissue (BAT) [7, 8, 12]. The activation of BAT promotes the oxidation of fats to produce heat, while an increased expression of the UCP1 protein is produced [8]. It is known that when stimulated by cold, white adipocytes can transdifferentiate into beige and brown-like adipocytes (a phenotype with increased expression of UCP1) in a process known as browning, leading to heat production [3]. It is important to note that during the browning process, the proliferation and differentiation of precursors of brown adipocytes also occurs, contributing to the growth of the population of heat-producing cells [14, 32]. A number of studies have shown that the activation of BAT in mouse models is able to prevent diseases such as obesity, type 2 diabetes and atherosclerosis [2, 6]. Thus, the study of the regulation of BAT was particularly important as a potential target for the treatment of obesity [14, 17]. It is known that adults have a different volume and amount of BWT, which decreases depending on age and BMI [11,33]. Studying the activation and browning of BZHT in humans is not easy due to several limitations. The most commonly used method available for this purpose is the study of the absorption of (18)F-FDG (2-deoxy-2-[18F]fluoro-d-glucose) by positron emission tomography-computed tomography (PET-CT), which, in addition to various technical limitations, is expensive and complex. In the search for alternative methods for assessing the activation of BAT in humans, we found the study of Palou and his colleagues, which was conducted on female rats, interesting. The results of this work showed that the expression of the regulators of the activity of BAT (CIDEA, Prdm16), browning of white adipose tissue (Hoxc9 and Slc27a1) and β-oxidation of fatty acids (Cpt1a) in both tissues correlates with the expression of the same markers in peripheral blood mononuclear cells (PBMC) when stimulated by cold [23]. The authors concluded that these genes can be considered suitable markers for assessing the activity of BAT in peripheral blood mononuclear cells (PBMC), avoiding the use of invasive procedures [23]. However, it was not clear whether the expression of these markers in the human PBMC is possible and whether it changes depending on exposure to cold. An earlier study conducted by us showed that adult indigenous residents of Yakutia exposed to cold showed greater beta-adrenergic activation and darkening of visceral fat depots compared to the comparison group living in thermoneutral conditions [12]. The aim of our study was to evaluate the expression of browning marker genes in the PBMC in cold-exposed adult indigenous residents of Yakutia compared with the control group, as well as to assess differences in metabolic status between the study groups.

Material and methods. The study was conducted in 2015 in the Verkhoyansk and Anabar districts of Yakutia in accordance with the guidelines of the Helsinki Declaration on the Ethical Treatment of People. The Protocol was approved by the Supervisory Board of the Ethics Committee of the YANC KMP (Protocol No. 46 of May 7, 2015).

Study participants. This study included 150 healthy male tunnellers of indigenous nationality engaged in open-pit diamond mining in the Anabar region of Yakutia (Polar zone, cold exposure group), and 29 healthy control subjects living in the city of Yakutsk (urban zone) in thermoneutral conditions. The subjects included in the cold exposure group spent an average of 8 hours a day working in the mine for 3 months (from

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December to February) at an average temperature of -45/-52 °C. A questionnaire interview was conducted to assess the amount of time spent in cold conditions, based on professional responsibilities. Twenty-nine healthy men were included in the control group in the summer (August), when the average air temperature ranged from +16 °C to +18 °C. Similarly, during blood sampling, the study coordinator conducted a questionnaire to make sure that none of the individuals belonging to the control group had been exposed to cold during the last three months before the study. A special questionnaire prepared by the research group was used for this purpose. Healthy control subjects were recruited through the clinic of the Yakut Scientific Center for Complex Medical Problems. Individuals with a documented diagnosis of a metabolic disease such as metabolic syndrome, type 2 diabetes, dyslipidemia or any other chronic disease, as well as those taking any medications that may affect glucose or lipid metabolism, were excluded from this study.Before registering for the study, all participants signed a written consent form.

Assessment of anthropometric parameters. The weight and height of the subjects were measured using standard weights and a stadiometer, respectively. BMI (kg/m2) was calculated by dividing weight (in kilograms) by height (in meters) squared. Waist circumference (cm) was measured in a standing position at the midpoint of the distance from the lower edge of the costal arch to the iliac crest of the iliac bone. Hip circumference (cm) was measured in a standing position at the level of the large femoral spits.

Blood sampling and biochemical studies. Blood samples were taken in the morning between 8:00 and 11:00 am from the subjects on an empty stomach. Sampling was carried out in February for the cold exposure group and in August for the control group, after 3 months of exposure to cold and thermoneutral conditions, respectively. After collection, the samples were immediately frozen at a temperature of -60 °C and delivered to the laboratory of the Yakut Scientific Center for Complex Medical Problems.

Glucose, triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol were determined using an automated biochemical analyzer Labio 200 (Mindray Medical International Limited, Nanshan, Shenzhen 518057, (China)) using Biocon kits (Biocon, Electronic City, 560100 Bangalore (India)). The cholesterol levels of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) were evaluated using the following formulas:

LDL = total cholesterol-VLDL-HDL;

VLDL = (tg)/2.2.

The atherogenicity coefficient (Ca) was calculated by the formula:

Ka = (total cholesterol-HDL)/HDL [16];

Collection of PBMC and quantitative PCR analysis. Whole blood samples were collected in vacutainers with EDTA. PBMC was isolated by gradient separation using the OptiPrepTM kit (D1556, Sigma-Aldrich, St. Louis, MO, (USA)) in accordance with the manufacturer's instructions and modifications previously described in the Palou study [19]. The quality of the obtained RNA samples was evaluated on the IMPLEN P-300 nanophotometer (Germany). After determining the quality, the RNA samples were stored at a temperature of -80 0C.

Gene expression in PBMC was determined by RT-PCR SFX96 (Germany). Reverse transcription was performed on a thermal cycler T-100 Thermal Cycler (Bio-Rad). The reaction conditions were as follows: 5 minutes at 25 °C, 30 minutes at 42°C, and 5 minutes at 85 °C. Each PCR sample consisted of a diluted cDNA sample (1:5), a direct and reverse primer (1 μm), a solution of SYBR Green PCR Master Mix (Bio-Rad) and DEPC water, the total volume was 20 μl.

PCR reaction conditions: 15 minutes at 95 °C, 1 minute at 60°C and 15 seconds at 95 °C. The primers used in this work are presented in Table 1.

Statistical analysis. The normality of the variables was assessed using the Kolmogorov-Smirnov criterion. Due to the small sample size, a nonparametric method was used. Median and interquartile ranges (IQR) were used to sum the variables. The Wilcoxon rank sum criterion between the two groups was used to assess the differences. To study the differences between the groups, a nonparametric ANCOVA with smoothed regression and the Young and Bowman test was used. Markers and biochemical variables were dependent variables, and age and BMI were covariates. One model was performed for each dependent variable. Nonparametric ANCOVA was also applied to evaluate the effect of the number of hours of cold exposure on the distribution of each marker and biochemical variables in subjects exposed to cold exposure, using BMI as a covariate. Based on the distribution of cold exposure time, four classes were considered: 1 or 2 hours, 4 or 4.5 hours, 8 or 10, 11 hours. To test the hypotheses, the Benjamin–Hochberg P-value correction method was used. For biochemical variables, the interaction between the classes of cold exposure and BMI was also considered. The significance level (p) when testing statistical hypotheses was taken to be $p<0.05$.

Results. A total of 179 people participated in this study: 29 of them belonged to the control group, and 150 subjects were in the group with cold exposure. The distribution of each variable was asymmetric, so a nonparametric statistical approach was chosen. The average age of the subjects was 32 years (IQR: 28; 38). Among those who were exposed to cold, 35% were exposed to cold for less than 5 hours, while 21% of the subjects were exposed to cold for less than 2 hours; 65% were exposed to cold for more than 5 hours and 55% were exposed to cold for 11 hours. There were no significant differences in age, waist circumference (FROM) and waist-to-hip circumference ratio (FROM/ABOUT) between the two groups. The parameters of weight, height, BMI and hip circumference were significantly lower in the cold exposure group compared to the control group (Table 2).

The study of gene expression in the PBMC revealed significantly lower levels of CIDEA expression and higher levels of HOXC9 expression in the cold exposure group compared to the control group, while no significant differences in the expression of other markers were found (Table 3). After age adjustment (as well as adjusted for BMI and without it), circulating total cholesterol, LDL, HDL, VLDL and triglycerides, as well as the atherogenicity coefficient were comparable between the two groups in analyses. However, in the group with cold exposure, the level of circulating glucose was significantly higher than in the control group (Table 3).

Table 4 shows the results of the analysis conducted in the cold exposure group, and due to the number of missed marker values, we compared subjects exposed to cold for less than 11 hours and subjects exposed to cold for 11 hours. There was no clear trend in the adjusted medians and statistically significant differences in the distribution of markers. This can be explained by the high variability of the distribution of markers in each study group. A statistically significant difference between the study groups was observed for such indicators as BMI, total cholesterol and atheroge-

nicity coefficient, for which the adjusted medians decreased with an increase in the number of hours spent in the cold.

This is the first study devoted to the study of gene expression of regulators of activation of brown, beige adipocytes and fatty acid oxidation in PBMC in people who have been chronically exposed to extremely low temperatures. Our data showed that the cold-exposed subjects expressed lower levels of the brown adipocyte marker CIDEA and higher levels of the beige adipocyte marker HOXC9 compared to the control, while the expression of other genes studied by us did not significantly differ between the groups. Interestingly, the people exposed to cold in this study had higher blood glucose levels, but lower body weight, BMI, and hip circumference compared to controls, which may have reflected a healthier metabolic status. The data obtained by us proved that certain markers of brown and beige adipocytes can be detected in the human PBMC and change depending on exposure to cold, potentially reflecting changes in the activation of BAT, browning process in white adipose tissue and the associated metabolic status of the subjects.

The increased energy costs associated with cold-induced activation of BAT and browning have attracted huge interest in its potential in the treatment of obesity and metabolic diseases [9, 11,14, 19,

Table 1

Sequence of nucleotides of primers used for real-time PCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Cidea	ATCGGCTCCTTAACGTGAA	AACCGCAGCAGACTCCTCA
Cpt1a	TCCACGATTCCACTCTGCTC	CAGCAACCCCGTGGCC
Hoxc9	CAGCAACCCCGTGGCC	CCGAGGTCCCTGGTTAAA
Prdm16	CCCAACAAGTACAGCCTGGA	GCGGATGAGGTTGGACTTCC
Slc27a1	GCGATATACCAGGAGCTGCA	TCTTGAAGGTGCCTGTGGTG
GAPDH (reference gene)	GTCGGAGTCAACGGATTTGGT	AGTGATGGCATGGACTGTGG

Note: Cidea, cell death-inducing DNA fragmentation factor-α-like effector A; Cpt1a, carnitine palmitoyl transferase 1a; Hoxc9, homeo box C9; Prdm16, PR domain containing protein-16; Slc27a1, solute carrier family 27.

Table 2

Anthropometric characteristics of the studied groups

	control group n=29	Cold exposure groupn=150	p
Age [years, median (IQR)]	34 (29;38)	32 (28;38.5)	0.522
Height [см, median (IQR)]	174 (171;176)	172 (168;176)	0.011
Weight [кg, median (IQR)]	75 (73;81)	70 (64;78)	<0.001
BMI [кg/м2, median (IQR)]	25.61 (23.84; 27.04)	24.06 (22.15; 26.51)	0.023
WC[см, median (IQR)]	92 (84;96)	85 (78;95)	0.082
HC[см, median (IQR]	100 (99; 102)	95 (92; 101)	0.001
WC/HC[median (IQR]	0.91 (0.85; 0.93)	0.89 (0.85; 0.95)	0.872

Table 3

Expression of browning and fatty acid utilization marker genes in PBMC and biochemical parameters when exposed to cold

	Control group n=29	Exposure in cold n=150			
Markers PBMC	Adj.Median (CI 95%)	Adj.Median (CI 95%)	RSE	Coefficient	p*
CIDEA	0.49 (0.43;0.58)	0.30 (0.22; 0.43)	0.467	0.019	0.042
PRDM16	2.97 (1.99;3.64)	1.74 (0.66; 3.42)	3.727	0.362	0.622
SLC27A1	1.30 (0.92; 1.88)	1.12 (0.73; 1.78)	3.772	0.125	0.761
HOXC9	0.96 (0.61;1.18)	1.75 (0.90; 2.42)	2.891	0.271	0.038
CPT1A4	2.40 (1.73; 2.83)	2.30 (0.55; 2.99)	4.167	0.155	0.931
	Biochemical parameters				
Glucose (mmol/l)	4.42 (4.35; 4.69)	5.29 (5.22; 5.36)	0.851	0.121	0.025
Triglycerides (mmol/l)	1.27 (1.00; 1.61)	1.22 (1.08; 1.47)	0.563	0.016	0.763
Total cholesterol (mmol/l)	5.12 (4.71; 5.54)	4.99 (4.81; 5.17)	0.728	0.019	0.888
LDL (mmol/l)	3.30 (2.95;3.63)	3.08 (2.79;3.20)	0.842	0.191	0.856
HDL (mmol/l)	1.21 (1.02; 1.41)	1.59 (1.13; 1.99)	0.534	0.011	0.557
VLDL (mmol/l)	0.71 (0.58; 0.84)	0.55 (0.49; 0.62)	0.474	0.021	0.899
Ka	3.42 (2.56; 4.51)	3.33 (2.61; 4.07)	0.062	0.062	0.878

Note: RSE - residual standard error; Adj. Mediana - adjusted median with smoothed regression; CI 95% - 95% confidence interval.
* nonparametric ANCOVA test using smoothing regression with correction of the p-value of the Benjamini-Hochberg; each model was adjusted for age and BMI. Ka is the coefficient of atherogenicity.

Table 4

MPCP markers and biochemical parameters in comparison groups depending on exposure time

Duration exposure in the cold									
	1-2 ч		4-4,5 ч		8-10 ч		11 ч		p
[Median (CI 95%)]	n=31 31	26.26 (23.51;28.54)	n=21 21	25.51 (24.57;26.39)	n=14 14	24.33 (23.70; 25.39)	n=82 82	23.12 (21.73; 24.49)	<0.05
Markers PBMC									
CIDEA [Adj. median (CI 95%)]	1	-	8	0.36 (0.11;0.82)	7	0.33 (0.01; 0.848)	22	0.30 (0.02; 0.58)	0.974+
PRDM16[Adj. median (CI 95%)]	15	0.64(0.12; 2.55)	16	2.57 (0.74; 4.39)	8	0.85 (0.15; 3.46)	43	1.77 (0.68; 2.89)	0.684+
SLC27A1[Adj. median (CI 95%)]	6	0.18 (0.01; 2.74)	7	1.68 (0.05; 4.14)	8	3.05 (0.78; 5.26)	20	1.20 (0.03; 2.59)	0.799+
HOXC9 [Adj. median (CI 95%)]	24	2.02 (0.61; 3.42)	14	1.78 (0.11; 3.50)	11	3.33 (1.24; 5.41)	52	1.68 (0.72; 2.64)	0.524+
CPT1A4 [Adj. median (CI 95%)]	9	0.27 (0.08; 3.44)	9	2.58 (0.15; 5.80)	8	4.25 (0.86; 7.64)	22	2.29 (0.26; 4.32)	0.668+
Biochemical parameters									
Glucose (mmol/l) [Adj. median (CI 95%)]	31	5.10 (4.74; 5.47)	20	5.28 (4.86; 5.70)	14	5.46 (4.91; 6.02)	82	5.20 (4.98; 5.42)	0.831
triglycerides (mmol/l) [Adj. median (CI 95%)]	31	0.96 (0.75; 1.18)	20	0.98 (0.74; 1.23)	14	1.29 (0.96; 1.61)	82	1.22 (1.09; 1.35)	0.217
Total cholesterol (mmol/l) [Adj. median (CI 95%)]	31	4.99 (4.71; 5.27)	20	4.63 (4.31; 4.96)	14	4.39 (3.96; 4.72)	82	4.43 (4.16; 4.68)	<0.05
LDL (mmol/l) [Adj. median (CI 95%)]	31	2.93 (2.58; 3.27)	20	3.22 (2.83; 3.62)	14	2.87 (2.36; 3.89)	82	3.10 (2.90; 3.31)	0.881
HDL (mmol/l) [Adj. median (CI 95%)]	31	1.78 (1.35; 2.16)	20	1.19 (0.95; 1.49)	14	1.26 (0.93; 1.68)	82	1.38 (1.25; 1.51)	0.679
VLDL (mmol/l) [Adj. median (CI 95%)]	31	0.45 (0.35; 0.55)	20	0.48 (0.36; 0.59)	14	0.56 (0.41; 0.72)	82	0.56 (0.50; 0.62)	0.558
[Adj. median (CI 95%)]	31	3.34 (2.97; 3.72)	21	3.04 (2.46; 3.46)	14	2.61 (2.04; 3.18)	82	2.64 (2.41; 2.86)	<0.05

32]. In fact, in recent decades, numerous studies have been undertaken to identify browning regulators and alternative markers responsible for the activation of BAT [11, 14, 15, 17, 20, 28, 32]. However, since only invasive methods are available to evaluate browning [18], the study of these processes in the human body has limitations. Therefore, the determination of browning markers or browning-associated metabolic changes is of scientific value. In the work of Villarroya and co-authors, circulating "batokines" were found [30], although none of them was recognized as a valid marker of the activation of BAT. Interestingly, the Palou data showed that cold-induced changes in the expression of several genes regulating browning and oxidation of fatty acids of BAT and white adipose tissue in rats are reflected by changes in the expression of the same markers in the PBMC, indicating attention to new potential analytical markers in the PBMC [23]. However, this discovery has never been investigated and confirmed in humans.

Our recent study showed that adult residents of Yakutia exposed to cold have a more intense darkening of visceral fat depots compared to people living in thermoneutral conditions [12].One of the objectives of the study was to find an answer to the question of whether residents of Yakutia belonging to the same population also show differential expression of PBMC markers identified by Palou and his colleagues. The cold-exposed subjects included in our study expressed higher levels of the beige adipocyte marker HOXC9 and lower amounts of the brown adipocyte marker CIDEA compared to the control. This conclusion is somewhat consistent with the conclusions of Palou and his colleagues, whose studies were conducted on female rats of different ages (1, 2, 4 and 6 months) exposed to cold for one week [23]. According to our results, in fact, cold exposure leads to a significant increase in the expression of HOXC9 in adult rats (4 and 6 months) [23]. It is believed that this marker is specific for beige fat depots, and it is known that its expression increases with browning stimulation (with the introduction of rosiglitazone) [31].

Thus, an increase in HOXC9 in the PBMC of people exposed to cold exposure may reflect changes in expression in their fat depots, which makes it a potential candidate for use as a browning marker. Our data are consistent with the results of RNA sequencing studies showing that human BAT has a gene expression signature resembling that of beige adipocytes [25]. On the other hand, Palou and his colleagues did not find significant changes in the expression of CIDEA in the PBMC in adult rats exposed to cold, but they revealed only a clear decrease

in the mRNA level of this marker in the BAT [23]. CIDEA is widely expressed on the surface of lipid droplets of brown adipocytes and is responsible for the formation of large lipid droplets through the stimulation of lipid metabolism between them [4]. The expression of adipocyte CIDEA increases under conditions conducive to triglyceride deposition [22], which is the opposite phenomenon compared to what happens when BAT is activated. According to some studies, it actually antagonizes UCP1 expression [26].

The lower expression of CIDEA in the PBMC in the studied group exposed to cold compared with the control may reflect similar differences in the expression of this marker of BWT, whose levels decreased after exposure to low temperatures in animal models [23]. Evaluation of CIDEA expression in the PBMC has the potential of great clinical significance in the study of human BAT activation and requires further study. In our study, we were unable to detect differences in the expression of Cpt1a4, Scl27a and PRDM16 in individuals exposed to cold when compared with the control group. This finding contrasts with the study of Palou and his colleagues, who observed increased expression of Cpt1a4 and Scl27 in female adult rats when exposed to cold [23]. The differences in our findings and Palou's findings can be explained by several factors, for example, different experimental models, gender, age, the conditions studied and the variability of these markers in the study groups. The group exposed to cold in the present study also had a lower body weight, BMI, and hip circumference, which may have reflected a healthier metabolic status [1]. Although we found no significant differences in cholesterol levels between our two groups, our data showed lower levels of BMI, total cholesterol, and atherogenicity coefficient with increasing daily exposure time to cold. This finding is consistent with data indicating an increase in lipid utilization and an improvement in the lipid profile induced by the activation of BAT caused by cold stimulation [5,6,10]. On the other hand, other reports have revealed a U-shaped relationship between ambient temperature and cardiovascular risk, with the latter increasing at temperatures below -1 °C and above 20 °C [21]. However, most of these studies analyzed temperatures ranging from ~-15 to -30°C, different from our study in which people exposed to cold were exposed to temperatures below -30°C. In addition, the experimental group exposed to cold had a higher fasting glucose level compared to the control group. Although this finding may seem counterintuitive, given that acute activation of BAT increases glucose uptake, plasma glucose levels do not change with acute exposure to cold in humans. Thus, it is possible that in individuals with normal glycemia, chronic stimulation of BAT requires higher basal glucose levels for its use, without leading to metabolic disorders (impaired fasting glucose or insulin resistance). It should be noted that the fasting glucose level in both our groups was within the normal range.

The group exposed to cold in the present study also had a lower body weight, BMI, and hip circumference, which may have reflected a healthier metabolic status [1]. Although we found no significant differences in cholesterol levels between our two groups, our data showed lower levels of BMI, total cholesterol, and atherogenicity coefficient with increasing daily exposure time to cold. This finding is consistent with data indicating an increase in lipid utilization and an improvement in the lipid profile induced by the activation of BAT caused by cold stimulation [5, 6].

Conclusion. In conclusion, it should be noted that this is the first study demonstrating that human peripheral blood mononuclear cells express markers of brown adipocytes and browning, CIDEA and HOXC9 mRNA levels vary depending on exposure to cold. Based on our results, we believe that the expression of CIDEA in human PBMC may reflect its expression in BAT in a state of chronic activation, while the expression of HOXC9 may reflect the expression of white adipocytes undergoing transdifferentiation from white to brown adipocytogenesis, which makes both markers potentially useful markers of BAT activation and browning process worthy of further study and validation.

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ANALYSIS OF THE CERVICAL CANCER INCIDENCE IN THE ARCTIC ZONE OF THE RUSSIAN FEDERATION TO IDENTIFY REGIONS IN FIRST NEED OF ITS PREVENTION

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To identify the territories of the Arctic in which urgent measures are needed for the cervical cancer prevention, including vaccination, a comparative analysis of cervical cancer incidence (CCI) was carried out among Arkhangelskaya and Murmanskaya Oblasts, republics Karelia, Komi, Sakha (Yakutia), Krasnoyarski Krai, Chukotski , Yamalo-Nenetski and Nenetski Autonomous Okrugs and Russia as a whole. The annual rates of CCI per 100 thousand populations, standardized by age, were analyzed. Multiple and paired rank analyzes, as well as the calculation of annual changes from baseline in CCI, showed that cervical cancer prevention is an important task in these state entities, and the population of the Nenetski Autonomous Okrug, which is characterized by a significant proportion of indigenous people and ethnic minorities, needs it most urgently.

Keywords. HPV, vaccination, immunization, ethnic groups, indigenous population, North.

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Introduction. Development and availability of a vaccine against human papillomavirus (HPV) has provided an exceptional opportunity for the prevention of HPV-associated cancers, such as cervical cancer, oropharyngeal, anal, vaginal, vulvar and penile cancer [1].

28 countries in the WHO European Region have added HPV vaccination to their routine immunization schedules [2], as the United States and Canada have [1,3].

In its global strategy for the period 2020-2030 the WHO recognized it is extremely important to include immunization against HPV in the National Health Programs of all countries of the world to accelerate the cervical cancer elimination. To eliminate cervical cancer the WHO emphasizes that all countries must achieve and maintain cervical cancer incidence at a rate of less than 4 cases per 100,000 women per year. Among the measures to eliminate cervical cancer the WHO notes the roadmap of complete vaccination of 90% of girls by the age of 15 years in all countries by 2030 [4].

In the Russian National Immunization Schedule named "Russian national preventive vaccinations and the vaccinations by epidemic indicators calendar" immunization against HPV has not yet been included. The Ministry of Health of the Russian Federation approved Order