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METABOLIC INDICES OF BLOOD IN CONDITIONS OF COLD EXPOSURE

ABSTRACT

We investigated 200 employees of the diamond mining company who worked in cold condition from 2 to 8 hours a day. Reliable correlations have been revealed to prove the effect of cold on weight, body mass index, waist volume and hip volume. Cold exposure increased expression of genes markers of browning. These results provide evidence pointing to PMBCs as an easily obtainable material.

Keywords: cold exposure, brown adipose tissue, lipid metabolism, obesity.

Introduction

The discovery of brown adipose tissue caused great interest in studying the origin of this thermogenetic tissue underlying the fight against obesity and complications associated with it.

Cold exposure is one of the strongest stimulators of brown adipose tissue (BAT) activation. The process of activation of brown adipose tissue is manifested in the growth of beige adipocytes in the depot of white adipose tissue, this process is called browning.

It is known that mammals have two types of adipose tissue, white and brown. White adipose tissue mainly consists of white adipocytes, which are a storehouse of the excess fat, designed to store energy, in the form of a large lipid drop. BAT consists of multi-compartment adipocytes that specialize in fat oxidation, producing heat during thermogenesis, in response to cold stimulation or consumption of a high-calorie diet during stimulation with B-adrenergic receptors.

The thermogenetic activity of BAT is associated with the presence of the mitochondrial protein UCP1 and represents an important part in the energy expenditure of the critical and total energy balance. The main thermogenetic stimulus in cold exposure is the stimulation of the sympathetic nervous system by cold and the control of B-adrenergic antagonists caused by the oxidation of fatty acids in adipose tissue and thermogenesis in BAT by the mediocre growth of the size of the BAT tissue, mitochondriogenesis, and expression of the UCP-1 protein and the activity of proteins to maintain body temperature [3, 5, 7, 15-17]. In addition, stimulation of B-adrenergic receptors by cold promotes such a process as browning, in which brown-like adipocytes (brite) form in the depot of typical white adipose tissue [1,4,5,6,7,10,25]. These adipocytes are called brite (brownish, beige, intermediate form between brown and white fat tissue), which prove specific gene expression and have some features characteristic of classical brown adipocytes, such as expression of UCP-1 protein in mRNA [1, 2, 9, 18, 19, 21, 22]. In the case of rodent studies, it is known that the Browning process can increase energy consumption and help maintain body weight [2,8,20]. Interest in the research of BAT appeared when the cases of active BAT in adults were proved [12]. At the moment, the main issue discussed by the world community is the possibility to activate or increase the mass of BAT in adults, since active BAT can play a significant role in controlling energy homeostasis and promote the development of drugs for the treatment of obesity.

Objective: to establish the usefulness of the use of peripheral blood mononuclear cells as a method for carrying out a study related to the activation of brown adipose tissue and the transition of white adipose tissue to brown tissue by analyzing the key markers of Browning in response to the main thermogenetic stimulus-the cold exposure.

Materials and methods

In 2016, biomaterial (blood) was collected for genetic and biochemical analyzes of 200 workers of the diamond mining company, mainly sinkers, who openly mined the diamond-bearing soil in the winter season. Workers spent on extraction from 4 to 9 hours a day depending on their professional duties.

The first group consisted of 76 workers, who conducted at low temperatures from 2 to 4 hours. The second group included 110 sinkers, the time of cold exposure was from 6 to 8 hours per day.

All subjects were examined for anthropometric measurements with determination of growth, body mass index (BMI), waist circumference (WC), hip circumference (HC), WC/HC ratio. The body mass index was calculated as the ratio of the body weight (kg) to the height (m). When evaluating the body mass index, the criteria of the World Health Organization (WHO) were used. The waist circumference was measured in a standing position midway from the lower edge of the costal arch to the crest of the abdominal bone. The hip circumference was measured in the standing position at the level of the greater trochanteres of the femurs.

Glucose, total cholesterol, high-density lipoprotein (HDL), triglycerides (TG) were determined by the enzymatic method on an automated Labio 200 analyzer using Biocon kits (Germany).

Total RNA was isolated from the

peripheral blood with the use of the Trizol reagent. The quality of the obtained RNA samples was evaluated on the IMPLEN P-300 nanophotometer. After determining the quality, RNA samples were stored at -80°C.

Gene expression in PBMC was determined on CFX96 qPCR thermal cycler. The reverse transcription was carried out using the iScript cDNA synthesis kit (Bio-Rad), on 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 diluted cDNA sample (1:5), forward and reverse primer (1μM), SYBR Green PCR Master Mix (Bio-Rad) and DEPC water, 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 study are shown in Table 1.

All studies were carried out with informed consent of the subjects in accordance with the ethical norms of the Helsinki Declaration (2000).

Statistical analysis of the study materials was carried out using the SPSS program (version 19). A check on the normality of the distribution of the quantitative indices studied was carried out according to the Kolmogorov-Smirnov test. The reliability of the differences between the mean values was estimated using Student's t-test for independent samples, the probability of a fair hypothesis was taken at p≤0.05. Correlation analysis was carried out according to Pearson. The data in the tables are presented in the form M±m, where M is the arithmetic mean, m is the error of the mean.

Results and discussion

In the statistical analysis of anthropometric data, we showed that workers with prolonged exposure in the cold (up to 9 hours a day) showed a significant decrease in weight (r = 0.359, p = 0.01) and, respectively, BMI (r = 0.435, p = 0. 00) (Table 2).

Also, with a prolonged cold exposure (up to 9 hours a day), significantly decreases RT (r = 0.263, p = 0.01) and OB (r = 0.171, p = 0.026). The results of our work confirm the study by Saito M. et al (2009), in which the relationship between the presence of active BAT and the thinness of the examined individuals is proved. According to the literature, the average mass of BAT in the body of a healthy adult is about 50 grams. Despite the small amount in the body, BAT can have a disproportionately large metabolic effect.

According to the International Diabetes Federation, 10.9 million patients in Russia suffer from diabetes mellitus, 11.9 people have impaired glucose tolerance and impaired fasting glucose. Obesity, usually preceded by diabetes, occurs as a result of changes in blood glucose. Indeed, adipocytes regulate insulin resistance, where half of the lipids from white adipose tissue are depot and regulate insulin signals. Also, the relationship between the presence of BAT with depletion [17] and euglycemia [13] has been proved. These researchers suggest that adipocytes of brown adipose tissue can affect metabolic processes, such as obesity and diabetes. A unique feature of BAT is the expected effects of obesity and the control of excess glucose in the mitochondria, which contain the splitting protein 1 (UCP-1). UCP-1 cleaves oxidative phosphorylation by exclusion protons when entering the mitochondrial matrix, independent of the synthesis of ATP, which produces heat instead of chemical energy [3]. The study of rodents showed that after stimulation BAT consumes glucose and free fatty acids for thermogenesis (heat production), which proves the regulatory role of BAT in glucose homeostasis and insulin resistance.

At present, the physiological role of BJT in the activation of metabolic indicators in humans has not been adequately studied. Researchers Orava J. et al [14] and Ouellet V. et al [13] found that with a cold exposure, a 12-fold increase in the glucose concentration in the adipocytes of BAT is observed, but not in other tissues. This suggests that after activation, BAT promotes the consumption of glucose in the blood plasma. But despite this, these researchers did not show differences in the content of free-circulating glucose between the groups with the presence of BAT and the absence of BAT, which raises the question of the ability of adipocytes of BAT to influence glucose metabolism. When comparing two groups with different exposure times, we did not find any significant differences, the blood glucose in the subjects was within the normal range (Table 3).

The study of BAT in humans requires invasive techniques, such as adipose tissue biopsy or the use of positron emission tomography, which involves the use of radioactive isotopes. Therefore, it is necessary to use a safe and accessible source of biomarkers. Peripheral blood mononuclear cells (PBMCs) are readily available biological materials that can be used to study brown fatty tissue with minimal invasion. PBMCs constitute a fraction of the blood, consisting mainly of lymphocytes and monocytes. These cells circulate in the body and are responsible for internal and external signals expressing a large number of genes that include tissue-specific transcription and reflect metabolic adaptation. PBMCs are capable of producing about 80% of the entire genome, including tissue-specific transcripts. Although these cells can not express the main key thermogenetic gene of UCP1, these cells have the ability to produce other brown / beige markers such as Cidea, Hoxc9, Prdm16 and Slc27a1. Our study is the first on the effect of cold exposure on the expression of genes in PBMC in humans. We have found that a prolonged cold exposure increases the expression of the Browning markers Slc27a (r = 0.421, p = 0.01), Hoxc9 (r = 0.164, p = 0.032), Cpt1a (r = 0.270, p = 0.00) and Cidea r = 0.187, p = 0.014). The data obtained show that PBMCs can reflect changes in the expression of genes in response to cold and prove the usefulness of PBMC as a method for performing a study related to activation of brown adipose tissue in humans.

Conclusions

1. A significant negative correlation was found between weight, waist volume and hip volume, depending on the duration of exposure in the cold.

Table 1

Sequences 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

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 studied groups

	I group N=77	II group n=107
	Cold exposure (h)	
	2 – 4	5 –8
Age (years)	32,16±1,41	31,29±0,66
Height (cm)	170,72±0,67	171,81±0,56
Weight (kg)	75,66±1,34	71,32±1,24
BMI (kg/m2)	25,97±0,42	23,96±0,36
WC (cm)	89,11±1,74	83,15±1,41
HC (cm)	96,15±1,50	93,62±1,37
WC/HC	0,91±0,01	0,83±0,02

Table 3

Biochemical indicators depending on the duration of exposure in the cold

Indice	I group n=76	II group n=110
	M±m	
Glucose, (mmol/L)	5,41±0,14	5,18±0,98
TG (mmol/L)	1,32±0,09	1,15±0,05
Cholesterol (mmol/L)	4,63±0,75	4,64±0,07
Cholesterol HDL (mmol/L)	1,19±0,28	1,39±0,03
Cholesterol LDL (mmol/L)	2,80±0,08	2,69±0,08
Cholesterol VLDL (mmol/L)	0,59±0,04	0,53±0,02
Atherogenic coefficient	3,13±0,14	2,55±0,11

2. The weight, waist and thigh size of the subjects were reduced with prolonged exposure in the cold.

3. At the analysis of biochemical parameters it is not revealed essential changes. We show reliable correlation links between the markers of the Browning process and the exposure in the cold.

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