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Features of Human Metabolic Reactions under Extreme Cold Exposure

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Abstract—We measured 45 biochemical indices of blood serum samples in six volunteers aged 23–35 years before and after 3-min exposure to air cryosauna at a temperature of –70°C. We observed an increase in the activity of glutamate dehydrogenase (by a factor of 3.4), aspartate aminotransferase (by 46%), lipase (52%), lactate dehydrogenase (16%), cholinesterase (18%), leucin aminopeptidase (12%), and prostatic acid phosphatase (45%). Moreover, we observed an increase in the level of free fatty acids (by a factor of 2.8), HDL-cholesterol (by 10%), creatinine (21%), glucose and β-hydroxybutirate (11%), high-sensitivity C-reactive protein (12%), potassium (14%), and chlorides (7%). In contrast, atherogenic index values decreased by 14% compared with background level. Most of changes in biochemical blood parameters mentioned above remained almost constant within 20, 60, and 180 min after 3 min of cryosauna; the values did not reach their initial levels. It is concluded that urgent adaptation of human body to low temperatures is associated with increased intensification of reactions in the respiratory chain of mitochondria, an increase in the rate of metabolic reactions due to the elevated production of energy substances resulting from activation of lipolysis and glycolysis.

Keywords: air cryosauna, cold exposure, cold stress, urgent adaptation to cold, blood biochemistry

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Cold is one of the natural environmental factors which affect the human body, especially at polar latitudes. At the present time, successfully addressing strategic and economical challenges of the natural resources development of the Arctic zone is possible only through the labor migration of people from the temperate climatic regions to the districts in the Far North. The need to develop a comprehensive set of measures on the formation of superior adaptation to cold in migrants who are going to work in polar regions on an ongoing basis or in the expeditionary regimen occurs due to the significant risk related to the negative effects of extreme cold exposure on the human body [1].

Addressing the issue of temperature environment adaptation is possible only through in-depth understanding of the natural mechanisms of resistance to cooling. In most cases, researchers study the mechanisms of formation of long-term adaptive responses to the prolonged exposure to stress-inducing factors [2–4]. At the same time, it has been shown that cold, as one of the key adaptogenic environmental factors, may set off an adaptive reaction even after a single cold exposure. In this case, changes usually occur in all systems and organs [5], many of which respond to cold exposure almost immediately [6]. Therefore, study of the early changes in biochemical parameters which characterize the formation of urgent adaptations to cold exposure is relevant.

The aim of the present study was to assess the effects of short exposure to extreme cold on biochemical parameters of the blood in healthy individuals during acute adaptation period.

METHODS

A group of six healthy male volunteers aged 23–35 years was selected for the prospective study. Written informed consent was obtained from all the participants. Experimental procedure was approved by the Biomedical Ethics Commission of the Institute for Biomedical Problems, Russian Academy of Sciences. Exposure to cold was performed in a CrioAir air cryosauna (Germany) at reference temperature of –70°C for 3 min. Blood samples were collected in the fasting state from the median cubital vein 30 min before the beginning of the experiment and 2, 20, 60, and 180 min after the termination of the experiment. The activities of aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyltransferase (GGT), alkaline phosphatase (AP), glutamate dehydrogenase (GLDH), cholinesterase (CE), α-amylase and its

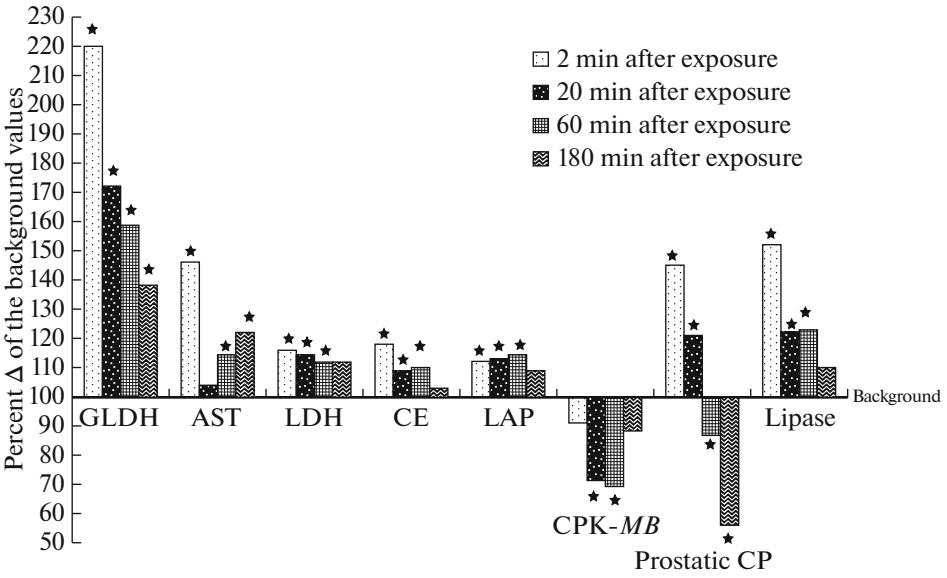

Fig. 1. Changes in the enzyme activities in the blood of the participants after cold exposure. The GLDH activity is shown in 1 : 2 scale. ★ Significant differences from the background values, $p < 0.05$.

pancreatic isoenzymes, pancreatic lipase, creatine phosphokinase (CPK) and creatine phosphokinase cardiac specific isoenzyme (CPK-*MB*), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (HBD), as well as total serum protein concentration and concentration of albumin, glucose, creatinine, urea, uric acid, cholesterine, high-density lipoprotein cholesterol (HDL-cholesterol), triglycerides (TG), free (non-esterified) fatty acids (FFA), phospholipids (PL), β-hydroxybutyrate, cystatin C, high-sensitivity C-reactive protein, iron, calcium, magnesium, inorganic phosphate, chlorides, and bicarbonates were determined in venous derived blood samples using DiaSys commercial kits (Germany). Total and direct bilirubin concentrations were measured by "Eco-service" commercial kits (Russia). Acid phosphatase (AP) and its prostatic isoenzyme activities were determined using the kits of Vital Diagnostics (Russia); the activities of leucine aminopeptidase and triacylglycerol lipase were measured with Randox (United Kingdom) kits. Measurements of all the above-mentioned parameters were performed on the Targa BT 3000 Biochemistry Analyzer manufactured by Biotecnika Instruments (Italy). Serum potassium and sodium levels were determined using selective EasyLite Na/K electrolyte analyzer manufactured by Medica (United States).

Activity of the muscular isoform of creatine phosphokinase (CPK-MM) was calculated as difference between the activities of CPK and CPK-*MB*. Concentrations of high-density lipoprotein cholesterol (HDL-cholesterol), very-low-density lipoprotein cholesterol (VLDL-cholesterol), and atherogenic index were determined by the formulas [7].

Statistical data processing was performed by the methods of variation statistics using the Statistica for Windows, Kernel Release 6.0 software (StatSoft, United States). Outlier values were excluded from the sampled population according to Dixon's Q test [8]. The results of the study were presented as the median (*Me*) and the lower and upper quartile ranges (LQr and UQr) for each series of measurements. The significance of the differences between the values obtained and the background values were assessed using Wilcoxon's matched pairs test ($p < 0.05$) [8].

RESULTS AND DISCUSSION

Twenty-seven out of forty-five measured and calculated biochemical parameters including ALT, GGT, AP, total and direct bilirubin, α-amylase, pancreatic amylase, pancreatic lipase, urea, uric acid, total protein, albumin, cystatin C, CPK and its muscular isoenzyme CPK-*MM*, HBD, CP, cholesterine, HDL-cholesterol, VLDL-cholesterol, TG, phospholipids, iron, calcium, inorganic phosphorus, sodium, and bicarbonates did not differ significantly from the background values at any of the examination periods after experimental exposure.

At the same time, significant changes in the activity of seven enzymes measured in the blood of the participants were observed 2 min after the termination of cold exposure. The activities of the mitochondrial enzymes were elevated to a greater extent (Fig. 1).

The activity of GLDH increased 3.4-fold, whereas AST activity increased by 46%; moreover, 180 min after the termination of the experiment, the values attained exceeded the initial values by 75% и 22%

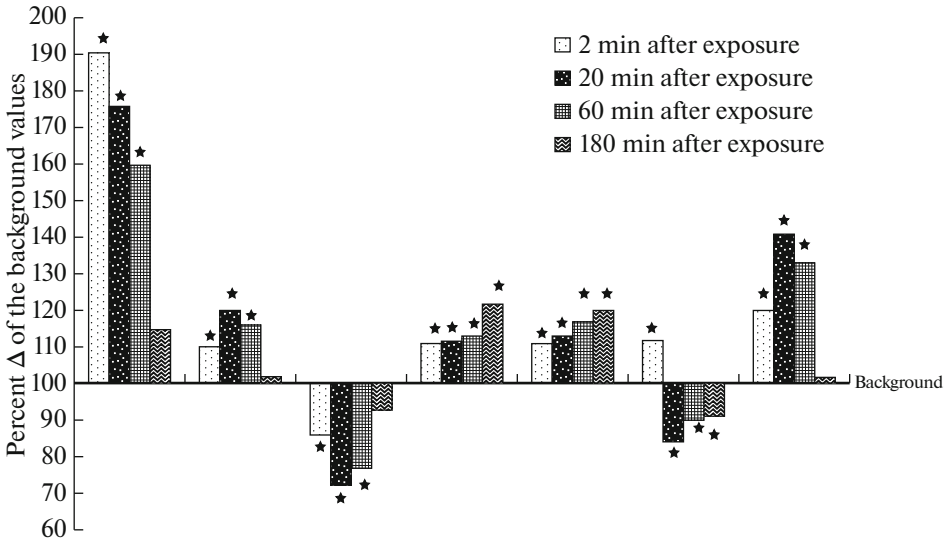

Fig. 2. Changes in the parameters of lipid, carbohydrate, and protein metabolism in the blood of the participants after cold exposure. The FFA concentration is shown in 1 : 2 scale. See Fig. 1.

respectively. Two minutes after the exposure, the activity of LDH increased by 16% and remained at approximately the same level during the following examination periods. We also observed a statistically significant increase in the activities of cholinesterase (CE) and leucin aminopeptidase (LAP) by 18% and 12% respectively 2 min after cold exposure. Twenty and 60 min later, their values exceeded the background level on average by 11%, and after 180 min they reached the initial values.

Persistent reduction in the CPK-*MB* activity together with the diverse changes in the activity of prostatic CP isoenzyme is worth noting. Twenty minutes after experiment termination, the activity of CPK-*MB* decreased by 29% compared to the background level. Sixty minutes later, the CPK-MB activity continued decreasing to a level of 31%; 180 min later the activity level tended to normalize. As for the prostatic CP isoenzyme, 2 min after cold exposure, its activity exceeded the initial level by 45%; 20 min later, by 21%; and after 60 min, it started gradually decreasing. It is noteworthy that 2 min after the termination of cold exposure, a 1.5-fold increase in the activity of triacylglycerol lipase was observed. Twenty and 60 min later, its values exceeded the initial values by 22% and 23%, respectively; after 180 min, these values almost returned back to the initial level.

In addition to the shifts in the enzymatic activities in the blood of the participants after cold exposure, pronounced changes in the parameters of carbohydrate, lipid, and protein metabolism were observed (Fig. 2).

Two minutes after the termination of cold exposure, FFA concentration increased by 2.8 times comparing to the initial values. Twenty and 60 min later, their levels exceeded the background level 2.5- and 2.2-fold, respectively, and after 180 min, 1.3-fold. Moreover, a statistically significant increase in the level of HDL cholesterol by 10% and, consequently, a decrease of atherogenic index by 14% were observed. Twenty and 60 min after the exposure, the aforementioned parameters continued changing in the same direction; however, 180 min later, their values approached the background level.

Significant changes in the carbohydrate metabolism parameters were observed together with changing indices of lipid metabolism. Two minutes after cold exposure, blood glucose and β-hydroxybutyrate levels increased by 11% and then continued progressively grow to the levels of 122% and 120% of the baseline respectively by the 180th minute of the acute adaptation period.

High-sensitivity C-reactive protein concentration significantly increased by 12% 2 min after cold exposure; 20, 60, and 180 min later, the protein level dropped to 16%, 10%, and 9% of the background concentration, respectively. Two minutes after the exposure, creatinine concentration increased by 21%; 20 min later, it increased by 41%, and then started decreasing. Creatinine level reached the background level 180 min after cold exposure.

We also found an increase in the magnesium, potassium, and chloride concentrations (Fig. 3).

Magnesium concentration in the blood of the participants significantly increased by 10% 20 min after the termination of the exposure. At the same time, 2 min after cold exposure, potassium and chloride concentrations significantly exceeded the initial values by 14% and 7% respectively and remained at approximately the same level 180 min after the exposure.

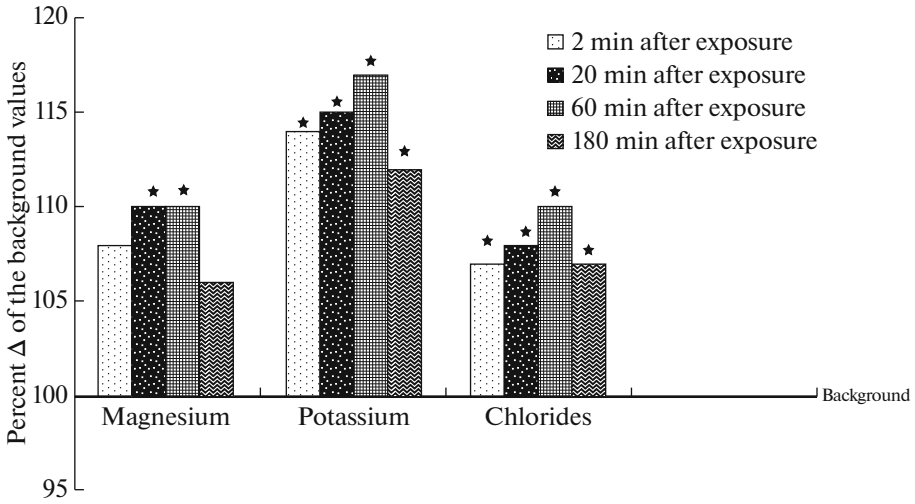

Fig. 3. Changes in the magnesium, potassium, and chloride concentrations in the blood of the participants after cold exposure. See Fig. 1.

Therefore, in healthy individuals, an increase of macroergic compound synthesis, together with the activation of the lipolysis and glycolysis processes and changes in the cell transmembrane potential, occurs during the acute cold adaptation period.

It has been indicated that the rate of reactions in the mitochondrial respiratory chain increases under acute cold exposure. It allows the body to rapidly generate and accumulate energy [9]. Studies by Bignucolo et al. (2013) demonstrated activation of the dehydrogenases in the citric acid cycle under the exposure to stress-inducing factor during the initial stages of adaptation (at early stages). Since these enzymes are NADP-dependent and involved in biosynthetic pathways, they increase the rate of energy production [10]. Research data indicate that the AST activity increases during the urgent adaptation to cold exposure; what is more, ALT activity increases only with the formation of long-term adaptation [11]. An increase in the LDH activity has also been demonstrated during the acute cold adaptation period and was greater when LDH catalyzed the production of pyruvic acid in the forward reaction [12]. It is known that LDH contributes to the accumulation of nicotinamide adenine dinucleotide (NAD+) which is essential for the ATP production through the glycolysis within the cells [13]. It is possible that a sharp increase in the activities of blood GLDH, AST, and LDH after cold exposure is associated with the need of providing the body with a quick burst of energy. Since cold stress causes impairments in the esterification processes in hepatocytes [14], changes in the functional state of the liver under cold exposure, probably, result in an increase in the LAP and CE activities.

The acute period of cold adaptation is characterized by activation of lipolysis; therefore, lipids become the predominant source of energy [15]. The activity of triacylglycerol lipase which cleaves lipids to glycerol and free fatty acids also increases in response to cold stress [9]. FFA are the main energy source for the heart muscle. 65 to 70% of ATP consumed by the cardiac muscle is produced through the mitochondrial β-oxidation. The remaining 20–25% is produced during the glycolysis [16]. Part of FFA is consumed by liver for lipoproteins synthesis, and the rest is metabolized to acetyl-CoA during the citric acid cycle. The excess of acetyl-CoA is converted to ketone bodies, particularly to β-hydroxybutyrate. Ketones, along with glucose, also provide the body with energy [17].

An increase in HDL cholesterol concentration and, therefore, a decrease in atherogenic index value are observed during acute cold adaptation [18]. In addition, some researchers observed a decrease in the CPK activity in response to cold stress [19]. The creatine kinase pathway responsible for the production and utilization of energy by subcellular structures plays a leading role in providing the cardiac muscle with energy for maintaining myofibrils and sarcoplasmic reticulum functioning [7]. It has been demonstrated that the cardiovascular system primarily reacts to cold exposure [20]. In this regard, a decrease in the activity of CPK-*MB* and in the atherogenic index value observed in healthy individuals during urgent cold adaptation may probably be considered to be cardioprotective factors.

An increase in blood creatinine level and high-sensitivity C-reactive protein concentration was observed at the initial stage of cold adaptation. Intensification of transamination and methylation reactions in response to cold exposure resulted in an increase in the rate of synthesis of creatine and creatine phosphate which, in turn, participates in the thermogenic processes of muscle contraction, thus providing muscle tissue with energy. Creatine breaks down into creatinine which

has no threshold concentration and is excreted by the kidneys [9]. C-reactive protein measured in the high-sensitivity range, probably, plays a role of a positive reactant of the acute phase reaction under exposure to cold. This is why its concentration grows during the initial stage of cold adaptation.

An increase in the activity of the prostatic CP isoenzyme is conceivably related to the changes in the ratio of the basic morphofunctional types of Leydig cells during cold stress. Thus, it has been shown that at the early stages of cold adaptation the percentage of Leydig cells increases and their size decreases. The changes observed acquire opposite direction under conditions of prolonged animals exposure to low temperatures [21].

Elevation of the blood potassium and magnesium levels reflects the body reaction to any kind of stress, including cold-induced reactions. As a result of the exposure to stress-inducing factors, we observed an intensification of catecholamines synthesis and activation of the glycolytic pathways that facilitate the leakage of potassium ions into the extracellular space and bloodstream as well as intracellular accumulation of the sodium ions. This process is regulated by Mg^{2+}-dependent Na^+/K^+-ATPase, which maintains the cell membrane potential. Magnesium ions involved in regulation of electrolyte balance can increase absolute refractory and decrease relative refractory periods in the myocardium, which is essential for the body in a stressed state [22].

During the initial period of cold adaptation, the volume of blood in the major vessels increases, which is associated with peripheral vasoconstriction [23]. Electrolyte concentration and osmolality of body fluids, including intravascular, extracellular, and intracellular fluids, increase during relative central hypovolemia [24]. Chlorides are known to be an essential component of osmolality and acid–base homeostasis of the intracellular fluid [7]. Therefore, chloride blood level increases within the first minutes of cold exposure.

CONCLUSIONS

In order to provide an adequate level of thermogenesis and gas exchange in healthy individuals during the period of acute adaptation to low temperatures, metabolic alterations aimed at intensification of macroergic molecules synthesis occur due to the activation of lipolysis and glycolysis.

Adaptation to the environmental conditions associated with the recurrent influence of stress factors is much more prevalent in nature than an immediate shift of the body to new conditions for a long-term period [25]. It is noteworthy that thermogenic process of muscle contraction and peripheral vasoconstriction decrease in response to periodic exposure to cold, whereas thermogenesis level remains constant [9]. Therefore, it is reasonable to use a consecutive series of cold exposures in an air cryosauna in order to develop superior adaptation of the body to low temperatures in healthy individuals preparing to work in polar latitudes.

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