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# ALTERED SUBCELLULAR REACTIONS TO STRESS AFTER A LONG-TERM HUMAN EXPOSURE TO ANTARCTIC CONDITION

**Ye. V. Moiseyenko, K. V. Rozova**

**Bogomoletz Institute of Physioligy National Academy of Sciences of Ukraine, Kyiv, Ukraine**

## Abstract

Medical research in Antarctica opens up new horizons to study individual health and reactions under stress and unusual environmental conditions. A long stay in Antarctica can evoke reactions associated with adaptation to hypoxia and mitochondrial dysfunction, determined by a set of molecular-genetic mechanisms. The aim was to study the changes in platelet ultrastructure in Antarctic winterers under hypoxic loading, and the ultrastructure of mitochondria (MT) of leukocytes in winterers, depending on the length of exposure to Antarctic conditions and number of expeditions participated. We included to the study 24 winterers, who once stayed in Antarctica during 1 year (group 1), 3-4 one-year stays (group 2), and 5-7 one-year stays (group 3). The control group included 8 men, who were not exposed to adverse environmental factors and never visited Antarctica. We assessed the ultrastructure of platelet and leukocytes before and after the hypoxic loading. The background characteristics of the ultrastructure of platelets and leukocytes of winterers. Significant changes developed in the platelet and leukocytes mitochondria ultrastructure after hypoxic loading. The severity of changes in leukocyte mitochondria correlated with number of expeditions participated, and manifested by development of autophagy, apoptosis up to irreversible organelle changes, a partial or complete vacuolization, excessive swelling of organelles after 1 year stay, 3-4, and 5-7 one-year

expeditions respectively. So, a long exposure to Antarctic conditions is associated with mitochondrial dysfunction.

**Key words: Antarctica; mitochondria; hypoxia; exposure to adverse conditions; stress; "Antarctic syndrome"; sub-cellular imaging; platelets; leukocytes; mitochondrial dysfunction.**

Medical research in Antarctica opens up new horizons in our understanding of the human being, particularly with research towards individual health conditions, a spectrum of psychological aspects, individual reactions towards stress and unusual environmental conditions.

The recent results and ideas for future research in the Ukrainian Antarctic station "Akademik Vernadsky" were published in The EPMA Journal [1, 2].

At the Ukrainian Antarctic scientific "Akademik Vernadsky" station it is possible to monitor health under the influence of environmental factors in a relatively undisturbed form without interference [1]. The landscape is characterized by its monochrome appearance, sterility and visual monotony. The stay during expedition to Antarctica (one year) is associated with social deprivation, a limited ambient gamma environment and changes in the electromagnetic spectrum. These factors have permitted the understanding of changes in body function and therefore the chance to develop new technologies that increases the adaptive capacity of the body.

*Mitochondrial (MT) dysfunction* has been implicated in the aetiology of many complex diseases, as well as the ageing process. Much of the research on mitochondrial dysfunction has focused on how mitochondrial damage may potentiate pathological phenotypes [3-8].

The cell adaptation to mitochondrial perturbations involves communication of stress to the cell and successful induction of responses, which include mitophagy, unfolded protein response, upregulation of antioxidant and DNA repair enzymes, morphological changes, and apoptosis if all else fails. The mitochondria is an inherently stressful environment and we speculate that dysregulation of stress signaling or an inability to switch on these adaptations during times of mitochondrial stress may underpin mitochondrial dysfunction and hence amount to pathological states over time [4, 9].

Long stay in extreme environmental conditions, with abrupt shift in heliogeophysical influences is a stress factor, negatively affecting the health, associated with disturbance in the balance of stress-realizing and stress-limiting systems, largely due to the development of abnormalities in the body, such as mitochondrial and endothelial (ED) dysfunction. The latter plays a leading role in the adaptation processes. Firstly, due to the metabolic activity of *mitochondria* (MT), the energy metabolism is maintained and increased, which plays an important role in adverse environmental effects including the hypoxic component; secondly, it is the adequate functioning of

the microcirculation system that helps to maintain optimal trophic processes in organs and tissues under extreme conditions [10, 11]. For this reason the study of mitochondrial apparatus and the microcirculatory bed in the body is of great importance for understanding the mechanisms of human adaptation to life in Antarctic conditions.

To study the structure and function of MT and other cellular organelles involved in adaptive processes, and also, indirectly, the functions of the vascular wall in humans, the blood cells, in particular, *platelets* (thrombocytes, T) and *leukocytes* (L) are almost the only objects (in the lack of surgical intervention). Platelets are highly specialized non-nuclear cells (or cell fragments) involved in many processes occurring in the body: in tissue regeneration, development of inflammatory and immune responses, maintenance of primary homeostasis [12-17].

Many researchers consider *platelets* as an example of the unity of structure and function [14-17]. In addition to a significant number of different granules, platelets contain glycoproteins, proteins, growth factors, ADP, ATP, calcium ions, serotonin, histamine, etc. Since a certain amount of MT inherited from *megakaryocytes, the* predecessors of platelets, the structure and functions of these organelles reflect the lasting changes in organism. In addition, leukocytes play an important role in the formation of adaptive reactions due to their leading role in specific and non-specific defense of the organism from any endo- and exogenous effects and are also a reliable site for studying the structure and function of MT in individuals, who have hibernated once or several times in Antarctica [18].

The main factors triggering development of `Antarctic syndrome` are considered stress and very unusual environmental conditions exposed during long time (even 1 stay = one year) in a close and relatively immutable environment.

*Hypoxia* is a contributing factor to `Antarctic syndrome` among others. The natural source of hypoxia in the Antarctic conditions is possible considering winds and sudden changes in barometric pressure (about 50 hPa or more per day), the fluctuation of oxygen partial pressure in the air, high physical activity, long polar days in the Antarctica. Furthermore a long term social and sexual deprivation affect an individual's psycho-physiological status and can alter normal biological rhythm, and likely decrease the tolerance to *hypoxic* conditions.

Thus our *hypothesis* is, that the specific type of stress, observed in Antarctica during long time evoke alteration in adaptation to stress *via* hypoxia signaling and increase sensitivity to hypoxia (tested via hypoxic loading).

***The aim of the study*** was: 1) to study the changes in platelet ultrastructure in winterers under hypoxic loading before and after a long stay in Antarctica; 2) to study the changes in the ultrastructure of mitochondria of leukocytes in winterers, depending on the length of exposure to Antarctic conditions.

**Materials and methods of research**

*Ukrainian Antarctic scientific "Akademik Vernadsky" station and Antarctic expeditions.*

The *geographical position* of the Ukrainian Antarctic station is defined by the coordinates 65° 15` south latitude and 64° 16` west longitude. It is located on the island of the Galindez archipelago near the Argentine Islands (strait 7 km) of the Antarctic Peninsula [1]. The island location of the station is characterized by a limited area (the island diameter of about 1 km), and two thirds of the island is covered with the spherical surface of the glacier and the rest of the territory—the rocks of volcanic origin—making it difficult for pedestrian movement and making it impossible to land aircraft.

Nearby islands are located within a radius of 35 km, to which members of the expedition in the summer may be reached by boat, and in the winter (in the case of formation of safe ice) on the ice surface, which is always accompanied by a certain risk. Regional climatic conditions make possible the maritime transport approach in the summer (February–March), when changing the station crew. The region of the station is characteristic of high latitudes photoperiodicity with long winter nights and extra-long summer days.

Precipitation in the form of snow and rain come in almost every day. In winter, there are snow drifts up to 2–3 m. However, total rainfall for the year is not higher than in Ukraine. The station area is always with a humidity increased to 80–90 %. The sun appears for about 35 days during the year. The duration of sunshine in Antarctica during the year is almost four times lower compared with that of Ukraine.

Strong hurricane winds and sudden changes in barometric pressure (55 hPa or more per day), which are stored for a long time, lead to the fluctuation of oxygen in the ambient air and reduces its partial pressure over 13 hPa.

In addition, infrasound test load (in the band 6–7 Hz) of the Antarctic station members of the expedition, which exceeds the background values in Ukraine, is almost doubled. The direction of the field lines of the magnetic field of the Earth in Antarctica is reversed, and helio-geomagnetic phenomenon is more pronounced, which is fixed by magnetometric equipment.

The *members of the expedition* (12–15 persons) (up to 13 months) are removed for a long time from familiar surroundings of the civilized world, working in a limited area in a small team environment under the conditions of social and psychological isolation, sensory deprivation, and sexual abstinence.

Territorial restrictions and the long Antarctic winter create the conditions for forced communication among a small team of members of the expedition and the development of a certain state of inactivity during the winter.

Living conditions at the station excludes the possibility of hypothermia, starvation, lack of water, and electricity. Environment in the station area is free of man-made sources of pollution and in neighborhoods formed a certain vicious bacterial microclimate without exogenous exchange outside of human viral and bacterial flora.

*The study groups*

I. The assessment of thrombocytes in the group of individuals, who prepared for wintering in Antarctica (n=6) was performed: before the hypoxic loading (subgroup 1) and after the hypoxic loading (subgroup 2), and also in the group that returned after wintering (n=11): before hypoxic loading (subgroup 3) and after hypoxic loading (subgroup 4). Hypoxic loading was carried out in two ways: breathing a gas mixture with oxygen content lowered to 12% and achieving a hypoxic state when performing hard physical work on a veloergometer (75% of the proper maximum oxygen consumption). The hypoxic loading tests were carried out *after* the return from Antarctica.

II. The study of the changes in the mitochondrial ultrastructure in leukocytes (L) was carried out in 24 winterers, who stayed during a long time in Antarctica 1 time (group 1), 3-4 times (group 2) and 5-7 times (group 3).

The length of their stay in the Antarctic conditions [1, 2] was measured as the number of the expeditions participated. The number of days of exposure to condition of Antarctica was assessed as follows: all winterers stayed one year during one expedition = 365 days; two and three stays / expeditions = 730 days and 1095 days accordingly; four stays = 1460 days. In this study the length of their stay and also the number of visits (shifts to the adverse conditions) was important.

The control group included 8 men, who were not exposed to adverse environmental factors.

*Sub-cellular imaging*

Studies of the ultrastructure of thrombocytes and leukocytes were performed after centrifuging the blood of the subjects. Platelets and leukocyte- enriched plasma was obtained by centrifuging whole blood at a room temperature for 15 min at 120g on a laboratory T-30 centrifuge (Ukraine). The plasma was neatly separated from the axial cells and centrifuged at 5000g for 15 min using a Vortecs Combispin FVL-2400N mini centrifuge (Latvia).

The preparation of samples for electron microscopy was carried out in accordance with the standard method for blood cells with double fixation of $OsO_4$ and *glutaraldehyde*, dehydration in alcohols of increasing concentration and filling in Epon-Araldit (reagents from Fluka, Switzerland). Ultrathin sections 40-60 nm thick were contrasted with 1% uranyl acetate solution and lead citrate solution (Sigma reagents, USA) and viewed in an electron microscope PEM-124C (Ukraine).

*Morphometric calculations* were performed using the software *Image Tool Version 3* (USA) [19] on 130-150 fields for each group of subjects with the determination of quantitative parameters of tissue structure according to the approach by Weibel ER [20, 21]. The determination of the total

number of studied objects was carried out on the basis of the method of direct estimation, for which it is sufficient to study 125 fields.

The software *Image Tool Version 3* (USA) designed for analysis and processing of images, has a spatial calibration that allows measurements in real quantities, can utilize the approaches by Weibel ER [20, 21]. This is suitable for studying structures at both the organ / tissue, and cellular / subcellular levels, and also provides reliable information about the quantitative properties of the structure of the object detected at various electron microscope magnifications. Determination of diameters and areas of objects was carried out by a stereological method, using planimetric analysis by the method of fields [21], the 95% reliability was achieved and proved at $m \leq 0.12$ μm (for linear values) and $m \leq 0.21$ $\mu m^2$ (for areas).

Statistical processing of the data was carried out using the Microsoft Excel, numeric data are presented as mean arithmetic values and their standard error of the mean (M±SEM) in accordance with the Shapiro–Wilk test, the obtained data fit into the normal Gaussian distribution.

The reliability of the results assessed using the Fisher's criterion and the Student's t test. Differences between mean values were considered statistically significant at $p<0.05$.

**Results**

*I. Sub-cellular imaging of platelets mitochondrial dysfunction*

Analysis of the obtained results showed that in persons of the subgroup 1 platelets in the most cases (more than a half) were the young forms (Table 1).

Table 1

Platelet area for winterers of the studied subgroups

| The surveyed winterers | Average platelet area, $\mu m^2$ | Number of young platelets according to their total number, % |
|---|---|---|
| Subgroup 1, n = 11 | 49.6±2.3 | 58.6±2.8 |
| Subgroup 2, n = 11 | 35.2±3.3* | 47.4±4.7* |
| Subgroup 3, n = 11 | 35.8±3.1* | 36.0±6.1* |
| Subgroup 4, n = 11 | 32.4±2.0* | 42.1±3.9* |

Notes: * - the differences are significant *vs* the control values of the 1st subgroup ($p <0.05$)

We have also revealed that platelets of the control subgroup contained optically dense corpuscles (δ-granules) in the amount of 5-12 (Table 2).

It is believed that such granules contain serotonin, ADP and ATP, and also accumulate $Ca^{2+}$. And the number of dense granules depends on the amount of serotonin, which seems particularly important, since the latter has a direct effect on the reduction of muscle cells in the walls of most vessels [22]. Consequently, the number of dense granules may, to a certain extent, be a marker of the contractility of the vascular wall. The presence of α-granules (3 - 5 per cell) (Table 2) is the

evidence that hydrolytic enzymes: acidic phosphatase, β-glucuronidase, cathepsin are preserved (and continue to be secreted, as these granules are considered secretory).

Table 2

Morphometric characteristics of thrombocytes in winterers of the studied subgroups

| The surveyed winterers | The number of delta-granules per cell | Number of alpha-granules per cell | Number of tubules per cell | Average diameter of MT, μm | Average area of MT, $*10^{-2}$ $\mu m^2$ |
|---|---|---|---|---|---|
| Subgroup 1, n = 11 | 7.5±0.8 | 4.9±1.1 | 6.8±0.9 | 0.60±0.04 | 48.8±3.9 |
| Subgroup 2, n = 11 | 4.1±0.3* | 5.7±1.3 | 6.0±1.2 | 0.47±0.05* | 38.6±2.4* |
| Subgroup 3, n = 11 | 3.3±0.5* | 8.6±1.4* | 9.4±1.1* | 0.30±0.03* | 31.2±1.8* |
| Subgroup 4, n = 11 | 3.9±0.7* | 7.6±1.6* | 8.9±1.5* | 0.69±0.05 | 51.3±2.2 |

Notes: * - the differences are significant *vs* the control values of the 1st subgroup ($p < 0.05$)

Platelets had a well-developed internal skeleton, as evidenced by the presence of a significant amount (5-10 per cell) of optically transparent tubules (the so-called tubules; Table 2). The location of a significant number of tubules at the periphery of T should be considered as the presence of a high intensity of metabolic processes, since the invagination of the membrane allow various substances to be absorbed or released by thrombocytes [14-16, 22].

The counting of the number of MT in T in the control group allowed making assumptions on the morphogenesis of MT that the intensity of energy exchange is inappropriate due to their small amount, as many as 2 (rarely 4) organelles were detected in the studied cells. However, it is possible to study the ultrastructure of MT, which to some extent reflects the morphology of MT in progenitor cells. The organelles differed in electron-dense matrix, they were well structured, which indicates the possibility of MT to efficiently synthesize macroergic compounds.

The average diameter of the MT was 0.60 ± 0.04 μm, i.e. the organelles were quite large (Table 2). This is also evidenced by the average area of MT was $48.8±3.9*10^{-2}$ $\mu m^2$.

After hypoxic loading (subgroup 2), the number of young platelets with an area of over 42 μm decreased by 11.2% (Table 1); platelets with the area of 35.2 ± 3.3 $\mu m^2$ dominated. It is generally believed that an geometric increase in the mean T size indicates the appearance of a higher percentage of active platelets in the circulation, while a decrease in optical parameters indicates the inadequacy or emptiness of dense granules. Indeed, the number of dense granules in the subgroup 2 decreased significantly (up to 3-5 per cell); some platelets did not contained such bodies (Fig. 1). Considering this indicator as directly related to the amount of serotonin, we can assume that it may reflect a decrease in its content, and, consequently, the contractility of the vascular wall. This conclusion could

likely be extended to at least some other cells, since T can be considered as "fragments" of megakaryocytes and, accordingly, give an idea of the changes that take place in the cells of the bone marrow. In addition, it is believed that a decrease in the optical density of these granules may indicate a suppression of thrombocytopoiesis and decreasing their functional activity.

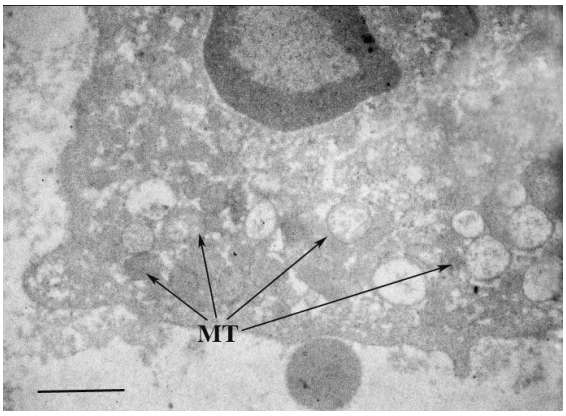



Fig. 1. Damage of the mitochondrial ultrastructure (MT) with partial or complete vacuolization, melting of the cristae, considered to be a manifestation of mitochondrial dysfunction, in leukocytes in winterers of the group 1. The *reference line* corresponds to *1 μm*

The total number of α-granules was maintained at the level of control values, i.e. T retained their secretory ability. The internal skeleton of platelets was also preserved.

Significant changes occurred in the ultrastructure of MT after hypoxic loading. Firstly, partially vacuolizated organelles, also MT with less coarse than in the control matrix, were detected, which often is regarded as a sign of activation of glycolysis. Such changes are characteristic of the reaction of the mitochondrial apparatus in cells of different tissues. Secondly, the MT diameter significantly decreased to 0.47±0.05 μm ($p<0.05$). Such process is considered ambiguously: either as a low activity of MT and, possibly, an initial stage of the mitochondrial path of apoptosis, or as adaptive changes leading to optimization of energy exchange, even with a decrease in the average MT area (Table 2).

The platelets in individuals who returned after wintering in Antarctica were mainly (by 64.0%) represented by plates of medium area as 35.8±3.1 $\mu m^2$ (Table 1). In this case, T were detected, which were densely covered with glycogen granules. In a significant number of cells, almost all of the surface area was occupied by the tubules of the endoplasmic reticulum, and the cytoplasm was only fragmented (Fig. 2). Such features of the ultrastructure are evidence of the overstrain of the functions of T precursor cells and the platelets themselves [14, 15]. Furthermore, the number of tubules of the inner T skeleton significantly increased (Table 2), which is an indirect confirmation of the increasing intensity of metabolic processes.

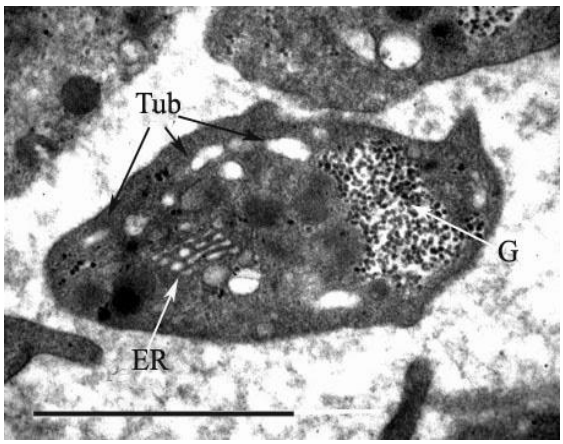



Fig. 2. Ultrastructure of the platelets in the individuals who returned after wintering in the Antarctica. Tub - tubules of the internal skeleton, G - granules of glycogen, ER - endoplasmic reticulum. The *reference line* corresponds to *1 μm*

The platelets in persons of three subgroups almost did not contain the dense corpuscles, at the same time the quantity of α-granules increased (Table 2). This dynamics of changes in the ultrastructure indicated: *firstly*, a decrease in serotonin, i.e., indirectly, a decreasing the contractile activity of the vascular wall; *secondly*, a significant depletion of ADP and ATP depots. In addition, since α-granules contain hydrolytic enzymes and play the role of lysosomes in platelets, it can be considered that platelets functioned in their sufficiently intense regime and are ready to activate metabolic processes [13-17].

Reducing the number of macroergic molecules at the voltage-gated channels of the cell requires the optimization (activation) of energy production. A confirmation of this possibility in platelets of individuals, who have been in unfavorable conditions during a long time is an increasing the number of MT to 5-7 (sometimes up to 10) per cell, which is not typical to platelets containing, as mentioned above, only few organelles. We detected mainly fairly small MT in T with an average diameter of 0.30±0.03 μm, lesser than the control values by 50%. This is typical for organisms exposed to hypoxic conditions during a long time (for several sessions). Naturally, the average area of such MTs was significantly smaller than the control values (Table 2). We follow the opinion of the researchers, considering this process as an element of adaptation, since a larger number of small organelles is energetically more beneficial for the cell [23].

After hypoxic loading, in individuals, who returned from Antarctica (subgroup 4), the morphometric characteristics of the T ultrastructure remained almost unchanged relative to those analyzed in the subgroups 3 (Table 2).

However, the conditions imposed on a cell could not be tolerated by the T mitochondrial apparatus. The mean MT diameter significantly increased due to moderate swelling, which is considered to be an indicator of an increase in functional activity with the participation of ATP-

dependent $K^+$ channels and the intensification of ATP synthesis [13-17]. The MT diameter averaged as 0.69±0.05 μm, the average area of the MT tended to exceed the control values (Table 2). Along with that, a number of organelles acquired pronounced signs of destruction (melting and loss of regularity of cristae, breach of the integrity of mitochondrial membranes) and partial vacuolization.

*Thus, the results obtained indicate that staying in the Antarctic conditions significantly modifies the ultrastructure of platelets.* The changes that occur indicate the intensity of the functioning of both T and, probably, precursor cells. The almost complete disappearance of dense granules in cells may indicate the depletion of the serotonin depot in T, which is essential for the function of the vascular wall. Such a dynamics is similar to the response of T to hypoxic loading in individuals, who never visited Antarctica.

*The response to hypoxia in individuals, who have spent a long time in Antarctica and those who have not been exposed to these conditions, was significantly different.*

This is strongly depicted in the mitochondrial apparatus of T, which reacted to the hypoxic loading in the opposite way: in the subgroup 2 the MT significantly decreased in size, but significantly increased in the 4th subgroup. Both these processes can be considered as compensatory/adaptive, however, the mechanisms involved in the development of adaptive reactions are different: firstly, the optimization of energy exchange by increasing the sum of the mitochondrial surfaces in a unit volume of tissue; secondly, an increasing the functional activity of the organelles due to moderate edema, can manifest not only the increasing energy production, but also the activation of glycolysis.

*II. Sub-cellular imaging of platelets mitochondrial dysfunction in leukocytes*

Analysis of the features of the mitochondrial apparatus of L in winterers of group 1 (single wintering) showed that the average number of MT per unit area of the cell did not differ from that determined in the control group and was 4-8 organelles (Table 3).

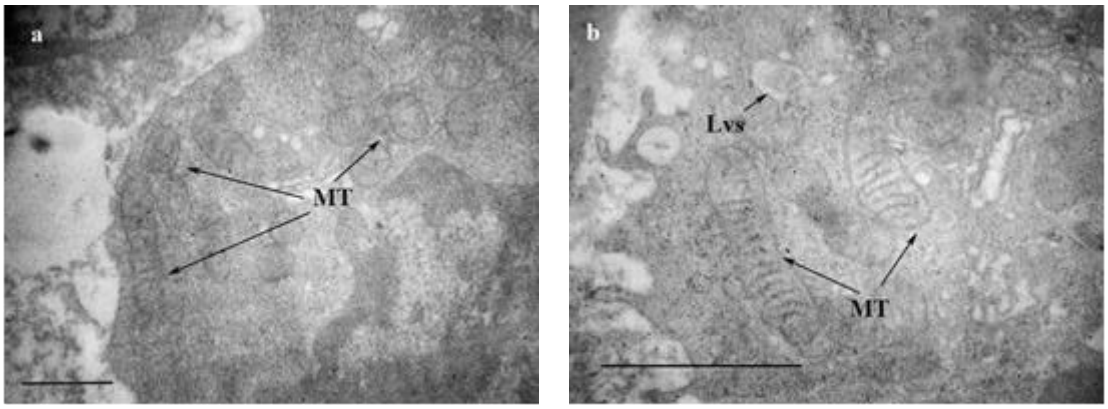
Table 3

Morphometric characteristics of the leukocyte mitochondria in winterers, depending on the number of their stay in Antarctica

| The surveyed winterers | Number of MT, Units/μm$^2$ | Average diameter MT, μm | Average area of MT, *$10^{-2}$ μm$^2$ |
|---|---|---|---|
| Controls, n = 8 | 6.8±0.3 | 0.50±0.05 | 38.2±2.6 |
| Group 1, n = 8 | 7.1±0.2 | 0.62±0.03* | 45.3±1.4* |
| Group 2, n = 8 | 13.4±0.9* | 0.56±0.04 | 40.7±2.0 |
| Group 3, n = 8 | 23.8±2.8** | 0.70±0.08* | 48.8±2.3* |

Notes: The data are presented as mean arithmetic values and their standard error of the mean (M ± SEM). The differences are significant *vs* the control values of the 1st subgroup: * - $p < 0.05$, ** - $p < 0.01$

The mean diameter of the MT was 0.62±0.03 μm, which exceeded the control values by 23% ($p<0.05$), the average area of the organelles was greater by 18.5% ($p<0.05$; Table. 3). Such dynamics of changes are considered as a moderate swelling associated, as mentioned above, with the voltage of the MT function, and, according to modern data, is provided by modulating the activity (in particular, activation) of ATP-dependent $K^+$ channels [37]. Such a process is probably compensatory, since about 30% of MT had a damaged ultrastructure with partial or complete vacuolization, melting of cristae (Fig. 3), which is considered as manifestations of mitochondrial dysfunction [10-12].




**A** **B**

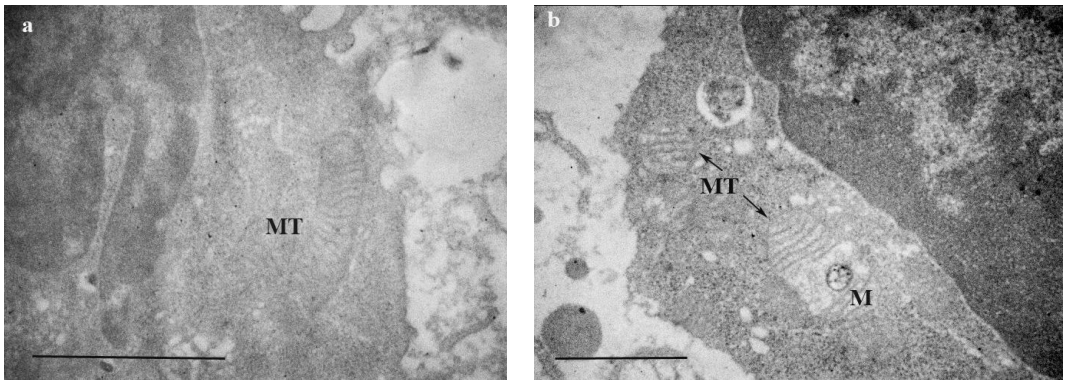
Fig. 3. Activation of the processes induced by TNF and/or $H_2O_2$-induced relevant to apoptosis (a) and autophagy (b) in the mitochondria (MT) of leukocytes of winterers of group 2. Lys - primary and secondary lysosomes. A pronounced compaction of MT membranes, which were often located near the lysosomes. Significant swelling of the organelles. The *reference line* corresponds to *1 μm*

We observed in winterers of group 2 (3-4 winterings) an increasing in the number of MT per unit area of L up to 10-15 units, i.e. by 2 times (see Table 5), which indicates the activation of MT morphogenesis. A pronounced compaction of MT membranes was revealed, which were often located near the lysosomes. Significant swelling of the organelles with an increasing their diameter and area was absent (Table 3, Fig. 3a, b) [6, 22].

On the other hand, the increase in the electron density of MT membranes and sometimes of the mitochondrial matrix is considered to be characteristic of apoptosis induced by TNF (soluble or transmembrane form of cytokines) and/or by H2O2. In addition, organelles were observed with diameter reaching 1-1.5 μm, and longitudinal association between MT (Fig. 4a) [22, 23].

The winterers of group 3 (5-7 wintering) demonstrated changes in MT, indicating the development of oxidative stress: an increase of the total number of organelles by 3.5 (Table 3), over 40% of MT had an altered ultrastructure in the form of loss of regularity of the crista, partial or

complete vacuolization: a spatial reorganization in some MT, disappearing the plate-shaped cristae with the formation of myelin-like structures (Fig. 4b).




**A** **B**

**Fig. 4.** Longitudinal association of mitochondria (MT) of leukocytes in winterers of the group 2 (a). The increase in the electron density of MT membranes and the mitochondrial matrix is a manifestation of apoptosis; the longitudinal association of MT indicates a pronounced hyperfunction of the organelle and the cell as a whole. The development of myelin-like structures (M) in the mitochondria (MT) of leukocytes in winterers of the group 3 (b). The changes in MT indicate the development of oxidative stress; over 40% of MT have demonstrated the loss of regularity of the crista, partial or complete vacuolization; in some MT a spatial reorganization of the membranes is observed: the correctly located plate-shaped cristae disappear with the formation of myelin-like structures against the background of electronically transparent hydrated regions of the matrix. *The reference line corresponds to 1 μm*

This ultrastructure of MT does not fully correspond to the existing classical morphological criteria of *apoptosis (mitoptosis)* [6] but they have a similar qualitative orientation. They reflect the widespread response of the cell to various types of adverse effects associated with inadequate energy production and are considered as partial cell necrosis. It is believed that the appearance of foci of partial necrosis, MT polymorphism and the formation of myelin-like structures in them - the result of their degradation - is associated with increased proteolysis due to activation of proteases in the cytosol and phospholipases in MT [22]. In addition, excessive swelling of MT was observed (see Table 3), which indicates the development of irreversible organelle changes. Such a set of changes is considered as the disintegration of a single MT system of the apparatus and, in the future, an increased death of MT, which will negatively affect the energy metabolism of the cell.

**Discussion**

*Vascular dys/regulation and hypoxia signaling*

Three major mechanisms are discussed to be involved in enhancing the PHD activity despite the lack of oxygen: “(1) NO mediated induction of a HIF-1α dependent feedback loop leading to

newly expressed PHD2 and enhanced nuclear localization, (2) $O_2$-redistribution towards PHDs after inhibition of mitochondrial respiration by NO, (3) reactivation of PHD activity by a NO mediated increase of iron and 2-oxoglutarate and/or involvement of reactive oxygen and/or nitrogen species".

*Endothelin-1 (Et-1)* is a vasoconstrictor peptide that plays an important role in the pathophysiology of hypertension, myocardial ischemia, and other diseases. The Et-1 gene contains HIF-1 binding hypoxia responsive elements which mediate transcriptional responses to hypoxia and cobalt in microvascular endothelial cells. *Genistein* (angiogenesis inhibitor and phytoestrogen) appears to inhibit this response by affecting the transcriptional activity of the HIF-1 complex, without significantly affecting its DNA-binding properties [30]. *Vascular endothelial growth factor (VEGF)* has important role in signaling hypoxia and linked with number of chronic diseases including bronchial asthma, diabetes mellitus, rheumatic disease and cancer. The microenvironment for erythropoiesis is regulated by *HIF-2α* [29, 30].

*Potential clinical utility of the study results for regenerative therapy*

The recent study demonstrating metabolic reprogramming by HIF-1 activation [31] provide insights to the alternative strategy for improving the efficiency of cell therapy. Thus, dimethyloxalylglycine (DMOG), a prolyl hydroxylase inhibitor, induces HIF-1 activation and metabolic reprogramming including decreased ROS, increased intracellular pH value, and enhanced glucose uptake and glycogen synthesis in adipose-derived stem cells (ADSCs) and therefore presents a positive impact on cellular angiogenesis and survival rate in an ischaemic environment after transplantation [31]. HIF-1 activates the transcription of diverse genes encoding angiogenic cytokines, including vascular endothelial growth factor (VEGF), stromal-derived factor 1 (SDF-1), placental growth factor (PLGF), angiopoietin 1 and 2, and platelet-derived growth factor B [31], therefore, it can improve vascularization for cell therapy [32] and increase overall efficacy for proved applications of regenerative tretments [33, 34] and platelets rich plasma (PRP)-based therapy [35].

The risk of *tumorogenesis* is known as significant limitation of using cell therapy. Thus PHD inhibitors may provide an appropriate environment for tumour growth and metastasis by stabilizing HIF-1, which plays a major role in tumorogenesis [36].

*Hormonal- and sex-related response on hypoxia*

It was reported that: 1) the male sex hormones play no role in the development of the excessive polycythemia during chronic hypoxic exposure; 2) the female sex hormones suppressed both the polycythemic and cardiopulmonary responses *in vivo* during chronic hypoxic exposure [37, 38].

*Role of spleen-associated markers in patient stratification*

Spleen structure and function are underestimated in medical profiling, since the bone marrow remains the most important erythropoietic organ under both resting and stimulated states,

but inordinate splenic erythropoiesis can be initiated e.g. during the development of chronic mountain sickness in chronic hypoxia [39]. Our preliminary results demonstrated changes in the spleen size in all participants with a tendency to deacrese after returning (this was also observed in the liver and thyroid gland size). The spleen and intestine are two major immune organs involved in the innate immune response to infection [40]. Spleen structure and size might be supposed as promising imaging biomarker for immunity- and stress-related conditions.

*Relevance of the data collected for cancer research and management*

Tumor microenvironment and hypoxia plays crucial role in the development of many types of cancer and metastatic diseases [27, 41]. Hypoxia is a key driver of tumour angiogenesis.

Extensive research during the last decades has revealed that oxidative stress can mediate cancer initiation and development by leading not only to molecular damage but also to a disruption of reduction–oxidation (redox) signaling [42]. Hence, as was discussed earlier, among the most frequently described pathways intracellular ROS/RNS generation organelles are mitochondria, endoplasmic reticulum and peroxisomes; NO is an important modulator which affects the activity of the cellular oxygen sensors.

Therefore, obtained and discussed data provide and interesting capacity for a novel exciting hypotheses and permits the study of conditions such as 'Flammer syndrome' (FS) in the Antarctic region amongst many others [42-45]. Thus, recently the "Flammer Syndrome" was supposed to update the "*seed and soil*" hypothesis suggesting the "pre-metastatic niches" in breast cancer to be created by or prior to the tumour onset [44]. It was supposed that the epi/genetic predisposition of individuals at risk to form the systemic hypoxic pre-metastatic niches in development and progression of the metastatic disease. The differences between Flammer syndrome and its "counterpart" endothelial dysfunction in the context of cardiovascular diseases were clearely delineated. At the molecular level, enhanced blood levels of endothelin-1 and metalloproteinases MMP-9 and MMP-2 have been described for the FS-affected individuals [46].

*Potential application for to the personalized sport medicine, physical therapy and pain management*

Preliminary analysis demonstrated changes of responses on Flammer syndrome questionnaire carried out before and after the expedition; medical examinations showed stress dysadaptation in alteration in thyroid, gut function, anxiety and pain sensation [47]. It was reported that exercise may enhance proliferative ability and decrease adipogenic ability of bone marrow stromal cells (BMSCs) and adipose-derived stem cells changed biological characteristics of both types; a positive effect was observed on the antiapoptotic ability of both mesenchymal stem cells (MSC) types. It is therefore believed that BMSCs derived from exercised organism on early passage may be a good cell source for bone tissue engineering [48].

We consider to apply an innovative approach via combining findings from the current research with novel tools like Computer Assisted Rehabilitation Environment (CAREN) [49] for modeling special situation, predictive testing and education. This virtual reality tool creates a simulated environment for physical therapy, and can be also used for diagnostic sessions to model conditions appearing under stress to define muscular patterns due to unusual physical activities (like mountain climbing, deep mining, cave exploration, etc.). This would provide benefits for stratification of patients, like candidates for regenerative treatments, and provide largely individualised preventive programs for occupational, sport medicine, etc.

*Microbiome and stress – the challenge for personalisation of dietary-based treatments and prevention*

The crucial role of microbiome for human health was extensively demonstrated [28, 50-52]. The emerging evidence implicating microbiota in stress-related disorders and links between lifestyle and microbiome [50], however causation links are still unclear [67-70]. The potential for precise therapeutic microbiome interventions can target microbial-mitochondrial metabolic communication [52]. Thus, the microbiome can be an essential supplier of metabolites that act at the level of resident mitochondria of host in skeletal muscle to stabilize host metabolism [52].

Our preliminary microbiome studies [1, 50] in the Antarctic setting have shown an increase in fungi on the skin in expedition participants, believed to be due to interferences with local immunity and dysbiosis of the normal skin microbiome due to stress, recycled air and antiseptic agents. The recent studies of different impact on health of microbiota of the indoor/outdoor environment, microbial habitat, geographic coverage showed that both bacteria and fungi from the same observational samples of indoor air has shown important differences between the sources for these microbes, influenced by geographic distance, the home type, and differences in resident activity [56].

Another challenge in Antarctica is recognizing Antarctic-specific microorganisms, number of species of this kind were reported [57-60]. The few of them demonstrated potentially beneficial properties for human health [61, 62]. Thus, among 25 isolated pure cultures obtained from the samples of mosses, lichens, soil and stones obtained during $18^{th}$-$20^{th}$ Ukrainian Antarctic expedition the strains *Pseudogymnoascus pannorum* and *Mucor circinelloides* demonstrated a pronounced activity to the synthesis of complex of biologically active lipids [63].

Developing the novel probiotics in addition to traditional and well-studied ones is a promising perspective [64]. Microorganisms found in Antartica or those cultivated in Antartcic conditions are likely promising for the *next-generation probiotics* and biotherapeutic products. This challenge needs involving multidiscilinary scientific efforts, the regulatory framework and more flexible nomenclature [65]. Lessons for the healthy nutrition and novel dietary interventions can be

learned in the Antarctica setting [66]. Promising is stratification of responders on microbiome-modulating interventions on hypoxia-associated mitochondria dysfunction for liver diasese and metabolic sydrome [67]. The recent studies provided knowledge for probiotic selection and elucidate greatest potential for supporting the crew health and well-being in a spaceflights and similar programs [68, 69].

**Conclusion**

The results obtained indicate that a long exposure to Antarctic conditions is associated with mitochondrial dysfunction. The stratification of the patients for stress and chronic disease is promising based on hypoxia-associated mitochondria dysfunction markers.

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**Data Availability Statement**

The data presented in this study are available on request from the corresponding author.

**Conflicts of Interest**

The authors declare no conflict of interest.