

ORIGINAL SCIENTIFIC PAPER

Reactive Changes of Buccal Epitheliocytes and Erythrocytes in Students with Different Somatic Health and Cardiorespiratory Endurance Levels

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Abstract

This paper aims to identify the specific features in buccal epitheliocytes' and peripheral blood erythrocytes' structural changes for students with different somatic health and cardiorespiratory endurance levels. Forty male students aged 17 were enrolled in this study, divided into three groups: 15 students with above-average and average somatic health levels, 15 students with below-average somatic health level, and a control group consisting of 10 healthy students. Cardio-respiratory endurance was determined using a 12-minute shuttle run test. Histological specimens were stained with azure and eosin and examined with a Leika DME light microscope. To obtain cell images, an Olympus Camedia C-480 ZOOM digital video camera was used. Determination of nucleus and cytoplasm structural perimeters on cytological medicine was performed using BioVision 4.0 software. Students with different somatic health levels were found to have regular reactive changes in the morphometric parameters of the buccal epitheliocytes and peripheral blood erythrocytes after cardiorespiratory endurance testing. As the proportion of discocytes decreases, and the contents of spherocytes and stomatocytes increases, a significant enrichment of microrelief and an increase of peripheral blood erythrocytes atypical forms occur, which indicates the low membrane resistance to the maximal aerobic physical activity in students with low somatic health. The revealed morphometric changes of buccal epitheliocytes and peripheral blood erythrocytes during physical activity are conditioned by the somatic health level, which affects the deformation degree of erythrocytes, limits the production of new, functionally active cells, and leads to a delay in the differentiation processes of cells within an increasing number of cells with raised core sizes, which closely correlates with the number of levels achieved during 12-minute shuttle run test.

Keywords: *epitheliocytes, erythrocytes, morphometry, core-cytoplasmic ratio, somatic health, cardio-respiratory endurance*

Introduction

Scientific literature indicates significant changes in the homeostasis of the human body at its maximum physical activity (Apanasenko, Volhina, & Bushuiev, 2000; Leshchak & Popel, 2012; Belykh, Eskov, & Fudin, 2015). In this case, the first impact element at the level of the whole body is the immune system, and at the outset in its nonspecific parts, such as local immunity (Dranik, 2006). The works of various au-

thors show the particular sensitivity of this element of the body's immune response to physical activity, not only in athletes (Zemska & Piatnychuk, 2017; Nedospasov, 2013) but also in people who do not engage in sports (Levytskyi, 2007; Leshchak & Popel, 2012) or are in a state of psycho-emotional or immobilizing stress (Duma, Popel', & Polyanskaya, 2016; Moshkin & Gerlinskaya, 2016; Popel & Mytskan, 2016; Gan & Popel, 2017), or have different organ diseases, and similar



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(Arutyunov, Pleskanovskaya, & Naumov, 2009; Rabinovich, Ostrovskij, & Abramova, 2013; Faichak, Popel, & Sluchyk, 2011).

In this regard, the oral cavity mucous membrane is of particular importance, because in many works it has been proven to be particularly sensitive in very different body conditions both in physiological (Arutyunov et al., 2009; Gasyuk, 2009) and in pathological states (Hande & Chaudhary, 2010; Popel, Mytskan, & Lapkovskiy, 2017; Prasad, Ramesh, & Balamurali, 2010).

The adhesion of bacterial flora and the penetration of other reagents under the oral cavity mucous membrane is impaired by a multilayered non-keratinized and partially keratinized epithelium (Dranik, 2006). Therefore, its functional state is of great importance in the development of recovery processes or the stabilization and maintenance of body homeostasis due to the combined involvement of activation mechanisms of nonspecific and cellular immunity. This is ensured by the fact that buccal epitheliocytes, except for their mechanical barrier function and participation in phagocytosis, can be antigen-presenting cells capable of secreting various biologically active substances, such as proinflammatory cytokines, chemokines, growth factors, and others (Gasyuk, 2009; Levytskyi, 2007; Rabinovich et al., 2013; Gan & Popel, 2017).

It should be noted that mucous membranes' epitheliocytes synthesize an additional secretory component "s", which attaches to immunoglobulin molecule A (IgA) produced by tissue lymphocytes that migrate from the bloodstream through haemocapillaries' walls in different parts of the oral cavity mucosa. As the IgA molecule passes through the epithelial cells, the sIgA complex is formed. Therefore, this complex of epithelial and plasma cells neutralizes bacterial toxins, localizes viruses, and stimulates phagocytosis, thus providing local resistance of the body at the oral cavity level (Dranik, 2006; Wandeur et al., 2011).

Considering that at maximal physical loading (PLmax) in different organs and tissues microcirculation disorders are observed (Baskevych et al., 2017; Popel & Mytskan, 2016; Popel et al., 2017), it is quite natural that they, first of all, concern rheological property disorders of peripheral blood erythrocytes (PBE). As such changes promote hypoxia and increasing of protein and lipid peroxidation (Faichak et al., 2011), we can suggest their influence on changes in cells' energy metabolism, which also results in functional changes of the buccal epitheliocytes (Hande & Chaudhary, 2010). Thus, according to some studies (Duma et al., 2016; Leshchak & Popel, 2012; Popel & Mytskan, 2016; Popel et al., 2017), one of the informative indicators of the state of cellular metabolism is morphological changes of PBE and buccal epitheliocytes, which are widely used in the diagnostics of various pathological conditions of the human body. Using the method of exfoliative cytology, the determining of the perimeter, core area and cytoplasm and their correlation in epitheliocytes, as well as their shape changes, can be evaluated comprehensively, which increases the level of the informativeness of these changes (Zemka & Piatnychuk, 2017; Levytskyi, 2007; Faichak et al., 2011).

It is known that morphometric changes in buccal epitheliocytes can occur not only under the influence of PLmax but also be an indicator of somatic health (SH) (Levytskyi, 2007). Therefore, the possibility of the interaction and cooperation of buccal epitheliocytes with immunocompetent cells and their ability to perform barrier function depends on their morpho-

functional state, which may be changed under the influence of exo- and endogenous factors, in particular, PLmax, SH level, and PBE state (Arutyunov et al., 2009; Zemka & Piatnychuk, 2017; Leshchak & Popel, 2012; Faichak et al., 2011; Popel et al., 2017).

The main goals of this research were to identify the specific features in buccal epitheliocytes' and peripheral blood erythrocytes' structural changes for students with different somatic health and cardiorespiratory endurance levels.

Methods

Participants and data collection

To achieve this goal, an assessment of the morphometric parameters of buccal epitheliocytes was conducted in forty 17-year-old male students of the Physical Education and Sports Faculty of Vasyl Stefanyk Precarpathian National University. At the time of the study, all the students (according to medical examination) had the following somatic health (SH) status verified on the basis of international criteria and diagnostic standards: above average (AA) (25.0%), average (A) (37.5%), and below average (BA) (37.5%). Students with high and low levels of SH were not identified. Based on these results, two groups were formed; the first group had 15 students with above-average and average cardiac somatic health levels; the second group had 15 students with below-average somatic health level; the control group (CG) had 10 healthy students of the same age. Cardio-respiratory endurance was determined using the 12-minute shuttle run test.

Students with inflammatory oral cavity phenomena, endocrine disorders (diabetes mellitus), and gastrointestinal tract diseases were not involved, because such conditions affect the morphofunctional buccal epitheliocyte state and, as a rule, these individuals receive pharmacotherapy, which also has some effect on the cytological picture of cavity mucous membrane and cavity peripheral blood state (Arutyunov et al., 2009; Gasyuk, 2009; Wandeur et al., 2011; Prasad et al., 2010).

Instruments and analysis

Peripheral blood erythrocyte morphological studies (PBE) were performed with a JEOL-25M-T220A (Japan) scanning electron microscope according to the conventional method.

After rinsing the oral cavity with saline, with a light pressure, we scraped the cheek epithelium inner surface in the area of molars with the end of a sterile dental trowel and transferred the obtained material to a slide with a smear, drying for 2-3 minutes outdoors.

Histological specimens were stained with azure and eosin and examined with a Leika DME light microscope. To the cell images, an Olympus Camedia C-480 ZOOM digital video camera (Olympus Corp., Japan) with 400-1200 magnification there was used.

Determination of core (Pc) and cytoplasm (Pcp) structural perimeters on cytological medicine was performed using a BioVision 4.0 software. The frequency of cells with anomalies was determined in terms of 1000 cells.

Correlation analysis of the core-cytoplasmic ratio (Sc/Scp) and (Pc/Pcp) was performed to identify the possible relationship of a single indicator changes with PLmax duration (determined by the level of performing the 12 min shuttle run).

Processing of the statistical study results was performed using the standard Statistica 5 for Windows software. For data processing, the correspondence analysis of obtained data to

the normal law of random variables distribution was carried out. The results are presented as mean values and standard deviation (M±SD). Averages were compared using Student's t-test. Statistical data analysis was performed at a given probability (0.95), and the obtained results were considered statistically significant at p<0.05. To determine the perimeters and core area ratio dependence to the cytoplasm perimeter and the area (Pc/Pcp and Sc/Scp) of buccal epitheliocytes after performing the 12-minute shuttle run test), a multiple linear

regression analysis was performed using a stepwise method (Venkataswamy-Reddy, 2019).

Results

Morphometric analysis of students' buccal epitheliocytes after performing the 12-minute shuttle run test revealed a significant perimeter and core area increase and a decrease of relevant cytoplasmic parameters of these cells. At the same time, the core-cytoplasmic ratio increase was established (Table 1).

Table 1. Changes in Morphometric Epitheliocytes Parameters in Students after Performing the 12-Minute Shuttle Run Test (M±m)

Indicators	1-st group (n=15) M±SD	2-nd group (n=15) M±SD	Control group (n=10) M±SD
Pcp (µm)	188.4±2.44*	179.8±3.20*	192.4±3.26
Pc (µm)	32.6±0.27*	35.2±0.36*	30.4±0.42
Pcp/Pc	0.17±0.003*	0.21±0.005*	0.15±0.001
Sc (µm ²)	61.4±2.52	71.4±1.28*	53.8±1.22
Scp (µm ²)	2032.5±60.11*	1918.7±68.53*	2156.3±73.41
Scp/Sc	0.03±0.001*	0.05±0.001*	0.02±0.001

Legend: Pcp–cytoplasm perimeter; Pc–core perimeter; Sc–core area; Scp–cytoplasm area; *-p<0.05

To determine core perimeters and area ratio dependence to cytoplasm perimeter and area (Pc/Pcp and Sc/Scp) of buc-

cal epitheliocytes after the 12 min shuttle run test, a multiple linear regression analysis was performed (Figure 1).

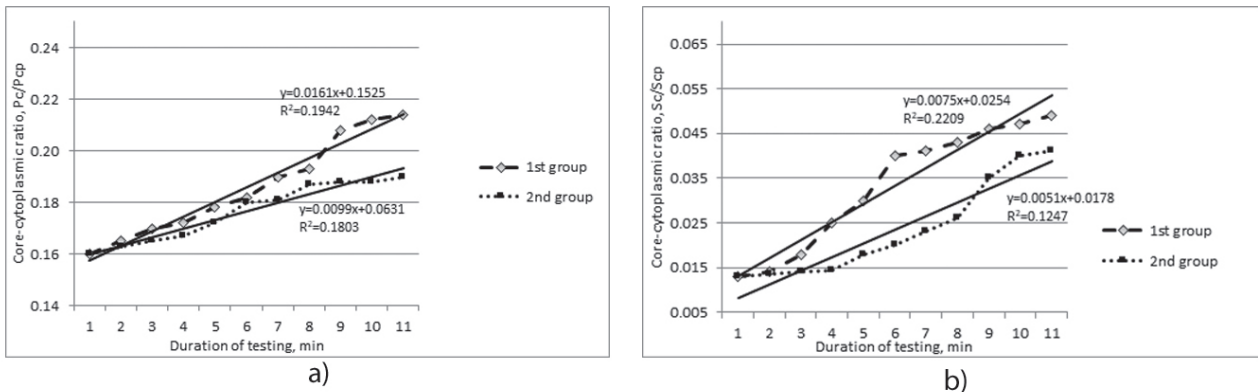


FIGURE 1. Specific features of the correlation between changes of core and cell perimeter ratio (a) and core and cytoplasm area (b) in students with different somatic health levels

Because of the results of the conducted studies, a correlation analysis of obtained relative indicators was also performed to identify possible correlations (Figure 2).

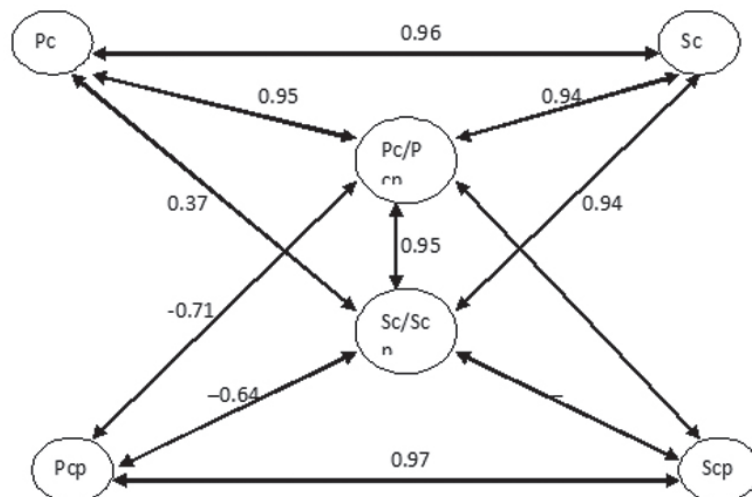


FIGURE 2. Correlation between indicators of morphometric epitheliocytes for students with above-average and average somatic health levels

In the first group, there was a strong positive correlation between such morphometric indicators as Pc and Sc ($rs=0.96$; $p<0.05$), Pcp and Scp ($rs=0.97$; $p<0.05$) as well as Sc/Scp to Pc/Pcp ($rs=0.95$; $p<0.05$).

An average inverse correlation was observed between Pcp and Pc/Pcp indices ($rs=-0.71$; $p<0.05$), Pcp and Sc/Scp ($rs=-0.64$; $p<0.05$), Sc and Pc/Pcp ($rs=0.34$; $p<0.05$), and Scp to Sc/Scp ($rs=-0.63$; $p<0.05$). During statistical data processing, the presence of a weak positive correlation between Pc and Pc/

Pcp ($rs=0.33$; $p<0.05$), Pc and Sc/Scp ($rs=0.37$; $p<0.05$) was determined. Sc and Pc ($rs=0.34$; $p<0.05$), and Sc and Sc/Scp ($rs=0.42$; $p<0.05$) (Figure 2).

The manifestation of the first reactivity signs of PBE in students of the 1st and 2nd groups was marked by the spherocytes proportional increase, and the discocytes' proportion progressive decrease (Table 2). After performing the 12-minute shuttle run test, an increase in the proportion stomatocytes was observed, while the ovalocyte number had no significant changes.

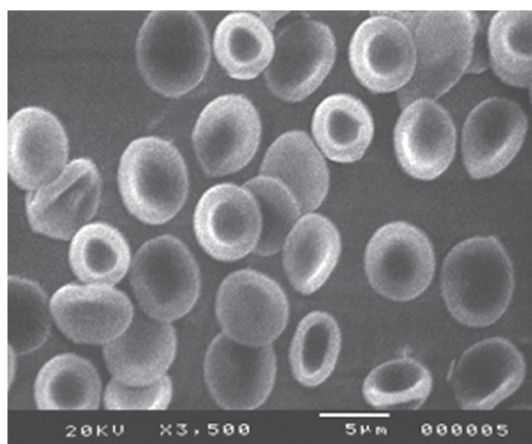
Table 2. Relative content (%) of various shapes of erythrocytes in the peripheral blood of students with different somatic health levels before and after the 12-minute shuttle run test

Erythrocytes shapes	Groups					
	1st (n=15)		2nd (n=15)		Control group(n=10)	
	before	after	before	after		
Discocytes	60.8	61.3	44.8	36.2*	65.4	
Discospherocytes	22.8	28.3	29.3	31.5*	34.3	
Spherocytes	3.3	3.7	7.3	12.9*	2.5	
Stomatocytes	2.4	4.8	4.6	6.1*	2.4	
Ovalocytes	2.1	2.3	2.7	3.9*	2.2	

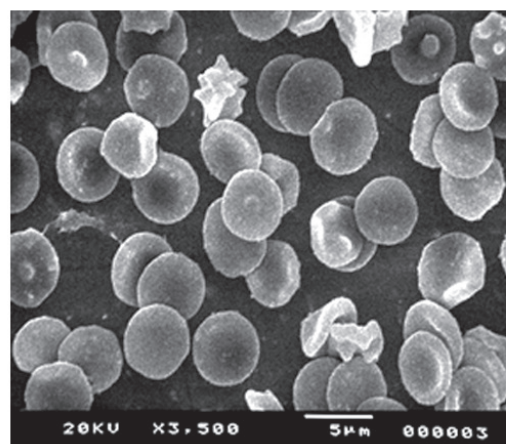
Legend: * - $p<0.05$

The students of the first group were dominated by discocytes (61.3%) and discspherocytes (28.3%). The atypical erythrocyte-form proportion was minimal: spherocytes – 3.7%, stomatocytes – 4.8% and ovalocytes – 2.3%.

In students of the 2nd group, significant deviations were observed, both from the control group indicators and 1st group students indicators (Table 2, Figure 3).



a)



b)

FIGURE 3. Erythrocyte geometry in students of the 1st (a) and the 2nd groups (b) after performing the 12-min shuttle test. Scanning electron microscopy (zoom: $\times 3500$)

Discussion

A considerable amount of research is devoted to the study of the body's response to PL including blood and various organs' epithelial cover (Duma et al., 2016; Levytskyi, 2007; Leshchak & Popel, 2012; Popel & Mytskan, 2016). These results are essential for modelling the PL volume, taking into account the SH level. This is confirmed by the cytological study results of buccal epitheliocytes in smear-imprints of the oral cavity mucous membrane (OCMM) of persons with poor health. It is known that one of the main mechanisms of nonspecific body protection forms is the microflora composition of OCMM (Faichak et al., 2011). It not only regulates and maintains the necessary colonization resistance level but also affects the whole body's metabolism and adaptation processes course (Belykh et al., 2015; Gan & Popel, 2017). Together with local

components (buccal epitheliocytes and leukocyte-lymphocytic cell pool of OCMM) of the immune system, the microflora maintains a sufficient enzymatic activity level that affects the secretion of various biologically-active substances that determine the nature of the reaction to different irritants, including PL (Popel et al., 2017). That is why in students with low SH level and cardio-respiratory endurance the number of cellular elements and autoflora components is statistically significantly increased, the adhesive epitheliocyte properties are disrupted, and the phagocytic properties of leukocyte-lymphocytic properties are reduced, which reveals local (nonspecific) protective properties. The fact that these parameters decrease under the PL influence confirms the need for a differentiated approach in training regime modelling to prevent and correct immunological disorders in students.

It is known that the stabilization of OCMM autoflora persistence indicators has a positive effect on microbial biocenosis as a whole (Rabinovich, 2013). Recent studies (Popel & Mytskan, 2016; Wandeur et al., 2011; Hande & Chaudhary, 2010; Prasad et al., 2010) show that the epithelium, including buccal, occupies a significant place in the cell-humoral homeostasis system. These cells react to intercellular communication molecules, change the gene expression and related phenotypic parameters (Wandeur et al., 2011). The specific OCMM reactions of students under PL influence showed a number of general changes in nonspecific protection indices, which show the possibility of local resistance data being used as an SH integral indicator and cardio-respiratory endurance.

Based on the works of Hans Selve (1977), the indirect indicator of stress development is the reactions in PBE by the type of poikilocytosis. In chronic stress, the number of buccal epithelium cells and PBE, changing in shape, does not go beyond norms limits (Bulgakova, 2006). According to current scientific studies in the red blood cells, the changes observed in PL are typical for stress reactions, which can be recorded in acute stress as an adaptation syndrome, and at PL maximum correspond to conditions observed in depleted adaptation potential (Vejn et al., 1981; Muruzyuk & Romanova, 2001).

It is known that a complex, multilevel stress-realization system functions in the body, but the stress hormones' effect on PBE has not been studied thoroughly (Volchegorskij et al., 1998). Our data on buccal epitheliocytes' cellular content and PBE indicate a change in autonomic tone and autonomic imbalance formation. A probable sign of autonomic balance formation is the increase in the number of differently shaped and sized cells. In this case, poikilocytosis and a possible decrease of the haemoglobin amount indicate that the body is adapting to new conditions. Therefore, changes in PBE should be considered in combination with other epitheliogram components.

According to Verkhoglyadova et al. (2005), poikilocytosis on the epithelial reaction background (anisocytosis and nuclear shift) usually indicates the progression of the fatigue processes, but they are not regarded as negative prognostic signs.

It is known that under adrenaline influence poikilocytosis occurs (Vejn et al., 1981; Hoffman et al., 2018), and at the depletion of sympathetic CNS section, the inhibitory process and leukopenia is observed. Therefore, we can suggest that a similar mechanism is the basis of buccal epithelium and PBE cellular responses, which we observed in students with low SH levels, which is probably due to the depletion of vascular and bone marrow cell reserves (Verkhoglyadova et al., 2005; Pseunok et al., 2000). Our previous studies have shown that under stress, there is a PBE redistribution between different organs, which is manifested by increased inversely altered PBE forms and the acceleration of their ageing (Faichak et al., 2011). The indication of this is the development of neutrophilic OCMM leukocytosis, which is mainly due to increased bone marrow neutrophils' flow into vascular beds, the recruitment of which is potentiated by glucocorticoids and catecholamines with increased concentration under PL.

It is observed that eosinophils' increase in OCMM is an ACTH-dependent process and is associated with these cells' release from the blood into connective tissue (Goldberg et al., 1997). Thus, in addition to nervous and endocrine components to modern ideas about induction mechanisms of stress response to PL, it is necessary to take into account the epithelial and haematological components. Therefore, they must be considered as

a node in the formation of hormonal and metabolic body status and as a manifestation of haematological stress syndrome's generalized reaction to acute and chronic stress (Vasilev et al., 1992; Gorizontov et al., 2013; Raushenbakh et al., 2012).

The change in PBE composition after the PL series probably occurs due to the influence on the hypothalamus. In fact, during PL due to reverse connection, the specific, time-stable, functional system of neural interactions is formed (Vasilevskij et al., 2013), which is an adaptation process coordinator and regulator, and is capable of forming new adequate reaction forms to internal and external factors.

The changing PBE form is very important and proves the dependence of erythrocyte homeostasis disorders on central regulation level functioning. This dependence is exceptional because it directly relates to the occurrence, course and result of haematological and cytological disorders and yet with the occurrence, the course of whole body is disordered. These deviations are reactive by nature, since blood indicators are restored upon CRC activity normalization (By'kov et al., 2012).

It can be assumed that the triggering mechanism of PBE composition changes and buccal epitheliocytes is the protective mechanisms' general mobilization to counteract endo- and exogenous factors' effect on the body. It is known that under PL, the body's need for oxygen increases, and it is quite logical that its haemoglobin amount increasing, which significantly reduces the whole body adaptation and has a positive effect on SH.

Blood is the transport medium for leukocytes from their formation sites (bone marrow, spleen and lymph nodes) to penetration sites into OCMM. Leukocyte output is associated with increased epithelial tissue permeability of OCMM, as well as their redistribution in the bloodstream, indicating a decrease in the body's immune resistance, which can occur in both acute and chronic physical fatigue, especially in individuals with low SH level and cardio-respiratory endurance.

It is important to emphasize once again that changes in EPC do not go beyond the normal parameters, which is distinct from the sharp jump of indicators of blood cells during acute stress.

Korneva (2015), states the importance of using tests that can identify "risk groups" and diagnostics of SH correction effectiveness by physical training. One of which can be PBE morphological composition changes control and buccal epitheliocytes.

Additionally, this study confirmed that morphometric analysis of buccal epitheliocytes revealed a probable increase in perimeter and core area, as well as a decrease in the above-mentioned parameters in students after performing the 12-minute shuttle run test. The morphometric changes of buccal epitheliocytes and peripheral blood erythrocytes during the aerobic endurance test were determined due to the health level. In particular, it affects the erythrocyte deformity degree in students with below-average health levels, limits the production of young, functionally active cells, and delays buccal epithelial cells' differentiation processes with an increase the number of heightened core-size ageing cells. The increase in the erythrocyte numbers of different forms compared with the increase of core-cytoplasmic ratio index in buccal epithelial cells of all health groups of students has a positive correlation with the number of achieved levels during the 12-minute shuttle test.

Further complex studies of changes in buccal epithelial cells and peripheral blood erythrocytes in students of different sexes after performing aerobic exercises of different power are recommended.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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