The Relationship of Saliva Microcrystalline Characterization and Contractile Duration of Skeletal Muscle in Medical Students

Maryana Zvir[1], Andriana Beliak[1], Yaryna Pohoretska[1], Maryana Savytska[1], Oksana Zayachkivska[1]

[1] Physiology Department, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Corresponding author: Oksana Zayachkivska, MD, PhD, DSc - ozayachkivska@gmail.com

Abstract

Aim: The aim of the study was to analyze the possibility of the diagnostic use of saliva investigation in relation to medical conditions among medical students, and provide a background of non-invasive physiological-based preventive measures for their health outcomes.

Methods: The study was conducted among 70 students from Danylo Halytsky Lviv National Medical University (Ukraine), who were asked for general information and interviewed about the subjects of lifestyle risk factors related to physical inactivity, circadian rhythmicity, the use of technical gadgets, and the presence of functional gastrointestinal disorders. Their saliva secretion and microcrystallization were evaluated. For detection of the microcrystallines of non-organic origin in saliva, X-Ray diffractional powder analysis was used.

Results: The results of the study indicated that 70% of students have decreased daily skeletal muscle contractile duration, circadian dysfunction and extended time of using gadgets all of which leads to changes in saliva secretion and the microcrystallization of biogeneous substances. About 30% of participants have signs of functional disorders of the upper gastrointestinal tract.

Conclusion: An integrative view on saliva microcrystalline changes could be a novel diagnostic tool for detection of early health disorders, and maintaining regular skeletal muscle contractile activity and normal circadian rhythmicity is a promising physiological approach to improve health outcomes in young people.

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Introduction

The modern lifestyle is characterized by an increasingly sedentary lifestyle, chronic stress overload, and sleep rhythm disorders as a result of intensive usage of various modern gadgets (tablets, smartphones, computer games, etc.), which cause circadian disruption (1,2,3). All these factors lead to additional negative impacts on health, early manifestations of lifestyle diseases,

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osteoporosis, metabolic disorders, obesity, and early aging. Prolonged sitting duration and the rapid progress of information technology lead to a sedentary lifestyle of young people, which adversely affects their health and subsequently leads to pathological processes in the body (4,5). The use of modern technological gadgets leads to the desynchronization of circadian rhythms, accompanied by sleep and appetite disturbance, worsening of mood, and initial mental disorders, autonomic nervous system (ANS) imbalance, which often stimulates different functional and organic diseases (3).

Young people are very sensitive to changes of lifestyle and have a tendency to an increased number of mental disorders as cognitive functions decrease, leading to a decline of natural resistance of the organism and initiating a wide range of mental and medical problems, including depression and suicide. All of these lifestyle factors have also been suggested as possible risk factors for upper functional gastrointestinal (GI) disorders (FGID), which are tightly linked to brain-gut interaction (6,7). To prevent health problems in young people and guarantee active aging, the need for the detection of these early risk factors attracted our attention.

Modern views on saliva show that it can be an important source for the early diagnosis of many diseases and the functional state of our body. Moreover, it is known that inorganic and organic components of saliva actively influence the cytoprotection of the esophagogastric region. The effects of normal salivation provide sufficient moisture of the mucous membrane of upper GI part that contains numerous growth factors, like the epidermal growth factor, EGF, which provides physiological regeneration, mucin – the formation of mucous-bicarbonate barrier, and specific and non-specific defensive bioregulators, which are crucial for the integrity of the GI epithelial barrier and a general resistance to extreme factors (8).

Noninvasiveness and simplicity make a saliva-sampling method attractive for microcrystalline investigations in terms of biogeneous mineral substances. The salivary biogeneous substances were studied using the X-ray diffractional powder analysis (XDPA) technique to determine all mineral phases of biogeneous minerals. Recently published data suggests the importance of the inorganic components of saliva, and the quantitative and qualitative state of saliva bioregulators, as determining factors of solid textures that are formed after dehydration (9,10,11).

Thus, we formed a hypothesis that the investigation of saliva as a non-invasive simple diagnostic tool could be used for detection of the early changes, associated with ANS imbalance, caused by physical inactivity (12,13).

In the present study, we aimed to provide an integrative view of saliva investigation, the screening of lifestyle risk factors, and the skeletal muscle contractility duration that occurs in healthy medical students.

Material and Methods

The study was conducted on a group of 70 participants who were second-year students of the medical faculty from Danylo Halytsky Lviv National Medical University (mean age 18.5 years, 38 women and 32 men) with 16 months of an academic lifestyle (during studying). Selected students were healthy—without any previous chronic diseases and addictions, including caffeine abuse or family-ancestral diseases—and also possessed good oral health—without dentures or dental restorations—according to the reports of the student's dental and orofacial examinations by dentists. Their daily fluid intake did not exceed 2.5 L. They were eligible to participate in this study. The exclusion criteria were medical students who refused to participate. The students who agreed to participate signed a consent form. The design of the study and the test procedures were approved by the ethics committee of Lviv National Medical University (15.02.2016; N2).

The subjective and objective evaluation of the students was divided into 3 parts. The first part of the study concerned general information: gender, previous general illness, administered medication, addictions, and an anthropometric examination. Anthropometric data included height, weight, body mass index (BMI), and age. Height was measured with a regular stadiometer, while weight and body composition, using the bioelectric impedance method, were evaluated with electronic scales (OMRON Corporation, Kyoto, Japan). BMI was calculated using the following parameters: fat content (% of body weight), visceral fat (%), and muscle mass (%).

The second part of the study was based on questionnaires: the International Physical Activity Questionnaire (IPAQ) (14), the Pittsburgh Sleep Quality Index (PSQI) (15), 10 closed questions on the usage of technological gadgets, and a determination of the daily time of physical inactivity (sitting time more than 60 minutes without a break), estimated as a mean value after evaluation every 5 days, and finally the assessment of the symptom manifestations of upper FGIDs through a standard self-report questionnaire, included in the Rome III consensus (7).

The third part of study concerned the saliva investigation by the examination of samples of saliva that were collected at the same time, not less than 2-3 hours after eating in the morning (between 9 and 11 a.m.), with a sterile pipette from the bottom of the oral cavity, in the volume of 2 ml into dry sterile test tubes. All women were examined in the estrogen phase of their menstrual cycle. The level of stimulated and non-stimulated saliva secretion was measured. Non-stimulated saliva was collected from students in the beginning. Later, students were asked to chew gum without sugar for 5 minutes and stimulated saliva was carried out in a similar manner into test tubes. Both deposition time and volume of deposited saliva were measured. The collected data made it possible to determine the rate of saliva secretion (ml/min). The morphological type of saliva crystallization was estimated by the dehydration of drops of mixed saliva in the air at room temperature for 24 hours on a sterile glass slide using light microscopic investigation with a Leica DM 750/4 microscope and Leica DFC 420 digital photo camera, both manufactured in Germany (Figure 1), and by phase contrast imaging for the evaluation of saliva samples without fixing and staining with Leica DM-2500 (Switzerland) microscopy and camera Leica DFT 450 with software application SUIT version 4.4 microscope Leica DM-2500 (Switzerland) (Figure 2). The evaluation of saliva crystallization was performed blind by two independent investigators and the estimation of the type was based on grading four main types according to Shatohina S.N., 2004 (16). The first (1^{st}) type is characterized by clear crystal tread-prismatic structures connected between themselves in a fern leaf and evenly placed; the second (2^{nd}) type is characterized by certain tree-crystal or small single crystals of various shapes placed evenly on the field of view in a grid; the third (3^{rd}) type is characterized by isometric-placed irregularly shaped structures; and the fourth (4^{th}) type is characterized by the absence of crystals. The rest of saliva was taken for further tests.

For detection of the microcrystallines of non-organic origin in saliva, X-Ray diffractional powder analysis was used. For this samples of saliva were selected with the 4^{th} type of crystallization. The samples were applied on the polymer coat and remained there until drying. The coat with dry substance was fixed and covered with the second coat. Experimental intensities and angles reflected from samples were obtained by an automatic diffractometer STOE STADI P ("STOE & Cie GmbH", Germany) with a linear position-precision detector. Primary processing of the experimental diffraction, the calculation of the theoretical diffraction, and the indexing parameters of unit cells were performed using the software package STOE WinXPOW and PowderCell with the method of comparing X-ray diffraction profiles (18,19).

Statistical data analysis was performed by calculating means with their standard errors and proportions. Differences in means were estimated by the Mann-Whitney U-test. All data was processed using the statistical package Statistica 10.0 (Statsoft, Tulsa, Oklahoma, USA).

Results

Among the 70 medical students of Lviv National Medical University who participated in the study

Table 1. *Study group's general information characteristics (n=70).*

Variable	**Results**
Gender	Male: 32 (45.7%) Female: 38 (54.3%)
Age	Mean: 18.5 years, range 17-19 years
Height	Mean: 171±9 cm, range 156-190 cm
Weight	Mean: 63±9 kg, range 47-82 kg
BMI	Mean: 21.5±1.4 kg/m², range 18-24.5 kg/m²
Body composition: fat content visceral fat muscle mass	 Mean: 25.1±6.8%, range 14.2–36.6% Mean: 6.14±1.2%, range 3.94–8.73% Mean: 29.7±6.8%, range 24.2–44.2%
Current smoker	Total: 13 (18.6%)

Table 2. *Types of physical activity among medical students (mean values in MET min/week).*

Type of activity	Gender		Total
	male	female	
moderate	748.1	545.6	632.4
intensive	938.7	456.5	618.2
walking	984.2	1024.1	992.2
total	2,671.0	2,026.2	2,242.8

Figure 1*. Light microscopy of samples of saliva by the type of microcrystallines in medical students (data from study group); x 120: A – 1st type; B – 2nd type; C – 3rd time; D – 4th type.*

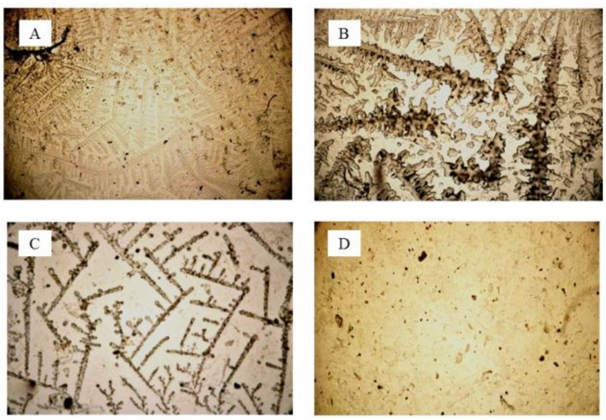


Table 3*. Characterization and duration of daily physical inactivity among medical students.*

Daily sitting time	
6-8 hours/day	48 (68.6%)
>8 hours/day	22 (31.4%)
Total usage of gadgets	
2-3 hours per day	24 (34.2%)
4-5 hours per day	31 (44.3%)
>5 hours per day	15 (21.5%)
Usage of gadgets for more than 2 hours without a break	
Never	8 (11.6%)
Only occasionally	12 (17.1%)
A few times per month	18 (25.6%)
A few times per week	21 (30.0%)
Almost every day	11 (15.7%)

Table 4. *Differences between distributions of types of saliva between groups of students with and without functional gastrointestinal disorders is statistically significant (p<0.001).*

Type of saliva microcrystalli-nes	Symptoms of upper functional gastrointestinal disorders			
	no		yes	
	n	%	n	%
1	0	0	12	24.5
2	1	4.8	24	49.0
3	8	38.1	11	22.4
4	12	57.1	2	4.1
All	21	100.0	49	100.0

Figure 2*. Micro photos of saliva samples from medical students by phase contrast imaging, ranging to four microcrystallines types: A – 1st type; x200; B – 2nd x200; C – 2nd x400; D – 3rd x200; E – 4th x200; F – 4th x400 (data from study group).*

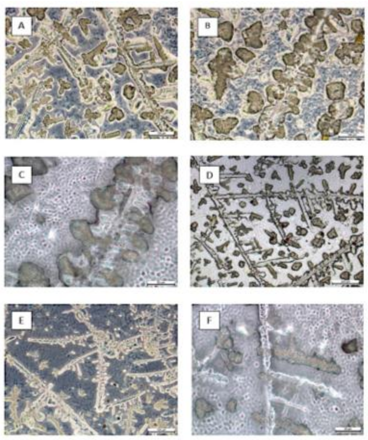
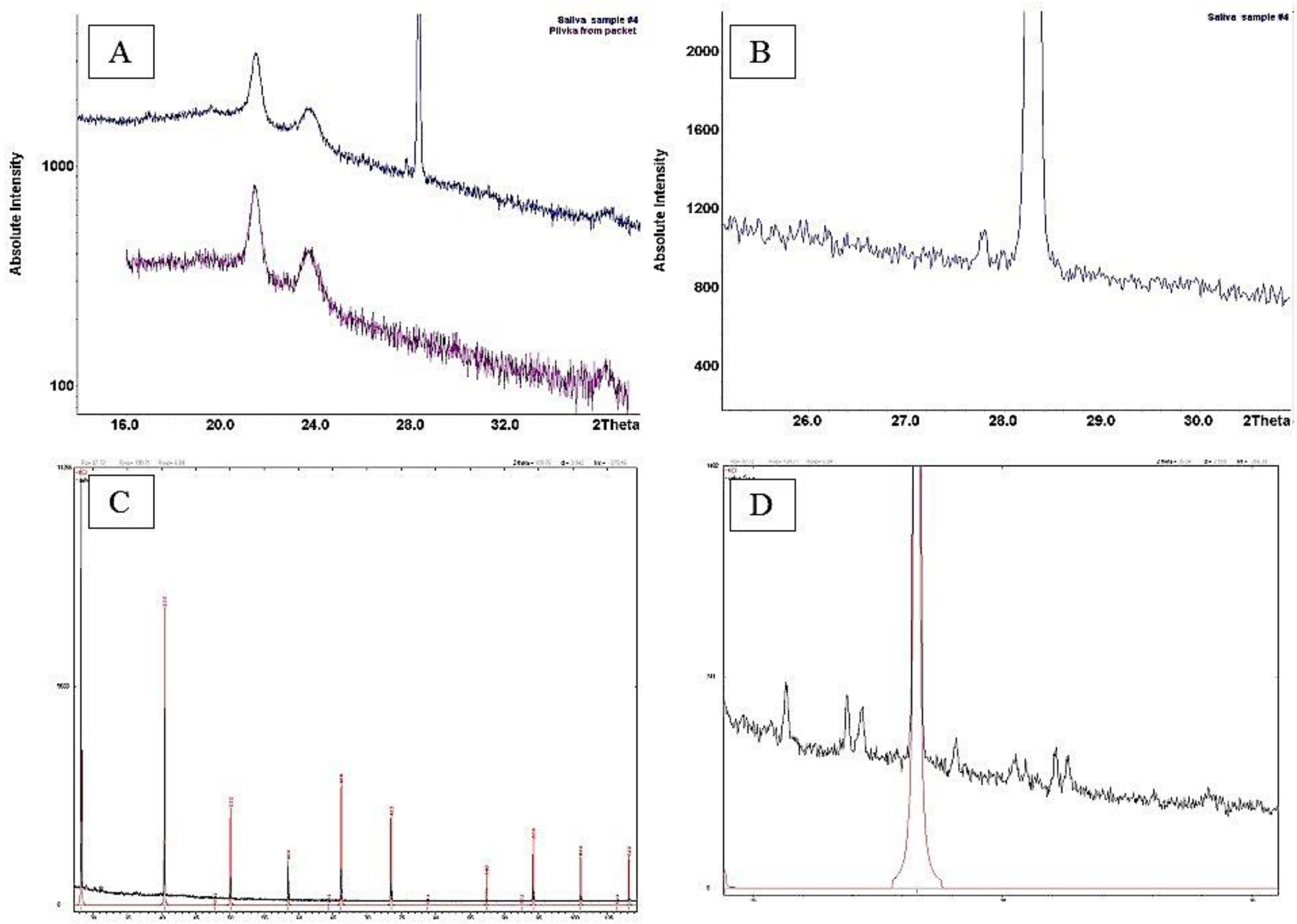


***Figure 3**. Results of investigation of saliva bio compounds by X-ray powder diffraction analysis: A – tested comparison of packet film and investigated dry precipitate of saliva samples; B – detection of two-phase KCl microcrystallines and undetected compound; C – standardization with theoretical diffractogram KCl; D – KCl detection with small traces of sodium.*



a mean age of 18.5. Table 1 presents the data for all participants, including age, gender, administered addictions, and anthropometric examination. Anthropometric data involved height, weight, BMI, body composition and general characteristics of study group represented in Table 1.

The physical activity of medical students involved in the study was estimated by IPAQ, expressed by arithmetic mean (MET min/week), to have reached the value of 2,242.8, with higher values observed in males (2,671.0) than in females (2,026.2). We also estimated the type of activity: moderate, intense, and walking. A larger number of participants confirmed walking as the dominant type of physical activity. Walking was the dominant type of physical activity in females (1024.1 vs. 984.2 in males) (Table 2).

The study assessed the level of physical activity that was calculated from the total physical activity and categorized it into three ranks: low, moderate, and high. A significant variation was observed at each level according to the gender, and the larger differences were in high level of physical activity in males (43.8%) vs. females (23.7%). Moderate physical activity was better obtained in females (50.0%), compared to males (34.4%).

It was observed that 78.5% of medical students use gadgets 3.6±0.5 hours/day and 21.5% of them spend more than 5 hours/day with technical gadgets. There were no significant differences in gender distribution in the collected data. More than half of participants do not take any breaks when they use computers, leading to an increased daily sitting time characterized as a physically inactive lifestyle (Table 3).

We also screened sleeping patterns by PSQI and figured out that poor sleep quality was in 67.2% of medical students with low physical activity vs. 25.7% with moderate or high physical activity. The study indicated that lost sleep because of late night computer use was very common among young men and women: almost every day – 11%, a few times per week – 15%, or a few times per month – 37% of participants.

To determine the prevalence and clinical-pathological characteristics of upper FGID, records of questionnaires were evaluated. The most commonly presented complaints were heartburn, indigestion, epigastric pain/burning, excess throat mucous, or a lump in throat etc. These symptoms were absent in 30% of enrolled participants, but among the students who fulfilled the criteria for FGID, were more common in males (65.5%), than in females (34.5%). Smoking causes a high risk for the prevalence of upper FGID.

The data of the microscopic analysis of human saliva, namely samples of non-stimulated saliva microcrystallines, detects different types of distribution according to FGID (Table 4). There were not any significant differences in saliva microcrystallines between the genders.

The 1st first type of saliva microcrystallines was dominant among participants with symptoms of upper functional GID, because increased gastric acidity entailed the increase of saliva crystallization. Both 2nd and 3rd crystallization types were the most common among the participants. It has been established that the 4th type of saliva microcrystallines is prevalent among medical students, who displayed both circadian disruption and physical inactivity. A growing amount of evidence argues that saliva could be a diagnostic tool for ANS imbalance, which is represented by decrease of general adaptive reserve (20-22). Microscopically, a large number of irregular crystal structures are seen in the 4th type of saliva crystallization (Figure 1D, and Figure 2E, F). Investigation of stimulated saliva secretion didn't show any significant differences in volumes and pH. The average pH of saliva was about 7.9, which is in the normal range, considered to be 5.6 to 7.9, and this keeps local balance in the oral cavity.

Figure 3 represents the crystal structure of the bio compounds in samples of saliva microcrystallines, which were determined using X-ray powder diffraction analysis according to standard procedure. At the beginning, the comparable characteristics of packet film and the dry precipitate of saliva samples with the 4th type of microcrystalline were tested in comparison for the elimination of wrong signals (Figure 3A). Phase analysis by X-ray diffraction confirmed that the microcrystalline samples were two-phase KCl (structural type NaCl, with group *Fm–3m*), parameter of unit cubic cell a = 6.3015(3) Å, its volume V = 250.227 (18) Å^3 (Figure 3B) with another compound with un-identified small reflex for 2 θ = 27,78˚. After standardization with theoretical diffractogram KCl (parameter of unit cubic cell *a* = 6.3060(4) Å, value *hkl* for first reflex 200 (Figure 3C) were a set of mild reflexes for the second phase (Figure 3D). Regarding several well-known data, parameter *a* for KCl could be from 6.26 to 6.3163 Å; for $K_{1-x}Na_xCl$, x= 0-1, a=6.2916–5.6400 Å. Thus, the main bio compound in dry microcrystalline is pure KCl. The traces of sodium are very small (x ~ 0.01 in $K_{1-x}Na_xCl$).

Discussion

This study confirmed that the intensive lifestyle of medical students related to their demanding educational process, modern behaviors of young people, and the lack of contractile duration of skeletal muscles, is quite common and could be a specific type of stress which causes imbalance in capacities for adaptation, leading to functional disorders in the very stress-sensitive gastrointestinal tract. Numerous data suggests impact dysfunction of brain-gut axis in induction FGID (23-26). Regarding the final data, saliva represents a potential fluid for diagnostics of various diseases (13, 16, 17) and our results have shown its microcrystallines could be a diagnostic tool for stress-related gastrointestinal disorders. It is well known that epigenetic modifications play a crucial role in maladaptation and chronic stress-associated health outcomes. Physiologically-based therapeutic implications of the student lifestyle should be included to recommendations of prevention and the treatment of FGID.

In conclusion, the above observations underline the obtained results in saliva microcrystalline samples and suggest that an integrative view of saliva investigation could be novel very promising diagnostic tool for the early detection of changes in the human body. Considering the obtained data about medical students' modern lifestyle, the disturbance of daytime/nighttime rhythmic changes, and the prevalence of risk factors, we can conclude that these factors are involved in the changes in saliva microcrystalline formation. The obvious changes in saliva microcrystallines in persons with higher physical inactivity and circadian disruption may present external stimuli for the induction of upper functional GI disorders. A physiologically based program of increased duration of skeletal muscle contractility will be helpful in the prevention of functional digestive disorders and their health outcomes.

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Competing interests. None to declare.

References

1. Hamilton M. T., Healy G. N., Dunstan D. W., Zderic T. W., Owen N. Too little exercise and too much sitting: inactivity physiology and the need for new recommendations on sedentary behavior. Current cardiovascular risk reports. 2008;2(4):292-298.
2. Karatsoreos I. N. Effects of circadian disruption on mental and physical health. Current neurology and neuroscience reports. 2012;12(2):218-225.
3. Stevens RG, Zhu Y. Electric light, particularly at night, disrupts human circadian rhythmicity: is that a problem? Phil Trans R Soc B 2015 May 5;370(1667):20140120.
4. Proper K. I., Singh A. S., Van Mechelen W., Chinapaw M. J. Sedentary behaviors and health outcomes among adults: a systematic review of prospective studies. Am J Prev Med. 2011;40(2): 174-182.
5. Thorp A. A., Owen N., Neuhaus M., Dunstan D. W. Sedentary behaviors and subsequent health outcomes in adults: a systematic review of longitudinal studies, 1996–2011. Am J Prev Med.. 2011;41(2):207-215.
6. Haruma K., Kinoshita Y., Sakamoto S., Sanada K., Hiroi S., Miwa H. Lifestyle factors and efficacy of lifestyle interventions in gastroesophageal reflux disease patients with functional dyspepsia: primary care perspectives from the LEGEND study. Internal Medicine. 2015;54(7):695-701.
7. Drossman DA. The functional gastrointestinal disorders and the Rome III process. Gastroenterology. 2006;130(5):1377-90.
8. Zayachkivska O.S. The role of salivary endogenic bioregulators in the formation esophagoprotection at experimental injury of the esophagus. Contemporary Gastroenterology 2006;30 (4):65-71. (In Ukrainian).
9. Bazin D, Letavernier E, Haymann JP. Biomineralization versus microcrystalline pathologies: Beauty and the beast. Comptes Rendus Chimie. 2016;19(11):1395-403.
10. Evdokimov F. A., Gamataev I. I., Iospa A. V., Yakushina O. A., Khozyainov M. S. Human Biolithos Concretions Study in X-rays using Computed Tomography and Powder Diffraction. Proceedings in GV-Global Virtual Conference. 2013; No. 1.
11. Mori M., Ishikawara F., Tomoda T., Yamada S., Okamoto M., Itabashi H., Sumino H. Use of capillary electrophoresis with dual-opposite end injection for simultaneous analysis of small ions in saliva samples from wrestlers undergoing a weight training program. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1012:178-185.
12. Choi M. Saliva diagnostics integrate dentistry into general and preventive health care. Int J Prosthodont. 2010;23(3):189.

13. Wong D.T. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. J Am Dent Assoc. 2006;137(3):313-21.
14. Booth ML, Ainsworth BE, Pratt MI, Ekelund U, Yngve AG, Sallis JF, Oja PE. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;195(9131/03):3508-1381.
15. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
16. Shatohina S.N., Razumova S.N., Shabalin V.N. Morphological picture of oral fluid: diagnostic possibilities. Stomatol. 2006; No. 4:14-17. (In Russian).
17. Lakhtin Y., Moskalenko P., Levkov A., & Smeyanov Y. Application of saliva crystalloscopy methods in the diagnosis of malignant tumours of salivary glands and Sjögren disease (Doctoral dissertation, Published by IASHE London), 2015.
18. Wriedt M, Näther C. Synthesis, Crystal Structures, Thermal and Magnetic Properties of New Selenocyanato Coordination Polymers with Pyrazine as Co - Ligand. Z Anorg Allg Chem. 2011;637(6):666-71.
19. Kraus W, Nolze G. POWDER CELL–a program for the representation and manipulation of crystal structures and calculation of the resulting X-ray powder patterns. J Appl Crystallogr. 1996;29(3):301-3.
20. Petrakova L, Doering BK, Vits S, et al. Psychosocial stress increases salivary alpha-amylase activity independently from plasma noradrenaline levels. Schmidt U, ed. PLoS ONE. 2015;10(8):e0134561. doi:10.1371/journal.pone.0134561.
21. Vineetha R, Pai K-M, Vengal M, Gopalakrishna K, Narayanakurup D. Usefulness of salivary alpha amylase as a biomarker of chronic stress and stress related oral mucosal changes – a pilot study. J Clin Exp Dent 2014;6(2):e132-e137. doi:10.4317/jced.51355.
22. Tominaga, K., Fujikawa, Y., Tsumoto, C., Kadouchi, K., Tanaka, F., Kamata, N., Arakawa, T. Disorder of autonomic nervous system and its vulnerability to external stimulation in functional dyspepsia. J Clin Biochem Nutr. 2016;58(2):161-5. doi: 10.3164/jcbn.15-140.
23. Bonaz B. Stress and the Gastrointestinal System. In Neuro-Immuno-Gastroenterology 2016 (pp. 123-156). Springer International Publishing.
24. Pellissier S, Bonaz B. Chapter Eleven-The Place of Stress and Emotions in the Irritable Bowel Syndrome. Vitam Horm 2017;103:327-54.
25. Brzozowski B, Mazur-Bialy A, Pajdo R, Kwiecien S, Bilski J, Zwolinska-Wcislo M, Mach T, Brzozowski T. Mechanisms by which Stress Affects the Experimental and Clinical Inflammatory Bowel Disease (IBD): Role of Brain-Gut Axis. Curr Neuropharmacol 2016;14(8):892-900.
26. Wiley JW, Higgins GA, Athey BD. Stress and glucocorticoid receptor transcriptional programming in time and space: Implications for the brain–gut axis. Neurogastroenterol Mot. 2016;28(1):12-25.