Diagnostics of Oxidative Stress by Laser Optical-Acoustic Spectroscopy

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Abstract. The results of the registration of oxidative stress volatile molecular markers (NOx, C_2H_6, and C_5H_{12}) in exhaled air by optical-acoustic spectroscopy are presented. Aerobic physical exercises were chosen as a method of oxidative stress activation. Three time points were studied: before exercises, immediately after exercises, and after 25 min rest. It was shown that there is a statistically significant increase in the studied markers concentrations in the exhaled air immediately after the exercises. After the rest, statistically significant differences are observed in relation to the initial state only for a part of these markers. This is most likely caused by different recovery of the subjects after the exercises and insufficient rest time. Thus, this instrumental approach is promising for non-invasive registration of markers of oxidative stress.

Keywords: oxidative stress; human exhalation analysis; non-invasive diagnostics; optical parametric oscillator; laser optical-acoustic spectroscopy.

Paper #3522 received 10 Sep 2022; revised manuscript received 16 Oct 2022; accepted for publication 17 Oct 2022; published online 12 Dec 2022. doi: 10.18287/JBPE22.08.040301.

1 Introduction

Oxidative stress (OS) is a disorder of the pro-oxidant-antioxidant balance, accompanied by an increase of reactive oxygen species (ROS) causing the oxidation of cells and their programmed death [1]. OS accompanies metabolic disorders, fatigue and headaches, skin aging [2], damage to internal organs and the development of chronic and oncological diseases [3].

The vast majority of approaches in OS control are indirect, since the main ROS are short-living compounds, and some of them are transitional products (intermediates) of fast biochemical reactions. Therefore, to detect OS, various biological markers are used, the presence of which at certain concentrations indicates OS. The standard methods of OS diagnosis are associated with monitoring the content of tyrosine and its derivatives in blood serum by liquid chromatography [4], in terms of the ratio of the concentrations of oxidized to reduced glutathione in the cellular material transformed in the solution state. This solution is further analyzed, using a photometric method [5]. OS can be determined through control by the chemiluminescence method in urine of the nucleosides or malondialdehyde appearing in blood and tissues in the result of lipid peroxidation (LPO) [6–8]. However, all these methods are invasive, time-consuming, and require preliminary physical or chemical manipulations with samples of a biological material.

The control of volatile molecules in exhaled air is a promising approach for the non-invasive express-analysis of metabolic processes in the body. The human breath sampling is simple, non-invasive, and comfortable for participants of similar studies. Concentrations of some components in exhaled air are closely correlated with their concentrations in the bloodstream, so when using the human breath, the need for blood sampling for analysis is eliminated [9]. Since small molecules, which are products of lipid peroxidation, as well as other reactions associated with an excess of reactive oxygen species, and excreted from the body through the lungs; the analysis of the exhaled air composition can be an alternative (or the first step) in diagnosing of OS in relation to a blood or cellular material tests. According to the literature, volatile molecular markers of OS include: CO [10, 11], pentane (C_5H_{12}), ethane (C_2H_6) [12], NO [13], hydrogen peroxide, and some others [14, 15].

The purpose of this work is to analyze volatile molecular markers of OS in the exhaled air caused by

aerobic exercises, using laser optical-acoustic spectroscopy.

2 Carrying out the Experiment

Criteria for inclusion and exclusion were as follows. The subjects should not have chronic diseases. Also, it was excluded:

- smoking;
- increased physical activity 72 h before the experiment;
- taking drugs 72 h before the experiment;
- intake of high-calorie foods 6 h before the experiment;
- taking tonic drinks 12 h before the experiment;
- taking light alcoholic drinks 7 days before the experiment;
- reception of strong alcoholic beverages 21 days before the experiment.

Students-volunteers were involved in the experiments. Taking into account the above criteria, a group of subjects of 7 males aged 23 to 24 years was formed. All participants of the experiments signed an informed consent.

OS can be initiated by emotional stress, intense physical or mental stress, prolonged starvation, alcohol [15–19]. Aerobic physical activity was chosen by us as the OS activation method [15, 17]. The advantages of this variant are the minimum discomfort for the subjects and the controllability of the process by varying the physical activity parameters (duration and intensity). The experimental procedure is illustrated in Fig. 1: the subject performs work on the exercise bike, the heart rate is controlled by a fitness tracker. An exhalation sample is collected into a disposable bag at several temporal points of the experiments. Then a laser gas analyzer was used for spectral analysis of the breath samples.

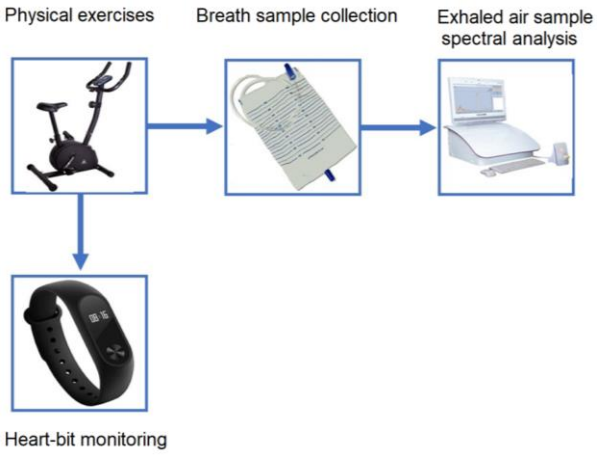

Fig. 1 The experimental procedure of OS study.

The experiment was carried out indoors, from 10:00 to 11:00 in the morning, at a room temperature (about +20 °C) and humidity (about 60%) not exceeding the permissible limits [20]. The subjects had to perform work on the DFC B40 exercise bike (see Fig. 1). The duration of the load was 25 min, the intensity of the load was regulated by the threshold heart rate (140 ± 5 beats/min). Immediately before the experiment, a warm-up was carried out, as well as work on an exercise bike for 5 min and a threshold pulse of 110 ± 10 beats/min [21]. The rest period after exercises was chosen to be 25 min [15]. The duration of the physical load and the pulse were recorded by the Xiaomi Mi Band 2 fitness tracker, which, in real-time, transmitted data to the smartphone. Exhaled air samples (EAS) were collected at the following stages of the experiment: (i) before the warm-up and the main physical load; (ii) ~ 1 min after load; (iii) after the rest with duration 25 min.

When EAS collecting, it should be taken into account that the part of a respiratory volume of a person does not involve in gas exchange, i.e. it does not carry useful information about current metabolism, filling the anatomically dead space of the respiratory tract (approximately 150 ml). The rest part reaches the alveoli, which comes into contact with the blood in the pulmonary capillaries, as a result, the blood is saturated by oxygen. Here, simultaneously, carbon dioxide and other molecular products of body metabolism are released into the exhaled air. The amount of alveolar air corresponds to the functional residual capacity of the lungs – the amount of air that remains in the lungs after a quiet exhalation, and is normally ~ 2500 ml. Therefore, the of EAS sampling was divided into two stages: preliminary exhalation (~ 3 sec) into the environment followed by exhalation without a pause into the disposable container of 150 ml volume (~ 10 sec).

Next, the samples were analyzed using a laser optical-acoustic gas analyzer LaserBreeze (Special Technologies LLC, Novosibirsk, Russia). This gas analyzer combines a resonant optical-acoustic detector designed to detect molecular components in a gas sample and an optical parametric oscillator (OPO) based on a Nd:YLF laser with a pumping wavelength of 1053 nm and PPLN and HGS nonlinear crystals, which are used to spectral tune the laser radiation in the range from 2.5 to 10.5 μm. The principle of operation of this spectrometer is described in detail in the paper [22]. At the entrance of gas analyzer, a bacterial filter was used to remove small particles of dust and aerosols in the EAS.

When measuring the concentrations of the target gases (NO, NO_2, C_2H_6, and C_5H_{12}), the possibility of their spectral overlap was analyzed. Absorption spectra of the target gases, CO_2, and H_2O are shown in Fig. 2. The calculations were implemented for room conditions (pressure 1 atm, temperature 25 ºC), spectral resolution 1 cm^{-1}, using data from Hitran [23] and NIST (C_5H_{12}) [24] spectral data bases.

Used spectral resolution is typical for OPOs. The benefit of OPO is that, due to wide spectral tunability, it allows one to provide a component analysis of complex gas mixtures with large amount of substances [25]. An example of decomposition of a model breath sample gas mixture containing CO_2 and H_2O at physiological concentrations; NO, NO_2, C_2H_6, and C_5H_{12} at ppm and sub-ppm concentration levels is presented in Tables 1, 2.

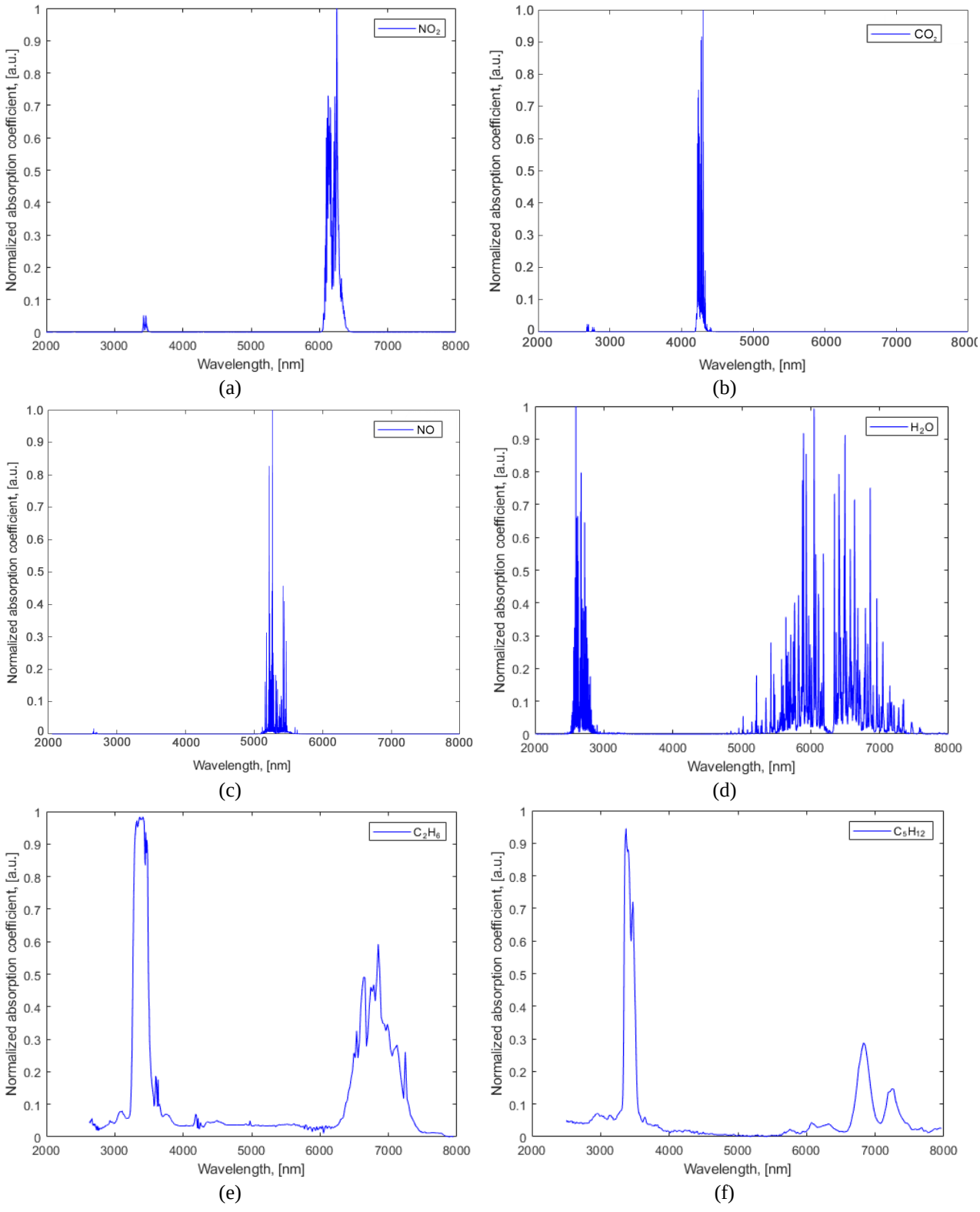

Fig. 2 Absorption spectra of (a) NO_2, (b) CO_2, (c) NO, (d) H_2O, (e) C_2H_6, (f) C_5H_{12}.

Table 1 An example of decomposition of a model breath sample gas mixture containing CO_2 and H_2O at physiological concentrations; NO, NO_2, C_2H_6, and C_5H_{12} at ppm concentration levels.

	H_2O	**CO_2**	**NO**	**NO_2**	**C_2H_6**	**C_5H_{12}**
Actual concentration, ppm	30000	30000	6	6	14	6
The relative error of concentration restoration, %. Zero noise	3.1×10^{-5}	2.0×10^{-6}	0.05	0.09	0.02	0.07
The relative error of concentration restoration, %. The noise amplitude is equal to 1% of mean absorption coefficient of tested gas mixture	3.1×10^{-5}	2.0×10^{-6}	0.06	0.14	0.10	0.2

Table 2 An example of decomposition of a model breath sample gas mixture containing CO_2 and H_2O at physiological concentrations; NO, NO_2, C_2H_6, and C_5H_{12} at sub-ppm concentration levels.

	H_2O	**CO_2**	**NO**	**NO_2**	**C_2H_6**	**C_5H_{12}**
Actual concentration, ppm	30000	30000	0.085	0.130	0.050	0.150
The relative error of concentration restoration, %. Zero noise	3.1×10^{-5}	2.0×10^{-6}	2.2	1.2	14.1	3.8
The relative error of concentration restoration, %. The noise amplitude is equal to 1% of mean absorption coefficient of tested gas mixture	3.1×10^{-5}	2.0×10^{-6}	4.9	17.9	19.9	18.2

3 Analysis of Experimental Data

For each individual EAS, the absorption spectrum was recorded in the wavelength ranges of 2600–4000 and 5800–8500 nm. An example of an exhaled air sample absorption spectrum is shown in Fig. 3.

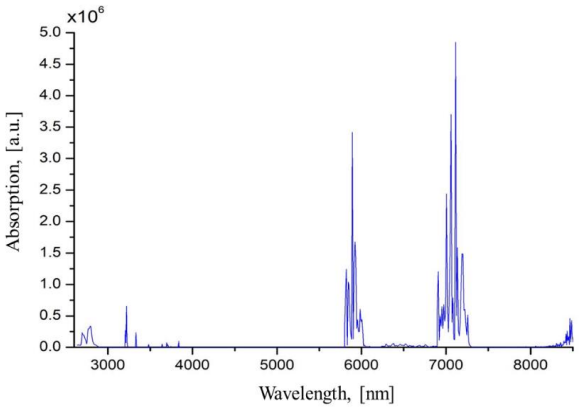

Fig. 3 An example of a measured EAS absorption spectrum.

Using the extension of software built into the gas analyzer [25], the concentrations of the OS biomarkers were determined from the measured EAS absorption spectra. The restored concentration values of the corresponding substances are shown in Fig. 4. The x-axis indicates the serial number of a participant, the y-axis indicates the concentration of a definite OS biomarker.

The values of the concentrations of the studied OS biomarkers were measured at the following temporal points of the experiments: “before exercise”, “after exercise” and “after the rest”. The estimation of the statistical significance of the concentration of OS biomarkers’ changes during the experiment was carried out using Student’s *t*-distribution for two dependent, related samples (paired *t*-distribution) for a significance level $\alpha = 0.05$ (see Fig. 5). Here, the OS biomarker concentration reference distribution corresponds to the “before exercise” state.

It can be seen that the concentrations of all OS volatile molecular biomarkers in the EAS are statistically significantly increased immediately after the physical load in relation to the initial state (before the physical load). After the rest, the content of NO and C_5H_{12} in the EAS significantly differs from the initial values (before the physical load). It indicates an incomplete recovery of the participants in the experiment after the physical load and (or) a significant variability in the data. At the same time, for NO_2 and C_2H_6, the empirical value of the *t*-criterion is either equal to or less than the critical one, which allows us to conclude that the differences in the average values of the concentrations of these substances are not statistically significant in relation to the initial ones. We can conclude that, during the rest, the concentrations of these biomarkers return to their initial values (before the physical load).

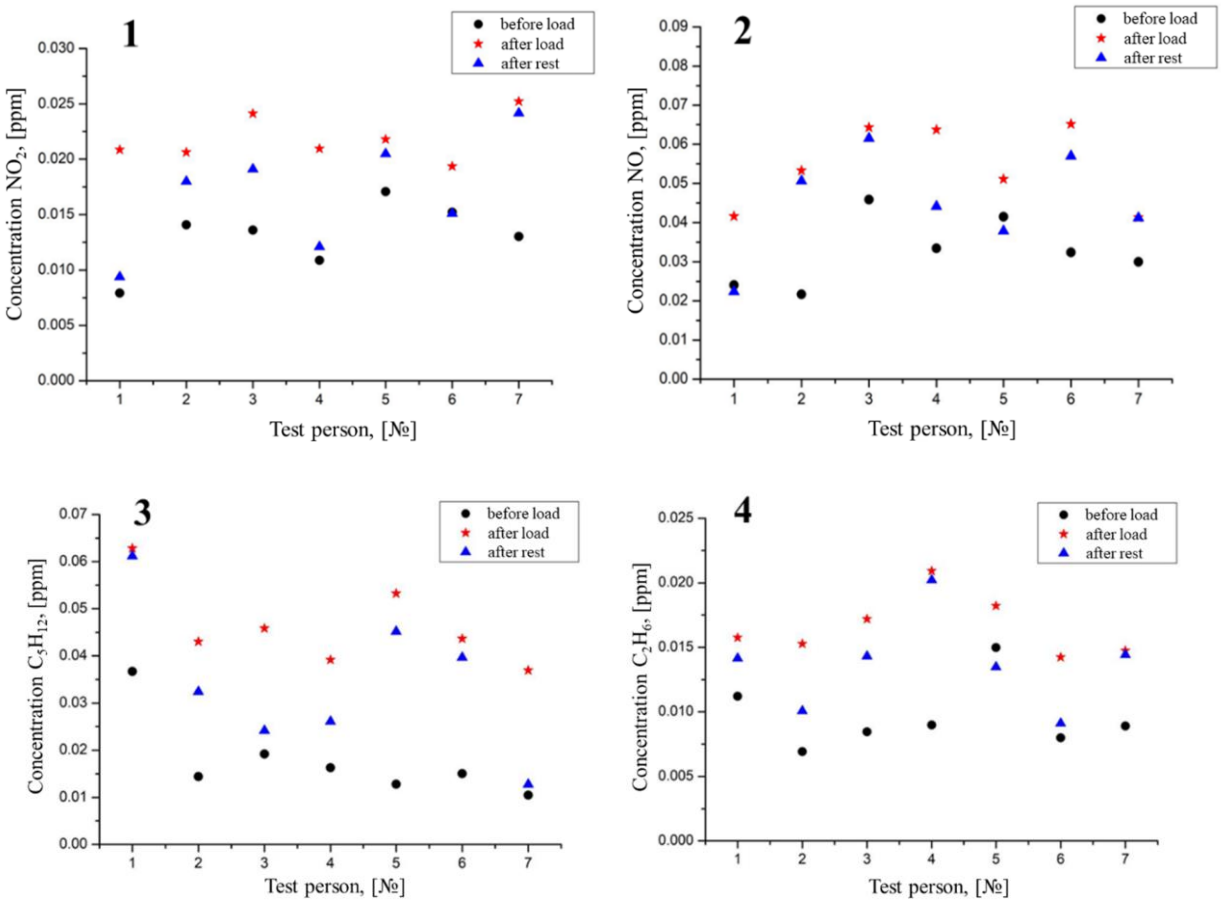

Fig. 4 Variations in the concentration of NO_2 (1), NO (2), C_5H_{12} (3), and C_2H_6 (4) in the EAS of participants during the experiment.

4 Conclusion

The aim of the work was to analyze OS volatile molecular biomarkers in exhaled air by optical-acoustic laser spectroscopy. Aerobic physical activity (work on an exercise bike) was used as an OS provocateur. Three states of the participants in the experiment were studied: "before physical exercises", "after the exercises", and "after the rest". Based on the results, the following conclusions were made. Immediately after the exercises, there is a statistically significant increase in the concentration of all the studied OS molecular biomarkers in the exhaled air, as evidenced by a clear separation of the groups of EAS "before exercises" and "after exercises", indicating the OS presence (Fig 5). After the rest, statistically significant differences are observed in relation to the initial state only for a part of these biomarkers. This is most likely due to the different recovery of the subjects after exercises and insufficient rest time (25 min). Thus, this approach is promising for non-invasive express control of OS.

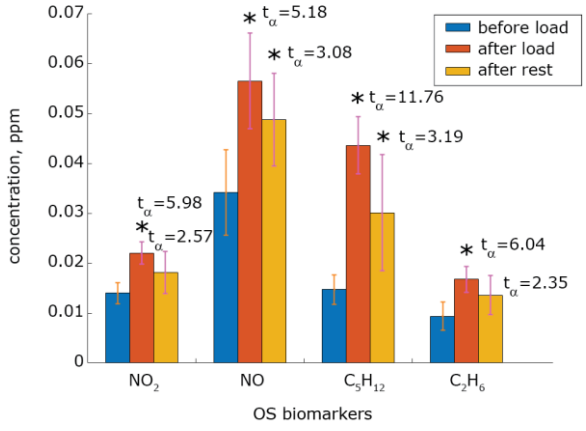

Fig. 5 Average concentrations of OS biomarkers for a group of participants before physical exercises, after exercises, and after the rest. Critical value of $t_\alpha = 2.57$.

Disclosures

The authors declare no conflict of interest.

Acknowledgments

A. A. Boyko thanks the RFBR for the financial support of the research (Project № 19-32-60055). A. V. Borisov, V. S. Zasedatel, Yu. V. Kistenev – Development Program of Tomsk State University (Priority–2030).

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