

„Our nature is the movement, the repose is death.” B. Pascal

INTRODUCTION

Living creatures have an essential characteristic: movement. Through it the body survives to the external environment changes [Ardelean, 2001].

The medical and pharmaceutically field have developed their knowledge level, reaching a detailed understanding of molecular interactions and changes that occur in the human body. Understanding of phenomena at the molecular level leads to more rational approach of the situations in which the body becomes exposed, potential imbalances are mainly removed from the beginning [Lucaciu Ionescu, 2006].

Deepening the knowledge about the organized practice of physical exercise can be done, among others, through the study of the molecular aspects underlying the effort's biochemistry. The biochemistry follows the sanogene aspects, but more at the theoretical level, while the motric activities are dealing with the practical level [Dumitru, 1980].

By combining the biochemical, physiological knowledge and the methodology for motric activities, generally, beneficial effects can be obtained, both at the performance sport level as well as at the level of amateur practitioners mass. In the vast performance no longer currently exists planning and empirical experimentation, everything transforming into strategies and scientific coordination of the activity, and we do not see why not, the same principles could be exposed, otherwise, in everyday practice of physical exercises.

The lifestyle greatly influences the health, morbidity and mortality of the mass population [Kujala et al, 1998; Wei et al, 1999; Cokkinides et al, 2009; White, 2007; Giovino, 2007]. The inactivity and smoking are another two causes of mortality that have gained momentum recently [Ferucci et al, 1999].

The promotion of physical exercise among young population and the adult one is an important step toward preventing a wide range of diseases, such as: diabetes, obesity, hypertension, heart disease, osteoporosis, colon cancer, lung cancer and some diseases of mental nature [Kaplan et al, 1996; Rockhill et al, 1999; U.S. Department Of Health And Human Services, 1996; Martinez-Gonzalez et al, 2001].

This paper refers to the oxidative stress that occurs during physical exertion in smokers and the possibility of improving this phenomenon by administering vitamin C before the effort. Given that smoking is an important factor in accelerating the oxidative stress, we consider that the pre-effort administration of an antioxidant will improve the negative effect at the cellular level (oxidative stress) occurred following the submission of a light aerobic effort.

OBJECTIVES OF THE RESEARCH

Physical inactivity poses a serious risk for the emergence and development of diseases, the most common being related to the functioning of the cardio-respiratory system [Morris et al., 1991; Chandrashekhara and Anand, 1991; Smith et al., 1995; Wenger et al., 1995; Paffenbarger et al., 1986].

Any type of physical effort requires energy consumption and its compensation through obtaining the energy from a balanced and healthy nutrition [Trofin and Cojocaru, 2012].

Studies relating to the biochemical and physiological effects of physical effort in untrained individuals are less numerous than those dedicated to performance sports, to these subjects the research on motric performance is more comprehensive in the specialized literature both in the country and abroad. On the other hand, the physical exercise used to overcome the normal human limits was and is extensively studied, while the prophylactic one, addressed to the untrained persons has a lower area of knowledge.

The organism produces some natural phenomena such as the oxidative stress, after which the cells and biological tissues suffers structural damage, losing some important functions. This occurs in both normal physiological conditions as well as in the ones of imbalance of the balance obtained in repose conditions. During physical effort the oxidative stress is increasing, so the problem of balance restitution occurs through nutrition intervention.

The general objective of the thesis is to study the changes of biochemical and physiological parameters in untrained subjects, smokers and nonsmokers, undergoing a quantified physical effort with relatively small intensity.

From the general objective we can detach the following two **objectives**:

1. Creating a general theoretical framework to support the research.
2. The determination and interpretation of the values trend of biochemical parameters in easy aerobic exercise.

Deepening the research details we can detach the following **specific objectives**:

1. The characterization of the aerobic physical effort from the biochemical and physiological point of view.
2. The general characterization of the generation mechanisms of oxidative stress.
3. Establishment of the concrete research direction.
4. Identification of parameters required in accurate interpretation of research results.
5. Valorisation of research results and identification of future research directions.

PART I - CURRENT STATE OF KNOWLEDGE

CHAPTER 1. GENERAL NOTIONS CONCERNING THE BIOCHEMISTRY AND PHYSIOLOGY OF THE EFFORT

1.1. General notions concerning the energetic metabolism

It is well known that during exercise the glucides and fat acids are the main energy supplier through their oxidation by the muscle tissue. The contribution of the two substrates can be influenced by: diet [Bergman and Brooks, 1999; Coyle et al., 2001; Horowitz et al., 1997], content of glycogen in musculature [Weltan et al., 1998; Weltan et al., 1998], the intensity of physical effort [Bergman și Brooks, 1999; Friedlander et al., 1998; Romijn et al., 1993; Romijn et al., 2000; Sidossis et al., 1997; Thompson et al., 1998], exercise duration [Romijn et al., 1993], and the individual level of training [Lawler et al., 1984; Jeukendrup et al., 1997; Van Loon et al., 1999].

Hans Krebs highlighted the energetic process stages that bears his name and which is also called the tricarboxylic acid cycle or citric acid cycle. Within it, acetyl-CoA (resulting from oxidative decarboxylation of pyruvic acid, formed in turn by the degradation of glucose through anaerobic glycolysis) is oxidized to CO₂. Is one of the fundamental biochemical processes of living organisms.

1.2. Theoretical aspects of physical effort in untrained individuals

1.2.1. Motric qualities necessary for untrained persons

Biological functions and constants of the human body in the physical effort vary between fairly wide limits. Exceeding these limits may occur in performance physical effort. The physical effort determines neuro-humoral reactions that start from the the somatic nervous system and ending with the vegetative functions as well as with the endocrine-metabolic ones (indispensable to ensure the energetic substrate of muscular contraction).

Muscle consumption of oxygen is directly proportional to the exercise intensity. This increase has a limit, beyond which, no matter how much the intensity (power) would increase, the oxygen consumption remains unchanged. The reached limit is referred to as the maximum oxygen intake or maximal oxygen consumption (VO₂max). The effort that exceeding VO₂max in terms of intensity, is excruciating and can be maintained only a few minutes.

In an muscular effort at constant working power, the oxygen consumption increases exponentially at first, followed that after 2 - 3 minutes it describes a "plateau" with its values [Apostol, 1998]. We can distinguish three phases (figure 5): the adaptation phase, the stable equilibrium phase (steady - state) and the recovery phase.

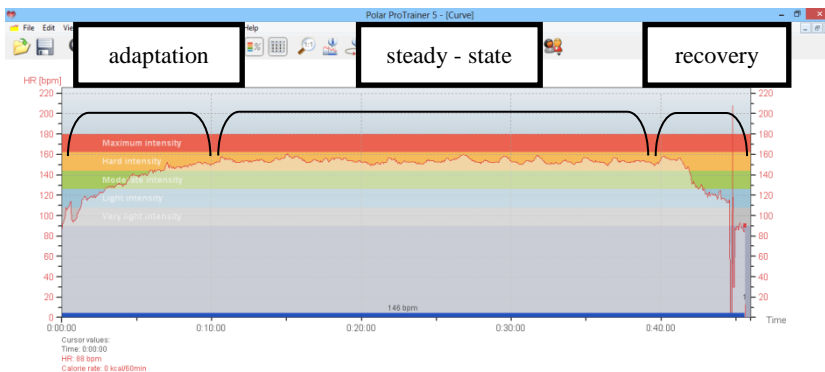


Figure 5 - The physical effort phases in a subject of this research (original)

Maximal aerobic power (MAP) reflects the maximum power developed based on aerobic metabolism and is achieved when the body uses the maximum amount of O₂ in metabolism. An increased VO₂max characterizes an increased physical resistance.

The resistance for the cyclic efforts can be divided [Bompa, 2002] depending on duration as follows:: long term resistance (> 8 minutes), medium term resistance (> 2 minutes to <6 minutes) and short-term resistance (> 45 seconds to <2 minutes). The influence factors [Bompa, 2002] of the resistance are: the central nervous system (CNS), the power of will, the aerobic capacity, the anaerobic capacity, the speed reserve etc.

1.2.2. The physical effort parameters

The physical effort is characterized by volume, intensity and complexity.

The volume is the total amount of effort made over a period of time.

The intensity is the qualitative component of the effort, considering the amount of work done per unit time. Along with the gradual increase of the physical effort intensity, the carbohydrate metabolism follows an upward path of intensity, and the lipid one an downward path.

The complexity is the degree of sophistication of an exercise. Through its high level we can increase the intensity of work, the request on the muscle.

1.3. Biochemical adaptations during aerobic physical effort

Ensuring the energy requirements of the human body is accomplished by the balance between exogenous energy intake through food ingestion and normal energy consumption [Lupea și Ardelean, 1997].

Through methodical training the following changes are produced [Lupea and Ardelean, 1997]:

- increasing the acid pH tamping capacity of the blood; the degree of training determines how quickly the pH returns to normal;
- the macroergic molecules degradation (carbohydrates, lipids and proteins) is accelerated;
- the ATP level is kept in constant concentrations even after training;
- the enzymatic activity increases of various metabolic processes.

It is normal that, during or after muscular labor, the muscle fatigue to make its presence felt. It is marked biochemical [Lupea and Ardelean, 1997]:

- the reduction or depletion of energy substrate;
- the decrease of enzyme activity intensity;
- the metabolites accumulation with negative effects on metabolic processes involved in muscle contraction.

The muscular contraction is "motorized" by two biochemical steps:

- anaerobic phase - without the O₂ participation in the reaction;
- aerobic phase - with the O₂ participation in reactions.

1.4. Physiological adaptations during aerobic physical effort

In the physical effort, two categories of physiological reactions of the body are more important and easily evaluated [Haulica, 2009]:

1. Cardiovascular reactions.
2. Respiratory reactions.

1.4.1. Cardiovascular adaptations during aerobic physical effort

Pumping the blood in the cardiovascular system is a consequence of rhythm presionale changes occurring in the cardiac tetracameral system, representing the result of contractions (systoles) and relaxation (diastole) of heart [Haulica, 2009]. The blood exerts pressure on the arteries walls, this is called blood pressure (BP), which undergoes fluctuations during the cardiac cycle. The blood pressure has two components: systolic pressure/tension (maximum) (TAS) and diastolic pressure/tension (minimum) (TAD).

Hemin transporters of oxygen in the blood are hemoglobin (Hb) and myoglobin (Mb) [Pui, 2008].

The mechanisms for increasing the oxygen transport are:

- the central mechanism (increased cardiac output);
- peripheral mechanisms (decreased muscle vascular resistance, distribution of local blood flow).

Heart rate (HR) is the fastest adaptive mechanism. Before the effort, the HR increases as a result of the psycho-emotional effect. During the exercise the HR growth is influenced by intensity, sex, age, level of training and reflects the body requirement. The HR can be considered an exercise intensity indicator. Restoring the HR is an important criterion for assessing cardiovascular functional status, its

time depending on the total volume of mechanic work and not on the intensity [Honceriu et al., 2012].

1.4.2. Respiratory adaptations during the aerobic physical effort

After the respiratory process, the oxygen is used inside the cell to obtain energy following some complex biochemical processes among whose products also includes biological "waste", whose disposal is important for the good functioning of the biological systems.

In humans, gas exchange takes place in several stages:

- external respiration (oxygen uptake and release of carbon dioxide);
- stage blood (gas transport using blood);
- cellular respiration (conducting reactions with energy production).

In association, the respiratory system and the cardiovascular one supply the tissues with oxygen, while removing carbon dioxide through the following processes [Predescu, 2009]: pulmonary ventilation, alveolo - capillary diffusion, the respiratory gases transport (blood) and the gas exchanges at the tissue level.

The hemoglobin exhibit affinity for the oxygen and the maximum volume of oxygen that it can be fix expresses its oxygenation capacity [Haulica, 2009].

1.4.3. The influence of motric activity on striated muscle

By training the body adaptations are produced of both the central nervous mechanisms and the peripheral muscles, thereby optimizing the recruitment of a large number of motor units in the effort [Filipaş and Ardelean, 2002].

Intense muscular efforts make the cardiovascular and respiratory system to create oxygen debt, which they will pay after their termination [Vâjială, 2007].

1.5. The homeostasis of the human body

The homeostasis involves physiological and biochemical processes that contribute to the maintenance of the balance in the body through adjustments actions of the internal environment parameters level.

The human body homeostasis refers to: the regulation of hidro-electrolyte balance and that of acid - base, maintaining constant the blood composition, maintaining a constant body temperature, regulating food intake, depending on the body's energy consumption etc.

1.6. The oxidative stress, damaging mechanism for the body during physical effort

About 3 billion years ago the oxygen appeared on Earth, and once with its increase in concentration the activation reactivity has increased (on the n*2p orbital level), forming reactive oxygen species (ROS). The term refers to both O₂-derived free radicals as well as reactive oxygen neradicalic derivatives (eg.

Hydrogen peroxide). At the cellular level, the oxygen is used at the mitochondria level: 95-98% for cellular respiration and 2-5% for the formation of ROS [Powers and Jackson, 2008].

1.6.1. The offensive oxidant system: pro-oxidants (PO)

Inevitably, the oxygen used in the cell leads to the formation of ROS or oxygen metabolites / catabolites, it having a low life and high toxicity. SRO are redox derivatives of O₂ and produced in cascade, their source being the superoxide anion (O₂⁻) [Powers and Jackson, 2008].

The oxidative stress term has been defined for the first time in 1985 as the imbalance that occurs between pro-oxidants and antioxidants in favour of the first category [itself, 1985; Himself and Cadenas, 1985].

In theory, lowering the non-enzymatic antioxidants level is an oxidative stress effect and measuring their decline in body tissues has been used as a biomarker of oxidative stress. It has not yet been discovered the ideal oxidative stress biomarker which faithfully reflect the real process and until then measuring as many indicator parameters is necessary for correct assessment of oxidative stress [Halliwell and Gutteridge, 2007].

Endogenous formation of SRON

SRON is formed in the body through: the univalent reduction in four steps, electronic transfer via small molecules, from various reductases and prooxidant action of endogenous antioxidants.

Exogenous formation of SRON

SRON may have an external source of the body, it can be: substances with oxidoreduction character, oxidative drugs, toxic substances, ionizing radiation [Powers and Jackson, 2008].

Role of reactive species

Paradoxically, SRON have both harmful as well as beneficial effects. The first occurs at high concentrations, and the beneficial ones at normal physiological concentrations [Powers and Jackson, 2008].

1.6.2. Antioxidant defense system: antioxidants (AO)

The antioxidants are substances that are sacrificed by the body to protect the DNA and the enzymes. They are present in low concentrations that decrease or inhibit the substrate oxidation. Protects the biological systems against the processes harmful effect or reactions causing excessive oxidation.

The antioxidants act enzymatic or non-enzymatic. AO have a low concentration in vivo, which increases in case of AO defense.

1.6.3. Vitamin C, a powerful antioxidant

The vitamin C is the most effective natural AO substance and represents the first line of AO defense in plasma. Interacts directly with oxidizing agents, OH •, lipid hydroperoxides. Contribute to the regeneration of vitamin E together with GSH and through its supplementation we can prevent/limit: diabetes, ischemic cardiopathy, cancer, aging etc. The ascorbic acid is widely distributed in mammalian tissues and has two roles [Packer et al. 1979]: direct purification of the superoxide, the hydroxyl radical and the hydroperoxide radical [Carr and Frei, 1999] and replenishing vitamin E stocks, with forming the vitamin C radical, semi-ascorbyl.

The ascorbic acid is not found in the human body as reserves, so it is indicated a continuous intake and as natural as possible [Cojocaru et al., 2010].

1.6.4. Total antioxidant capacity (CAOT)

Also known as total antioxidant status (SAT), it is a parameter that must be reported to the body oxidative stress. The report SRO/CAOT reflects the oxidative stress, more objective than the separate values of the two factors [Muresan et al., 2006].

1.6.5. Evaluation of oxidative stress

The SRO measurement can be done through two approaches [Muresan et al., 2006]: RL fixing by the "spin traps" obtaining the combinations that can be evaluated later on and measuring the injuries caused by the circulation of RL - the OS appreciation itself.

Direct methods

The electron spin resonance (ESR) - detects unpaired electrons of RL and identifies the RL in real specific time. Spin traps – through some agents that form stabil RL compounds that can be further analysed [Muresan et al., 2006].

Indirect methods

The determination of aldehydes that are produced by the decomposition of lipid peroxides. A particular importance has the malondialdehyde (MDA) and 4-hydroxynonenal [Muresan et al., 2006]. The antioxidant enzyme measurement involves measuring the major antioxidant enzymes (SOD, CAT, GPX), as well as the ones of secondary line (glutathiontransferaze, glutathione reductase, glucose-6-phosphate dehydrogenase and others).

1.6.6. The role of mitochondria in oxidative stress

In addition to the energetic role, the mitochondria interferes in the oxidative stress. The mitochondrial reactions are considered an important source of O₂⁻, which is obtained in steps [Lupea, 2007]. During aerobic physical effort the oxygen consumption increases, and with it the mitochondrial activity also increases

resulting in an increase of 50-100 times of the superoxide generated by skeletal muscles [Kanter, 1994; Urso and Clarkson, 2003].

PART 2 – PERSONAL CONTRIBUTIONS

CHAPTER 2. THE MOTIVATION OF CHOOSING THE THEME AND THE PURPOSE OF THE RESEARCH

2.1. The Motivation of Choosing the Theme

As a young person interested in the motor activities I chose the theme as a result of active involvement in the field, of the wish to deepen interdisciplinary knowledge that occurred at the intersection of biochemistry and physiology on the one hand and the human motor skills on the other hand, as a concern for the implementation of this knowledge. This research aims to solve an important aspect of exercise in smokers: accelerated oxidative stress relieve stress by taking vitamin C, a powerful antioxidant.

2.2. The Purpose of the Research

Our purpose is that, by realizing this research, to highlight the changes that occur biochemically and physiologically as a result of easy aerobic exercise, in subjects whose lifestyle do not perform regular daily exercise. At the same time we aim to bring to your attention some aspects of specific training for people with a sedentary lifestyle, which may have benefic and directed effects if planned "biochemical".

The overall aim of the thesis is to study changes in biochemical and physiological parameters in untrained subjects, smokers and nonsmokers, undergoing intensity quantified exercise of a relatively small intensity. This major objective shall coordinate the following specific objectives:

1. Creating a general theoretical framework for research support.
2. Determination and interpretation of the trend values of biochemical parameters in easy aerobic exercise.

2.3. Research Hypotheses

Underlying conducting research are the following assumptions:

Hypothesis 1: Assume that moderate aerobic exercise induces untrained persons, biochemical and physiological oxidative stress of different magnitude from those of athletes, compared with the data we collected from the literature oriented in sports.

Hypothesis 2: *We assume that if we introduce in the nutrition of smoking individuals a tablet of vitamin C with slow absorption (12 hours) before an exercise (with 18 hours) dosed at 50% of maximal oxygen consumption, then we*

could get oxidative stress relief after exercise, in the way that it may manifest statistically insignificant differences compared with the same indicators evaluated in smoking individuals after the same type of effort.

CHAPTER 3. MATERIAL AND METHODS

3.1. Time, place and material conditions of conducting research

The research aims to highlight the manner and extent to which the vitamin C, before a light aerobic exercise in individuals who smoke, improves the effects of oxidative stress during exercise comparing to smoking individuals, both groups being represented by individuals with low or moderate daily motor activity.

The practical part of the research took place in February 2012 - April 2013. The stage in which the subjects were put to exercise, was held at the University "Alexandru Ioan Cuza".

The subjects were initially evaluated physically, for determining the study group specific features, then the data had been used in the dosage of effort for the stage of the collection of biological samples.

During the research, atmospheric parameters were monitored because recent studies have revealed that ambient temperature may influence oxidative stress. [Gomes et al., 2011; Laitano et al., 2010; McAnulty et al., 2005; Salo, 1991].

3.2. The subjects of the research

Volunteers involved in research are male and aged between 19 and 30 years. They were divided, by means of a questionnaire, which they completed prior to assessment in two groups: one of smokers, and one of non-smokers. Each group consists of 14 volunteers. Their characteristics are shown in Table 7.

A subject is considered active or passive smoker if he inspires every day, the smoke from burning tobacco. Also, the smoker subject inhales the smoked from the burning of at least one cigarette per day.

Tabelul 7 – Characteristics of experimental groups
(average and range of reference)

Characteristics	Non-smokers	Smokers
Age(years)	$21,79 \pm 1,18$	$23,64 \pm 1,66$
Number of subjects	14	14
Hight (cm)	$177,93 \pm 4,19$	$176,29 \pm 3,69$
Weight (kg)	$71,97 \pm 5,52$	$75,16 \pm 8,82$
Level of exercise (1 – 5)	$2,43 \pm 0,49$	$2,14 \pm 0,38$

3.3. Physical Evaluation of Subjects

The 28 subjects went through anthropometric, somatoscopic and physiological evaluations. We tried removing the disturbant factors (as much as possible) of the research using: rigorous selection of subjects (sex, age range, able to exercise, untrained), the formation of two groups, each of 14 subjects that provide statistical certainty, ensuring batch uniformity in terms of the criteria selection and the use of standard analytical techniques in determining the observation of biochemical parameters.

Subjects have been evaluated in the sense of: body composition, VO₂max on bike with electromagnetic brake, blood pressure and resting heart rate, chest elasticity, forced vital capacity - spirometry, maximum voluntary ventilation - spirometry, vital capacity - spirometry, lumbar muscle force, force abdominal muscles, joint mobility, strength and plyometric anaerobic exercise capacity.

3.4. Determination of biochemical indicators of oxidative stress

Since there is not an evaluation technique of producing free radicals in each cell compartment yet, the quantitative assessment of oxidative stress in the human body will be further indirect [Powers et al., 2011].

Subjects were tested twice in aerobic exercise, corresponding to 50% of VO₂max, for 40 minutes. The temporal distance between the two effort sessions is of 7 days. The effort was dosed on PMA determined after physical assessments. The efforts were called "experimental efforts". The first experimental effort was preceded by a biological sample (blood), which was analyzed in the laboratory to establish basal biochemical parameters. Before the biological blood sample subjects did not make effort, and maintained a normal daily rhythm of activity.

Determining the biochemical values of the observed parameters was performed in the Laboratory of Biochemistry and Molecular Biology Faculty of Biology (University "Alexandru Ioan Cuza") and the Center for Biomedical Research of the Romanian Academy, Iași branch.

One experimental effort (EE1) involved pedaling a bike Vision Fitness E3200, after a joint Gymnastics (6 minutes) standard, in front of a laptop that ran a video file. The duration of exercise was 40 minutes, and the intensity was 30% of VO₂max for the first 3 minutes, increasing to 50% of VO₂max for the next 37 minutes. Immediately after the experimental effort, the subject has been collected biological samples for determination of biochemical parameters followed. Immediately after the experimental effort in January, the subject has been collected biological samples for determination of observed biochemical parameters. The experimental effort 2 (EE2) has the same dosage as the first one, with the following differences:

➤ 18 hours before was administered one capsule of Vitamin C 1000 mg with rosehips Vitaking. The capsules contain traces of hidroxypropilmetilceluloză,

microcrystalline cellulose, stearic acid (vegetable origin), silicon dioxide, magnesium stearate (vegetable origin);

- biological samples were collected only after the experimental effort.

Throughout the research was provided specialized medical care to prevent immediate health problems and to awaken a sense of safety for the subjects. After collection of biological samples, they were taken to the laboratory and prepared for analysis. In our research we used as a source of enzyme the blood serum obtained according to standards.

3.4.1. Determination of superoxide dismutase specific activity of Winterbourne method, Hawkins, Brian and Carroll, adapted by Vlad Artenie

Superoxide dismutase activity is determined by the ability of the enzyme to inhibit the reduction of Nitro Blue Tetrazoles (NBT) by superoxide radicals generated in the reaction medium by photoreduction of riboflavin.

In addition, some experimental conditions were used to determine the activity of superoxide dismutase by means of chemiluminescence of lucigenin. A special kit was used to determine SOD, produced by Fluka. Several methods have been used to determine the specific activity of SOD, because of its low values, errors of the method are relatively high, with repercussions on research rigor.

3.4.2. Determination of the specific activity of glutathione peroxidase method with DNTB (Cojocar 2005)

The method measures the activity of glutathione peroxidase in serum and plasma, using as a substrate for the enzyme hydrogen peroxide (H₂O₂) and reduced glutathione (GSH). The color reaction is carried out with hydrogen dithio-bis-dinitro-benzoic acid (DNTB) and the maximum absorbance is measured spectrophotometrically at 412 nm against the reagent blank.

3.4.3. Determination of the specific activity of catalase in the blood by the titrimetric method with potassium permanganate

The method is based on the dosage of hydrogen peroxide remaining after cessation of the enzyme on it. Hydrogen peroxide is dosed in the sample and blank titration with potassium permanganate in acid medium. The difference between the amount of potassium permanganate used for titration of hydrogen peroxide in the sample and blank, is the one that appreciate catalase activity.

3.4.4. Determination of malondialdehyde concentration

At high temperatures and in an acid medium malonic dialdehyde (MDA) resulting from decomposition of the lipid peroxide reacts with 2-thiobarbituric acid

(TBA), forming an adduct trimetinic TBA2 MDA-pink colored that has a maximum absorption at 532 nm.

3.4.5. Determination of glucose by the ortho-toluidine colorimetricăcu micromethod (Artenie et al, 2008)

Glucose and other aldohexoze (from the blood) dehydrate by heating in the presence of concentrated acetic acid at furfural derivatives. The 5-hydroxymethyl furfural formed is condensed with ortotoluidina (2 aminotoluenul) resulting a green colored compound with absorption maximum at $\lambda = 630$ nm. The reaction with ortho-toluidine is stabilized by the addition of thiourea.

3.4.6 Determination of lactate dehydrogenase activity by colorimetric method (Cojocaru, 2005)

The method is based on the reduction of pyruvate to lactate in the presence of NADH, under the catalytic action of the lactate dehydrogenase. The untransformed pyruvate reacts with 2,4-dinitrophenylhydrazine resulting the corresponding phenylhydrazone, which is colorimetrically determined in the alkaline environment. The sample to be analyzed is represented by heparinized serum or EDTA-plasma, freshly collected blood, and without hemolysis. It is necessary to pre-dilution (1 + 4) with physiological saline solution (NaCl 0.9% isotonic saline) before use.

3.4.7. Quantitative determination of creatinine in the blood (Artenie and Tanase, 1981)

Creatinine reacts with the enolic form of the picric acid, forming the creatinine picrate, that under alkaline conditions, is converted into red tautomeric form. The color intensity is proportional to the creatinine concentration in the sample.

3.5. Statistical tools used

Research evaluation results were recorded on individual cards and then pooled using statistical processing software (Microsoft Excel 2010 and GraphPad Prism 6).

3.5.1. Descriptive Statistics

Statistical indicators used are those of central tendency (arithmetic average) and of the dispersion (deviation / standard deviation, standard error, confidence interval, coefficient of variation).

3.5.2. Tests of significance

Were used as tests of significance Student-test and ANOVA-test (Tukey HSD post-hoc analysis).

3.5.3. Statistical data processing

.Statistical processing has led to the synthesis of data through tables and graphical representation.

CAP. 4 RESULTS AND DISCUSSION

4.1. Anthropometric, physiological parameters and exercise capacity

4.1.1. Body composition

The method used to determine body composition is bioelectrical impedance (IBE), that assumes completion of the body by alternating currents of different frequencies [Bedogni G. et al., 2006].

The elements of body composition of the subjects did not show significant differences between groups ($p > 0.05$).

4.1.2. Joint mobility

Joint mobility was assessed by the fact that its high values lead to more efficient motor act performed by the specific muscles, also to reducing energy consumption and increasing the local resistance. The coxal-femoral, knees, ankles, spine and chest mobility had not statistically significant differences.

4.1.3. Respiratory parameters of subjects at rest

Regarding respiratory parameters, research groups did not differ statistically, which proves that smoking does not significantly alter the respiratory act.

4.1.4. Circulatory parameters at rest and in physical assessment

TA has normal values for the characteristics of the research groups, as well as FC [Apostol, 1998]. There were found statistically insignificant differences between TA and FC of the two groups at rest, also during maximal aerobic exercise test.

4.1.5. Aerobic exercise capacity

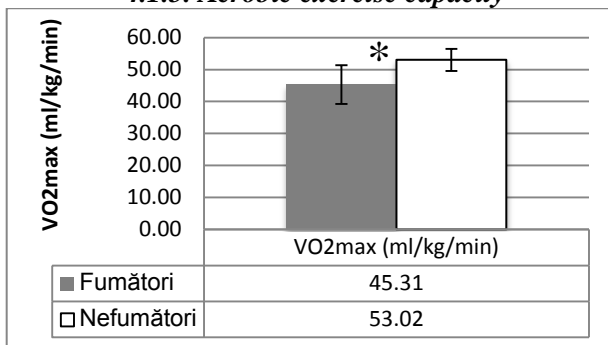


Figura15 – Maximum oxygen consumption average on subjects (values are mean \pm confidence interval, * $p = 0.028$)

It was found (figura15) a statistically significant difference between the groups ($p = 0.028$), non-smokers having a VO_{2max} of $53.02 \text{ ml / kg / min} \pm 4.87 \text{ ml / kg / min}$, higher with $7.71 \text{ ml / kg / min}$ than that of the smoker. This result demonstrates that smoking lowers the maximum aerobic capacity, even if the body remains the same physical characteristics.

4.1.6. Anaerobic exercise capacity

A difference was found (not statistically significant) for each sample, non-smokers with higher values of plyometric strength, in particular. The isometric and isotonic force, and the anaerobic exercise capacity, are more developed in the smoking group.

4.1.7. Circulatory parameters in the experimental efforts

After the physical assessment of the subjects, followed the second part of the research, in which the biological samples were collected for analysis of characteristic parameters of oxidative stress (SOD, CAT, GPX and MDA) that appears in efforts with an intensity of 50% of the PMA. Two experimental efforts have been sustained at 50% of MAP for 40 minutes at an interval of a week. The first effort was conducted under normal conditions, and the second with administration of vitamin C 18 hours before the time of the planned effort. In the first effort the biological sampling has been made before (min0) and after exercise (min40), and in the second effort after its completion (min40), given that the oxidative stress level markers did not significantly alter under basal conditions.

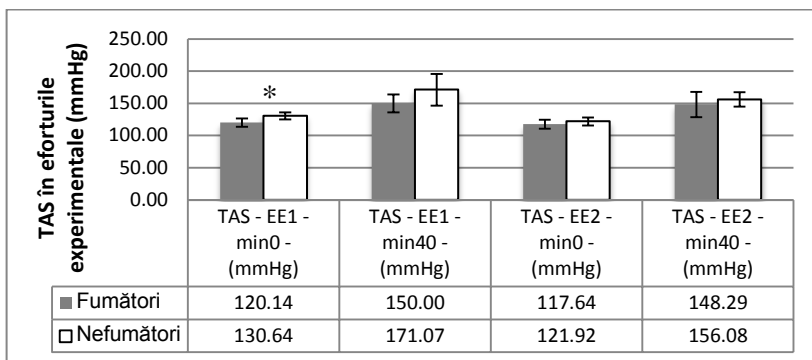


Figure 16 - Average systolic blood pressure (SBP) of the subjects in the first experimental effort (EE1) and the second experimental effort (EE2), at the beginning of the efforts (min0) and at the end of them (min40) (the values represent the average \pm the confidence interval, * $p = 0,012$)

The figure 16 graphically depicts the systolic blood pressure of the research subjects at the beginning the experimental effort 1. The difference of 50.93 mmHg shows a stronger systole in non-smokers, probably being caused by restlessness, given that smoking has a soothing effect on the body, and this was restricted before the experimental efforts.

4.2. Biochemical parameters

It was demonstrated that physical effort leads to accelerated production of ROS but nevertheless, there are still enough unknowns about both the interaction that is established between the two and the need for a diet to improve OS [Muaz and Hakki, 2006].

It is known that the redox reactions are disturbed by exercise, the oxidative stress being a result of the unbalance created [Gomez-Cabrera et al. 2005; Gomez-Cabrera et al. 2008; Hudson et al., 2008; McAnulty et al., 2005; McAnulty et al., 2007; Quindry et al., 2008; Quindry et al., 2003]. At the same time the oxidative stress causes tissue adaptations, this is beneficial for the body [Gomez-Cabrera et al., 2005; Gomez-Cabrera et al., 2008; Ristow et al., 2009; Vina et al., 2006; Vina et al., 2000].

The oxidative stress mechanism is still unknown in detail, the current research is going towards sending some specific antioxidants in mitochondria in order to meet some questions whose answer is still unknown [Smith et al., 2003; Brown et al., 2007; Murphy and Smith, 2007; Adlam et al., 2005; Zhao et al., 2004].

On the untrained subjects, the administration of vitamin C is beneficial, especially when they submit easy aerobic exercise (50% VO₂max) for 40 minutes. As a result of the effort mentioned, prior to the administration of vitamin C slowly absorbed, the oxidative stress is greatly reduced in comparison with that occurred after exercise without intake of vitamin C [Trofin et al., 2014 - A]. It is thus interesting to see if the same vitamin C is effective for improvement of oxidative stress appeared in smokers after a light aerobic exercise (50% VO₂max) for 40 minutes, the reporting being made to oxidative stress occurred in non smokers after the same effort.

Table 17 contains the results of this study, schematic arranged to achieve an overview.

Table 17 – The evolution of oxidative stress markers within the evaluations (average ± confidence interval)

		EE1 – min0	EE1 – min40	EE2 – min40
N E F U M	SOD (U/ml)	1,21 ± 0,40	1,53 ± 0,56	1,82 ± 0,77
	GPX (U/ml)	0,06 ± 0,02	0,05 ± 0,01	0,09 ± 0,02
	CAT (U/ml)	11,96 ± 4,63	24,55 ± 8,59	13,37 ± 4,60
	MDA (nM/ml)	43,15 ± 8,30	82,94 ± 24,87	34,11 ± 7,09
F U M	SOD (U/ml)	1,06 ± 0,46	2,01 ± 0,68	1,60 ± 0,65
	GPX (U/ml)	0,07 ± 0,01	0,05 ± 0,01	0,08 ± 0,01
	CAT (U/ml)	14,43 ± 5,30	17,27 ± 5,27	14,07 ± 4,59
	MDA (nM/ml)	41,12 ± 7,98	94,76 ± 24,45	35,09 ± 10,52

In the research hypothesis testing, we will use all six data sets analyzed (non smokers - min 0, smokers - min 0, non smokers - min 0, smokers - min 40, non smokers - min 40 + vitamin C, smokers - min 40 + vit C - Table 17).

It must be taken into the account the fact that smokers are exposed to hundreds of reactive chemicals and trillions of radicals and particles at the consumption of each cigarette and harmful changes can be reached at the lungs level [Genç et al., 2014].

There were determined, in addition to oxidative stress markers, three biochemical parameters: glucose, the specific activity of lactate dehydrogenase and creatinine concentration. It represents important indicators of glucose metabolism as well as the proteic one. The recaled parameters values are shown in table 18.

Table 18 – The evolution of biochemical markers within the assessments (average \pm confidence interval)

		EE1 – min 0	EE1 – min 40	EE2 – min 40
S M O K E	Glucose (mg%)	113,79 \pm 11,42	87,83 \pm 7,82	88,99 \pm 8,24
	Creatinine (mg%)	1,02 \pm 0,09	1,24 \pm 0,11	1,17 \pm 0,13
	Lactate dehydrogenase (U/l)	165,9 \pm 33,58	203,8 \pm 24,97	237,05 \pm 28,20
N O N	Glucose (mg%)	108,92 \pm 8,13	74,64 \pm 7,50	84,20 \pm 10,00
	Creatinine (mg%)	1,08 \pm 0,13	1,21 \pm 0,14	1,16 \pm 0,18
	Lactate dehydrogenase (U/l)	155,80 \pm 28,46	209,38 \pm 25,58	255,60 \pm 29,57

4.2.1. The influence of aerobic exercise on the specific activity of superoxide dismutase at untrained persons

The first aspect studied concerning the evaluation of biochemical parameters evolution in the exercise at the two groups of untrained subjects was the superoxide dismutase activity determination. The experimental results are summarized in Table 19 and plotted in Figure 17.

Through the intervention of superoxide dismutase, the superoxide radical can be split into less reactive compounds such as hydrogen peroxide. Thus, SOD is the first line of defense in the face of oxidative stress that occurs in the human body. Its activity improves the destructive effect of the chain generating SRO, on through the intervention on the superoxide radical.

Table 19 – The evolution of superoxide dismutase activity in the experimental efforts (avarege \pm confidence interval)

SOD (U/ml)	EE1 – min 0	EE1 – min 40	EE2 – min 40
Non - smokers	1,21 \pm 0,40	1,53 \pm 0,56	1,82 \pm 0,77
Smokers	1,06 \pm 0,46	2,01 \pm 0,68	1,60 \pm 0,65

As the data shows, at the non-smoking group of subjects, the superoxide dismutase activity was on average of 1.21 U/ml blood serum before experimental effort one, so that after 40 minutes from the achievement of the motric activity to suffer a moderate growth, up to an average of 1.53 U/ml that remains inferior to

the enzyme activity at the same group of subjects, but after 40 minutes from the second experimental effort (mean 1.82 U/ml).

For the group of smoking subjects, the superoxide dismutase activity dynamics is different in that they initially showed a catalytic activity average of 1.06 U / ml, by approximately 12% less than that recorded in non-smoking subjects so that after 40 minutes of making the effort to register a much stronger increase up to a value almost double (2.01 U / ml). After 40 minutes of the completion of second experimental effort in this group of subjects the mean activity of this enzyme has relatively modestly decreased up to 1.60 U / ml.

These different evolutions of superoxide dismutase activity in the two groups of subjects in the study could be explained perhaps by the fact that in the smoking subjects case, the oxidative stress generated by the motoric activity stack with the one caused by toxic substances in cigarette smoke whereas, although after 40 minutes after the exercise realization in both groups the enzymatic activity is approximately 50% higher than that recorded at the starting time, in smokers it is lower than that recorded in the non-smoking group, it increases more strongly after 40 minutes of motoric activity performance and reduces much stronger at 40 minutes after the second experimental effort.

After applying the ANOVA test it was necessary to apply a non-parametric test. The result of the ANOVA test ($F(3,52) = 1.49$, $p = 0.227$) showed a statistically insignificant difference between all data sets analyzed.

The Kruskal-Wallis test application generates an approximate value of $p (= 0.210)$ that leads to the conclusion that the specific activity of SOD is not statistically significantly influenced by the factors put into question (effort, smoking and effort, effort, smoking and vitamin C administration). This demonstrates that the appearance and development of oxidative stress in the given conditions are of such a magnitude as the body's response to be primed to the level of superoxide dismutase activity.

In the figure 17 it is visible that the difference between the groups and tracked moments is statistically insignificant.

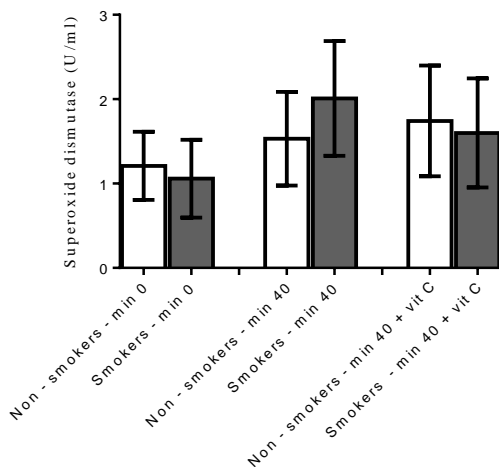


Figure 17 - The dynamic of specific activity values of superoxide dismutase in the experimental efforts (the values represent the average \pm 95% confidence interval)

Modifying the specific activity of SOD was studied over time, the findings of the done research having different directions. Some studies show that SOD do not show changes after intense endurance efforts [Alessio et al., 1988; Hellsten et al., 1996; Lambertucci et al., 2007; Laughlin et al. 1990], others show increases between 20 and 112% of the activity of SOD1 and SOD2 in muscle [Criswell et al., 1993; Higuchi et al. 1985; Lawler et al., 2006; Bourgeois Leeuwen et al., 1994; Leeuwenburgh et al. 1997; Oh-Ishi et al. 1997; Powers et al. 1994; Quintanilha, 1984; Vincent, 1999; Vincent, 2000]. Differences that occur between the studies are quite large and can be explained by: the methods used to determine the enzyme activity, different types of effort used in research, different types of fibers involved in the studied efforts etc.

4.2.2. The influence of aerobic exercise on the the specific activity of glutathione peroxidase in untrained persons

The GPX is the enzyme in the second line of defense, interfering in the reduction of H₂O₂ or organic hydroperoxide in water and in alcohol or uses glutathione (GSH) reduced, thioredoxin or glutaredoxina as electron donors. The experimental results obtained from the determination of glutathione peroxidase activity in the subjects of the two investigated groups are summarized in Table 23 and plotted in Figure 18.

Table 23 - The evolution of glutathione peroxidase activity in the experimental efforts (average \pm confidence interval)

GPX (U/ml)	EE1 – min 0	EE1 – min 40	EE2 – min 40
Non-smokers	0,06 \pm 0,02	0,05 \pm 0,01	0,09 \pm 0,02
Smokers	0,07 \pm 0,01	0,05 \pm 0,01	0,08 \pm 0,01

At the initial moment, the enzymatic activity in the two groups of subjects averaged at 0.06 and respectively 0.07 U / ml, remaining at the same level at 40 minutes after the first experimental effort while the second experimental effort results in a relatively pronounced increase in enzymatic activity recorded 0.09 and respectively 0.08 U / ml.

After applying the ANOVA test it was recommended applying a post hoc test (Tukey HSD).

Applying the Tukey HSD conduce post hoc test leads to signaling two statistically significant differences ($p < 0.05$). The first ($p = 0.007$) is established between the values of the specific activity of GPX in the non-smokers after the first experimental effort and those determined in the group of smokers after the second experimental effort, before which vitamin C was given.

The second difference is determined within the same group. The GPX in smokers modifies statistically significant ($p = 0.011$) after submitting an effort of 50% of VO_{2max} for 40 minutes, preceded by the administration of vitamin C, compared to performing the same effort without administering an antioxidant, which means that the administration of ascorbic acid acts synergistically with the glutathione peroxidase action.

Figure 18 highlights the differences determined according to the tests of significance applied to the data series tracked.

Thus, it appears that vitamin C has an effect on the specific activity of GPX, in the sens of intensifying its activity significantly after the administration of vitamin C to the smokers and depositing an easy aerobic effort for 40 minutes, as compared to the same group without administration of vitamin C as well as the group of non-smokers after a similar effort in intensity and duration.

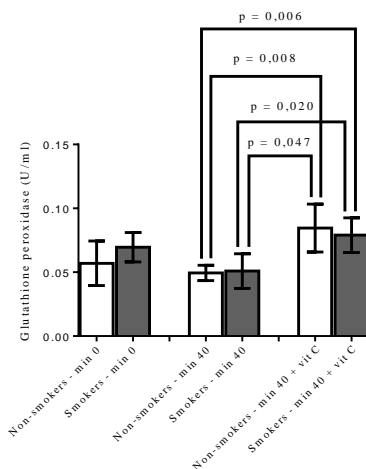


Figure 18 - The values dynamic of the specific activity of glutathione peroxidase activity in the experimental efforts (the values represent the average \pm 95% confidence interval)

The GPX growth reported in studies varies between 20 and 117% [Criswell et al., 1993; Hellsten et al., 1996; Ji et al., 1988; Karanth et al., 2004; Lambertucci et al., 2007; Lawler et al., 2006; Leeuwenburgh et al. 1994; Leeuwenburgh et al. 1997; Powers et al. 1994; Powers et al. 1994; Powers et al. 1992; Sen et al. 1992; Venditti and Di Meo, 1996; Venditti and Di Meo, 1997]. The endurance exercises stimulate enzyme activity in both the cytosol and the mitochondria. With the increase in exercise intensity the GPX activity increases [Powers et al., 1994]. This happens in the case of muscle fibers type I and IIa [Powers and Jackson, 2008].

The differences from the values determined in the performance athletes could be explained primarily by the fact that their antioxidant defense systems present a more effective adjustment system, the physical effort being part of "normal" activity of an athlete, on the other hand, by the fact that the motric activity done in professional sports does not cause an important increase in the concentration of some peroxide, the decomposition of which is ensured by catalase.

4.2.3. The influence of aerobic exercise on the specific activity of catalase in untrained persons

Catalase is involved in the annihilation of free radicals when GPX fails to cope with H₂O₂, its level being increased.

Before the experimental efforts, the specific activity of catalase in non-smoking subjects was averaged at 11.96 U / ml while in those smoking was higher with more than 20% (14.43 U / ml), which reflects the existence of a higher concentration H₂O₂ due to smoking.

Tabelul 27 – The evolution of catalase activity in the experimental efforts (average ± confidence interval)

CAT (U/ml)	EE1 – min 0	EE1 – min 40	EE2 – min 40
Non-smokers	11,96 ± 4,63	24,55 ± 8,59	13,37 ± 4,60
Smokers	14,43 ± 5,30	17,27 ± 5,27	14,07 ± 4,59

Although at 40 minutes from the experimental effort it recorded a significant increase in both groups (24.55 U / ml and respectively 17.27 U / ml), the increase is much more pronounced in non-smoking subjects (more than doubled in non-smoking subjects compared with an increase of 1.2 times in the smokers). After 40 minutes from the experimental effort 2, in non-smoking subjects the catalase activity returns to a value very close to the initial one (13.37 U / ml), while in smokers it lies slightly below the value at the zero moment (14 , 07 U / ml). This means that at the untrained non-smoking subjects, the physical effort causes a relatively strong intensification of the oxidative metabolic sequences forming hydrogen peroxide corresponding to the occurrence of oxidative stress, whereas at smoking subjects the oxidative stress due to exercise is of a lower intensity than that due to smoking and it cumulates with it.

The result of the ANOVA test ($F(3.52) = 3.93$, $p = 0.013$) shows that between the data sets discussed are established statistically significant differences, their particularity is to be identified by the post hoc test.

Tukey HSD post hoc test application leads to signaling two statistically significant differences ($p < 0.05$). A first difference ($p = 0.012$) is established between the values of the CAT specific activity in non-smokers before the experimental exertion (basal conditions) and those of the same group after the first experimental effort. The second difference is established between the research groups, CAT having different values ($p = 0.048$) of the specific activity between the non-smoking group after the first experimental effort and the smokers group after the experimental effort preceded by administration of vitamin C.

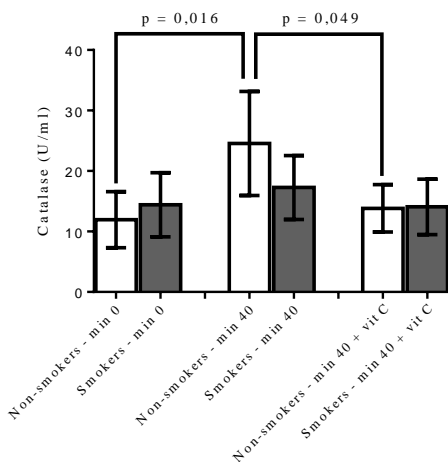


Figura 19 –The specific activity values dynamics of catalase in experimental efforts (the values represent the average \pm 95% confidence interval)

Figure 19 highlights the differences determined for the specific activity of catalase following the tests of significance applied to data sets traced. It is obvious that both light aerobic exercise and vitamin C have significant effect on the specific activity of catalase in non-smokers, respectively smokers. Following the first experimental effort CAT has intensified its specific activity in non-smokers. Through the administration of vitamin C was assessed a specific activity of CAT in the smokers group statistically different from that of the non-smokers group after the first experimental effort.

4.2.4. The influence of aerobic exercise on the concentration of malon dialdehyde in untrained persons

By lipid peroxidation can theoretically degenerate cell membrane structure and lose the functions of membrane protein [Girotti, 1985]. These disorders lead to hardening of the membrane and its willingness to further attacks.

Before carrying out the motric activity, the concentration of this metabolite was sensitive close in the two categories of subjects (mean 43.15 nM / ml in non-smokers and respectively 41.12 nM / ml in smoking subjects) after which the evolution is similar, registering almost double the amount of malon dialdehyde after 40 minutes after the end of the experimental effort 1 (82.94 nM / ml and respectively 94.76 nM / ml) so that at 40 minutes after the experimental effort 2 to decrease slightly below the initial values (34.11 nM / ml in non-smokers and 35.09

nM / ml in smoking subjects) (Table 31). This leads us to assume that exercise causes equally strong acceleration of lipid peroxidation in both groups of subjects, which demonstrated that during exercise hydrogen peroxide formed is not completely decomposed by catalase and peroxidase. This explains the increasing concentration Malon dialdehyde equally in all subjects studied.

Tabelul 31 –The evolution of malon dialdehyde concentration in experimental efforts (the mean ± confidence interval)

MDA(nM/ml)	EE1 – min 0	EE1 – min 40	EE2 – min 40
Non-smokers	43,15 ± 8,30	82,94 ± 24,87	34,11 ± 7,09
Smokers	41,12 ± 7,98	94,76 ± 24,45	35,09 ± 10,52

The result of the ANOVA test ($F(3,52) = 11.48, p < 0.0001$) between the data sets show that raised significant differences are established, their particularity is to be identified by the post hoc test.

Tukey HSD post hoc test application leads to signaling four statistically significant differences ($p < 0.05$). The first difference is reported between the non smokers before the first experimental effort and non-smokers after the first experimental effort ($p = 0.01$), the light aerobic exercise modifying the malon dialdehyde concentration in non-smokers. The second significant difference is established between non smokers before the first experimental effort and smokers after the first experimental effort ($p = 0.0006$), smoking and light aerobic exercise significantly changing the malon dialdehyde concentration. The third significant difference ($p = 0.0015$) was established between the the values measured in the non smoking group after the first experimental effort and those from smokers after the experimental effort that was preceded by the administration of vitamin C. Between the latter set of data and the values determined in the samples collected from the same group (smokers) after the first experimental effort is established the fourth statistically significant difference ($p < 0.0001$).

It is confirmed that by submitting a light aerobic exercise (50% VO₂max) for 40 minutes the lipid peroxidation is increasing. The same thing happens when you add the smoking factor. However by administering vitamin C in smokers, the lipid peroxidation is significantly reduced (Figure 20) compared with the level evaluated after making the experimental effort proposed in the case of this research.

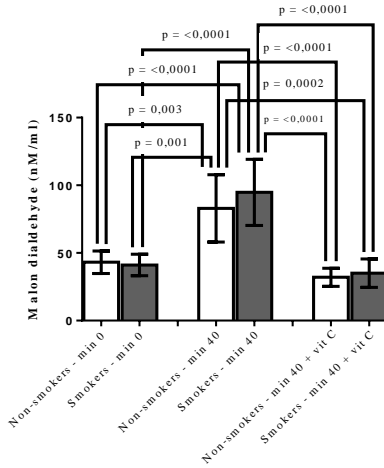


Figura 20 – The malon dialdehyde concentration values dynamics within the experimental efforts (the values represent the mean \pm 95% confidence interval)

4.2.5. The influence of aerobic exercise on blood sugar in untrained persons

Initially, the blood sugar registered average values of 113.79 ± 11.42 mg% in the non-smoking group, respectively 108.92 ± 8.13 mg% in the group of smokers. After submitting the first experimental effort, the blood sugar decreased significantly in both groups of the research which demonstrates an acceleration of degradation of glucose through anaerobic glycolysis in order to form energy (ATP) required for muscle contraction. The same thing also happened after the second experimental effort, only at the group of smokers, in relation to basal values which means that at this category of subjects, smoking induces a slowdown of glucose homeostasis installation. Interesting is that the blood sugar decreases statistically insignificant after the experimental effort preceded by the administration of vitamin C probably due to the antioxidant protective effect of ascorbic acid.

Tabelul 35 – The evolution of glucose in the experimental efforts (the mean \pm confidence interval)

Blood sugar (mg%)	EE1 – min 0	EE1 – min 40	EE2 – min 40
Non-smokers	$113,79 \pm 11,42$	$87,83 \pm 7,82$	$88,99 \pm 8,24$
Smokers	$108,92 \pm 8,13$	$74,64 \pm 7,50$	$84,20 \pm 10,00$

Brown-Forsythe and Bartlett test results are negative, which indicates the the need to apply a non-parametric test. The result of the ANOVA test ($F(5, 78) =$

13.28, $p < 0.0001$) showed statistically significant differences between all data series, so that a specific post hoc test (Dunn) will be applied, to see where it occurs. The Kruskal-Wallis test application generates an approximate value of $p (< 0.0001)$ which leads to the conclusion that the glucose level in blood is statistically influenced by the factors put into question (effort, smoking and effort, effort, smoking and administration to the vitamin C). This demonstrates that the blood sugar changes are irregular (biochemical individuality) and statistically significant.

Dunn post hoc test results highlighted five statistically significant differences ($p < 0.05$) between the data sets analyzed. The first difference ($p = 0.03$) is established between blood sugar levels in non-smokers after the first experimental effort, and those determined in the same group after the second the experimental effort. The second significant difference ($p < 0.0001$) is established between the basal values of the non-smoking group and those of smokers after the first experimental effort. The third difference calculated ($p = 0.03$) occurs between basal values of non-smokers blood sugar and smokers glucose level after the experimental effort preceded by administration of vitamin C. The last two differences ($p < 0.0001$, $p = 0.01$) is found between the basal values of the smoking group and those determined after sustaining the two experimental efforts.

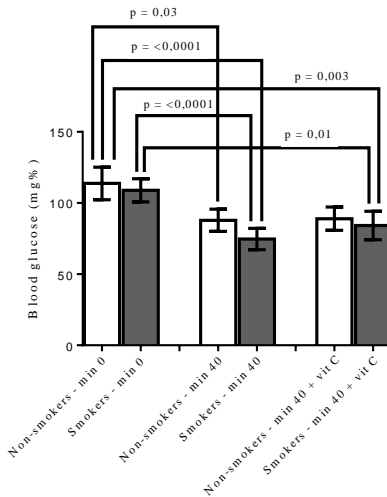


Figure 21 – Dynamics of blood glucose values in experimental efforts (values represent the mean \pm 95% confidence interval)

Figure 21 graphically highlights the differences determined from tests of significance applied to data sets traced to blood sugar levels.

The blood sugar of both groups is statistically significant modified, downwards after the first experimental effort. In this case, only the blood sugar of the smoking group manifests significant differences from baseline values of both experimental groups, while that of non-smokers decreased significantly from baseline values of same group, the difference from baseline value of smokers not being significant.

It seems that vitamin C administered before a light aerobic exercise affects the glucose level in blood, in relation to the basal values of smokers and non-smokers, in smokers only, the non-smoking group values, at the same moment, being slightly modified compared to the same reference values.

4.2.6. The influence of aerobic exercise on the specific activity of lactate dehydrogenase in untrained persons

Lactate dehydrogenase is involved in the conversion of pyruvic acid into lactic acid.

Tabelul 40 – The lactate dehydrogenase evolution in the experimental efforts (the mean ± confidence interval)

Lactate dehydrogenase (U/l)	EE1 – min 0	EE1 – min 40	EE2 – min 40
Non-smokers	165,9 ± 33,58	203,8 ± 24,97	237,05 ± 28,20
Smokers	155,80 ± 28,46	209,38 ± 25,58	255,60 ± 29,57

The specific activity of lactate dehydrogenase shows statistically insignificant differences between the two research groups for each moment of biochemical evaluation. In basal conditions, the lactate dehydrogenase activity reached 165.9 ± 33.58 U / l in the non-smoking group, respectively 155.80 ± 28.46 U / l in the smoking group (Table 40). The experimental effort determined a slight increase of enzyme activity while the vitamin C pre-administration and experimental efforts have statistically significant altered its specific activity, in relation to basal values. This means that ascorbic acid causes an acceleration of the conversion of lactate into pyruvate, followed by its degradation through oxidative decarboxylation, the Krebs cycle, etc.

The result of the ANOVA test (F (5, 78) = 8.66, p <0.0001) showed statistically significant differences between all data series.

Dunn post hoc test results highlights four significant differences (p <0.05) between the data sets analyzed. The first two differences are established between lactate dehydrogenase activity in non-smokers under basal conditions and that registered in the same group after the second experimental effort (p = 0.02) respectively the smoking group at the end of the same type of effort (p = 0.001). The next two statistically significant differences were observed between the

smoking group values under basal conditions and that recorded at the non-smoking group after the second experimental effort ($p = 0.003$) respectively the smoking group at the end of the same type of effort ($p = 0.0001$).

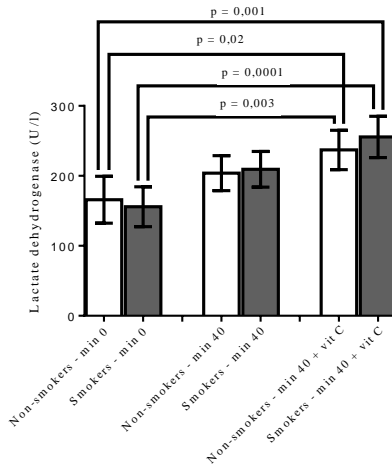


Figura 22 – The dynamics of lactate dehydrogenase specific activity in experimental efforts (the values represents the mean \pm 95% confidence interval)

Lactate dehydrogenase enhances its specific activity after any type of effort that has as energy substrate the glucose. The enzyme activity changes significantly only as a result of pre-administration of vitamin C, together with the experimental effort, in relation to baseline values of the two groups (Figure 22). Each group significantly increases the specific activity of lactate dehydrogenase, both to its own baseline values as well as the basal ones to the other. Thus the administration of vitamin C before a light aerobic exercise affects the specific activity of lactate dehydrogenase, in relation to basal values of smokers and non-smokers.

Following a 4-week training program that consisted of pedaling on the cycle ergometer for 2 hours a day, six days a week, lactate dehydrogenase showed a significant decrease in its activity in eight male subjects [Messonnier et al, 2006].

4.2.7. The influence of aerobic exercise on the concentration of creatinine in untrained persons

Through the removal of inorganic phosphate from the creatine phosphate structure and the cyclization of the remaining structure the creatinine is formed.

Tabelul 45 – The evolution of creatinine concentration within the experimental efforts (the mean \pm confidence interval)

Creatinine (mg%)	EE1 – min 0	EE1 – min 40	EE2 – min 40
Non-smokers	1,02 \pm 0,09	1,24 \pm 0,11	1,17 \pm 0,13
Smokers	1,08 \pm 0,13	1,21 \pm 0,14	1,16 \pm 0,18

The basal conditions of the two groups have recorded, for the creatinine concentration values of 1.02 ± 0.09 mg% in non-smoking group, respectively 1.08 ± 0.13 mg% in the smoking group (Table 45). After submitting the first experimental effort, the creatinine concentration increased statistically insignificant in both groups of research. The same thing happened after the second experimental effort, but with a lower amplitude. The result of the ANOVA test ($F(5,78) = 1.74, p = 0.13$) showed a statistically insignificant difference between the data sets analyzed. The Kruskal-Wallis test application generates an approximate value of $p (= 0.09)$ indicating a lack of influence of factors put into question (effort, smoking and effort, effort, smoking and administration of vitamin C) on the concentration of creatinine (Figure 23).

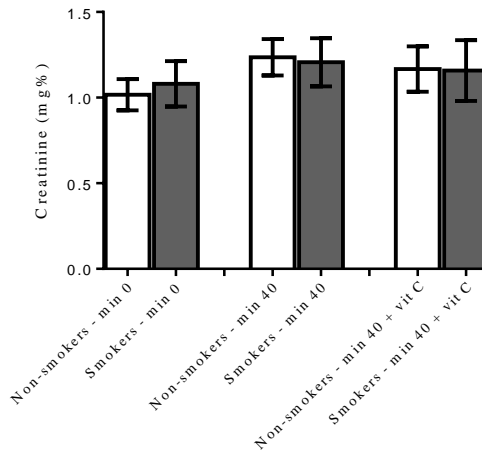


Figura 23 – The creatinine concentration dynamics within the experimental efforts (the values represent the mean \pm 95% confidence interval)

Conclusions and perspectives

The motric activities field is one that interacts with that of molecular biology because all physical actions undertaken by the body of leisure physical activity practitioners and athletes of high performance are conditioned by its biological status.

The specialized literature contains a lot of data concerning biochemical and physiological changes that occur in the human body in the case performance athletes that intense physical activity is a daily constant. However, there are fewer the researches that had as objective the study of these changes in untrained individuals, for whom the physical effort is outside the scope of daily activities, given the lack of time for such an occupation.

The experimental results obtained in our research regarding the biochemical and physiological parameters change as a result of motric activities performed by smoking and non-smoking subjects with a sedentary lifestyle allow us to formulate the following conclusions.

1. In terms of body composition, the groups of this study do not differ statistically significant, but better results are observed in the non-smoking group, which reflects the higher levels of physical activity declared at the beging of the evaluations.

2. Without recording a statistically significant difference in height or thoracic elasticity, the research groups have similar values in terms of the respiratory system functioning.

3. The evaluated circulatory parameters (blood pressure and heart rate) in various stages of the research are not statistical different between the two groups. The heart rate recorded in repose indicates for both groups the untrained person status.

4. From the physiological point of view, the subjects belonging to the two groups in the study are behaving differently in terms of the maximum oxygen consumption and systolic blood pressure at the start of effort. The maximum oxygen consumption is higher in non-smokers compared to smokers, due to active and regular inhalation of cigarette smoke by the latter. This means that smoking can be a factor that led to some differences (not statistically significant) between smokers and nonsmokers in all the assessments demonstrated by the difference found in maximum oxygen consumption. It is interesting that maximal aerobic power has not registered statistically significant differences, although it is related to the maximum oxygen consumption.

5. The anaerobic capacity of the two groups has similar values. The greater difference in particular is determined for the anaerobic lactacid effort capacity, which has connections with the body oxygen rezerves.

6. During the experimental efforts, the circulatory parameters had normal values, undifferentiated for the research batches. The heart rate and blood pressure have undergone increases, which are physiologically normal and indicating the expected normality.

7. The somatic parameters and effort capacity assessed in the groups of this study presents two statistically significant differences in the systolic blood pressure and the maximum oxygen consumption. The first difference occurred, probably, as a result of state of agitation, the subjects not being used to such an evaluation. The second difference is caused, definitely, by smoking, being already demonstrated that this habit leads to a decrease of aerobic effort capacity.

8. Superoxide dismutase specific activity is lower under basal conditions in smoking subjects, probably due to the continuous tension undergone by the their body through inhalation of cigarette smoke, but has no significant differences between the two groups. After the first experimental effort the specific activity further increases in smokers, which confirms the higher resistance to oxidative stress of those with greater aerobic effort capacity. Similar to changes registered in athletes, superoxide dismutase activity dynamics is not visible in statistical terms.

9. Between the level of superoxide dismutase activity in the non-smoking group after the first experimental effort and the group of smokers after the second experimental effort, a statistically significant difference does not register, the corresponding values of the two moments are close. Thus, the vitamin C administered to smokers helps the superoxide dismutase in the antioxidant activity, keeping them close to the level of non-smokers.

10. The glutathione peroxidase does not have statistically different values between research groups for each moment of its evaluation. Vitamin C leads to significant intensification of its activity after the second experimental effort, thus contributing to the strengthening of the second antioxidant line for both groups.

11. The comparative results for the testing of the second hypothesis (non smoking group after the first experimental effort and smoking group after the second experimental effort) show a statistically significant difference to increase specific glutathione peroxidase activity in the group of smokers after exercise before which administered vitamin C.

12. The catalase modifies its specific activity statistically significantly only at the non smoking group. The vitamin C and the experimental aerobic exercise to which the subjects were subjected did not have influence at the catalase action level for smokers. It is possible that the hydroxyl radical production to have been slowed by the vitamin C, through preventive effect.

13. The smoking group after the second experimental effort registered significantly lower values at the specific activity level of catalase, compared to the non-smokers group after the first experimental effort, resulting that the administered vitamin led to a reduction of enzymatic activity monitored. Just like in the case of glutathione peroxidase, vitamin C was able to facilitate the antioxidant fight of catalase, if the reporting is done to smokers who did not benefit from additional non-enzymatic antioxidant for the same exercise conditions.

14. The lipid peroxidation significantly increases following the experimental exertion, as evidenced by the malon dialdehyde concentration recorded for both groups of the study. The light aerobic physical effort carried for 40 minutes leads to significantly increase of lipid degradation in cells, while vitamin C removes this process, managing to bring the phenomenon at quotas close to basal value.

15. In accordance with the changes of catalase and glutathione peroxidase in the two research groups, the malon dialdehyde concentration is reduced by the administration of vitamin C to smokers after the experimental effort, under the level of that determined at nonsmokers undergoing exercise without non-enzymatic antioxidant administration.

16. A direct comparison between the results of this research and those in the literature is difficult to achieve, on the one hand because the methods for determining the biochemical parameters followed are different and secondly the data in the literature refers mainly to performance athletes. In the analyzed subjects the enzyme activity dynamics and the malon dialdehyde concentration is different in the sense that the changes are more pronounced in those that are untrained. This confirms our first hypothesis.

17. The vitamin C, through its powerful antioxidant effects, manages to optimize the enzymes activity (GPX, CAT) involved in fighting the oxidative stress and reduces the destructive effect of the presence of free radicals in the body - lipid peroxidation, at a general level. In concrete terms the smokers are helped through the pre administration of vitamin C to reduce the oxidative stress level under that manifested by non-smokers who did not take antioxidant supplements and are less exposed to continuous oxidative stress by avoiding direct contact with fumes from burning tobacco. Thus the second hypothesis made is confirmed.

Our research can be continued by studying the biochemical and physiological changes manifested in untrained subjects in other types of efforts (maximum aerobic, anaerobic, etc.) dosed at different intensities. We can also study female subjects, the dynamics and evolution of biochemical parameters being more sensitive in interpretation because the changes in the female body are

much more complex. The research is needed for public awareness regarding the risks to which it is involuntarily exposed.

Selective bibliography

1. Adlam V.J., Harrison J.C., Porteous C.M., James A.M., Smith R.A., Murphy M.P. and Sammut I.A. (2005) „Targeting an antioxidant to mitochondria decreases cardiac ischemia–reperfusion injury”. *FASEB J.* 19: 1088-1095.
3. Alessio H.M., Goldfarb A.H. and Cutler R.G. (1988) „MDA content increases in fast- and slow-twitch skeletal muscle with intensity of exercise in a rat”. *Am J Physiol Cell Physiol.* 255: 874-877.
8. Apostol I. (1998) *Ergofiziologie: curs*. Iași: Editura Universității „Alexandru Ioan Cuza” din Iași. pp. 11-14; 25-26; 149-157; 212-238.
10. Ardelean G. (2001) *Biochimia și energetica contracției musculare*. Satu Mare: Editura Bion. pp. 11-16.
17. Bedogni G., Brambilla P., Bellentani S. and Tiribelli C. (2006) „The Assessment of Body Composition in Health and Disease”. *Journal of human ecology.* 14: 21-25.
18. Bergman B.C. and Brooks G.A. (1999) „Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men”. *J Appl Physiol.* 86: 479-487.
23. Bompa T. (2002) *Periodizarea: teoria și metodologia antrenamentului*. București: Editura EX PONTO, C. N. F. P. A. p. 65-75; 364-367.
29. Brown S.E., Ross M.F., Sanjuan-Pla A., Manas A.R., Smith R.A., Murphy M.P. (2007) „Targeting lipoic acid to mitochondria: synthesis and characterization of a triphenylphosphonium-conjugated α -lipoyl derivative”. *Free Radic. Biol. Med.* 42: 1766-1780.
34. Carr A. and Frei B. (1999) „Does vitamin C act as a pro-oxidant under physiological conditions?”. *FASEB J.* 13: 1007-1024.
37. Chandrashekar Y. and Anand IS. (1991) Exercise as a coronary protective factor. *Am Heart J.* 122:1723-1739.
43. Cojocaru D.C., Ciornea E. și Cojocaru S. I. (2010) *Biochimia vitaminelor și a hormonilor*. București: Editura Academiei Române. pp. 296-314.
45. Cokkinides V., Bandi P., McMahon C., Jemal A., Glynn T., Ward E. (2009) „Tobacco control in the United States–recent progress and opportunities”. *CA Cancer J Clin.* 59: 352-365.
46. Coyle E.F., Jeukendrup A.E., Oseto M.C., Hodgkinson B.J. and Zderic T.W. (2001) „Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise”. *Am J Physiol Endocrinol Metab.* 280: 391-398.
48. Criswell D., Powers S., Dodd S., Lawler J., Edwards W., Renshler K. and Grinton, S. (1993) „High intensity training-induced changes in skeletal muscle antioxidant enzyme activity”. *Med Sci Sports Exerc.* 25: 1135-1140.
55. Dumitru I.F. (1980) *Biochimie*. București: Editura Didactică și Pedagogică. p. 21-232; 267-892.
61. Ferucci L., Izmirlian G., Leveille S., et al. (1999) „Smoking, physical activity and active life expectancy”. *Am. J. Epidemiol.* 149: 645-653.
62. Filipaș I. și Ardelean G. (2002) *Biochimia efortului*. Satu Mare: Editura Bion. p. 16-44; 153.

65. Friedlander A.L., Casazza G.A., Horning M.A., Buddinger T.F. and Brooks G.A. (1998) „Effects of exercise intensity and training on lipid metabolism in young women”. *Am J Physiol Endocrinol Metab.* 275: 853-863.
70. Genç A., Üçok K., Şener U., Koyuncu T., Akar O., Çelik S., Ünlü M. (2014) „Association analyses of oxidative stress, aerobic capacity, daily physical activity, and body composition parameters in patients with mild to moderate COPD”. *Turk J Med Sci.* 44: 1308-1315.
71. Giovino G.A. (2007) „The tobacco epidemic in the United States”. *Am J Prev Med.* 33(6): 318-326.
72. Girotti A.W. (1985) „Mechanisms of lipid peroxidation”. *J Free Radic BiolMed.* 1: 87-95.
73. Gomes E.C., Stone V. and Florida-James G. (2011) „Impact of heat and pollution on oxidative stress and CC16 secretion after 8 km run”. *European Journal of Applied Physiology.* 111(9): 2089-2097.
74. Gomez-Cabrera M.C., Borrás C., Pallardo F.V., Sastre J., Ji L.L. and Vina J. (2005) „Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats”. *The Journal of Physiology.* 567: 113-120.
75. Gomez-Cabrera M.C., Domenech E. and Vina J. (2008) „Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training”. *Free Radical Biology & Medicine.* 44(2): 126-131.
79. Halliwell B. and Gutteridge J. (2007) *Free Radicals in Biology and Medicine.* Oxford, UK: Oxford Univ. Press.
80. Häulică I. (2009) *Fiziologie umană.* Ediția a III-a. București: Editura Medicală. pp. 243-368; 385-433; 1014-1021.
81. Hellsten Y., Apple F.S. and Sjodin B. (1996) „Effect of sprint cycle training on activities of antioxidant enzymes in human skeletal muscle”. *J Appl Physiol.* 81: 1484-1487.
83. Higuchi M., Cartier L.J., Chen M. and Holloszy J.O. (1985) „Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise”. *J Gerontol.* 40: 281-286.
86. Honceriu C., Popescu L. and Trofin P.F. (2012) „Comparative study regarding the developing method of the football players' aerobic capacity and power”. *Gymnasium: Scientific Journal of Education, Sports & Health.* 13(1): 162-165.
87. Horowitz J.F., Mora-Rodriguez R., Byerley L.O. and Coyle E.F. (1997) „Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise”. *Am J Physiol Endocrinol Metab.* 273: 768-775.
88. Hudson M.B., Hosick P.A., McCaulley G.O., Schrieber L., Wrieden J., McAnulty S.R. and Quindry J.C. (2008) „The effect of resistance exercise on humeral markers of oxidative stress”. *Medicine and Science in Sports and Exercise.* 40(3): 542-548.
90. Ionescu C. și Lucaciu R. (2006) *Căi fundamentale în metabolismul uman.* Cluj – Napoca: Editura Medicală Universitară „Iuliu Hațieganu”. pp. 11; 137-180.
92. Jeukendrup A.E., Mensink M., Saris W.H. and Wagenmakers A.J. (1997) „Exogenous glucose oxidation during exercise in endurance-trained and untrained subjects”. *J Appl Physiol.* 82: 835-840.
94. Ji L.L., Stratman F.W. and Lardy H.A. (1988) „Antioxidant enzyme systems in rat liver and skeletal muscle. Influences of selenium deficiency, chronic training, acute exercise”. *Arch Biochem Biophys.* 263: 150-160.

100. Kanter M.M. (1994) „Free radicals, exercise, antioxidant supplementation”, *Int J Sport Nutr.* 4: 205-220.
101. Kaplan G.A., Strawbridge W.J., Cohen R.D. and Hungerford L. R. (1996) „Natural history of leisure-time physical activity and its correlates: associations with mortality from all causes and cardiovascular disease over 28 years”. *Am. J. Epidemiol.* 144: 793-797.
102. Karanth J., Kumar R. and Jeevaratnam K. (2004) „Response of antioxidant system in rats to dietary fat and physical activity”. *Indian J Physiol Pharmacol.* 48: 446-452.
109. Kujala U.M., Kaprio J., Sarna S. and Koskenvuo M. (1998). „Relationship of leisure-time physical activity and mortality: the Finnish twin cohort”. *JAMA.* 279: 440-444.
111. Laitano O., Kalsi K.K., Pook M., Oliveira A.R. and Gonzalez-Alonso J. (2010) „Separate and combined effects of heat stress and exercise on circulatory markers of oxidative stress in euhydrated humans”. *European Journal of Applied Physiology.* 110(5): 953-960.
112. Lambertucci R.H., Levada-Pires A.C., Rossoni L.V., Curi R. and Pithon-Curi T.C. (2007) „Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats”. *Mech Ageing Dev.* 128: 267-275.
114. Laughlin M.H., Simpson T., Sexton W.L., Brown R., Smith J.K. and Korthuis J.R. (1990) „Skeletal muscle oxidative capacity, antioxidant enzymes, and exercise training”. *J. Appl. Physiol.* 68 (6): 2337-2343.
115. Lawler J.M., Kwak H.B., Song W., Parker J.L. (2006) „Exercise training reverses downregulation of HSP70 and antioxidant enzymes in porcine skeletal muscle after chronic coronary artery occlusion”. *Am J Physiol Regul Integr Comp Physiol.* 291: 1756-1763.
116. Lawler J.M., Holloszy J.O. and Coyle E.F. (1984) „Adaptations of skeletal muscle to endurance exercise and their metabolic consequences”. *J Appl Physiol.* 56: 831-838.
119. Leeuwenburgh C., Fiebig R., Chandwaney R., Ji L.L. (1994) „Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems”. *Am J Physiol Regul Integr Comp Physiol.* 267: 439-445.
120. Leeuwenburgh C., Hollander J., Leichtweis S., Griffiths M., Gore M. and Ji L.L. (1997) „Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific”. *Am J Physiol Regul Integr Comp Physiol.* 272: 363-369.
123. Lupea A.X. și Ardelean A. (1997) *Biochimia efortului*. Arad: Editura Universității de Vest „Vasile Goldiș”. pp. 1-6; 35-38; 92; 171-185.
126. Martinez-Gonzalez M.A., Varo J.J., Santos J.L., De Irala J., Gibney M., Kearney J. and Martinez J. A. (2001) „Prevalence of physical activity during leisure time in the European Union”. *Med. Sci. Sports Exerc.* 33(7): 1142-1146.
129. Mărușteri M.Ș. (2006) *Noțiuni fundamentale de biostatistică: note de curs*. Târgu – Mureș: Editura University Press.
130. McAnulty S.R., Hosick P.A., McAnulty L.S., Quindry J.C., Still L., Hudson M.B., Dibarnardi A.N., Milne G.L., Morrow J.D. and Austin M.D. (2007) „Effect of pharmacological lowering of plasma urate on exercise-induced oxidative stress”. *Appl. Physiol. Nutr. Metab.* 32: 1148-1155.

131. McAnulty S.R., McAnulty L., Pascoe D.D., Gropper S.S., Keith R.E., Morrow J.D. and Gladden L.B. (2005) „Hyperthermia increases exercise-induced oxidative stress”. *International Journal of Sports Medicine*. 26(3): 188-192.
136. Messonnier L., Freund H., Denis C., Féasson L. and Lacour J.-R. (2006) „Effects of Training on Lactate Kinetics Parameters and their Influence on Short High-Intensity Exercise Performance”. *Int J Sports Med*. 27(1): 60-66.
141. Morris C.K. and Froelicher V.F. (1991) „Cardiovascular benefits of physical activity”. *Herz*. 16: 222-236.
143. Muaz B. and Hakki G. (2006) „Acute exercise induced oxidative stress and antioxidant changes”. *Eur J Gen Med*. 3(3): 126-131.
144. Mureșan A., Tache S. și Orășan R. (2006) *Stresul oxidativ în procesele fiziologice și patologice*. Cluj – Napoca: Editura Todesco. pp. 1-39.
145. Murphy M.P. and Smith R.A. (2007) „Targeting antioxidants to mitochondria by conjugation to lipophilic cations”. *Annu. Rev. Pharmacol. Toxicol*. 47: 629-656.
150. Oh-ishi S., Kizaki T., Nagasawa J., Izawa T., Komabayashi T., Nagata N., Suzuki K., Taniguchi N. and Ohno H. (1997) „Effects of endurance training on superoxide dismutase activity, content and mRNA expression in rat muscle”. *Clin Exp Pharmacol Physiol*. 24: 326-332.
152. Packer J.E., Slater T.F. and Willson R.L. (1979) „Direct observation of a free radical interaction between vitamin E and vitamin C”. *Nature*. 278: 737-738.
155. Paffenbarger R.S.Jr, Hyde R.T., Wing A.L. and Hsieh C.C. (1986) „Physical activity, all-cause mortality, and longevity of college alumni”. *N Engl J Med*. 314: 605-613.
161. Powers S.K. and Jackson M.J. (2008) „Exercise-Induced Oxidative Stress: cellular mechanisms and Impact on Muscle Force Production”. *Physiol Rev*. 88: 1243-1276.
162. Powers S.K., Criswell D., Lawler J., Ji L.L., Martin D., Herb R.A. and Dudley G. (1994) „Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle”. *Am J Physiol Regul Integr Comp Physiol*. 266: 375-380.
163. Powers S.K., Criswell D., Lawler J., Martin D., Ji L.L., Herb R.A. and Dudley G. (1994) „Regional training-induced alterations in diaphragmatic oxidative and antioxidant enzymes”. *Respir Physiol*. 95: 227-237.
165. Powers S.K., Nelson W.B., Hudson M.B. (2011) „Exercise-induced oxidative stress in humans: Cause and consequences”. *Free Radical Biology & Medicine*. 51: 942-950.
166. Powers S.K., Lawler J., Criswell D., Lieu F.K. and Martin D. (1992) „Aging and respiratory muscle metabolic plasticity: effects of endurance training”. *Journal of Applied Physiology*. 72: 1068-1073.
167. Predescu C. (2009) *Fiziologia sistemelor funcționale vegetative*. București: Editura Moroșan. pp. 53-67.
169. Pui A. (2008) *Chimia oxigenului*. Iași: Editura Tehnopress. pp. 119-130.
171. Quindry J.C., McAnulty S.R., Hudson M.B., Hosick P., Dumke C., McAnulty L.S., Henson D., Morrow J.D. and Nieman D. (2008) „Oral quercetin supplementation and blood oxidative capacity in response to ultramarathon competition”. *Int. J. Sport Nutr. Exerc. Metab*. 18: 601-616.
173. Quindry J.C., Stone W.L., King J. and Broeder C.E. (2003) „The effects of acute exercise on neutrophils and plasma oxidative stress”. *Medicine and Science in Sports and Exercise*. 35(7): 1139-1145.

174. Quintanilha A.T. (1984) „Effects of physical exercise and/or vitamin E on tissue oxidative metabolism”. *Biochem Soc Trans.* 12: 403-404.
179. Ristow M., Zarse K., Oberbach A., Kloting N., Birringer M., Kiehnopf M. and Bluher M. (2009) „Antioxidants prevent health-promoting effects of physical exercise in humans”. *Proceedings of the National Academy of Sciences of the United States of America.* 106(21): 8665-8670.
180. Rockhill B., Willett W.C., Hunter D.J., Manson J.E., Hankinson S.E. and Colditz G.A. (1999) „A prospective study of recreational physical activity and breast cancer risk”. *Arch. Intern. Med.* 159: 2290-2296.
183. Romijn J.A., Coyle E.F., Sidossis L.S., Gastaldelli A., Horowitz J.F., Endert E. and Wolfe R.R. (1993) „Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration”. *Am J Physiol Endocrinol Metab.* 265: 380-391.
184. Romijn J.A., Coyle E.F., Sidossis L.S., Rosenblatt J. and Wolfe R.R. (2000) „Substrate metabolism during different exercise intensities in endurance-trained women”. *J Appl Physiol.* 88: 1707-1714.
186. Salo D.C., Donovan C.M. and Davies K.J. (1991) „HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise”. *Free Radical Biology & Medicine.* 11(3): 239-246.
190. Sen C.K., Marin E., Kretschmar M. and Hanninen O. (1992) „Skeletal muscle and liver glutathione homeostasis in response to training, exercise, immobilization”. *J Appl Physiol.* 73: 1265-1272.
192. Sidossis L.S., Gastaldelli A., Klein S. and Wolfe R.R. (1997) „Regulation of plasma fatty acid oxidation during low- and high-intensity exercise”. *Am J Physiol Endocrinol Metab.* 272: 1065-1070.
193. Sies H. (1985) *Oxidative Stress.* London: Academic.
194. Sies H. and Cadenas E. (1985) „Oxidative stress: damage to intact cells and organs”. *Philos Trans R Soc Lond B Biol Sci.* 311: 617-631.
197. Smith R.A., Kelso G.F., Blaikie F.H., Porteous C.M., Ledgerwood E.C., Hughes G., James A.M., Ross M.F., Asin-Cayuela J., Cocheme H.M., Filipovska A. and Murphy M.P. (2003) „Using mitochondria-targeted molecules to study mitochondrial radical production and its consequences”. *Biochem. Soc. Trans.* 31: 1295-1299.
198. Smith S.C.Jr., Blair S.N., Criqui M.H., Fletcher G.F., Fuster V., Gersh B.J., Gotto A.M., Gould K.L., Greenland P., Grundy S.M., Hill M.N., Hlatky M.A., Houston-Miller N., Krauss R.M., LaRosa J., Ockene I.S., Oparil S., Pearson T.A., Rappaport E., Starke R.D., and the Secondary Prevention Panel. (1995) „Preventing heart attack and death in patients with coronary disease”. *Circulation.* 92: 2-4.
207. Thompson D.L., Townsend K.M., Boughey R., Patterson K. and Bassett D.R.Jr. (1998) „Substrate use during and following moderate- and low-intensity exercise: implications for weight control”. *Eur J Appl Physiol.* 78: 43-49.
210. Trofin P.F. și Cojocaru D. (2012) „Impactul utilizării spirulinei asupra efortului fizic”. *Sesiune de comunicări științifice: Sportul _ de la concepte fundamentale la dimensiuni inovatoare în societatea cunoașterii, ediția a 4- a.* Iași. pp 28-32.
212. Trofin P.F., Chirazi M., Honceriu C., Drosescu P., Șerban I.L., Grădinaru G., Vorniceanu A., Cojocaru D., Ciobica A., Ciornea E. and Cojocaru S. (2014 – A) „Pre-administration of vitamin C reduces exercise-induced oxidative stress status in untrained

subjects". *Archives of Biological Sciences Belgr.* 66(3). Factor de impact pentru 2012 = 0,791.

217. U.S. Department Of Health And Human Services. (1996) „Physical activity and health: a report of the Surgeon General". *Atlanta, GA: U.S. Department of Health and Human Services, Centers for*

218. Urso M.L. and Clarkson P.M. (2003) „Oxidative stress, exercise, antioxidant supplementation". *Toxicology.* 189: 41-54.

219. Van Loon L.J., Jeukendrup A.E., Saris W.H. and Wagenmakers A.J. (1999) „Effect of training status on fuel selection during submaximal exercise with glucose ingestion". *J Appl Physiol.* 87: 1413-1420.

220. Văjială G.E. (2007) *Biochimia efortului.* Ediția a III-a. București: Editura Fundației România de Măine. pp. 32-113.

221. Venditti P. and Di Meo S. (1996) „Antioxidants, tissue damage, endurance in trained and untrained young male rats". *Arch Biochem Biophys.* 331: 63-68.

222. Venditti P. and Di Meo S. (1997) „Effect of training on antioxidant capacity, tissue damage, endurance of adult male rats". *Int J Sports Med.* 18: 497-502.

224. Vina J., Borras C., Gomez-Cabrera M.C. and Orr W.C. (2006) „Part of the series: From dietary antioxidants to regulators in cellular signalling and gene expression. Role of reactive oxygen species and (phyto) oestrogens in the modulation of adaptive response to stress". *Free Radical Research.* 40(2): 111-119.

225. Vina J., Gomez-Cabrera M.C., Lloret A., Marquez R., Minana J.B., Pallardo F.V. and Sastre J. (2000) „Free radicals in exhaustive physical exercise: Mechanism of production, and protection by antioxidants". *IUBMB Life.* 50(4-5): 271-277.

226. Vincent H.K., Powers S.K., Demirel H.A., Coombes J.S. and Naito H. (1999) „Exercise training protects against contraction-induced lipid peroxidation in the diaphragm". *Eur J Appl Physiol Occup Physiol.* 79: 268-273.

227. Vincent H.K., Powers S.K., Stewart D.J., Demirel H.A., Shanely R.A. and Naito H. (2000) „Short-term exercise training improves diaphragm antioxidant capacity and endurance". *Eur J Appl Physiol.* 81: 67-74.

228. Wei M., Kampert J.B., Barlow C.E., et al. (1999) „Relationship between low cardiorespiratory fitness and mortality in normal weight, overweight and obese men". *JAMA.* 282: 1547-1553.

230. Weltan S.M., Bosch A.N., Dennis S.C. and Noakes T.D. (1998) „Influence of muscle glycogen content on metabolic regulation". *Am J Physiol Endocrinol Metab.* 274: 72-82.

231. Weltan S.M., Bosch A.N., Dennis S.C. and Noakes T.D. (1998) „Preexercise muscle glycogen content affects metabolism during exercise despite maintenance of hyperglycemia". *Am J Physiol Endocrinol Metab.* 274: 83-88.

232. Wenger N.K., Froelicher E.S., Smith L.K., Ades P.A., Berra K., Blumenthal J.A., Certo C.M., Dattilo A.M., Davis D., DeBusk R.F., et al. (1995) „Cardiac Rehabilitation as Secondary Prevention. Clinical Practice Guideline". *Clin Pract Guidel Quick Ref Guide Clin.* 17: 1-23.

233. White W.B. (2007) „Smoking-related morbidity and mortality in the cardiovascular setting". *Prev Cardiol.* 10(2): 1-4.

238. Zhao K., Zhao G.M., Wu D., Soong Y., Birk A.V., Schiller P.W. and Szeto H.H. (2004) „Cell-permeable peptide antioxidants targeted to inner mitochondrial

membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury". *J. Biol. Chem.* 279: 34682-34690.

Own scientific papers from the thesis theme published during the doctoral internship

A. Scientific papers published in ISI indexed journals with impact factor

1. **Trofin P.F.**, Chirazi M., Honceriu C., Drosescu P., Șerban I.L., Grădinaru G., Vorniceanu A., Cojocaru D., Ciobica A., Ciornea E. and Cojocaru S. (2014 – A) „Pre-administration of vitamin C reduces exercise-induced oxidative stress status in untrained subjects”. *Archives of Biological Sciences Belgr.* 66(3). **FI = 0,791**. Acceptat spre publicare (nr. 070/13).

2. **Trofin P.F.**, Ciobica A., Cojocaru D., Chirazi M., Honceriu C., Trofin L., Șerban I.L., Cojocaru S., Aton E. (2014 – B) „Increased oxidative stress in rat after five minutes treadmill exercise”. *Central European Journal of Medicine.* 9(5): 722-728. **FI = 0,262**.

B. Scientific papers published in journals acknowledged by CNCISIS

1. **Trofin P.F.**, Honceriu C., Ciobica A. and Cojocaru D. (2014 – C) „The Influence of Vitamin C on the Oxidative Stress in Untrained Smoking Subjects”. *Modeling and Optimization of the Aerospace, Robotics, Mechatronics, Machines-Tools, Mechanical Engineering and Human Motricity Fields - OPTIROB 2014*. Mangalia, pp. 713-722.

2. **Trofin P.F.**, Chirazi M., Honceriu C. and Cojocaru D. (2013 – A) „Study regarding the validation of an assessment protocol of VO₂max on cycle ergometer”. *Analele Științifice ale Universității „Alexandru Ioan Cuza” din Iași, s. Biologie animală, Tom LIX:* 139-146.

3. **Trofin P.F.**, Abalașei B., Drosescu P. and Cojocaru D. (2013 – B) „Study on the correlation between self-esteem, physical capacity of effort and the somatometric parameters”. *Sp Soc Int J Ph Ed Sp.* 14(2): 170-178.

4. **Trofin P.F.**, Honceriu C. and Cojocaru D. (2013 – C) „Comparative study on the assessment of VO₂max by ergospirometrie or field test”. *Sp Soc Int J Ph Ed Sp.* 14(2): 111-124.

5. **Trofin P.F.** și Cojocaru D. (2012) „Impactul utilizării spirulinei asupra efortului fizic”. *Sesiune de comunicări științifice: Sportul _ de la concepte fundamentale la dimensiuni inovatoare în societatea cunoașterii, ediția a 4- a.* Iași. pp 28-32.

6. Honceriu C., Popescu L. and **Trofin P.F.** (2012) „Comparative study regarding the developing method of the football players' aerobian capacity and power”. *Gymnasium: Scientific Journal of Education, Sports & Health.* 13(1): 162-165.

Other papers published

1. **Trofin P.F.** și Honceriu C. (2011) „Preferințele sportive ale copiilor cu vârste între 5 și 17 ani”. *Sesiune de comunicări științifice: sportul – de la concepte fundamentale la dimensiuni inovatoare în societatea cunoașterii.* Iași: Editura StudIS. ISBN 978-606-8242-85-9.

2. **Trofin P.F.** și Abălașei B. (2011) „Valențe cathartice ale activităților fizice”. *VoxStud.* Bacău: Editura Alma Mater. nr. 1. ISSN 2068-455X.

3. **Trofin P.F.** and Abălașei B. (2011) „Study on potential aggressive tension discharge of amateur athletes”. *Scientia movens.* Praga: Editura Univerzita Karlova v Praze. ISBN 978-80-86317-84-7.

4. **Trofin P.F.** și Cojocariu A. (2010) „Analiza statistică a eficacității luptătorilor din cadrul turneului K1 World Grand Prix, în perioada 2007 – 2009”. *VoxStud.* Bacău: Editura Alma Mater. nr. 1. ISSN 2068-455X.

5. **Trofin P.F.** și Cojocariu A. (2010) „Dezvoltarea rezistenței prin mijloacele artelor marțiale în ciclul gimnazial”. *VoxStud.* Bacău: Editura Alma Mater. nr. 1. ISSN 2068-455X.

6. **Trofin P.F.**, Chirazi M. și Honceriu C. (2009) „Dinamica relațiilor socio – afective în cadrul Liceului cu program sportiv din Iași”. *Sport și societate.* Iași: Editura Universității „Alexandru Ioan Cuza”. nr. 2. ISSN 1582-2168.

7. **Trofin P.F.**, Chirazi M. și Honceriu C. (2009) „Influența practicării sportului asupra relațiilor sociale”. *Revista Sesiune de comunicări științifice: dimensiuni ale educației sportive.* Iași: Editura AS'S. ISBN 973-7846-05-2.