

TO A SCIENTIFIC JURY,
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S T A T E M E N T

from

professor Julia Nikolova, DM, PhD

Department of Physiology, MF, MU-Plovdiv

of a dissertation for awarding the educational and scientific degree "Doctor"

Professional field: Medicine,

Doctoral program: Human Physiology.

Author: **Veselin Atanasov Vassilev, MD.**

Form of doctoral study: doctoral student of independent training.

Department: Department of Physiology at the Faculty of Medicine,
Medical University - Plovdiv.

Topic: **"Effect of selective androgen receptor modulators (SARM) on
physical work capacity and some side effects in an experimental
model"**

Scientific supervisor: **Prof. Nikolay Petrov Boyadzhiev, DM, PhD,**

Department of Physiology of the Faculty of Medicine at
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The set of documents presented by Dr Veselin Atanasov Vassilev is in accordance with the requirements specified in the Regulations for the implementation of the LDASRB and Article 67 of the Regulations for the acquisition of the ESD "Doctor" at the Medical University - Plovdiv.

Dr. Veselin Atanasov Vassilev was born in the city of Plovdiv on June 4, 1993. He completed his secondary education in 2012 at the Plovdiv Language High School, profile "Foreign Language". Acquired the qualification "Master in Medicine" in 2018 at the Medical University - Plovdiv. After successfully passing the competition, from January 22, 2019, Dr. Vassilev is an assistant at the Department of Physiology at the Faculty of Medicine, MU - Plovdiv. From 15.02.2023, Dr. Vasilev is enrolled as a doctoral student of independent training at the Department of Physiology at the MU-Plovdiv Medical University. After successfully passing the exam, from 01.01.2023 he has acquired a specialty in Human Physiology. Dr. Vassilev is a member of the Bulgarian Society of Physiological Sciences and the Bulgarian Medical Union, Regional Medical Union - Plovdiv. He is fluent in English (level C1) and has computer skills in MS Office: Word, Excel, Power Point SPSS.

The dissertation work and the submitted abstract contain structurally the necessary elements specified in the regulations of MU-Plovdiv, arranged in the generally accepted sequence. The dissertation is written on 174 pages, well illustrated with 53 tables and 90 figures.

The **topic** chosen by Dr. Veselin Vasilev is currently actual and the reason for this is the following facts:

- The non-steroidal group of selective androgen receptor modulators (SARMs) has pronounced anabolic and less pronounced androgenic effects;
- There is a growing trend for the use of non-steroidal selective androgen receptor modulators (SARMs) not only by training individuals for health but also by professional athletes;

- There is little information on the effects of the non-steroidal group of selective androgen receptor modulators (SARMs) during exercise;
- There is little information on the side effects of the non-steroidal group of selective androgen receptor modulators (SARMs) when used for a long period of time;
- There is growing clinical interest in the non-steroidal group of selective androgen receptor modulators (SARMs) due to the possibility of their application in the treatment of socially significant diseases such as hypogonadism, osteoporosis, benign prostatic hypertrophy, cachexia with different etiopathogenesis, breast carcinoma, amyotrophic lateral sclerosis.

Why did Dr. Vasilev directed his scientific pursuits to the non-steroidal group of selective androgen receptor modulators (SARMs) - with their pronounced anabolic action, they have an advantage over anabolic androgenic steroids and it is possible a number of their adverse side effects to be avoided.

The **literature review** is comprehensive and entirely focused on the topic of dissertation work. It is based on 256 literary sources, most of them from the last ten years. The cited Bulgarian sources are only six and this illustrates the limited information on the problem in the country. The literature review up-to-date reflects the discussion regarding the chemical structure and mechanism of action of SARMs, their effects on various organs and systems, as well as their observed adverse effects. On the other hand, the inclusion of SARMs in the list of prohibited substances of the World Anti-Doping Agency (WADA) has been commented on due to their pronounced anabolic properties and their registration in different concentrations in food supplements and their possible use by both professional athletes and training individuals for health.

The conclusions arising from the literature review are a logical prerequisite for forming the **purpose** of the dissertation work. It is subordinated to the idea to follow the effects of non-steroidal SARMs in combination with systemic exercise on functional, morphological and clinicochemical indices in sexually mature male rats, and in parallel to register their possible adverse effects.

The **tasks** logically derived from the set goal are specifically justified in six points: to investigate the role of SARMs in increasing aerobic performance, sprint speed and maximal oxygen consumption; their effects on endurance, some markers of muscle oxidative capacity and carbohydrate metabolism; luteinizing hormone, follicle stimulating hormone and testosterone concentrations during submaximal training and combined with non-steroidal SARMs; some adverse effects of non-steroidal SARMs, the effects on lipid profile, hematological and metabolic indices, as well as changes in gene expression in m.gastrocnemius of myostatin, IGF-1 and VEGF-A when combined with submaximal training.

The **material** and **methods** for realizing the set tasks are adequate and appropriately tailored to the set goals and tasks. The scientific research was conducted on the basis of two experiments. The merit of the dissertation work is the number of experimental animals used - 100 sexually mature male Wistar rats, bred in individual metabolic cells at a temperature of 22-24°C, light/dark cycle, controlled humidity and access to standard food and water ad libidum, in conditions complying with the recommendations of the European Commission for the protection and humane treatment of laboratory animals, the Declaration of Helsinki on ethics in science and the Bulgarian laws and regulations for carrying out scientific research and experiments with animals. For training purposes, each experiment was preceded by a session of a duration that did not induce adaptive changes. For each experiment, experimental animals were divided into 4 groups. In each experiment, experimental rats were subjected to dosed systemic submaximal exercise on a treadmill. The substances used in the experiments are the non-steroidal SARMs ostarine and ligandrol at a dose of 0.4mg/kg daily 5 days a week or placebo. At the end of both experiments, rats were decapitated with a small animal guillotine after anesthesia with Ketamine and Xylazine. *Anthropometric measurements* were performed - body mass, body mass index, length, Lee index, abdominal circumference, weight of heart, liver and striated muscles. The presence of glycogen was established in heart and liver material by the *histological technique PAS-reaction*. Clinicochemical indices were examined with a *clinical chemistry analyzer* Konelab 60i Thermo Fisher Scientific Vantaa Finland. An *ELISA sandwich immunoenzymatic method* was used to quantify plasma levels of luteinizing, follicle-stimulating hormones, and testosterone. The number of erythrocytes, leukocytes and thrombocytes, as well as the erythrocyte indices in the

serum were measured with a *hematology analyzer* ADVIA 2120i (Siemens Germany). Analysis of myogenic expression was performed based on real-time *quantitative polymerase reaction (PCR)*. The measured expression of myostatin, IGF-1 and VEGF-A were calculated by the *2- $\Delta\Delta$ CT method*. *Functional tests, indirect calorimetry and dynamometry* were used to measure maximal sprint speed, submaximal endurance and maximal time to exhaustion, as well as maximal oxygen consumption (VO_{max}), running economy, respiratory quotient (RQ), energy expenditure and grip strength. *Statistical methods - parametric and graphic analysis, two-way ANOVA, one-way ANOVA and Tukey or Games-Howell post hoc tests* are consistent with the nature of the tasks and provide certainty for the reliability of the data at p-value<0.05. *SPSS version 19.0 and MS Excel 2010* were applied.

The own **results** are described correctly, they are illustrated through 53 tables and 90 figures and correspond to the tasks set. The first experiment examined the effects of the non-steroidal SARM *ostarine*, submaximal exercise and their combined effects. Submaximal training had a significant effect on abdominal circumference, muscle mass, and grip strength. It was found that ostarine does not significantly affect the parameters mentioned above. Administration of ostarine, however, did not significantly increase heart mass. The maximal time to exhaustion at the beginning of the experiment was similar in all experimental groups. At the end of the experiment, it increased significantly in the experimental animals of submaximal exercise, but not in those treated with ostarine. Sumaximal training showed a tendency to increase the number of leukocytes, but did not affect the number of platelets. Administration of *ostarine* did not affect the number of either leukocytes or platelets. Blood level was significantly decreased by *ostarine* but not significantly affected by submaximal workout. Neither ostarine nor submaximal exercise significantly affected plasma HDL-cholesterol levels. Follicle-stimulating hormone and testosterone did not significantly change their plasma concentrations in either ostarine-treated or submaximal-trained experimental animals. A significant increase in the time spent in sleep was found when ostarine was administered. The *second experiment* looked at the effects of another nonsteroidal SARM, ligandrol, as well as submaximal training and their combined action. Ligandrol did not significantly affect abdominal circumference, liver mass, maximal sprint speed, endurance exercise, maximal oxygen consumption, but

significantly increased respiratory quotient and sleep time, and decreased VGF-A expression in m.gastrocnemius. Ligandrol did not significantly affect the number of erythrocytes and the plasma level of hemoglobin, but significantly increased the values of the parameters reflecting the lipid profile. Ligandrol does not significantly change the plasma level of luteinizing hormone, but a pronounced tendency to decrease that of testosterone is established. Results of all measures are presented as mean \pm standard deviation of each quantity.

The **Discussion** analyzes the results of the two conducted experiments and makes a good comparability with other studies and practical messages. Dr. Veselin Vasilev interprets the obtained data with ease, showing a free handling of the information on the discussed issues. An adequate understanding of the matter enables him to analyze and synthesize his own data, comparing them with those discussed in the commented literature data. The dissertation is the first study of its kind studying the effects of the non-steroidal SARM *ostarine* alone or in combination with submaximal training on the economy of running, maximal sprint speed, submaximal endurance and maximal time to exhaustion, maximal oxygen consumption, respiratory quotient and energy expenditure values. Ostarine did not alter maximal oxygen consumption, a result similar to the administration of nandrolone decanoate, and probably related to the lack of effect on the oxygen-carrying effect of the blood. Through his experiments, Dr. Vassilev found that *ostarine* increased the gene expression of myostatin in m.gastrocnemius – known as an inhibitor of muscle growth. Dr. Vassilev suggests that the tendency for elderly people to have a negative effect on submaximal endurance is precisely related to the increased gene expression of myostatin. The reduced blood sugar level with the administration of *ostarine* is supported by a three-month clinical study in 60 adult men and women. *Ostarine* does not affect the lipid profile. Dr. Vassilev looks for an answer in the different cellular response to the different classes of non-steroidal SARMS. Regarding the plasma levels of gonadotropic hormones, the data are still conflicting and more studies are needed to elucidate the exact mechanisms. It turns out, commented Dr. Vassilev, that more experiments are needed regarding the influence of *ostarine* on the time spent in sleep – *ostarine* increases it significantly, other non-steroidal SARMS and anabolic androgenic steroids disrupt it. Regarding *ligandrol*, Dr. Vasilev concludes that, like *ostarine*, it is not suitable for improving performance in sports where sprinting is a major component. *Ligandrol*

significantly increased the value of the respiratory quotient during the maximal oxygen consumption test and favorably affected maximal sprint speed.

The **conclusions**, 11 in number, reflect in detail the results presented by the doctoral student and are in accordance with the tasks set.

Contributions: The dissertation work is a pioneering, representative not only on a national scale study on the effects of the non-steroidal androgen receptor modulators *ostarine* and *ligandrol* on indices of physical work capacity. The effects of the above-mentioned non-steroidal SARMs on submaximal endurance in conditions of submaximal training, lipid profile, plasma levels of gonadotropic hormones, gene expression of myostatin, as well as on the duration of sleep, established by Dr. Vasilev are also of a scientific and applied nature.

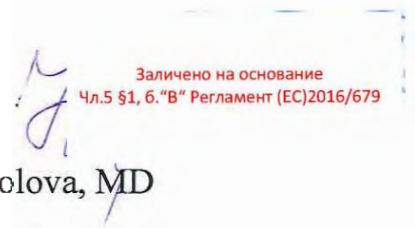
Based on the presented dissertation work, as well as my personal impressions, I express my satisfaction with Dr. Vassilev. He, as a doctoral student and free preparation, has built the ability to analyze and synthesize scientific problems, to formulate scientific judgments and conduct scientific experiments, adequately applying the relevant methodology.

The **list of publications** on the dissertation includes 5 publications - four in international journals and one in a national journal. Dr. Vassilev has participated in 5 scientific forums - four international and one - national.

Conclusion: The dissertation work of Dr. Veselin Atanasov Vassilev, a doctoral candidate in free preparation at the Department of Physiology at the Faculty of Medicine of the University of Medicine - Plovdiv, "*Influence of selective androgen receptor modulators (SARM) on physical work capacity and some side effects in an experimental model*" is fully completed, methodologically sound, with scientific and clinical application, and meets the requirements for obtaining the scientific and educational degree "DOCTOR".

The set of current debatable problems, the correct research methods underlying the planned scientific development, as well as the formulated conclusions - of a scientific-applied nature and with a socially significant sound are a prerequisite for my convinced recommendation to the respected Scientific Jury to

vote positively for awarding Dr. Veselin Vasilev Educational and Scientific Degree "DOCTOR" in the doctoral program "Human Physiology".



20.03.2024

Cityof Plovdiv

(Prof. Dr. Julia G. Nikolova, MD

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