The Chairman of the Scientific Jury

At the Medical University - Varna ,

Appointed by order No. P -109-520/1 6. 12. 2025
On The Rector of MU – Varna , by procedure

for defense of a dissertation for acquisition

of the ONS "DOCTOR" in the Department of Physiology and Pathophysiology

at the Faculty of Medicine of the Medical University of Varna

with a candidate Dr. Diana Asparuhova Kyuchukova.

REVIEW

By Prof. Dr. Blagoy Ivanov Marinov, MD, PhD

Medical University of Plovdiv

SUBJECT : Dissertation of Dr. Diana Asparuhova Kyuchukova " *INVESTIGATING THE RELATIONSHIP BETWEEN OBESITY AND CARDIOVASCULAR DAMAGE IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME* **" with scientific supervisor Assoc. Prof. Dr. Kamelia Zhechkova Bratoeva** for the acquisition of the educational and scientific degree "DOCTOR" in the scientific specialty "Pathophysiology" at the Department of Physiology and Pathophysiology at the Faculty of Medicine of MU - Varna. I declare that I have no joint scientific works with the dissertation or any other form of conflict of interest.

Brief biographical data

Dr. Diana Asparuhova Kyuchukova was born on 23.01.1991 and completed his secondary education at the High School for Foreign Languages, Pleven in 2010, and in 2016 she acquired the professional qualification "Physician" in a Master's program at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. Her professional path began at the Department of Physiology and Pathophysiology of MU-Varna, while in parallel, Dr. Kyuchukova also worked at the Emergency Medical Care Center - Varna. In 2020, she was

enrolled as a full-time doctoral student at the Department of Physics and Pathophysiology of MU-Varna. Her interests are mainly in the field of pathophysiology of cardiovascular diseases and metabolic syndrome, some of which are also the basis of the present dissertation work. Dr. Diana Kyuchukova is a member of a number of specialized and professional organizations, including Bulgarian Medical Union and Bulgarian Association of Pathophysiology (BAP). She is fluent in English and Russian.

Relevance of the problem

The dissertation presented by Dr. Diana Kyuchukova is in an interdisciplinary field, as the topic requires experimental experience and consistency in processing the result. The obesity epidemic in the 21st century seems to be directly related to the development of various cardiovascular risk factors, such as dyslipidemia, type 2 diabetes, atherosclerosis, arterial hypertension, which are the main components of the metabolic syndrome. Of particular importance for the pathogenesis is the association of obesity with cardiovascular diseases with endothelial dysfunction, insulin resistance, chronic disease and oxidative stress. A more in-depth clarification of the relationship between obesity and cardiovascular damage can serve as new therapeutic strategies and programs for interprevention.

Structure of the dissertation

As the entire structure of the dissertation is preserved, I would recommend separately displaying *Contributions* and *List of publications and participation related to the dissertation* after the bibliography. The total volume of the manuscript covers 170 pages (including Bibliography with 445 sources, of which only one is in Cyrillic!). The dissertation is extensively annotated, and the bibliography is systematized alphabetically.

The literature review is thorough, but slightly disproportionate to the other sections of the dissertation. At the moment it has a too propaedeutic sound, which is reflected in its entire volume. The general impression is that the dissertation demonstrates a thorough ability to summarize and analyze information, as well as a high degree of awareness in developing a problem. The author's justification for developing this work makes a very good impression and does not logically lead to the presentation of the goal and objectives.

The whole is formulated clearly and precisely in its first part and is sufficient to legitimize the result of the study. The assessment of the "effect of the application of antioxidants in a fructose-induced experimental model" could be studied separately. The memory tasks are **6** in number and are adequate for the implementation of the whole.

Material: The study includes 18 male laboratory white rats of the Wistar line weighing 120 – 160 g. The procedures for conducting experiments and treating laboratory animals are carried out in compliance with the requirements of the Commission on Ethical Treatment of Experimental Animals of the Bulgarian Food Safety Agency (BFSA) with order number 272/20.07.2020.

Patients were divided into 3 groups: **Group One** – control (C), **Group Two** – obesity model with high fructose diet (HFD), **Group Three** – obesity model with high fructose diet and treated with S-adenosinemethionine (HFD+SAM)

The methods are presented sequentially and in great detail in a separate chapter. The division of the methods into groups: zoometric, laboratory, immunological, morphological, immunohistochemical, and the methods used are presented with a clinical focus makes a very good impression. The statistical processing of the data was carried out with modern statistical tools, and descriptive statistics and graphical analysis were applied for visualization of the indicated results .

The results are presented logically and are well illustrated with 74 figures and 5 tables, some of which are original and others confirmatory. High fructose intake significantly increases the development of various metabolic changes, such as hyperglycemia, dyslipidemia, chronic low-grade disease, oxidative stress and the risk of insulin resistance. It was found that despite the lack of difference between the total caloric intake of the average experimental animals, at the end of the experiment in rats, fructose-fed animals showed changes in zoometric indicators and an increase in body weight compared to control animals (demonstrative increase in the Lee index in the fructose group). At the end of the study, higher TG, VLDL and LDL values were observed in the fructose group in the direction of the control. On the other hand, supplementation with S-AMe has a beneficial effect on lipid metabolism by statistically significantly reducing TG values, which approaches the results in the control group and lowering VLDL and LDL levels. A similar relationship was found for uric acid levels in the VFD group relative to the control. The results of the study also showed a very high negative correlation between the degree of

decrease in serum vitamin D3 levels and the increase in the TyG index in the VFD group, suggesting that VFD causes a decrease in vitamin D3 (25-OH), through oxidative stress, stress and true damage. Other important findings of the dissertation are that:

- The higher serum levels of TNF-α in the fructose group compared to the control
- Higher levels of C-reactive protein in the fructose group versus the control group

This is explained by the fact that it is probably a consequence of specific fructose metabolism, oxidative stress and hypertrophy of visceral adipose tissue.

The discussion is focused and competent. The ability of the dissertation candidate to analyze her own results in the context of the known data in the literature is evident. The thesis is well defended that high fructose intake significantly increases the development of various metabolic changes, such as hyperglycemia, dyslipidemia, chronic low-grade inflammation, oxidative stress and the risk of insulin resistance. Therefore, it is necessary to search for biomarkers for non-invasive study of necroptosis activity and monitoring even at the earliest stages of vascular and cardiomyocyte damage. In conclusion, the thesis is substantiated that the intake of S-AMe is able to effectively prevent all changes associated with the improvement of metabolism induced by a high-fructose diet in rats. Supplementation with S-AMe not only reduces body weight, but also improves insulin resistance, dyslipidemia, hyperglycemia and lowers the levels of built-in mediators.

Dr. Diana Kyuchukova has performed 12 **the conclusion** in response to the 6 tasks set. The conclusions are comprehensive, although I have some objections regarding the verbosity presented in the facts.

The developed dissertation has **contributions** of a scientific, scientifically applied and confirmed nature, namely:

- A comprehensive study was conducted on changes in the expression of SOD-1, VCAM-1, NOS3 and RIP3, and their relationship with pathomorphological changes in endothelial cells of coronary vessels, interlobar arteries and cardiomyocytes in experimental animals submitted to VFD.
- A comprehensive study was conducted on the effect of SAM supplementation on morphometric and morphological parameters, and on the expression levels of SOD-1, VCAM-1 and NOS3 in coronary vessels, preglomerular arteries and cardiomyocytes in experimental animals presented to VFD.

- The intake of VFD leads to changes in zoometric indicators - an increase in body weight, weight of retroperitoneal fat tissue and Lee index, development of oxidative stress, chronic low-grade decrease and endothelium dysfunction, thickening of the left ventricular wall and pathomorphological changes in the wall of the interlobar branches of the renal artery, which are manifested in thickening and remodeling of the wall.
- The experimental model tested in the dissertation is useful for studying the pathogenic mechanisms of metabolic syndrome in humans, as well as for developing effective therapeutic methods for prevention, diagnosis, and treatment of this, all in the context of a growing global pandemic.
- Supplementation with exogenous S-AMe in VFD increases the value of isometric indicators, such as body weight, RPMT weight, Lee index and TyG index, making it a potential tool for the treatment of obesity and insulin resistance.
- The demonstrated antioxidant effects of exogenous S-AMe on fructose-induced morphological and morphometric changes in endothelial cells and cardiomyocytes, as well as changes in NOS3, VCAM-1, RIP3, and SOD-1 levels, demonstrate its potential as an effective tool for the expression of endothelial dysfunction, oxidative stress, inflammation, and necroptotic changes associated with fructose-induced obesity.

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The dissertation work presented to me for evaluation shows very good professional qualities of the doctoral student and the ability to conduct a scientific, as well as to make an adequate analysis of the results obtained and to formulate the conclusions arising from them. It is evident that the work is the fruit of the author's long-term efforts. 3 articles have been published in Bulgarian journals on the comments on the problem, and the results have been presented at 4 international scientific congresses and conferences and 1 part of the national scientific forum.

In conclusion, I would like to emphasize that the considerations I have outlined and the critical remarks I have made above are in a way that does not at all diminish the merits of the dissertation work. I believe that the dissertation work submitted to me for evaluation,

MODEL OF METABOLIC SYNDROME " meets the requirements of the Higher Education Act, the Act on the Development of Academic Affairs in the Republic of Bulgaria and the Regulations for their application, as compiled in the Regulations of the Medical University - Varna for the acquisition of the educational and scientific degree "DOCTOR". Considering the positive aspects and the undoubted contributions of the presented dissertation, I will vote with conviction "YES" for awarding the educational and scientific degree " *DOCTOR* " to **Dr. Diana Asparuhova Kyuchukova .**

04.02.2026 The review i

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Plovdiv Prof. Dr. B. Marinov, MD, PhD