

REVIEW

by **Assoc. Prof. Armine Vardani Grigoryan, MD, PhD**

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Regarding: Dissertation titled: **“Investigation of the Relationship between Obesity and Cardiovascular Damage in an Experimental Model of Metabolic Syndrome”**, prepared by Dr. Diyana Asparuhova Kyuchukova, doctoral student in the PhD programme Pathophysiology, Faculty of Medicine, Medical University “Prof. Dr. Paraskev Stoyanov” – Varna, for the award of the educational and scientific degree “Doctor (PhD)” in the field of higher education 7. Health and Sport, professional field 7.1. Medicine.

According to Order No. R-109-520/16.12.2025 of the Rector of the Medical University “Prof. Dr. Paraskev Stoyanov” – Varna, I have been appointed as a member of the Scientific Jury in the procedure for awarding the educational and scientific degree “Doctor (PhD)”, conducted in accordance with the Academic Staff Development Act in the Republic of Bulgaria and its Implementing Regulations.

I. BIOGRAPHICAL DATA OF THE DOCTORAL CANDIDATE

Dr. Diyana Asparuhova Kyuchukova was born on 23 January 1991. In 2016, she completed her higher education and obtained the educational and qualification degree “Master” in Medicine at the Medical University “Prof. Dr. Paraskev Stoyanov” – Varna. After graduation, she directed her professional development toward areas in which clinical practice and academic medicine intersect, consistently building a profile that combines work in a real clinical environment with teaching engagement.

Since 2017, Dr. Kyuchukova has been working as a physician at the Center for Emergency Medical Care – Varna, where she has acquired significant practical experience under dynamic and highly demanding clinical conditions. Alongside her clinical activity, as of 01 September 2017, she has held the position of full-time

Assistant Professor at the Department of Physiology and Pathophysiology at the Medical University “Prof. Dr. Paraskev Stoyanov” – Varna and actively participates in the teaching process in Pathophysiology.

Within this professional development, Dr. Kyuchukova maintains a sustained interest in research activity focused on experimental models and pathophysiological mechanisms of cardiometabolic diseases. Proficiency in English and Russian at a high level ensures continuous access to current international scientific literature and enables full participation in academic and scientific communication.

II. GENERAL CHARACTERISTICS OF THE DISSERTATION

The dissertation was developed at the Department of Physiology and Pathophysiology of the Medical University “Prof. Dr. Paraskev Stoyanov” – Varna and is devoted to a topical and socially significant problem – the relationship between obesity, as a key component of metabolic syndrome, and the development of cardiovascular damage.

The topic fully corresponds to contemporary scientific priorities in the field of pathophysiology and cardiometabolic diseases, as it analyzes the complex interactions between metabolic, inflammatory, oxidative, and vascular mechanisms underlying endothelial dysfunction, vascular remodeling, and cardiovascular complications. The dissertation comprises 168 pages, is illustrated with 74 figures and 5 tables, and includes 445 literature sources, which demonstrates in-depth theoretical preparation and a solid orientation in current scientific literature.

The structure of the dissertation is logically consistent and includes an introduction, literature review, aim and objectives, materials and methods, original results and discussion, conclusion, scientific contributions, and a list of publications, fully meeting the requirements for a dissertation for the award of the educational and scientific degree “Doctor”.

III. RELEVANCE AND SCIENTIFIC SIGNIFICANCE OF THE TOPIC

Obesity and metabolic syndrome represent a complex clinical and pathophysiological problem with a clearly pronounced impact on cardiovascular morbidity and survival. The expansion of visceral adipose tissue is accompanied by metabolic and inflammatory processes that progressively impair normal vascular regulation. Insulin resistance, dyslipidemia, and disturbances in glucose metabolism are associated with chronic inflammation and increased oxidative stress, which compromise nitric oxide-mediated endothelial regulation and facilitate the development of endothelial dysfunction and vascular remodeling. In turn, these mechanisms promote adverse structural and functional changes in the cardiovascular system and increase the risk of complications over time.

In this context, the dissertation is well substantiated and methodologically consistent, tracing interconnected levels of damage—from anthropometric and biochemical alterations to structural changes in the myocardium and vascular wall. The fructose-induced experimental model employed enables reproducible investigation of key components of metabolic syndrome under controlled conditions and provides a suitable framework for mechanistic interpretation of the observed changes. An additional emphasis is placed on the evaluation of S-adenosylmethionine as a potential protective intervention, which adds further scientific and applied value to the study and outlines meaningful perspectives for subsequent research in this field.

IV. AIM, OBJECTIVES AND METHODOLOGY

The aim of the dissertation is clearly formulated—to investigate the relationship between obesity and cardiovascular damage and to assess the effect of an antioxidant intervention with S-adenosylmethionine in a fructose-induced experimental model of metabolic syndrome.

The objectives address the main pathophysiological levels of analysis:

- Zoometric assessment of obesity;
- Biochemical and clinical laboratory evaluation of the metabolic profile;
- Analysis of inflammatory and metabolic status using immunological methods;
- Morphological, morphometric, and immunohistochemical evaluation of the myocardium and blood vessels.

The methodological approach is appropriate and up to date. A well-validated in vivo model using male Wistar rats was applied, allocated into three experimental groups under a 12-week protocol. Standard biochemical, histological, morphometric and immunohistochemical methods were employed, along with appropriate statistical analysis consistent with the study design.

V. ORIGINAL RESULTS AND DISCUSSION

The results are presented precisely, systematically, and in a logical sequence that follows the clearly formulated aim and objectives of the study. Data from the zoometric parameters (body weight, anthropometric indices, and Lee index), as well as from the biochemical and immunological investigations, convincingly confirm the successful induction of an experimental model of metabolic syndrome. The observed metabolic disturbances are interpreted comprehensively and in close association with subsequent structural and tissue alterations, which lends pathogenetic coherence to the presentation and enhances the scientific value of the analysis.

A significant strength of the dissertation is that it goes beyond a purely descriptive presentation of the metabolic profile and traces cause–effect relationships between dyslipidemia and disturbances in carbohydrate metabolism, indicators of low-grade chronic inflammation, and the morphological manifestations of organ damage. In this respect, the morphological, morphometric, and immunohistochemical analyses directed at the evaluation of the myocardium and vascular wall are of particularly high scientific value. Through these approaches, the author contributes to a more detailed elucidation of the mechanisms underlying cardiovascular damage in metabolic syndrome, including the roles of oxidative stress, endothelial dysfunction, and processes of cell death.

The immunohistochemical assessment of the expression of the investigated proteins related to antioxidant defense, adhesion activity, and endothelial regulation is well substantiated and corresponds to the main hypotheses of the study. The presentation of the results is accompanied by an adequate interpretation of their pathophysiological significance, considering possible mechanistic links between changes in expression and the observed structural alterations. This adds further depth to the analysis and allows the formulation of broader conceptual conclusions.

The effect of S-adenosylmethionine is analyzed consistently and in a well-argued manner across all principal levels of evaluation—metabolic, inflammatory, and tissue-related. Trends toward normalization of selected parameters and reduction in the severity of structural damage are demonstrated, and probable mechanisms of action are discussed, including antioxidant and anti-inflammatory potential, as well as a possible protective effect on endothelial function. This approach strengthens the validity of the conclusions drawn and outlines perspectives for further research on the topic.

The discussion is written in a logically structured scientific style and demonstrates a solid understanding of contemporary pathophysiological concepts. The author critically compares her own findings with data from current literature, discusses similarities and differences with published experimental models, and interprets the results without unjustified generalizations. This reflects analytical thinking and the ability to integrate experimental findings into the broader context of cardiometabolic pathology.

VI. SCIENTIFIC CONTRIBUTIONS

The scientific contributions of the dissertation can be summarized as follows:

- Confirmation and detailed characterization of a fructose-induced experimental model of metabolic syndrome through combined assessment of zoometric parameters (including the Lee index and visceral adipose tissue) and

biochemical indices of carbohydrate and lipid metabolism (including the TyG index).

- Establishment of pathogenetic interrelationships between metabolic disturbances and the systemic inflammatory response (TNF- α , CRP), as well as their association with structural and morphometric changes in the myocardium and the vascular wall.
- Demonstration of changes in the expression of key proteins reflecting processes of oxidative stress, endothelial dysfunction, adhesion activity, and cellular injury, identified by immunohistochemical analysis in cardiomyocytes and endothelial cells (SOD-1, NOS3, VCAM, RIP3).
- Substantiation of a protective effect of S-adenosylmethionine on metabolic disturbances and structural alterations observed in cardiovascular damage.

VII. PUBLICATIONS AND SCIENTIFIC ACTIVITY

The results of the dissertation have been reflected in scientific publications and presented at scientific forums, which testifies to focused and consistent research activity on the topic. The publication output includes three scientific papers thematically related to the dissertation—experimental models of obesity and metabolic syndrome, vascular and cardiac damage in cardiometabolic disorders, as well as the role of necroptosis as a mechanism of cellular injury. There is clear continuity between the published findings and the presented dissertation research.

Scientific activity is further complemented by participation in international and national scientific forums (oral presentations and posters), where data corresponding to the main emphases of the dissertation have been presented—morphological and functional changes in the vascular wall in a fructose-induced model, vascular remodeling, and organ damage in the context of metabolic disturbances. The indicated publication and conference activity demonstrates skills in scientific communication and the ability to integrate the obtained experimental findings into the contemporary framework of cardiometabolic and vascular pathophysiology.

VIII. CONCLUSION AND EVALUATION

The dissertation is written in a clear, precise, and professional scientific style, with a logically consistent structure and correctly applied specialized terminology. The presentation of the results is systematic and well-substantiated, while the discussion is analytical, with the obtained data critically compared with the current international scientific literature.

Overall, the dissertation represents a completed, independent, and methodologically sound experimental study with distinct scientific merit and clearly formulated original scientific contributions. The conducted experimental investigations, the application of

contemporary methods, and the in-depth analysis of the results demonstrate that the author possesses the necessary scientific knowledge and practical skills to assess the significance of a scientific idea, to plan and conduct experimental research, and to critically interpret the obtained results.

The presented dissertation contains scientific and applied scientific results of an original nature that contribute to the advancement of knowledge in the field of pathophysiology and cardiometabolic diseases and fully comply with the requirements of the Academic Staff Development Act of the Republic of Bulgaria, the Regulations for its implementation, as well as the specific regulatory and academic requirements adopted at the Medical University of Varna.

Dr. Diyana Asparuhova Kyuchukova demonstrates very good theoretical training, confidence in working with an experimental model, and the ability to integrate experimental findings within the framework of contemporary pathophysiological concepts, which fully corresponds to the requirements for awarding the educational and scientific degree "Doctor".

In view of the above, I give my positive evaluation and propose that the esteemed Scientific Jury award Dr. Diyana Asparuhova Kyuchukova the educational and scientific degree "Doctor" in the PhD programme "Pathophysiology", within the field of higher education 7. Health and Sport, professional field 7.1. Medicine.

Date: 3.02.2026

Signature:

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
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Reviewer: Assoc. Prof. Armine Vardani Grigoryan, MD, PhD