

## **REVIEW**

by Prof. Margarita Stefanova Velikova, MD, PhD  
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of a dissertation submitted for the acquisition of the educational and scientific degree “Doctor”  
under the doctoral program “Pathophysiology”, professional field: 7.1. Medicine

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*Dissertation title:* “Investigation of the relationship between obesity and cardiovascular damage  
in an experimental model of metabolic syndrome”

*Scientific supervisor:* Assoc. Prof. Dr. Kamelia Bratoeva, MD, PhD

This review has been prepared in accordance with Order No. R-109-520/16.12.2025 of the Rector of MU–Varna, by which I was appointed as a member of the Scientific Jury. All necessary materials have been provided to me in both hard copy and electronic form, in compliance with the requirements of the Regulations for Academic Staff Development at MU–Varna.

### **Brief biographical data**

Dr. Diana Kyuchukova graduated from the Medical University – Varna and obtained a master’s degree in medicine in 2016. During the period 2017–2025, she worked as a physician at the Emergency Medical Care Center. In 2017, she was appointed through a competitive process as an Assistant in the Pathophysiology Sector at the Department of Physiology and Pathophysiology, MU–Varna. In 2020, she was enrolled as a full-time doctoral student in the doctoral program “Pathophysiology.”

### **Scientometric analysis and evaluation of the dissertation**

The dissertation comprises 170 pages, including: Introduction – 2 pages; Literature review – 41 pages; Aim and objectives – 1 page; Materials and Methods – 7 pages; Results – 38 pages; Discussion – 26 pages; Conclusions and Contributions – 4 pages; References – 34 pages, containing 445 literature sources.

### **Relevance of the topic**

The dissertation by Dr. Diana Kyuchukova presents research on a timely and significant scientific topic, aimed at establishing the relationship between obesity and cardiovascular damage in an experimental model of metabolic syndrome. The topic is of high social and clinical importance, given the global rise in obesity and the central role of cardiovascular diseases as a leading cause of morbidity and mortality worldwide.

The use of an experimental model of metabolic syndrome in the present study is particularly valuable, as it allows for controlled investigation of key pathological processes underlying the interconnection between obesity and cardiovascular dysfunction, namely chronic inflammation, oxidative stress, insulin resistance, dyslipidemia, endothelial dysfunction, and cardiac remodeling. The model enables analysis of molecular and cellular pathways that are difficult to study in humans due to the influence of various factors, such as genetic and epigenetic factors and comorbidities. Understanding the relationship between the processes of obesity and cardiovascular damage could contribute to the identification of early biomarkers of cardiovascular risk and to the development of preventive and therapeutic strategies aimed at reducing obesity-related cardiovascular complications.

The study is scientifically well-grounded and has the potential to make a significant contribution to both fundamental and clinical metabolic and cardiovascular research.

### **Literature review. Knowledge of the problem**

The literature review presents an in-depth analysis of adipose tissue dysfunction in obesity, describing the endocrine, inflammatory, and metabolic changes underlying systemic metabolic disturbances. The doctoral candidate demonstrates excellent knowledge of the scientific literature related to the research topic and an ability to systematize and analyze scientific information.

The presented literature data, appropriately supplemented with 17 figures and 2 tables, demonstrates conceptual understanding and a convincing presentation of adipose tissue pathology and its relationship to cardiovascular risk. The mechanisms of obesity development are discussed, distinguishing between metabolically healthy and high-risk obesity phenotypes, highlighting the role of visceral adipose tissue and ectopic fat in the development of cardiometabolic disorders. The relationship between fructose consumption and obesity is examined, supported by experimental and clinical evidence. The specific metabolic and endocrine effects of fructose are convincingly outlined, including its role in hepatic lipogenesis, insulin resistance, and hormonal dysregulation.

This section also provides a solid rationale for the chosen experimental model, namely a high-fructose diet as a model of metabolic syndrome. Contemporary theories regarding the role of oxidative stress, endothelial dysfunction, and inflammation are examined in detail as interrelated mechanisms leading to cardiovascular damage in obesity. Special attention in the review is devoted to necroptosis—an alternative form of regulated cell death and a pathogenic mechanism in cardiovascular injury—highlighting its role in myocardial remodeling, atherosclerosis, and heart failure. The molecular pathways involved in the development of necroptosis, as well as certain target molecules for its inhibition, are described in detail.

The literature review is comprehensive, logically well-structured, and demonstrates a high level of scientific rigor and critical thinking. Dr. Kyuchukova has successfully presented current knowledge on obesity, adipose tissue dysfunction, metabolic syndrome, fructose metabolism, and the pathophysiological mechanisms of oxidative stress, endothelial dysfunction, inflammation, and necroptosis. This integrative approach provides a solid theoretical foundation for the experimental work and clearly justifies the research hypotheses.

#### **Aim, Objectives, Methods**

The aim and objectives of the dissertation are clearly and specifically formulated, and the experimental model and research methods used are appropriately selected to achieve the stated goal.

From a methodological perspective, the dissertation is conducted at a high level and includes a wide range of analytical methods. Particularly impressive is the use of diverse types of investigations—zoometrical, biochemical, clinical-laboratory, macroscopic, histological, and immunohistochemical—which enable a precise analysis of the pathophysiological changes occurring in the body under a high-fructose diet. Descriptive and analytical statistical methods were applied, contributing to the objectivity of the results.

#### **Results, Discussion**

The scientific results are interesting and original, presented in an informative and comprehensive manner, and illustrated with 50 figures and 3 tables. The data from the three groups of experimental animals (controls on a standard diet; a high-fructose diet group [HFD]; and a group subjected to a high-fructose diet treated with S-adenosylmethionine) are competently and thoroughly interpreted with respect to changes in zoometric, biochemical, and clinical-laboratory parameters; morphological changes; and immunohistochemical analysis of the expression of key

mediators (SOD1, RIPK3, VCAM-1) in the pathways of cellular inflammation, oxidative stress, and necroptosis. The presentation of the results is systematic, characterized by precision and depth, and reveals the effectiveness of the chosen research approach.

The doctoral candidate presents a well-structured and evidence-based discussion of metabolic syndrome as a major factor in cardiovascular morbidity, in the context of the available information from contemporary scientific sources. Both in the review and in the discussion, Dr. Kyuchukova demonstrates excellent analytical skills in working with literature sources and scientific data.

The 12 formulated conclusions are well substantiated, logical, and adequately summarize the results.

1/ It has been established that the application of a high-fructose diet (HFD) in rats:

- induces changes in zoometric, biochemical, and clinical-laboratory parameters that meet the criteria for metabolic syndrome in humans;
- leads to a decrease in vitamin D3 levels, with a functional relationship identified with the TyG index (triglycerides/glucose ratio), which reflects insulin resistance and impaired lipogenesis under HFD;
- causes morphological and morphometric changes in arterial endothelial cells corresponding to the diagnostic criteria for endothelial dysfunction;
- induces morphological and morphometric changes in cardiomyocytes, with evidence of left ventricular hypertrophy;
- leads to changes in inflammatory and antioxidant status and to activation of necroptosis in arterial endothelial cells and cardiomyocytes.

2/ It has been established that supplementation with S-adenosylmethionine (SAM) in rats on HFD:

- improves zoometric parameters, reduces levels of pro-inflammatory markers, and increases vitamin D3 levels, interpreted as a protective effect against the development of fructose-induced metabolic disturbances
- prevents oxidative damage to endothelial cells and cardiomyocytes
- suppresses the progression of necroptosis, thereby improving redox homeostasis
- prevents the development of endothelial dysfunction.

## **Contributions**

I accept the stated contributions of the dissertation, among which I would like to highlight those of an original and applied nature.

A valuable contribution of the dissertation is the new, comprehensive investigation of the expression of SOD-1, VCAM-1, NOS3, and RIP3 in arterial endothelial cells and cardiomyocytes and their relationship to pathomorphological changes in animals subjected to HFD. The study of these markers allows for a comprehensive assessment of oxidative stress, inflammation, endothelial dysfunction, and cell death, which is essential for understanding the mechanisms of cardiovascular damage in obesity and metabolic syndrome.

Also original are the data on the effects of supplementation with S-adenosylmethionine on morphometric and morphological parameters and on the levels of expression of superoxide dismutase – SOD-1, vascular cell adhesion molecule-1 – VCAM-1, and nitric oxide synthase – NOS3 in arterial vessels and cardiomyocytes in animals subjected to HFD. The established changes indicate angio-protective and cardio-protective effects of SAM with respect to the development of necroptotic cell death and endothelial dysfunction.

Of applied significance is the validation of HFD as a human-relevant model of metabolic syndrome. Since the metabolic disturbances observed in rats induced by HFD (obesity, dyslipidemia, hyperglycemia, and insulin resistance) meet the diagnostic criteria for metabolic syndrome in humans, the model can be considered suitable for studying the pathogenetic mechanisms of the syndrome, as well as for developing new approaches to its prevention, diagnosis, and treatment.

An important applied aspect is the demonstrated therapeutic potential of S-adenosylmethionine in reducing the disorders accompanying fructose-induced obesity. It could be used as a potential agent for the prevention and treatment of obesity, insulin resistance, and the associated cardiovascular damage.

## **Publications related to the dissertation**

Three full-text publications in peer-reviewed Bulgarian scientific journals have been presented in connection with the dissertation. The results and some theoretical aspects of the dissertation have been disseminated through participation in five Bulgarian and international scientific forums.

## **Abstract**

The abstract, consisting of 84 pages, has been prepared in accordance with regulatory requirements and accurately, precisely, and concisely reflects the entire dissertation.

## **CONCLUSION**

The dissertation has been prepared in accordance with the standards for its development in the respective professional field and meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB), its Implementing Regulations (IR), and the IR of the Medical University – Varna.

The conducted up-to-date and in-depth study of the relationship between obesity and cardiovascular damage in an experimental model of metabolic syndrome has high scientific and practical significance. A thorough and high-quality literature review of current information on the topic has been carried out, which effectively supports the experimental design. The integration of metabolic, morphological, and molecular evidence contributes to the quality of the research and its translational relevance. The obtained results contribute to a better understanding of pathogenetic mechanisms and open perspectives for the development of preventive and therapeutic approaches. The dissertation demonstrates the doctoral candidate's in-depth theoretical training, competence in the use of research methods, ability to plan and organize experimental research, and to present and interpret the obtained results. I believe that Dr. Kyuchukova possesses the necessary theoretical and professional knowledge and skills to conduct high-quality scientific research.

I give a positive evaluation of the dissertation and propose to the Scientific Jury to award the educational and scientific degree "Doctor" to Dr. Diana Asparuhova Kyuchukova in the doctoral program "Pathophysiology."

6.02.2026

Reviewer:

Заличено на основание чл. 5,  
§1, б. „В“ от Регламент (ЕС)  
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/Prof. Margarita Velikova, MD, PhD/