To Assoc. prof. Mila Georgieva,

Head of department “School for PhD students”

According Protocol No.1/18.10.2020

and Order No. R-109-378/06.10.2020,

By the Rector of the Medical University-Varna

Prof. Valentin Ignatov, MD, PhD

REVIEW

by Prof. Maria Mitkova Orbeztova, MD, PhD
Scientific specialty ”Endocrinology and metabolic diseases”
Head of the Clinic of Endocrinology and metabolic diseases, UMHAT “St. Georgy”
Head of Department Endocrinology, Faculty of Medicine, MU –Plovdiv

of dissertation for awarding educational and scientific degree "**PhD**"

to **Dr. Elena Stoyanova Marinova**

doctoral student on a self-study basis, Second Department of Internal Medicine, Faculty of Medicine, Medical University “Prof. d-r Paraskev Stoyanov” Varna

On the topic: “**NON-INVASIVE ASSESSMENT OF ARTERILA STIFFNESS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS – CORRELATION WITH SOME BIOMARKERS”**

in the scientific specialty „**Endocrinology**“
with scientific tutors: **Assoc. Prof. Mila Boyadzhieva, MD, PhD**
Prof. Branimir Kanazirev, MD, PhD

I. Procedural requirements.

I have been elected as an external member of a Scientific Jury by order No. R-109-378/06.10.2020 by the Rector of the Medical University “Prof. d-r Paraskev Stoyanov” - Varna and for a reviewer according to a protocol No. 1/18.10.2020. The review was prepared in accordance with the requirements of the Rules for the structure and activity of MU Varna. The doctoral student of independent training in the program "Endocrinology" in a professional field 7.1. Medicine, field of higher education: 7. "Health and sport" from Second Department of Internal diseases, Faculty of medicine, Medical University “Prof. d-r Paraskev Stoyanov” Varna presents all necessary materials for review in accordance with regulation for obtaining the educational scientific degree "Doctor" printed dissertation and abstract and administrative folder.

II. Relevance of the topic

The dissertation is devoted to the macrovascular complications of type 2 diabetes mellitus in relation to the cardiovascular system and in particular the occurrence and development of arterial stiffness, which refers to non-traditional atherogenic cardiovascular risk factors. Arterial stiffness is thought to develop in parallel with endothelial damage due to insulin resistance and correlates with aortic stiffness, i.e. it may be an early marker of increased risk of cardiovascular morbidity and mortality. A modern non-invasive echo-tracking methodology for assessment of arterial stiffness of the carotid arteries has been selected, which has not been purposefully and systematically applied in patients with type 2 diabetes mellitus in our country in order to assess its predictive value. Data in type 2 diabetics without a previous history, resp. proven clinical manifestation of macrovascular complications are important for the realization of the idea and goals of the dissertation.

The dissertation aims to explain the relationship between the current bone protein for study in recent years - osteocalcin and type 2 diabetes mellitus, given the accumulating data on the participation of osteocalcin in carbohydrate and lipid metabolism, in parallel with its intervention in vascular calcification and atherosclerosis process. Data in the literature in patients with type 2 diabetes are scarce, largely divergent, and no distinction or comparison is made between the carboxylated and non-carboxylated forms of osteocalcin in most studies in experimental animals and humans. In this sense, the accumulation of data from various ethnic and population clinical trials is valuable and especially relevant.

The second main object of study in the dissertation, namely changes in arterial stiffness and serum levels of total osteocalcin and its two forms after 4 weeks of treatment with Vitamin K2 (involved in the processes of carboxylation of proteins, including osteocalcin) are also relevant and can generate both scientific-theoretical and clinical-applied conclusions and contributions. The role of Vitamin K2 has been confirmed in the treatment of osteoporosis. The accumulation of data on possible beneficial effects of vitamin K2 supplementation and in type 2 diabetes mellitus in the direction of metabolic control and vascular changes would be particularly useful for clinicians.

In summary, the idea of the dissertation is relevant, innovative and with clinical significance.

III. Structure of the dissertation: purpose and tasks; material and methods; results, discussion, conclusions.

The dissertation covers 136 standard typewritten pages and is properly structured in terms of the required components. The individual chapters and subchapters are reflected in detail in the content, which brings clarity and clarity to the material.

The ***literature review*** (40 pages) is purposeful and covers the available data on markers of development of early vascular damage in type 2 diabetes mellitus with a focus on arterial stiffness of the carotid arteries and the influence of metabolic disorders on its development. Known cardiovascular risk factors are considered in detail - endothelial dysfunction, hyperglycemia and insulin resistance, end products of advanced glycation, hemodynamic factors, etc. Significant place is given to the methods for assessment of arterial stiffness, and the echo-tracking selected for application is considered in depth with a description of its individual measurable indicators. A comprehensive review of data on the role of osteocalcin in carbohydrate metabolism, insulin signaling and action, the relationship with major adipocytokines, changes in its levels in type 2

diabetes on the one hand, and on the other - its participation in the processes of vascular calcification and development of arterial stiffness, resp. possible association with the atherosclerotic process and development of macroangiopathy in type 2 diabetes mellitus.

In the review, the role of Vitamin K2 is only marked, rather as participation in the carboxylation processes of its dependent proteins, including osteocalcin and some possible effects on the development of arterial stiffness in type 2 diabetes mellitus. In this regard, the review could be more extensive, given the inclusion of tracking the effects of Vitamin K2 supplementation on ultrasound changes and osteocalcin levels as one of the main objectives of the dissertation. However, some literature data are commented on in the discussion, and in general they are scarce, so the lack of a more detailed description in the review I cannot make as a significant remark.

In summary, the review is built logically, its content is completely related to the motivation and conceptual design of the dissertation and outlines the further discussion and interpretation of the results.

The aim of the dissertation is clearly formulated and concerns the application of echo-tracking methodology for assessing the local arterial rigidity of the carotid arteries in patients with type 2 diabetes mellitus and its relationship with clinical, including anthropometric and hemodynamic, glucometabolic and lipid parameters and serum osteocalcin. An additional goal is the complex effect of Vitamin K2 supplementation on arterial rigidity and osteocalcin levels.

The tasks are formed in 8 numbers, arising from the main objectives, as they contain the individual aspects of the analyzes, specified according to a study of: 1. Local arterial stiffness of the two carotid arteries by echo-tracking methodology, levels of osteocalcin - carboxylated and non-carboxylated form and correlation analyzes of the markers of arterial stiffness with the provided clinical and chemical indicators, including osteocalcin. 2. Monitoring the effect of Vitamin K2 treatment on serum osteocalcin and arterial stiffness.

The approaches used in the construction of ***the design*** of clinical trials***, the material and the methods*** are adequate to the purpose of the development and the set tasks, their description occupies 6 pages.

The criteria for inclusion and exclusion of the participants in the study, the methods used, including the instrumentals are strictly represented. 100 patients with type 2 diabetes mellitus (52 women and 48 men) and 30 healthy controls (both sexes) were examined. 18 patients (7 men and 11 women) underwent Vitamin K2 supplementation and were followed up after 4 weeks.

The statistical processing is at a modern high level and the analyzes are properly described.

The results are presented on 44 pages. The data are illustrated with 31 tables, 20 diagrams and 11 graphs, which contain in a synthesized form the information from the statistical analyzes and supplement the presentation of the material. The results are presented specifically according to each task, with skill and in a complete form, which shows that the goals and objectives of the development are fulfilled in full.

The discussion occupies a volume of 16 pages. It is built logically, following the structure of the presentation of the results, showing the very good knowledge of the subject by the author, insight into the analysis of the indicators set in the dissertation for evaluation and interpretation.

The conclusions are 11 - they reflect in a synthesized form the results related to the main and most important aspects of development.

The bibliography is comprehensive and up-to-date, meets the requirements - it includes 207 basic and contemporary literary sources, including publications in Cyrillic (1) and Latin (2) by 2 Bulgarian groups.

IV. Contributions

The results of the present dissertation enrich the established knowledge about the overall clinical picture of macrovascular of complications in type 2 diabetes mellitus and especially with regard to the pathology of the carotid arteries. An interesting research plan and extremely important for clinical practice aspect of the study is related in principle to the design of the study - a detailed echocardiographic assessment of type 2 diabetics without a history of cardiovascular complications through the introduced modern echo-tracking methodology. The present study is the first in Bulgaria to assess arterial stiffness of the carotid arteries in patients with type 2 diabetes mellitus without proven macrovascular complications, using this method - found significantly increased local arterial stiffness compared to clinically healthy controls, increasing with age. The data show that the study is fast, reliable and suitable for everyday clinical practice as non-invasive and accessible. It has sufficient value as an early diagnosis for the prevention and timely treatment of macrovascular complications. The tendencies found for greater sensitivity of the right carotid artery parameters are also a valuable conclusion for the practice, which, however, must be confirmed in a larger sample in order to establish possible statistical significance.

Important and with scientific-theoretical contribution are the conclusions about the correlations of markers of arterial stiffness with certain clinical indicators (anthropometric and hemodynamic, smoking) and biochemical indicators (glucometabolite and lipid). Most are expected, as impaired glycemic control, low HDL-cholesterol, smoking, hypertension are proven risk factors. Their relationship with the indicators of early vascular damage, established by echo-tracking methodology, is confirming their role in the pathogenesis of macroangiopathy in type 2 diabetes mellitus.

Pioneering and innovative are the development data on changes in serum concentrations of total, carboxylated and non-carboxylated osteocalcin. The lower levels of total and non-carboxylated osteocalcin (considered a hormone) in type 2 diabetes have been confirmed. The differences are significant for the carboxylated form, which positively and independently correlates with the β-stiffness index (R). The association of osteocalcin with the markers of arterial rigidity is studied for the first time in a Bulgarian clinical study and can be a starting point and basis for subsequent targeted ones on larger samples of patients with varying degrees of carbohydrate disorders and vascular damage.

The present study is the only one of its kind in Bulgaria, covering the effect of vitamin K2 supplementation as a cofactor of carboxylase in patients with type 2 diabetes mellitus without previously established macrovascular cardiovascular pathology. There was a significant increase in baseline reduced carboxylated osteocalcin, which is indicative of the action of the vitamin and leads to a significant change in the ratio of non-carboxylated to carboxylated form. The study also found an effect of supplementation on hemodynamic parameters - a significant increase in central aortic systolic pressure and a tendency to increase other markers of arterial stiffness. The dissertation makes an attempt to explain this effect, but I think that the sample is relatively small, the treatment period is insufficient to establish the final clinical effect and the result of the application of Vitamin K2 cannot be interpreted definitively as unfavorable for vascular processes. In general, the study of the complex role of osteocalcin in type 2 diabetes mellitus in metabolic control and the development of vascular pathology is very important in terms of therapeutic

strategies, such as the use of Vitamin K2, and the present dissertation contributes to initial data on this area of research.

I accept Dr. Marinova's self-assessment of the contributions of the dissertation as a national pioneer in terms of these scientific and theoretical ones, the practical orientation of those related to diagnostic procedures, and the confirmatory nature of changes in the markers osteocalcin and PWV in type 2 diabetes, which supports the sustainability of design and research.

In addition, clinical findings can be drawn from the descriptive statistics of the study groups regarding hemodynamic and lipid parameters in type 2 diabetics compared to controls, some gender differences in correlation analyzes, etc., which do not are directly set in the objectives, but derive from the design of the study.

In summary, the dissertation provides valuable databases and conclusions, which enriches the information from our national studies, while an international contribution can be realized.

V. Publication activity

In connection with the dissertation Dr. Elena Marinova points out 3 publications, 2 of which are in Latin, the first author of all publications. She has 2 participations in international scientific forums as a first author. I recommend increasing the publishing activity based on the multifaceted results of the development

VI. Abstract

The presented abstract is properly structured, contains all the required details and reflects the most important aspects of the dissertation, which meets the requirements of the Rules of MU Varna.

VII. Conclusion

Based on the above, I believe that the dissertation of Dr. Elena Stoyanova Marinova is relevant and practically focused and completed research work with a contributory nature, meeting all the requirements of the Law for Development of the Academic Staff of the Republic of Bulgaria (LDASRB), the regulations for application of LDASRB and the Rules for the structure and activity of MU Varna for acquiring the educational scientific degree "Doctor" under the doctoral program "Endocrinology"

I give a positive review and I strongly recommend the members of the esteemed Scientific Jury to give a positive vote for the implementation of the procedure for awarding a doctoral degree to Dr. Elena Stoyanova Marinova

20.11.2020. Prof. M. Orbetzova, MD, PhD

Plovdiv