REVIEW

by Prof. Ruzha Pancheva-Dimitrova, PhD
Department of Hygiene and Epidemiology, Faculty of Public Health,
Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

of the dissertation by **Dr. Ivan Georgiev Enev**
titled ***"Micronutrient Supplementation in Patients with Type 2 Diabetes Treated with Metformin"***
for the award of the educational and scientific degree "PhD"
in Higher Education Field 7. Healthcare and Sports,
Professional Field 7.1. Medicine, Scientific Specialty Hygiene

Following Order No. R-109-171/28.03.2025 of the Rector of the Medical University – Varna, Prof. Dr. Dimitar Raykov, D.Sc., I was appointed as a member of the Scientific Jury, and in accordance with Protocol No. 1 of the Jury meeting, I was assigned to prepare a review for the defense procedure of Dr. Ivan Georgiev Enev's dissertation for the award of the PhD degree.
The documentation submitted in the procedure meets the requirements of MU-Varna. No procedural violations were identified.

1. Brief Information on the Professional Development of Dr. Enev

Dr. Ivan Georgiev Enev is a specialist with long-standing experience in pediatrics, general medicine, and integrative approaches to the treatment of chronic diseases. Born in 1965 in the city of Vratsa, he completed his secondary education at the First Language High School "Henri Barbusse" with a German language profile, and graduated as a medical PhDr in 1992 from the Medical Academy – Sofia. He acquired a specialty in pediatrics in 1998 and in general medicine in 2005.

Since 2000, his professional interest has turned toward classical and clinical homeopathy, completing a full certification program organized by the European School of Clinical Homeopathy and the Center for Continuing Education at the Medical University – Sofia. He holds diplomas from prestigious international centers for homeopathy – CEDH in France and the London International College of Homeopathy in the UK, where he received training with an emphasis on pediatric and integrative medicine.

Between 1996 and 1998, Dr. Enev worked in the Neonatology Department of the "Hristo Botev" Hospital – Vratsa. He then started his own practice as a general practitioner. Since 2000, he has been managing and serving as the lead clinician at an individual primary healthcare practice focused on personalized pediatric care, chronic disease prevention, and the application of complementary and alternative therapeutic approaches. Since 2011, he has been an official lecturer in clinical homeopathy at the Training Center of MU – Sofia, and since 2012 he has been the regional coordinator for the training program in Northwestern Bulgaria.

He actively participates in organizing courses, masterclasses, and seminars for physicians interested in integrative medicine.

Since 2021, he has been a PhD student at the Department of Hygiene and Ecology at the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna. During the preparation of his dissertation, Dr. Enev applied a multidisciplinary approach to the research, integrating aspects of nutrition science, endocrinology, and preventive medicine.

He has authored over 40 scientific and popular-scientific publications in Bulgarian, English, and German, and has participated in national and international congresses in medicine, homeopathy, and nutrition science. He is a member of the Bulgarian Medical Association, the Bulgarian Medical Homeopathic Organization (BMHO), and the National Association for Practical Dietetics and Integrative Medicine (NAPDIM), where he actively contributes to expert and training initiatives.

Dr. Enev's clinical, scientific, and teaching activities are characterized by interdisciplinarity, patient-centered orientation, and innovative solutions in the context of modern preventive and integrative medicine.

2. Relevance of the Dissertation Topic

Type 2 diabetes mellitus (T2DM) is one of the most prevalent and socially significant chronic diseases in today's world, with its incidence continuing to rise. According to the data presented in the dissertation, the number of diagnosed individuals in Bulgaria exceeds half a million, with the real number likely being higher due to the high prevalence of undiagnosed cases. Despite established clinical practices for diagnosing and managing the disease, recent decades have emphasized the need for additional efforts in preventing and treating T2DM-related complications.

Of particular importance is the role of micronutrient status in patients with T2DM, especially those treated with metformin—a first-line drug widely used both as monotherapy and in combination therapies. Scientific literature increasingly documents the link between metformin use and deficiencies in certain vitamins, particularly vitamin B12 and folic acid, which can exacerbate patient conditions by contributing to cognitive impairments, polyneuropathies, and anemias. Nevertheless, there is a lack of systematic studies addressing this issue in Bulgaria. With his dissertation, Dr. Ivan Georgiev Enev addresses this significant gap in Bulgarian medical science and practice. Through his research, he not only identifies various micronutrient deficiencies in T2DM patients treated with metformin but also interprets these findings in the context of the clinical and metabolic management of the disease. Such an approach is particularly valuable as it provides opportunities for timely correction of nutritional status and

prevention of complications without the need to alter the primary therapy. The relevance of the topic is further emphasized by the fact that micronutrient supplementation in this patient group remains outside the scope of routine medical practice and is not included as a mandatory component in the guidelines and recommendations of health authorities. This makes the study both timely and potentially translatable into clinical practice. Dr. Enev's work aligns with contemporary trends in preventive and personalized medicine, underscores the need for a multidisciplinary approach in managing chronic diseases such as T2DM, and offers concrete guidance for screening, monitoring, and intervention. It presents a compelling case for including nutritional status as a key component of diabetes care and addresses one of the major challenges of modern medicine—improving patients' quality of life with limited resources.

3. General Characteristics of the Dissertation

The dissertation consists of 164 standard pages and includes 30 tables, 16 figures, and 9 appendices. The bibliography contains 315 references, more than 70% of which are from the last five years. The dissertation meets the structural requirements and includes the following sections: Introduction – 3 pages, Literature Review – 53 pages, Methodology – 7 pages, Results and Analysis – 37 pages, Discussion – 11 pages, Conclusions – 1 page, Final Conclusions and Recommendations – 3 pages, Contributions – 2 pages, Appendices – 25 pages, References – 15 pages

4. Evaluation of the Content of the Main Structural Components of the Dissertation

Evaluation of the Literature Review

The literature review presents a thorough and analytical overview of the role of micronutrients in the pathogenesis, clinical presentation, and therapeutic modulation of type 2 diabetes mellitus (T2DM). It provides detailed data on calcium, phosphorus, magnesium, zinc, chromium, and iron, along with their interactions with key physiological processes such as insulin secretion and sensitivity, electrolyte and bone metabolism, vascular homeostasis, inflammatory responses, and oxidative stress.

The cited evidence from literature sources, meta-analyses, and interventional studies unequivocally demonstrates that T2DM should not be viewed solely as a disorder of glucose metabolism. Rather, it is a systemic disease involving multiple deficiencies and imbalances of vitamins and minerals—some of which may precede the onset of diabetes or significantly worsen its course.

Key conclusions from the literature review include:

1. The most frequent deficiencies in T2DM involve magnesium, zinc, and vitamin D, and in certain populations, chromium and iron. These deficiencies are associated with impaired insulin signaling, β-cell dysfunction, increased inflammation, vascular complications, and dyslipidemia. Particular attention should be paid to patients with longstanding diabetes, hospitalized individuals, and those taking metformin, diuretics, and proton pump inhibitors.
2. Excessive levels of certain micronutrients (e.g., phosphorus and iron) also carry risks, particularly in patients with chronic kidney disease, where elevated phosphate levels may cause vascular calcification and iron overload may lead to insulin resistance and β-cell apoptosis. This underscores the need for precise balance, rather than indiscriminate supplementation.
3. Supplementation is not universally effective. Data from meta-analyses and interventional trials indicate that the effects of supplements such as vitamins C, E, D, and minerals like chromium, zinc, and magnesium vary widely depending on dosage, duration, baseline deficiency, and concurrent factors (e.g., diet, age, medication). Findings on chromium, in particular, are contradictory, with effects ranging from negligible to moderately significant—especially at higher doses approaching the toxic threshold.
4. A large proportion of diabetic patients are not routinely screened for micronutrient deficiencies, and reference ranges often fail to reflect individual needs—especially when based solely on serum levels without considering intracellular concentrations or functional metabolic indicators. For example, hypomagnesemia often goes unrecognized, despite its role in worsening insulin sensitivity and its resistance to spontaneous correction, even after achieving glycemic control.
5. There are significant gaps in clinical practice related to the absence of standardized screening algorithms for assessing nutritional status in T2DM patients. Existing clinical guidelines and recommendations (including those of the ADA and EFSA) often advise against supplementation in the absence of overt deficiency, but do not address the reality that such deficiencies are rarely proactively investigated.

Clinical and Practical Recommendations

- A systematic approach to nutritional assessment is required in patients with type 2 diabetes (T2DM), especially in specific subgroups (e.g., elderly patients, those with cardiovascular complications, nephropathy, or receiving specific therapies).

- Supplementation should be based on confirmed or high-risk deficiencies, rather than being used as a universal remedy.
- Sensitive and reliable biomarkers should be used for identifying nutritional deficiencies, rather than relying solely on serum levels, whenever possible.
- Screening protocols for assessing micronutrient status should be developed and integrated into clinical practice for patients with T2DM.

Research Perspectives and Future Needs

The review highlights a serious need for large-scale, well-controlled studies to address unanswered questions: which supplements, for which patients, in what doses, for how long, and with what expected benefits.

It is also important to investigate new potential micronutrients and compounds with biomodulatory effects on insulin sensitivity and β-cell function. Additionally, precise, personalized strategies should be employed in patients with T2DM, based on their genetics, microbiota, dietary patterns, and metabolic status.

Micronutrients should not be considered merely as auxiliary measures in T2DM but as essential modulators of the disease, with potential roles in both primary prevention and long-term management to prevent complications.

At present, an individualized, evidence-based, and clinically tailored approach to supplementation in patients with type 2 diabetes remains the best possible strategy.

Evaluation of the Methodology

1. *Definition of Aim and Objectives*

   The aim of the dissertation is clearly defined: to determine the micronutrient status of ambulatory patients with type 2 diabetes mellitus (T2DM) undergoing monotherapy with metformin, and to identify specific dietary habits and the need for supplementation. This aim is realistic, meaningful, and addresses a current clinical problem with high practical relevance.

   It leads to seven specific research objectives, logically sequenced—from identifying deficiencies and analyzing their relationship to metabolic control, to evaluating the intervention and creating practical tools for self-monitoring by patients. The scope of the objectives covers diagnostic, interventional, and educational aspects, providing breadth to the study.

2. *Study Design*

   The research is designed as a prospective, open-label, single-arm study without a control group. The pre-post design is suitable in the context of real-world clinical practice and

allows for monitoring the effects of the intervention within the same patients. While the absence of a control group limits the strength of causal conclusions, the approach is ethically acceptable and justified considering the outpatient nature of the studied population. The context of real-world clinical practice is well considered, which is particularly important for the applicability of results.

3. Sample Selection

The inclusion of 48 patients from a total of 190 with T2DM in the physician's practice is justified by clearly defined inclusion and exclusion criteria.
The patients were selected to form a homogeneous group—all were on monotherapy with metformin, had no significant comorbidities that could influence micronutrient absorption, and lived in a defined geographical area. Signed informed consent and voluntary participation enhance the reliability of the collected data.
While some tests were self-financed by the participants, which limited the scope of parameters studied, this also added realism to the sample and reflected genuine willingness for self-care and health responsibility.

4. Data Collection Methods

Data collection was carried out in a structured manner, integrated into four scheduled outpatient visits, including clinical assessment, laboratory testing, food diaries, questionnaires, and consultations.
All procedures were conducted during routine medical visits, which enhanced participant adherence and minimized dropouts.
The tools used—a three-day food diary and a food frequency questionnaire—are validated in clinical practice and were completed under supervision by trained medical staff. This ensured the high reliability of the nutritional data.
Anthropometric measurements were carried out by trained staff using a strictly defined protocol, guaranteeing reproducibility.

5. Laboratory Tests

The panel of biochemical and hematological tests included both standard markers for diabetic control and indicators of nutritional status—such as serum levels of vitamin D, B12, folate, iron, and magnesium. The tests were performed in licensed laboratories under contract with the National Health Insurance Fund (NHIF), ensuring standardization and quality control.

Due to logistical reasons, some participants declined testing for homocysteine and osteocalcin. This missing data was correctly reported and does not compromise the primary aims of the analysis.

6. Supplementation Intervention

When deficiencies were identified, supplementation was provided with licensed medications available in Bulgaria, using appropriate starting doses. In cases of persistent deficiency, dosage adjustments were made, including switching to parenteral forms when necessary.

This approach reflects good medical practice and was individualized for each patient. The staged therapeutic adjustment and subsequent follow-up demonstrate a systematic and consistent intervention protocol.

7. Statistical Analysis

Both parametric (independent t-test, paired t-test) and non-parametric methods (Mann-Whitney U test, Wilcoxon test) were used depending on distribution characteristics. Effect size was calculated using appropriate indicators (Cohen's d, rank correlation), and relationships between variables were analyzed using Pearson's correlation. The statistical software used (Jamovi) is modern and accessible, offering good visualization and analytical precision.The overall statistical analysis meets the standards for medical research and enables the reliable drawing of conclusions.

Evaluation of the Results

1. Participant Characteristics

The study involved 48 patients with type 2 diabetes mellitus (T2DM) treated with metformin. There was a slight female predominance—56.3% (n=27) women versus 43.8% (n=21) men. The mean age at the first visit was 61 years (range: 25 to 84 years); at the second visit, the mean age was 63 years. Most participants had multiple comorbidities, most commonly hypertensive heart disease, ischemic heart disease, and diabetic polyneuropathy. This reflects the typical outpatient population in Bulgaria and increases the validity of the findings. Graphical representations of gender, age distribution, and comorbidities aid in comprehending the sample characteristics.

2. Analysis of Micronutrient Deficiencies at First Visit

A high prevalence of deficiencies was observed at baseline: vitamin D (58.3%), vitamin B12 (68.8%), and magnesium (66.7%). Some patients exhibited severe vitamin D deficiency (<10 ng/ml). Folic acid deficiency was found in 16.7% of patients, and iron deficiency in 14.6%—significantly higher than rates reported in the general population.

These findings highlight the need for routine screening in this group. The distributions are presented in both tables and figures to enhance understanding of the extent of deficiencies.

3. Effect of Interventions on Biochemical Markers

After 3 months of supplementation, there was a statistically significant increase in the levels of vitamin D (p=0.025), vitamin B12 (p=0.004), and magnesium (p=0.014). The effect sizes were moderate, suggesting clinical relevance. Vitamin D deficiency was corrected in 46.4% of those initially deficient, and vitamin B12 in 40%. Folic acid showed partial improvement without reaching statistical significance. Iron and HbA1c were unaffected by the intervention, suggesting that isolated supplementation is insufficient to influence all metabolic parameters.

4. Impact of Supplementation on Vitamin and Mineral Levels

Despite the statistically significant increase in vitamin D and B12 levels, full correction was not achieved in all patients. For example, the target level of 40 ng/ml for vitamin D was rarely reached, and 31.3% (n=15) of patients remained deficient. This underscores the need for individualized dosing. Four new cases of B12 deficiency emerged, suggesting a need for long-term monitoring.

5. Analysis of Dietary Patterns and Awareness

Comparative analysis of food diaries between the first and second visits showed statistically significant improvements in dietary habits—increased intake of protein, fiber, and vegetables, and decreased consumption of high glycemic index foods. However, no significant changes were seen in the monthly frequency of intake for most food groups, possibly indicating socially desirable behavior during the second assessment. Half of the patients felt they did not need further education on therapeutic nutrition. Vitamin B12 supplementation was associated with lower intake of meat and processed meats.

6. Interpretation of Results

- A high frequency of micronutrient deficiencies in T2DM patients on metformin was confirmed through biochemical screening.
- Supplementation led to partial but clinically relevant correction.
- Important associations were observed between diet and the need for supplementation—especially for vitamin B12 and magnesium.
- Patients with higher consumption of fruits and nuts had better magnesium levels.
- Correction of B12 deficiency did not result in a statistically significant improvement in diabetic neuropathy symptoms, but the etiological relevance of the deficiency justifies preventive monitoring.

Evaluation of the Discussion

The discussion is thorough, multilayered, and demonstrates excellent scientific reasoning based on both the study's findings and a comprehensive, up-to-date literature review. The author systematically addresses key aspects of metformin therapy in T2DM patients, moving beyond the usual focus on glycemic control to emphasize the often-overlooked role of micronutrient status—vitamins D and B12, magnesium, folic acid, and iron.

A logical transition is made from the effects of metformin on body weight and visceral fat to the need for individualized dietary regimens. This conclusion is not only based on food diaries but validated with biochemical data. The study shows that metformin does not significantly reduce weight in the real-world clinical population, and its effect on fat mass is limited without additional dietary or physical interventions.

Deficiencies were assessed conservatively and critically, with attention to the etiology of each. The discussion rightly notes that supplementation with vitamins B12, D, and magnesium did not achieve full correction in all cases. The emergence of new B12 deficiencies also justifies the recommendation for long-term monitoring. A commendable aspect is the detailed comparison of B12 forms—cyanocobalamin vs. methylcobalamin—along with the clarification that there is no conclusive evidence supporting the metabolic superiority of methylcobalamin.

Regarding vitamin D, the discussion reflects a deep understanding of the issue: the high prevalence of deficiency, the need for individualized dosing, the stronger correlation in women, and the importance of maintaining levels above 40 ng/ml for effective diabetes prevention and management. The inclusion of both Bulgarian and international references enhances the contextual relevance of the findings.

The author skillfully explores the metabolic roles of vitamins and minerals, including interactions between vitamin D and magnesium, and the role of genetic polymorphisms in folate absorption. The use of methylated folate (5-MTHF) is appropriately suggested in non-responders.

The discussion of dietary habits, including discrepancies between reported behavior and actual intake frequency, is critical and appropriate. The author highlights the likelihood of socially desirable reporting and calls for more objective dietary assessment tools. The inclusion of a patient awareness screening questionnaire and educational materials is especially valuable.

The link between micronutrient status and diabetic neuropathy is handled with appropriate scientific caution. Although a pathophysiological link between B12 deficiency and neuropathy

is acknowledged, no statistically significant symptom improvement was observed—likely due to the small sample size and short follow-up duration.

The final part of the discussion is particularly impactful, offering practical guidance on supplement types, dosages, and the role of overall diet in T2DM management. The recommendations are well-reasoned, realistic, and clinically applicable.

Evaluation of Conclusions, Recommendations, and Contributions

The conclusions are logically derived from the empirical data and demonstrate a systematic, analytical approach to assessing micronutrient status in T2DM patients treated with metformin. The study documents a high prevalence of deficiencies in vitamins D, B12, and magnesium—values significantly exceeding those reported in national and international sources. This underscores the clinical importance of the issue and the need to revise screening practices in outpatient care.

A valuable strength of the dissertation is that the conclusions go beyond simple observations to offer realistic recommendations for supplementation regimens and clinical strategies. The effectiveness of standard supplement doses is evaluated, and a clear recommendation is made for individualized approaches—e.g., increasing vitamin D to 5000 IU/day if target levels are not achieved, or switching to parenteral B12 if oral treatment is ineffective. These strategies are evidence-based and supported by the biochemical and behavioral data collected.

Particularly important is the analysis of patient awareness and dietary behavior. Results show significant knowledge gaps about therapeutic nutrition in T2DM, which are not spontaneously corrected and require targeted education. In this context, the development of three practical tools—a knowledge questionnaire, a dietary habits survey, and a brief dietary guide—represents an original and highly relevant contribution, especially given the time and resource constraints in primary care.

The recommendations to institutions such as the Bulgarian National Association of General Practitioners and the NHIF are evidence-based and realistic. The proposal to include micronutrient screening in the diabetes care package for patients on metformin is well-justified. There is a clear link between timely identification and correction of deficiencies and the prevention of complications—metabolic, neurological, and cardiovascular. The proposal is also economically sound, with potential to reduce long-term healthcare costs.

The dissertation's contributions are clearly distinguished as scientific and practical. Scientific contributions include the first study in Bulgaria assessing micronutrient status in routine outpatient care and identifying clinically significant differences from published data. Practical

contributions include the development of clinical tools, policy recommendations, and a call for general practitioners to consider new dimensions in T2DM management.

The limitations of the study are clearly stated and critically analyzed. Despite the small sample size and lack of a control group, the results are consistent, valid, and of high practical value. Rather than diminishing the impact of the study, these limitations highlight its importance as a foundation for larger future research efforts.

The author's Abstract book consists of 71 pages and accurately reflects the dissertation content.

Critical Remarks

Dr. Enev's dissertation addresses an important and practically relevant topic but has some methodological limitations. The sample size (48 patients from a single GP practice) is small and not representative. The absence of a control group and data on the influence of other medications (e.g., diuretics, PPIs) limits the ability to establish a causal link between metformin therapy and observed deficiencies.

The discussion is comprehensive and well-reasoned but occasionally overloaded with literature, blurring the line between original results and external sources. The proposed questionnaires and guides have not been validated or tested for applicability, which raises questions about their reliability. Some of the stated contributions are more descriptive than innovative. Nonetheless, these issues do not diminish the overall quality of the dissertation.

6. Conclusion

The dissertation titled *"Micronutrient Supplementation in Patients with Type 2 Diabetes Treated with Metformin"* is of high public health and clinical relevance, with an original approach and systematic analysis. The research goal and objectives are clearly defined and consistently implemented. The methodology is appropriate for the research aim, with well-selected biochemical markers and well-justified criteria for deficiency. The result analysis is thorough and contextualized with current literature, enhancing the scientific value of the discussion.

The conclusions are clearly articulated, with strong practical orientation. The recommendations for screening and supplementation in T2DM patients on metformin have direct applicability in outpatient care. The developed tools for dietary assessment and patient awareness support individualized treatment. The dissertation's contributions highlight the need for an integrated approach that goes beyond glycemic control to include proactive management of nutrient status in chronically ill patients.

I highly appreciate the scholarly work of the PhDral candidate and conclude that the dissertation fully meets the requirements for awarding the educational and scientific degree

“PhD” according to the Law on the Development of the Academic Staff and the regulations of MU–Varna.

I give a **positive evaluation** and recommend that the members of the Scientific Jury vote in favor of awarding Dr. Ivan Enev the educational and scientific degree **"PhD"** in Higher Education Field 7. Healthcare and Sports, Professional Field 7.1. Medicine, Scientific Specialty: Hygiene.

May 8th, 2025 **Member of the Scientific Jury:**

Prof. Dr. Ruzha Pancheva, PhD