MEDICAL UNIVERSITY
"Prof. Dr. Paraskev Stoyanov" – Varna

FACULTY OF PUBLIC HEALTH
Department of Hygiene and Epidemiology

Ivan Georgiev Enev, MD

Micronutrient Supplementation in Type II Diabetes Patients Treated with Metformin

THESIS SUMMARY

of a PhD Thesis
For awarding the educational and scientific degree Philosophy Doctor
Scientific Speciality – Hygiene (incl. Nutrition and Dietetics)

Research Supervisor:

Prof. Darina Naydenova Hristova, MD, PhD

Varna, 2025

The doctoral thesis is presented on 163 pages and is illustrated with 30 tables, 15 figures, and 9 appendices.

The bibliographic reference includes 315 sources, 4 in Cyrillic and 311 in Latin.

The thesis was discussed at the Department Council of the Department of Hygiene and Epidemiology at the Medical University "Prof. Dr. P. Stoyanov" on 27/02/2025. It was approved and referred for public defence before a Scientific Jury consisting of:

External Members:

1. Prof. Veselka Laleva Duleva, MD, PhD
2. Prof. Maria Mitkova Orbetsova, MD, PhD
3. Prof. Lyudmila Borislavova Ivanova, MD, PhD

Reserve External Member: Assoc. Prof. Vanya Atanasova Boycheva-Birdanova, MD, PhD

Internal Members:

1. Prof. Rusha Zlatanova Pancheva-Dimitrova, MD, PhD
2. Assoc. Prof. Rositsa Hristova Stancheva-Chamova, MD, PhD

Reserve Internal Member: Assoc. Prof. Svetlana Hristova Hristova, MD, PhD

The thesis defence will take place on **06/06/2025 at 9.30 AM** in the Doctoral School hall of the Medical University – Varna, 55 Marin Drinov Street.

The documentation for the defence is available in the Scientific Department of MU-Varna and is published on the University website.

Contents

Thesis Summary ……1

Abbreviations Used……...4

Introduction ……...5

Aim and Tasks..…… 9

Materials and Methods…… 10

Results ……17

Discussion.……...53

Conclusions.……61

Conclusions and recommendations for the institutions ….……62

Contributions.…….....65

Publications and participation in scientific forums……68

Abbreviations Used

BMI - Body Mass Index

EFSA – European Food Safety Authority (European Food Safety Authority)

DPP4 – dipeptidyl peptidase 4

GLP-1 – glucagon-like peptide

HbA1c – glycated haemoglobin

HOMA-IR – homeostasis model assessment – estimated insulin resistance

IR – insulin resistance

MTHFR - methylenetetrahydrofolate reductase

K+ – potassium

Na+ – sodium

T2DM – Type 2 diabetes mellitus

BDA – Bulgarian Drug Agency

NHIF – National Health Insurance Fund

NAGPBG – National Association of General Practitioners in Bulgaria

NSAIDs – Non-steroidal anti-inflammatory drugs

GP – General Practitioner

HF – Heart Failure

Introduction

Diabetes mellitus (Type 2 diabetes) is a metabolic disease characterised by hyperglycemia, which results from a disruption in insulin secretion, insulin action, or both **[Alberti K.G.M.M, 1998]**.

It is a socially significant disease, and in Bulgaria, diabetes mellitus affects about 9% of the population, or about 500 000 patients. The vast majority of them (about 90%) suffer from T2DM.

T2DM is characterised by the development of insulin resistance (IR) and is often but not always associated with overweight or obesity, arterial hypertension, dyslipidemia and other markers of metabolic syndrome.

T2DM is a chronic disease that requires significant expenses, control, and modern treatment of its progression, as well as prevention and treatment of the late complications. According to various expert assessments, healthcare for people with diabetes costs at least 2.5 times more than for individuals of the same age without diabetes.

The main goals of treatment and monitoring T2DM patients include controlling hyperglycemia and preventing complications – dyslipidemia, macroangiopathy, microangiopathy, nephropathy, and neuropathy.

The main approaches in diabetes treatment include:

- Patient education about the nature of the disease, setting personal health goals, motivation for lifestyle and dietary changes.

- Recommendations for measured physical activity and nutrition.

- Medication therapy with oral antidiabetic medications (metformin, sulfonylurea preparations, and thiazolidinediones).

- When oral therapy options are exhausted, treatment transitions to DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, insulin, and insulin analogues.

Preventive and therapeutic nutrition for T2DM (medical nutrition therapy) is based on consuming diverse foods providing all necessary micronutrients. Studies have found a connection between inadequate vitamin intake, glycemic control, and the progression of type 2 diabetes. Different vitamins intervene at various stages of the metabolic processes in diabetes. At the same time, the nutritional status of T2DM patients and levels of fat- and water-soluble vitamins often escape the attention of both physicians and their patients.

Metformin remains the "gold standard" as a first-choice medication for mono- and combination therapy, including with insulin, for T2DM patients.

When used correctly, metformin treatment has favourable pleiotropic effects, low hypoglycemia risk, and minimal risk of lactic acidosis. However, chronic metformin treatment can induce vitamin B_{12} and folic acid deficiency. Vitamin B_{12} deficiency can lead to or deepen and worsen cognitive disorders, depression, peripheral polyneuropathy, and in some cases, macrocytic anaemia. Diabetic patients on metformin treatment show worse cognitive indicators compared to diabetics not on metformin and compared to non-diabetic individuals.

Folic acid deficiency leads to megaloblastic anaemia, worsening of cardiovascular diseases, senile dementia, and cancer. Folic acid deficiency is particularly adverse for patients with proliferative diabetic retinopathy.

With combined vitamin B_{12} and folic acid deficiency, homocysteine levels rise, which is associated with toxicity to the vascular endothelium, especially the retinal endothelium. Even short-term metformin treatment leads to decreased folic acid and vitamin B_{12} levels and increased homocysteine levels.

In type 2 diabetes, several other micronutrient deficiencies have been described – vitamin D, magnesium, and vitamin K, which influence the metabolic control of the disease.

In Bulgaria, there are no targeted and comprehensive studies on the micronutrient status in T2DM patients and this chronic disease in general, especially in recent years. Diabetes melitus itself is a condition where a deficiency of certain microelements is observed, but this issue has not been studied systematically. Replacing the shortage (deficiency) of necessary micronutrients remains outside the treatment consensus guidelines for type 2 diabetes mellitus (T2DM).

According to the National Health Insurance Fund (NHIF) requirements, the supervising physician (general practitioner) who monitors the T2DM patient orders annual creatinine and microalbuminuria tests and glycated haemoglobin tests every 6 months, as well as a blood sugar profile every 6 months at their discretion.

The general practitioner refers the patient for an endocrinologist consultation once a year if the glycated haemoglobin (HbA1c) level is ≥ 8%. In other cases, the endocrinologist consultation referral is made at the discretion of the physician. If the disease progresses with complications, the NHIF pays for an additional consultation with the relevant specialist (neurologist, ophthalmologist, vascular surgeon, or surgeon) once every two years.

Global perspectives of research data on the frequency of various micronutrient deficiencies, especially for patients undergoing metformin treatment, are contradictory, and such systematic studies are lacking in Bulgaria.

Currently, there is no universally accepted supplementation model for T2DM patients – neither regarding the search and diagnosis of potential micronutrient deficiencies nor the type of supplementation – whether it should be with mono-component or multi-component ingredients.

The most adequate and effective approach would be an individual one when the patient must be evaluated and examined for micronutrient deficiency risks.

Personal assessment of nutritional and health status should dominate clinical practice.

Considering the chronic course of the disease and its long duration, deepening micronutrient deficiencies over time could be a serious factor for both the onset and progression of T2DM complications and reduced quality of life. In view of the above and due to the increasing prevalence of type 2 diabetes mellitus, conducting this study is imperative.

Aim and Tasks

The thesis aims to establish the micronutrient status of outpatients with type 2 diabetes undergoing metformin monotherapy, to reveal the characteristics of their nutritional habits, and the need for supplementation.

In this context, the following tasks were formulated:

1. To establish the presence of deficiencies in vitamin D, vitamin B_{12}, folic acid, magnesium, and iron in type 2 diabetes patients on metformin monotherapy.
2. To analyse the correlation between micronutrient status and diabetes metabolic control (glycated haemoglobin, lipid profile, kidney and liver function).
3. To evaluate the impact of dietary regimen on micronutrient status.
4. To establish the correlation between vitamin B_{12} levels and diabetic neuropathy manifestations.
5. To assess the effectiveness of standard supplementation for correcting micronutrient deficiencies and its association with the clinical picture.
6. To develop recommendations for general practitioners and endocrinology specialists for monitoring and correcting micronutrient status in type 2 diabetes patients.
7. To create questionnaires and a self-monitoring nutrition manual for educating type 2 diabetes patients.

Materials and Methods

From 20/03/2021 to 26/03/2024, a prospective, open study was conducted, encompassing patients diagnosed with type 2 diabetes on metformin treatment and diet.

The methods and procedures related to this study were implemented during the patients' regular follow-up check-ups – once every 3 months.

Clinical examinations and procedures were carried out in the outpatient medical facility of the principal investigator at the Diagnostic and Consultative Center 2 (DCC), 1 Nikola Vaptsarov Street in Vratsa.

Subject of the Study

The study subjects are patients over 18 years old with type 2 diabetes, recommended Diet No. 9 by an endocrinologist, on metformin monotherapy prescribed by an endocrinologist.

Out of 190 type 2 diabetes patients in the medical practice, 48 patients were selected. The study subjects:

- Are undergoing metformin monotherapy;
- Have expressed and completed informed consent forms to participate in the study;
- Agree to pay for the necessary tests and fill out the required documents (nutritional diaries and food intake frequency questionnaires).

A total of 1 410 follow-up check-ups were conducted for all 190 T2DM patients. For the selected study participants, 172 examinations were conducted during the investigation period.

Study Design

The study is prospective, open (non-blinded), and experimental, without a control group (single-arm). The main objective is to track the effects of metformin therapy on the micronutrient status and metabolic control in type 2 diabetes patients.

The study uses a comparative design **before** and **after** treatment, with each group of participants serving as its own control. Patients undergo a series of regular follow-up check-ups, measuring various metabolic and biochemical indicators, including micronutrient levels and glycated haemoglobin, at the beginning and end of the study period. Since the study has no control group, its design is experimental, focused on observing changes resulting from treatment and investigating therapy effects on patients in a real clinical setting. Individuals were personally recruited by the principal investigator within his practice as a general practitioner in Vratsa. Participation was voluntary. Inclusion and exclusion criteria were personally evaluated by the principal investigator.

Inclusion Criteria

- Type 2 diabetes diagnosis on metformin monotherapy;
- Age over 18 years;
- Residence in Vratsa, Glavatsi, and Kravoder village (Vratsa region);
- Signed informed consent for participation.

Exclusion Criteria

- Proven and diagnosed hereditary anaemia;
- Patients with resections of the upper gastrointestinal tract;
- Patients on chronic treatment with proton pump inhibitors (PPI) such as esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole;

- Patients on chronic treatment with H2-blockers such as ranitidine and famotidine;
- Patients with chronic small intestine diseases preventing micronutrient absorption (celiac disease, Crohn's disease, chronic ulcer-hemorrhagic colitis, etc.);
- Patients with autoimmune diseases leading to accelerated metabolism - lupus erythematosus, Graves' disease;
- Patients with severe musculoskeletal diseases limiting ambulatory examination;
- Pregnant and breastfeeding women;
- Absence of T2DM diagnosis on metformin treatment;
- Transition to diabetes therapy other than metformin;
- Age outside the specified range;
- Residence outside the specified area;
- Lack of signed informed consent.

From the group that was reached out and briefed via an informative brochure, 48 patients signed informed consent – 27 women and 21 men.

During routine follow-up check-ups, after explaining the study objectives and obtaining informed consent, the following tests were prescribed:

- Tests included in T2DM follow-up regulations: Complete blood count, creatinine, urea, microalbuminuria, lipid profile, blood sugar profile, glycated haemoglobin;
- Laboratory tests for electrolyte disorders – sodium, potassium, phosphorus, calcium;
- Additional laboratory tests for micronutrient deficiency biomarkers: serum iron, total iron-binding capacity, vitamin D, vitamin B12, folates, homocysteine, magnesium, and osteocalcin.

Due to the COVID-19 pandemic and additional logistical challenges (test cost, intake restrictions), most patients refused homocysteine and osteocalcin testing. Therefore, no statistical processing of results for these two indicators was performed.

For each study participant, in addition to the clinical examination required by the follow-up care for chronic diseases, the following was performed:

- Nutritional status assessment, anthropometry, and nutritional diary-keeping (three-day recall);
- Assessment of food intake frequency for specific food groups.

All methods and procedures were carried out in the following order:

Initial Examination

At the initial examination, a clinical assessment is performed, and laboratory tests are appointed. The patient is educated to complete a food diary and food intake questionnaire within 15 minutes. Laboratory results assigned in this examination are later referred to as "first visit".

Second Examination

During the second examination, there is a discussion on the results obtained. When micronutrient deficiencies are detected, appropriate supplementation is prescribed. When a deficiency of a particular micronutrient is detected, supplementation with a medicinal product registered with the Bulgarian Drug Agency (BDA) is prescribed as follows:

- In case of Vitamin D deficiency: Initial supplementation with 2000 UI vitamin D_3 (Cholecalciferol) daily, equivalent to 50 µg cholecalciferol;

- In case of Vitamin B_{12} deficiency: Initial supplementation with 1000 μg vitamin B_{12} (Cyanocobalamin) orally;
- In case of Magnesium deficiency: Initial supplementation with 500 mg magnesium orotate, equivalent to 1.35 mmol or 32.8 mg magnesium;
- In case of Folic acid deficiency: Initial supplementation with 5 mg daily (5000 μg);
- In case of Iron deficiency: Initial supplementation with 325 mg ferrous sulfate (extended-release tablets), providing 105.0 mg elemental iron.

All patients received explanations about therapeutic nutrition for type 2 diabetes after analysing the results of their nutritional diaries and food preferences.

Third Examination

The third examination is conducted during the next follow-up examination for Type 2 Diabetes, to be more convenient for the patient and to ensure a sufficient degree of cooperation. Clinical assessment, anthropometry, and the appointment of reference laboratory examinations were conducted. Second completion of a nutritional diary and questionnaire follows, along with advices on hygiene and diet regimens. Laboratory results assigned in this examination are subsequently reflected as "second visit".

Fourth Examination

Evaluation of the results from the repeated examination, therapy correction, advice on hygiene and diet regimen is performed. In the course of this assessment, discussions were once more held with every patient concerning nutritional habits and preferences, macro- and micronutrient composition of consumed food, control of laboratory indicators (metabolic status, glycated haemoglobin), and control of fat mass (body weight, body mass index, waist circumference). Cardiovascular risk factors were discussed.

In case of repeated identification of deficiencies in the respective microelements, the supplementation dose was adjusted accordingly:

- In case of vitamin D deficiency – supplementation with 5000 UI vitamin D_3 (Cholecalciferol) daily, equivalent to 125 μg cholecalciferol;
- In case of vitamin B12 deficiency – supplementation with 2000 μg vitamin B_{12} (Cyancobalamin) orally, or transition to parenteral administration of vitamin B_{12} 1000 μg intramuscularly once weekly;
- In case of magnesium deficiency – initial supplementation with 3x2 tablets of magnesium orotate, equivalent to 196.8 mg magnesium, followed by a maintenance dose of 65.6 mg magnesium orotate (2 tablets daily);
- In case of folic acid deficiency, dose correction by increasing the dose to 10 mg daily.

In case of iron deficiency – continuation of supplementation without changing the dosage. If a micronutrient deficiency is first detected in the patient during the fourth examination, supplementation is prescribed in doses used for the initially identified deficiency.

Clinical tests and procedures were carried out in the outpatient medical facility of the principal investigator at DCC 2, 1 Nikola Vaptsarov Street in Vratsa.

Laboratory tests were ordered during follow-up examinations. Additional tests not covered by the National Health Insurance Fund were paid for personally by the patient.

Laboratory tests were conducted in three certified laboratories which are under contract with the National Health Insurance Fund – "Ramus", "Tsibalab", and at DCC1 in Vratsa.

Methods

The following methods were used for the purposes of this study:

1.1. Clinical Examination

- Medical history, general clinical status, blood pressure measurement, ECG, and anthropometry (patient's height, weight, and waist);
- **Laboratory Methods:** complete blood count, serum glucose, HbA1c, lipid profile, uric acid, creatinine, urea, microalbuminuria, liver transaminases (AST, ALT, GGT), electrolytes (sodium, potassium, calcium, magnesium, phosphorus, serum iron, IBC;
- Vitamin B_{12}, folate, vitamin D.

1.2. Nutritional Status Assessment

- Body Mass Index (BMI), calculated using the standard formula BMI = Weight (kg) / height² (m²)

1.3. Questionnaire Method – developed by the principal investigator

A nutritional diary provided to the patients, after which patients are trained on how to answer the questions as accurately as possible, and a Questionnaire on the frequency of consumption of different types of foods and food products. The training for filling out the nutritional diary takes approximately 10–15 minutes.

1.4. Statistical Methods

The statistical methods used include independent t-test (Independent Samples t-test) for comparing mean values between two independent groups and its non-parametric analog – Mann-Whitney U test**.** For comparing dependent samples, paired t-test (Paired Samples t-test) and its non-parametric analog – Wilcoxon Signed-Rank test were applied. Effect size assessment for parametric tests was

performed using Cohen's d for independent and dependent samples, and for non-parametric tests – using Rank-Biserial Correlation. To analyse correlations between variables, Pearson's correlation (Pearson's r) was calculated.

Statistical analyses were conducted using jamovi version 2.6.

Results

General characteristics of the patient sample – gender, age, percentage of deficiencies (data from the first visit)

Age and gender distribution of patients

The age and gender distribution of patients included in the study are presented in Fig. 1.

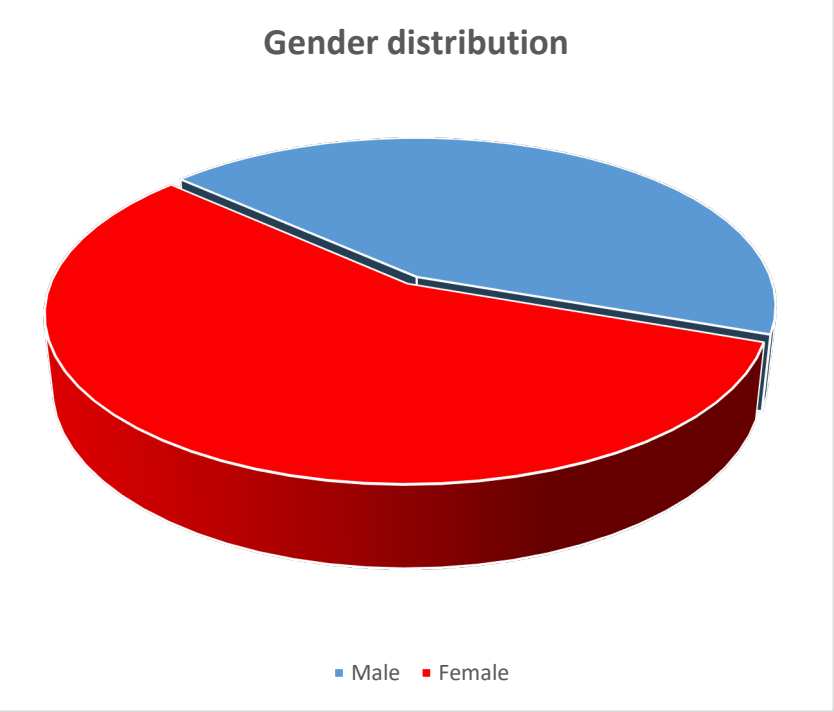


Fig. 1. Distribution of study participants by gender

The gender distribution shows a slight predominance of females – 56.3% (27 patients) are women, and 43.8% (21 patients) are men.

The average age of participants at inclusion in the study was 61 years. The youngest female participant at inclusion was 34 years old, and the oldest was 79 years old. The youngest male participant was 25 years old, and the oldest was 84 years old. The average age of participants at the conclusion of the study was 63 years.

The youngest female participant at the study's conclusion was 35 years old, and the oldest was 80 years old. The youngest male participant was 26 years old, and the oldest was 85 years old.

Almost all patients have between one to five comorbidities (hypertensive heart disease, coronary heart disease, cerebrovascular disease, diabetic polyneuropathy, diabetic angiopathy), for which they receive corresponding therapy (Fig. 2).

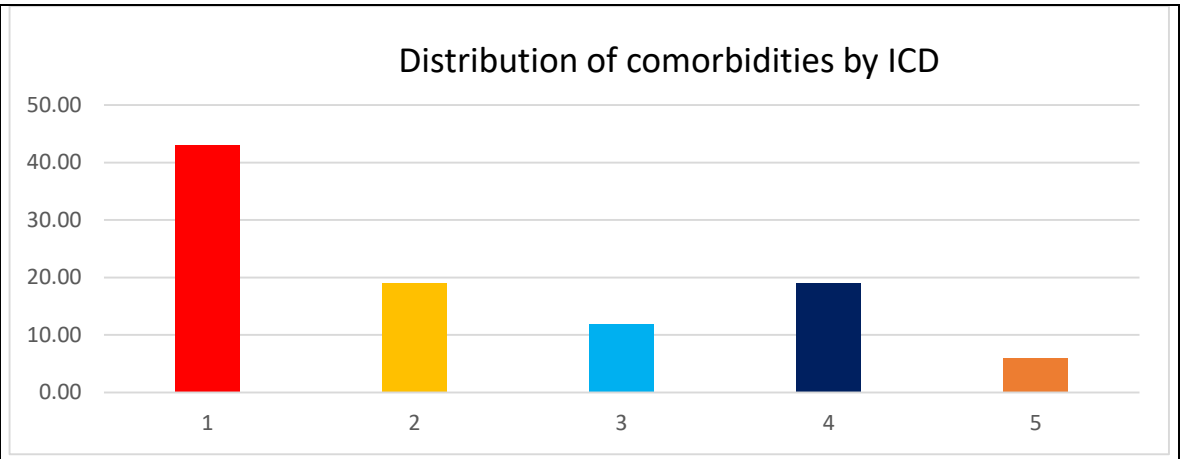


Fig. 2. Comorbidities [1 - hypertensive heart disease, 2 - cerebrovascular disease, 3 - CHD, 4 - diabetic polyneuropathy, 5 - diabetic angiopathy]

By nosological diagnoses, hypertensive heart disease is most frequent, followed by coronary heart disease and diabetic polyneuropathy.

Micronutrient Deficiencies Identified after the First Visit

At the first visit, vitamin D, vitamin B_{12}, folic acid, magnesium, and iron levels were assigned and examined for all 48 study participants. The results are reflected in Fig. 3, 4 and 5, as well as in Tables 2, 3, 4, 5, and 6.

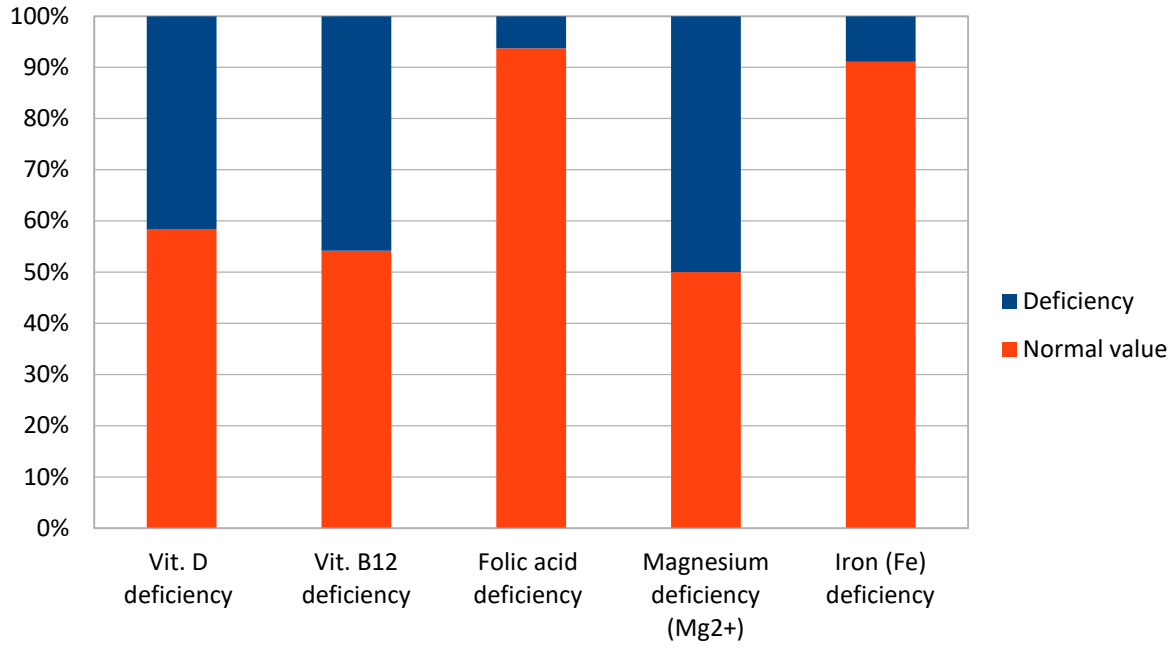


Fig. 3. Micronutrient deficiencies in patients at first visit

Vitamin D Deficiency

At the first visit, vitamin D levels were measured for 48 participants, with a mean value of 18.6 ng/ml (median 18.3 ng/ml, SD 7.88), ranging from 7.10 to 45.5 ng/ml. Adequate vit. D levels (> 20 ng/ml) were found in 41.7% of the study participants (n=20 patients). Vit. D concentrations in the blood were adequate.

Distribution of patients according to vitamin D levels at first visit		
	Number	Percentage
Have no deficiency	20	41.7%
Have a deficiency	28	58.3%
Total	48	100.0:

Table 1

Vitamin D deficiency was found in 28 patients (58.3%). The distribution of serum vitamin D concentrations is uneven. In 7 patients, vitamin D values between 7.20 and 9.60 ng/ml were measured, which indicates a severe deficiency. All patients with vitamin D deficiency (values < 20 ng/ml), regardless of the specific measured value (mild or severe deficiency), were prescribed supplementation with 2000 UI Cholecalciferol. Additional dietary and sunlight exposure guidance was provided.

Vitamin B_{12} Deficiency

At the first visit, the mean measured vitamin B_{12} value was 278 pg/ml (SD=122), ranging from 98 to 615 pg/ml. Adequate vitamin B_{12} levels at the first visit (values > 300 pg/ml) were found in 31.25% of participants (15 patients). Vitamin B_{12} deficiency (values < 300 pg/ml) was detected in 68.75% of the study participants (33 patients), (Table 2).

Distribution of patients according to vit B_{12} levels at first visit		
	Number	Percentage
Have no deficiency	15	31.3%
Have a deficiency	33	68.8%
Total	48	100.0%
Table 2		

The distribution of measured vitamin B_{12} values per patient is uneven. Most patients have vitamin B levels slightly below the lower reference value (< 300 pg/ml), but some patients have extremely low vitamin B_{12} levels. Severe deficiency (values < 200 pg/ml) was found in 13 patients, with values varying widely – from 98.00 to 185 pg/ml.

All patients with vitamin B_{12} deficiency (values < 300 pg/ml) were prescribed oral supplementation with 1000 μg Cyanocobalamin. Additional dietary guidance was provided.

Folic Acid Deficiency

At the first visit, folic acid deficiency was found in 8 patients, or 16.67% of study participants. All patients with confirmed folate deficiency were prescribed supplementation with 5 mg folic acid. Besides these 8 patients with proven folic acid deficiency, supplementation was also prescribed to patients whose folate values were close to the lower reference limit, though not in a state of deficiency. Thus, after the first visit, folic acid supplementation was prescribed to 13 patients (27.1%). 35 patients from all study participants (72.9%) remained without supplementation. Additional dietary guidance was provided.

Magnesium Deficiency

We found magnesium deficiency in 32 out of 48 participants, i.e., in 66.7% of all individuals included in the group we studied. All patients with confirmed magnesium deficiency were prescribed supplementation with 500 mg magnesium orotate, equivalent to 1.35 mmol or 32.8 mg magnesium. Additional dietary guidance was provided.

Iron Deficiency

Iron deficiency (values < 6.0 μmol/L) was found in 7 patients, or 14.59% of the study participants. All patients with serum iron deficiency were prescribed supplementation with 325 mg ferrous sulfate (ferrous sulphate), extended-release tablets providing 105.0 mg of elemental iron. Additional dietary guidance was provided.

Micronutrient Deficiencies after the Second Visit

The results show statistically significant and clinically relevant improvement in vitamin D, vitamin B_{12}, and magnesium levels between the two visits, with a moderate effect size. This suggests that the interventions between the two visits had a positive effect on these parameters. A control examination of serum levels of vitamin D, vitamin B_{12}, folic acid, magnesium, and iron was performed for all 48 study participants at the second visit.

The results of the examinations are reflected in Figures 4 and Tables 3.

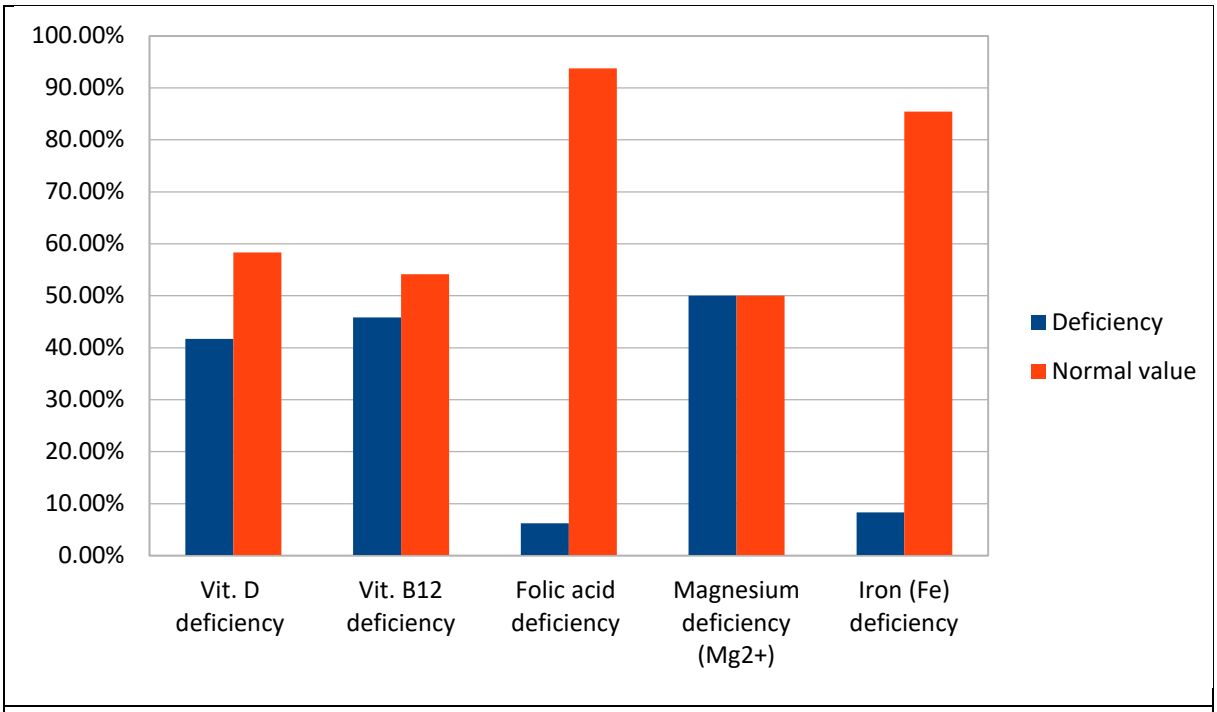


Fig. 4. Micronutrient deficiencies identified after the second visit

Vitamin D Deficiency

Despite the prescribed supplementation of 2000 UI vitamin D_3, a vitamin D deficiency was still found at the second visit, albeit in fewer patients. Almost none of the patients achieved the target of vit. D value of 40 ng/ml recommended for T2DM patients. The number of patients with vitamin D deficiency in the studied group decreased from 28 to 20, i.e., from 58.3% at the first visit to 41.7% at the

second visit. Compensation of the deficiency with a dose of 2000 UI vitamin D occurred in 8 out of 28 patients with a deficiency. In a cross-comparison of vitamin D levels at the second visit compared to the first visit, it was found that of the 28 patients with vitamin D deficiency registered after the first visit, after the prescribed supplementation of 2000 UI Cholecalciferol, the deficiency was overcome in 13 patients (Table 3).

Vitamin D deficiency – first versus second visit					
			Vit. D deficiency – second visit		Total
			There isn't	There is	
Vit. D deficiency - first visit	There isn't	Number	15	5	20
		% of total	31.3%	10.4%	41.7%
	There is	Number	13	15	28
		% of total	27.1%	31.3%	58.3%
Total		Number	28	20	48
		% of total	58.3%	41.7%	100%
Table 3					

In 15 patients, the vitamin D deficiency was not overcome, and therefore, values < 20 ng/ml were registered at the second visit. This necessitates continuing supplementation or reconsidering the dose. For these patients who had a deficiency at the first visit and continue to have such after the second visit and prescribed supplementation with 2000 UI vitamin D, the supplementation dose was increased to 5000 UI daily. A vitamin D deficiency was newly identified in 5 patients, so the total number of patients with a deficiency at the second visit is n=20. These 5 patients with newly discovered vitamin D deficiency were prescribed the initial supplementation of 2000 UI Cholecalciferol.

At the second visit, the distribution of supplementation changed – 16 patients (33.3%) did not receive supplementation, while 32 patients (66.7%) received a supplementation prescription. Nevertheless, the mean vitamin D levels at the second visit were similar between the groups – 21.1 ng/ml for non-supplemented (median 22.5 ng/ml, SD 8.21) and 21.0 ng/ml for supplemented (median 21.6 ng/ml, SD 7.17).

The t-test shows no statistically significant difference between the two groups ($t(46) = 0.0714$, $p = 0.943$), with the effect being negligibly small (Cohen's $d = 0.0209$).

Vitamin B_{12} Deficiency

Vitamin B_{12} levels increased significantly between the first and second visits (mean value at first visit: 277.67 ± 121.5; at second visit: 386.00 ± 285.0; $p = 0.004$), with a moderate effect size (–0.4812). This suggests that the interventions between the two visits had a positive effect on vitamin B_{12} levels.

Conversely, vitamin B_{12}-related haemoglobin and mean erythrocyte volume (MCV) levels showed no clinically significant changes. Haemoglobin levels remained stable between the two visits (mean value at first visit: 144.33 ± 14.6; at second visit: 144.48 ± 17.3; $p = 0.894$), with an insignificant effect size (0.0229). This indicates no significant change in erythropoiesis. There was also no significant difference in MCV (mean value at first visit: 90.53 ± 5.21; at second visit: 91.72 ± 5.96; $p = 0.268$), with a small effect size (-0.1845), suggesting no significant change in mean erythrocyte corpuscular volume.

After the first visit, 37.5% (n=18) of participants did not take additional vitamin B_{12} because they had levels > 300 pg/ml. Supplementation was prescribed to 62.5% (n=30) of study participants. Three patients with a deficiency (6.25% of

total participants) did not take the prescribed supplementation because their vitamin B_{12} values were close to the lower reference value.

At the second visit, vitamin B_{12} deficiency continued to exist, but the percentage of patients with a confirmed deficiency decreased from 68.75% to 45.83%.

At the first visit, 33 patients (68.8% of total examined) had a vitamin B_{12} deficiency. At the second visit, a vitamin B_{12} deficiency was found in 22 patients (45.83% of total examined). Not all patients with a vitamin B_{12} deficiency registered at the second visit had such a deficiency at the first visit. In some patients, this is a newly emerged vitamin B_{12} deficiency (Table 4).

Vitamin B_{12} deficiency – first versus second visit					
			Vitamin B_{12} deficiency – second visit		Total
			There isn't	There is	
Vit. B12 deficiency - first visit	There isn't	Number	10	5	15
		% of total	20.8%	10.4%	31.7%
	There is	Number	16	17	33
		% of total	33.3%	35.4%	68.8%
Total		Number	26	20	48
		% of total	54.2%	45.8%	100%
Table 4					

Compensation, reaching levels > 300 pg/ml, was achieved in n=9 patients out of all patients with a deficiency (40% of the patients with a deficiency, or 18% of the total study participants). An increase in values, but without reaching the lower reference limit (300 pg/ml), was found in n=6 patients who also had a deficiency at the first visit. Thus, incomplete compensation of the deficiency was found in 27.27% of the patients with a deficiency, or 12.5% of the total study participants. In n=9 of the study participants (18.75% of total participants), a decrease in vitamin B_{12} levels was found at the second visit compared to the first visit. Of

these, in n=4 patients (8.33% of total participants), low vitamin B_{12} levels were found for the first time, after having values above the reference 300 pg/ml at the first visit.

At the second visit, the share of non-supplemented patients decreased to 27.1% (n=13), while that of supplemented patients increased to 72.9% (n=35).

Folic Acid Deficiency

Folate levels showed no significant change (mean value at first visit: 9.38 ± 4.91; at second visit: 9.90 ± 4.62; p = 0.369), with a small effect size (-0.1309). The confidence interval (-0.414 to 0.1539) confirms that the difference is minimal. Out of 8 patients with folic acid deficiency, correction of the deficiency occurred in 6 (12% of total study participants), while in 2 patients (4.2% of the total number of patients), a folic acid deficiency continued to be present at the second visit, necessitating the continuation of supplementation. For folic acid, the distribution of supplementation remained unchanged between the first and second visits – 72.9% (n=35) of the participants did not take supplements, while 27.1% (n=13) were supplemented (Table 5).

Folic acid deficiency – first versus second visit					
			Folic acid deficiency – second visit		Total
			There isn't	There is	
Folic acid deficiency - first visit	There isn't	Number	39	1	40
		% of total	81.3%	2.1%	83.3%
	There is	Number	6	2	8
		% of total	12.5%	4,2%	16.7%
Total		Number	26	20	48
		% of total	93.8%	6.3%	100%
Table 15					

Magnesium Deficiency

Magnesium levels show a significant increase (mean value at first visit: 0.76 ± 0.12; at second visit: 0.82 ± 0.12; p = 0.014), with a moderate effective size (-0.4073). This suggests that the change is clinically significant. At the second visit, despite prescribed supplementation, magnesium deficiency was found in 50% of the study participants. For most patients, values were reached above the recommended minimum for the general population (0.62 mmol/l), but the minimum values (0.80 mmol/l) recommended for T2DM patients were not reached. When cross-comparing magnesium values between the second and first visits, it was found that at the first visit, 32 patients (66.7% of study participants) had a magnesium deficiency. At the second visit, magnesium deficiency was found in 24 patients (50% of study participants). Of these, 14 patients had a deficiency at the first visit, indicating unsatisfactory supplementation. Ten patients had a **newly established** magnesium deficiency. For 18 out of the 32 patients with a deficiency described at the first visit, compensation was achieved, and no deficiency was found (Table 6).

Mg^{2+} deficiency – second versus first visit					
			Mg^{2+} deficiency - second visit		Total
			Няма	Има	
Mg^{2+}– first visit	There isn't	Number	6	10	16
		% of total	12.5%	20.8%	33.3%
	There is	Number	18	14	32
		% of total	37.5%	29.2%	66.7%
Total		Number	24	24	48
		% of total	50.0%	50.0%	100%
Table 6					

Iron Deficiency

In the analysis of iron levels, there was no significant difference (mean value at first visit: 14.08 ± 6.01; at second visit: 13.56 ± 5.43; p = 0.316), with a small effective size (0.1462). The confidence interval (-0.139 to 0.4299) confirms that the difference is statistically and clinically insignificant. After the first visit, 89.6% (n=43) of the participants did not receive supplementation because iron levels were above the lower reference value (6 µmol/l); 10.4% of the participants (n=5) were supplemented. Except for one patient, where serum iron decreased despite supplementation, iron deficiency was overcome in the remaining patients. However, new patients with borderline serum iron values experienced a drop in their iron levels below the reference deficiency values (<6 µmol/l) during the observation period. By the second visit, the proportion of supplemented patients increased to 16.7% (n=8). The mean iron levels at the first visit were significantly higher in the non-supplemented group (Mean = 15.4 µmol/L, SD = 5.70) compared to the supplemented group (Mean = 7.62 µmol/L, SD = 2.23), with a statistically significant difference (t = 3.76, p = 0.001, Cohen's d = 1.46). This result reflects the fact that restoring serum iron in the presence of a deficiency requires sufficient time, which may be more than the three months of patient observation.

Correlation between Micronutrients and Metabolic Control

Correlations from the first visit

At the first visit, some significant correlations were observed between different biochemical indicators (Table 7).

The strongest correlation is between glucose and glycated haemoglobin – HbA1c (r = 0.779, p < 0.001), which is expected since HbA1c reflects long-term blood

glucose control. Haemoglobin shows a moderate positive correlation with glucose ($r = 0.379$, $p = 0.008$).

Significant correlations between different indicators at first visit		
Indicators	**Correlation coefficient (r)**	**p-value**
Glucose ↔ HbA1c	0.779	< 0.001
Glucose ↔ Haemoglobin	0.379	0.008
MCV ↔ Vitamin B12	-0.380	0.008
Folate ↔ Vitamin B12	0.394	0.006
Iron ↔ Haemoglobin	0.477	< 0.001
IBC ↔ Iron	-0.707	< 0.001
Table 18		

Between MCV and vitamin B_{12}, a moderate negative correlation was observed ($r = -0.380$, $p = 0.008$). This can be explained by the fact that vitamin B_{12} deficiency often leads to macrocytosis.

In this study, we examined the presence or absence of a correlation between MCV and vitamin B_{12} deficiency. In this context, the hypothesis is that as vitamin B_{12} levels decrease, the mean erythrocyte volume should increase. Using Pearson correlation analysis, a weak negative correlation between the two indicators was found at the first visit and no such correlation at the second visit (Fig. 5).

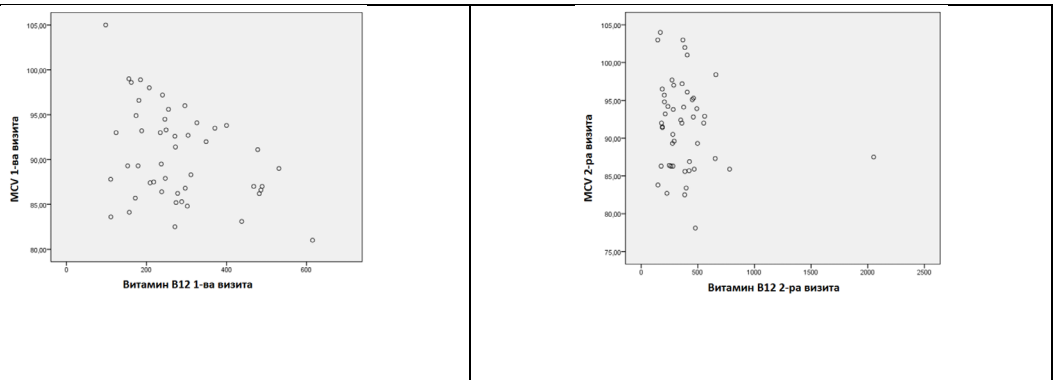


Fig. 5. Correlation between vitamin B_{12} levels and mean corpuscular volume (MCV)

There is a weak inverse correlation between the studied variables. A statistically significant increase in the mean erythrocyte volume (MCV) was found in patients with vitamin B_{12} deficiency, as established by laboratory tests. The correlation coefficient is statistically significant: r = -0.380.

Conclusion: Detecting an increase in MCV > 96 fL in a type 2 diabetes patient on metformin treatment can serve as a marker for probable vitamin B_{12} deficiency and provide grounds for additional laboratory testing. At the second visit, the correlation coefficient is negative, indicating an inverse correlation between variables. However, due to the weak connection and small number of study participants, the statistical significance of the correlation coefficient cannot be proven. With this small number of study participants, the mean erythrocyte volume (MCV) could not be used as an indirect indicator that vitamin B_{12} deficiency in a specific patient is decreasing. A control testing of vitamin B_{12} levels is necessary.

Folate and vitamin B_{12} show a moderate positive correlation ($r = 0.394$, $p = 0.006$). This is logical, as both nutrients are important for proper blood formation and DNA synthesis.

Iron and haemoglobin have a strong positive correlation ($r = 0.477$, $p < 0.001$), which is expected since iron is a key component in haemoglobin synthesis. Iron-binding capacity (IBC) and iron show a strong negative correlation ($r = -0.707$, $p < 0.001$). This can be explained by their interrelation in metabolic pathways related to blood formation.

Correlations from the Second Visit

At the second visit, some observations from the first visit were confirmed (Table 8).

Significant correlations at second visit		
Indicators	**Correlation coefficient (r)**	**p-value**
Glucose ↔ HbA1c	0.760	< 0.001
Folate ↔ Vitamin	0.476	< 0.001
Iron ↔ Haemoglobin	0.562	< 0.001
FBC ↔ Iron	-0.753	< 0.001
MCV ↔ Iron	-0.294	0.043
Table 8		

Glucose and HbA1c show a strong positive correlation ($r = 0.760$, $p < 0.001$), confirming the stability of this correlation. Folate and vitamin B_{12} show a strong positive correlation as well ($r = 0.476$, $p < 0.001$), confirming their interdependence in metabolic processes. Iron and haemoglobin again show a strong positive correlation ($r = 0.562$, $p < 0.001$), consistent with iron's role in haemoglobin synthesis. Iron-binding capacity and iron again show a strong negative correlation ($r = -0.753$, $p < 0.001$), confirming the observation from the first visit. A new observation at the second visit is a weak negative correlation between MCV and iron ($r = -0.294$, $p = 0.043$). This can be explained by the fact that low iron levels can lead to a reduction in red blood cell size (microcytosis).

Changes in participants' anthropometric indicators during the study

The study examined changes in participants' anthropometric indicators – height, weight, waist circumference, and body mass index (BMI). We analysed whether the combination of recommendations for following Diet No. 9 and metformin treatment affects the anthropometric indicators of the patient group (indirectly affecting fat mass). A statistical test of differences between two interdependent samples was applied (Paired t-test) (Table 9).

Anthropometric indicators at first and second visit				
	Average	Number	Standard deviation	Standard error
Weight – first visit	90.34	48	15.750	2.273
Weight – second visit	90.72	48	19.128	2.761
Waist – first visit	106.65	48	11.307	1.632
Waist – second visit	105.71	48	11.984	1.730
BMI – first visit	32.661	48	5.56189	0.80279
BMI – second visit	33.233	48	10.02118	1.44643
Table 9				

Weight: p-value (0.855/2 = 0.4275) > α; the difference is statistically insignificant.

Waist Circumference: p-value (0.280/2 = 0.140) > α; the difference is statistically insignificant.

Body Mass Index (BMI): p-value (0.524/2 = 0.262) > α; the difference is statistically insignificant.

Conclusion: Metformin treatment has a weak, statistically non-significant influence on the anthropometric indicators of T2DM patients participating in the study. Neither body weight, waist circumference, nor body mass index (BMI) decreased statistically significantly, despite the blood glucose control in follow-up examinations and stable glycated haemoglobin indicators. This outcome corresponds with the medication's characteristics, and specifically, it maintains a neutral effect on weight during chronic use.

Correlation between Micronutrient Levels and the metabolic control indicators for T2DM

The current analysis compares the mean values of biochemical parameters between the first and second visits, using the Wilcoxon signed-rank test for non-parametric data and the Paired t-test for parametric data. Beyond statistical significance (p-value), the assessment of effect size provides information about the practical significance of observed differences. Effect size is measured by Cohen's d for t-tests and Rank biserial correlation for Wilcoxon tests.

The interpretation of effect size is based on the following criteria: 0.2 – small effect; 0.5 – moderate effect; 0.8 – large effect (Table 10).

Variable	**Arithmetic Mean/Standard Deviation (1st visit)**	**Arithmetic Mean/Standard Deviation (2nd visit)**	**Wilcoxon rank/ Paired t-test**	**P - value**	**Rank biserial correlation (RBC)/ Cohen's d**	**95% CI**
Weight (kg)	90.34 ± 15.750	90.72 ± 19.128	W = 386	0.855	Rank biserial correlation: 0.3761	-7.39; 6.15
BMI (kg/m^2)	32.36 ± 5.562	33.23 ± 10.021	W = 405	0.524	RBC: 0.2841	-0.91; 1.49
Cholesterol (mmol/l)	5.55 ± 1.519	5.30 ± 1.361	W = 700	0.266	RBC: 0.2411	-0.33; 0.15
HDL (mmol/l)	1.19 ± 0.406	1.15 ± 0.320	W = 659	0.326	RBC: 0.1684	-0.05; 0.12
LDL (mmol/l)	3.33 ± 0.956	3.23 ± 1.031	t = 0.563, df = 47	0.576	Cohen's d: 0.0813	-0.05; 0.12
Triglycerides (mmol/l)	1.88 ± 1.384	2.34 ± 2.835	W = 561	0.228	RBC: -0.0459	-0.37; 0.11
Uric acid (μmol/l)	313.31 ± 97.613	317.47 ± 113.765	t = -0.329, df = 47	0.744	Cohen's d: -0.0475	-8.47; 13.43

Table 10

Conclusion: For most of the indicators, there was no statistically significant association between micronutrient levels and metabolic indicators between the first and second visits. The exception is the correlation we found between vit. D, weight and BMI in women and between vit. D, HDL and triglycerides also in women.

The participants' lipid profiles show no statistically significant changes between the first and second visits. The levels of total cholesterol, HDL, LDL, and triglycerides remain stable during the observed period, with no substantial differences (p-values significantly above 0.05). There are no significant fluctuations in participants' lipid profiles, suggesting that the therapeutic regimen involving metformin and diet does not lead to changes in lipid parameters during the examined period.

During the first visit, no correlation was found between vitamin D levels and any indicators (weight, lipid profile values, uric acid). At the second visit, in women vitamin D correlates with several indicators. For the female participants in the study vitamin D showed a statistically significant negative correlation with weight ($r = -0.555$, $p = 0.003$) and BMI ($r = -0.477$, $p = 0.012$). This indicates that higher vitamin D levels are associated with lower weight and body mass index (Fig. 6).

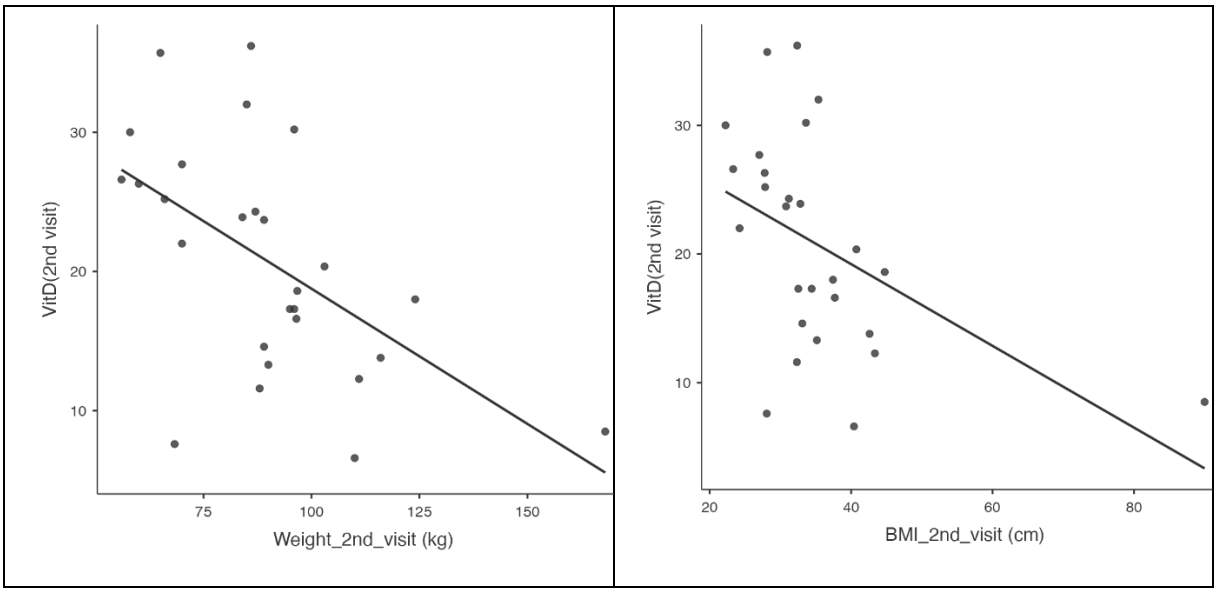
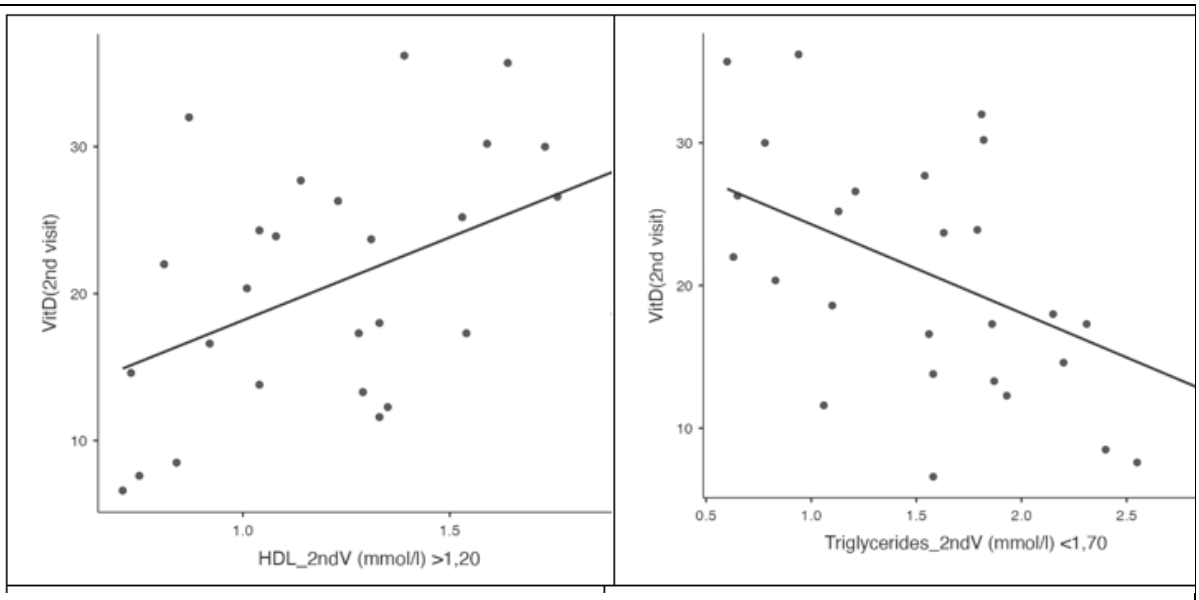


Fig. 6. Correlation between vitamin D levels, weight and BMI in women

Vitamin D also showed a statistically significant positive correlation with HDL cholesterol (r = 0.461, p = 0.016), suggesting an association between higher vitamin D levels and higher HDL levels. The correlation with triglycerides was statistically significant and negative (r = -0.467, p = 0.014), suggesting that higher vitamin D levels were associated with lower triglyceride levels (Fig. 7).



Vit. D and HDL

Vit. D and triglycerides

Fig. 7. Correlation between vitamin D, HDL and triglycerides levels

Correlations with LDL cholesterol (r = 0.042, p = 0.836) and uric acid (r = 0.160, p = 0.426) were not statistically significant.

No statistically significant correlations were found between vitamin D, weight, BMI, and lipid profile in the male participants of the study group.

According to data from the literature review, there could be an association between vitamin D levels and glycaemic control as reflected by the glycated haemoglobin (HbA1c) index.

In this regard, in our study, we hypothesized that as serum cholecalciferol concentration decreases below normal, HbA1c increases. To prove this hypothesis, we used correlation analysis (Pearson's coefficient).

We found a weak inverse correlation between the two parameters: a decrease in vit. D levels was associated with a rise in glycated haemoglobin. Correlation coefficient: r = -0.237; H_0: $\rho \geq 0$ ('ro' = correlation coefficient for the population); H_1: $\rho < 0$; p-value = 0.052 < **$\alpha = 0.10$;** p-value = 0.052 > **$\alpha = 0.05$;** r = -0.237.

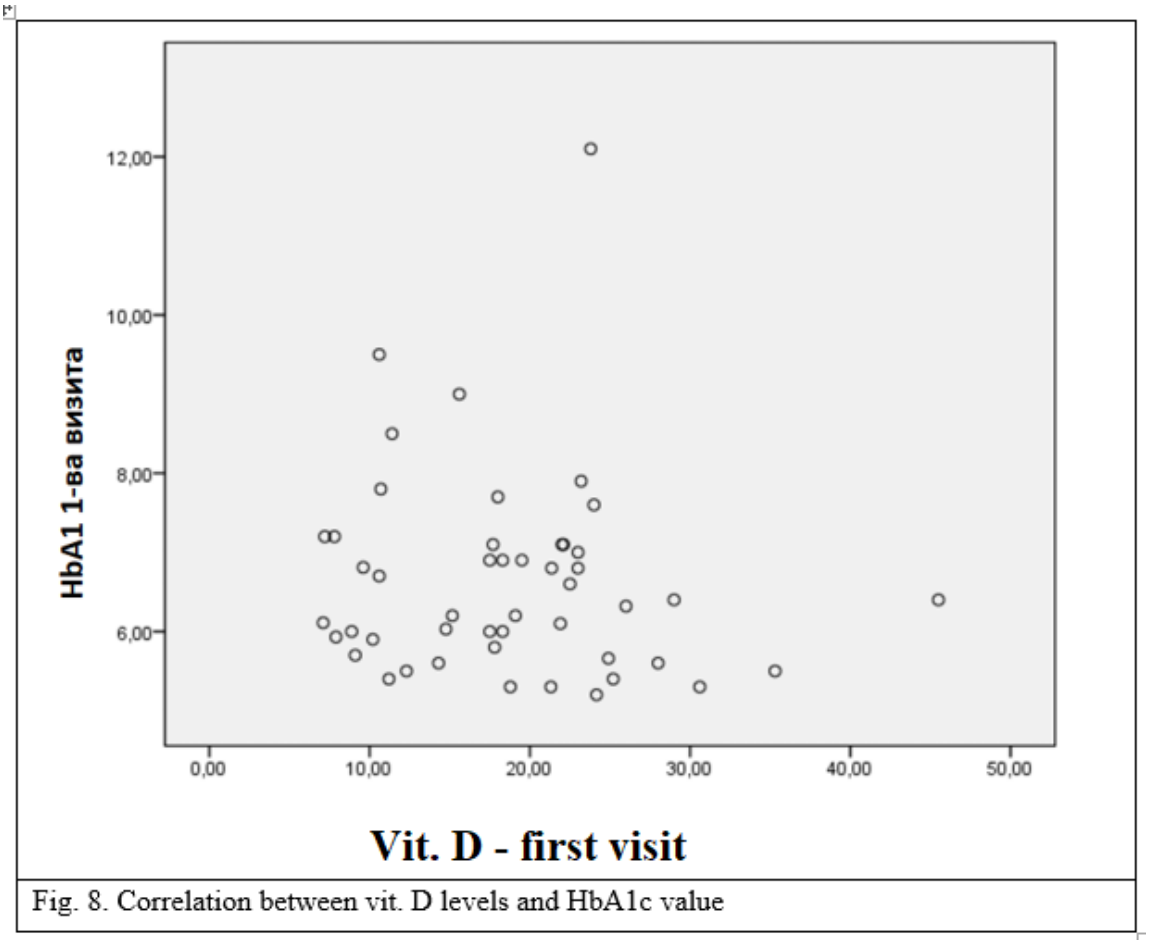


Fig. 8. Correlation between vit. D levels and HbA1c value

Conclusion: At significance level (0.05), i.e. assuming a 5% error, there is no reason to reject the assumption that the change is statistically insignificant. Therefore, at a 5% risk of error, no statistically significant inverse correlation is found. However, at a significance level of $\alpha = 0.10$, the null hypothesis (H_0) has reason to be rejected, and the correlation coefficient is statistically significant.

With the small number of participants in the study, T2DM patients may have a low vitamin D level and a normal glycated haemoglobin level (good glycemic control). However, in a larger group of participants, it is possible to confirm the hypothesis that vitamin D deficiency is a factor that autonomously and independently impairs glycemic control.

Nutritional Pattern Analysis in T2DM Patients in whom the micronutrient status was examined

It is widely acknowledged that there is currently no successful approach for managing type 2 diabetes without patients changing their eating habits [**Petroni et al. 2021**]. In this regard, we attempted to analyse the eating patterns of the patients included in the study. The study participants completed a three-day diary of foods consumed (types of food, approximate quantity, times of meals) and a questionnaire on the frequency of foods consumed within a month.

Nutritional Pattern Analysis Based on Three-Day Recall

The patients submitted twice a diary of food intake over at least three days. From the submitted diaries, we analysed the protein intake, fibre intake, vegetable consumption, carbohydrate quality (normal or high glycemic index), and eating pattern (single, double, triple, or multiple meals). Data analysis was performed using a non-parametric Chi-square test.

From the food diaries after **the first visit**, it can be concluded that 41.7% of the patients did not consume food with a sufficient amount of protein, and 83.3% of them consumed foods with a high glycemic index. Regarding the consumption of vegetables, fruits and fibre, it can be concluded that 58.3% of patients do not consume a sufficient amount of fresh vegetables and fruits, and 56.3% of patients do not consume foods with a sufficient amount of dietary fibre (Table 11).

Assessment of dietary protein, vegetable, fibre and carbohydrate consumption – first and second visits			
χ^2=3.86, df=1, p=0.049		**Protein**	
		Insufficient amount in the diet	**Sufficient amount in the diet**
First visit	n	20	28
	%	41.7%	58.3%
Second visit	n	11	37
	%	22.9%	77.1%
χ^2=16.2, df=1, p=0.001		**Carbohydrates**	
		Normal GI	**High GI**
First visit	n	8	40
	%	16.7%	83.3%
Second visit	n	27	21
	%	56.3%	43.8%
χ^2=12.5, df=1, p=0.001		**Vegetables/fruits**	
		Insufficient	**Sufficient**
First visit	n	28	20
	%	58.3%	41.7%
Second visit	n	11	37
	%	22.9%	77.1%
χ^2=11.2, df=1, p=0.001		**Fibres**	
		Insufficient	**Sufficient**
First visit	n	27	21
	%	56.3%	43.8%

Second visit	n	11	37
	%	22.9%	77.1%
Table 11			

After **the second visit**, the analysis of nutritional diaries shows an increase in the proportion of patients eating protein-rich foods (77.1%), a decrease in the consumption of high glycemic index foods (from 83.3% to 43.8%), and an increase in the share of patients who have included sufficient vegetables and fibre in their diet – 77.1%. However, these changes should be viewed critically because during the second examination, patients know what is expected of them, and it is very likely that they are adjusting their daily diet in the context of the results expected by the physician. For this purpose, it is necessary to compare the data from the three-day nutritional diary with the food frequency questionnaire. It turns out that when analysing the consumption of certain food products on a monthly basis, there is no statistically significant change in the consumption of vegetables and fruits. The only statistically significant change is observed in the consumption of legumes and meat (increase in the frequency of intake of these products).

Regarding the dietary regimen, there is a statistically significant increase in the proportion of patients who have breakfast between the first and second visits (Table 12).

Dietary change – first and second visits			
χ^2=0.487, df=1, p=0.001		**Breakfast**	
		No	**Yes**
First visit	n	14	34
	%	29.2%	70.8%
Second visit	n	11	37
	%	22.9%	77.1%
		Lunch	

		No	**Yes**
First visit	n	1	47
	%	2.1%	97.9%
Second visit	n	1	47
	%	2.1%	97.9%
		Dinner	
		No	**Yes**
First visit	n	0	48
	%	0.0%	100.0%
Second visit	n	1	47
	%	2.1%	97.9%
χ^2=0.253, df=1, p=0.615		**Night meal (after 10 pm)**	
		No	**Yes**
First visit	n	39	9
	%	81.3%	18.8%
Second visit	n	37	11
	%	77.1%	22.9%
χ^2=0.04, p=0.837		**Intermediate meals**	
		No	**Yes**
First visit	n	28	20
	%	58.3%	41.7%
Second visit	n	27	21
	%	56.3%	43.8%
Table 12			

In contrast, during the second visit, the share of patients reporting night eating (after 10 PM) increased from 18.8% to 22.9%.

Analysis of Patient Awareness Regarding Dietary Regimen

Due to the contradictory data from the three-day nutritional diary (recall) and the food frequency-intake questionnaire, as well as the lack of significant changes in the anthropometric indicators of the studied patients, an additional survey was conducted. Patients were asked to self-assess their awareness of the dietary regimen for type 2 diabetes. Moreover, they were asked whether they wanted to be additionally trained in proper dietary practices, or in the event of an unfavourable disease progression, whether they were more inclined to rely on changes in the medication treatment.

The survey analysis was performed using a non-parametric Chi-square test.

The conclusion is that more than half of the patients believe they have sufficient information about therapeutic nutrition for diabetes and **do not wish** to be additionally trained on how to eat properly. At the same time, almost half of them think that if their disease is not controlled well enough, they would rather change their medication therapy than modify their eating pattern. During the second visit, there was a slight but statistically insignificant increase in the number of patients who were more inclined to change their eating patterns rather than switch medication therapy.

During the second visit, an assessment was made of the extent to which each patient had changed their eating behaviour following the strategies employed during their sessions (Fig. 9).

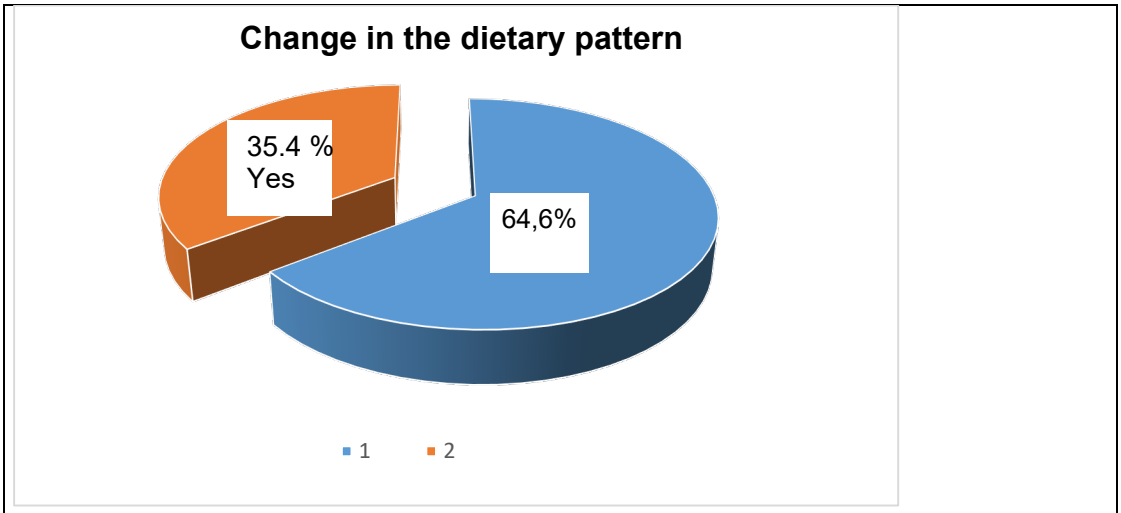


Fig. 9

Cooperation, expressed in changes to the dietary regimen and composition of consumed products, was observed in 17 patients (35.4%). In 31 patients (64.6%), no change in the eating patterns and composition of consumed products was observed.

Analysis of Nutritional Habits and Patients' Preferences Based on the Monthly Intake Questionnaire

For this purpose, a comparison was made between the two questionnaires (visits) using a non-parametric Chi-square test for categorical data. The assumption is that after the analysis of nutritional diaries and advice for changing the intake of certain foods, such a change will occur and will be recorded in the next survey. Differences are considered statistically significant at $p < 0.05$. Overall, a significant difference was observed only in the consumption of legumes and meat. The other changes in the frequency of food consumption between the two visits were not statistically significant.

Change in the food consumption – first versus second visit			
Products	χ^2	df	p-value
Pulses (beans, lentils, peas)	11.00	df=4	0.027
Meat (pork, veal, lamb, poultry)	12,94	df=4	0.012
Table 13			

Analysis of Nutritional Habits and the Presence or Absence of Vitamin B_{12} Deficiency

In this study, we attempted to analyse the presence or absence of vitamin B_{12} deficiency in patients who consume less than the recommended amount of meat products or do not consume meat, sausages, and fish at all.

The research hypothesis is that B_{12} levels are positively correlated with the frequency of consumption of meat products – meat, sausages, and fish. A Spearman correlation test was used – a non-parametric measure of dependency, where the average hierarchy of observations is calculated, and differences are squared and included in the formula. Classified correlations are a non-parametric alternative as a measure of dependency between two variables when we cannot apply the Pearson correlation coefficient.

When comparing vitamin B_{12} levels with the consumption of meat, fish, and sausages, no statistically significant correlations were found. This is likely due to the unchanged eating habits of the patients, which is evident from the survey on the consumption of various food products (Table 14).

Correlations between frequency of meat, fish, and sausage consumption and vitamin B_{12} levels			
Variable	**Meat**	**Fish**	**Sausages**
Meat	1.000	0.277	0.390**
Fish	0.277	1.000	-0.034

Sausages	0.390**	-0.034	1.000
Vitamin B12 (1st visit)	0.233	0.175	0.096
Vitamin B12 (2nd visit)	-0.245	-0.197	-0.270

Table 14

Vitamin B_{12} **supplementation** was significantly negatively associated with meat (rho = -0.381, p = 0.007) and sausage (rho = -0.384, p = 0.007) consumption. This suggests that meat-eaters are less likely to need vitamin B_{12} supplementation (Table 15).

Correlations between the frequency of meat, fish, sausage consumption and the need for vitamin B_{12} supplementation

Variable	**Meat**	**Fish**	**Sausages**
Meat	1.000	0.277 (p = 0.052)	0.390** (p = 0.004)
Fish	0.277 (p = 0.052)	1.000	-0.034 (p = 0.788)
Sausages	0.390** (p = 0.004)	-0.034 (p = 0.788)	1.000
Vitamin B_{12} (1st visit)	-0.381** (p = 0.005)	-0.221 (p = 0.126)	-0.384** (p = 0.005)
Vitamin B_{12} (2nd visit)	-0.187 (p = 0.213)	-0.041 (p = 0.746)	-0.091 (p = 0.490)

Table 15

After the second visit, correlations with meat (rho = -0.187, p = 0.202) and sausages (rho = -0.091, p = 0.538) are no longer significant, which may mean that factors such as overall diet and adaptation to supplement intake reduce this dependency.

Analysis of Nutritional Habits and the Presence and/or Absence of Magnesium Deficiency

Consumption of fruits and nuts has a positive correlation with magnesium levels during the first visit (rho = 0.297, p = 0.041 for fruits; rho = 0.328, p = 0.023 for nuts), which supports the idea that these foods are an important source of magnesium (increased consumption of these foods raises magnesium levels) (Table 16).

Correlations between frequency of consumption of fruits, vegetables, nuts, legumes and magnesium levels				
Variable	Vegetables	Fruits	Nuts (almonds, walnuts, etc.)	Pulses (beans, lentils, etc.)
Vegetables	1.000	0.469**	0.178	0.126
Fruits	0.469**	1.000	0.186	0.142
Nuts	0.178	0.186	1.000	0.155
Pulses (Bean foods)	0.126	0.142	0.155	1.000
Mg2+ (1st visit)	0.200	0.297*	0.328*	0.196
Mg2+ (2nd visit)	0.101	0.218	0.169	0.113
Table 16				

Correlation between Vitamin B_{12} Levels and Diabetic Polyneuropathy Control

Verification of the Hypothesis about the Correlation between B_{12} Deficiency and Diabetic Polyneuropathy

Vitamin B_{12} deficiency can manifest with peripheral neuropathy symptoms. Pathophysiologically, vitamin B_{12} deficiency can cause demyelination, manifested by white matter brain damage and peripheral neuropathy. Symptoms

vary depending on the affected nerve: pain, tingling, numbness, loss of sensation, reduced motor activity, or reduced muscle mass.

In this context, our study aimed to investigate the extent to which patients in the studied group with diagnosed diabetic neuropathy (registered in the outpatient sheet with code G63.2 with prescribed alpha-lipoic acid) have a registered vitamin B_{12} deficiency.

Hypothesis testing that diabetic polyneuropathy is associated with vitamin B_{12} deficiency

The number of patients with clinical manifestations of diabetic neuropathy was compared with the number of patients with vitamin B_{12} deficiency. The results are shown in Table 17.

Diabetic neuropathy versus patients with B_{12} supplementation – first visit					
			Vit. B_{12} supplementation First visit		Total
			There isn't	There is	
G63.2 First visit	There isn't	Number	15	19	34
		Estimated number	12.8	21.3	34
	There is	Number	3	11	14
		Estimated number	5.3	8.8	14.0
Total		Number	18	30	48
		Estimated number	18,0	41.7%	100%
Table 17					

At the first visit, 14 patients were registered with diabetic polyneuropathy. Of these, 11 were found to be vitamin B_{12} deficient and were prescribed supplementation. Vitamin B_{12} deficiency was not found in 3 patients, and supplementation was not prescribed.

Statistical analysis showed that the two variables were independent (Table 18).

χ^2 tests for independence: to compare the correlation between diabetic polyneuropathy (G63.2) and vitamin B12 deficiency

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,178[a]	1	,140		
Continuity Correction[b]	1,318	1	,251		
Likelihood Ratio	2,300	1	,129		
Fisher's Exact Test				,196	,125
Linear-by-Linear Association	2,133	1	,144		
N of Valid Cases	48				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.25.

b. Computed only for a 2x2 table

Table 18

Of the 14 patients registered with diabetic polyneuropathy, vitamin B_{12} deficiency was found in 11, while three patients had no vitamin B_{12} deficiency despite having registered manifestations of diabetic polyneuropathy.

A Chi-square test for independence was applied in the statistical analysis. According to the null hypothesis (H_0), the two variables are statistically independent, meaning a patient can have diabetic polyneuropathy (G63.2) and simultaneously have or not have vitamin B_{12} deficiency. According to the alternative hypothesis (H_1) – the two variables are statistically dependent – i.e., if there is diabetic polyneuropathy (G63.2), there will also be vitamin B_{12} deficiency. A significance level of $\alpha = 0.05$ was chosen, meaning a 5% probability of error; p-value = $0.140 > \alpha = 0.05$.

Result: The two variables are independent, and there is no connection between them.

Conclusion: Diabetic neuropathy can occur with vitamin B_{12} deficiency, but such a deficiency is not mandatory for diabetic neuropathy to manifest. Actually, this result was expected, considering the multifactorial pathogenesis of diabetic neuropathy.

Verification of the Hypothesis for Reducing Diabetic Polyneuropathy Symptoms upon Correcting Vitamin B_{12} Deficiency

There is no clarity in the research community on whether vitamin B_{12} supplements can help in the treatment of already-diagnosed diabetic neuropathy. A small, double-blind, placebo-controlled study [**Didangelos T, et al., 2021**] did not find a convincing difference in diabetic neuropathy symptoms among patients who took vitamin B_{12} at a dose of 1000 μg/daily for one year. In this context, our study attempted to verify the hypothesis that correcting vitamin B_{12} deficiency affects diabetic neuropathy symptoms.

The results of the statistical analysis are shown in Table 19.

Diabetic neuropathy versus patients with B12 supplementation – second visit					
			Vit. B12 supplementation Second visit		Total
			Няма	Има	
G63.2 First visit	There isn't	Number	8	23	31
		Estimated number	8.4	22.6	31.0
	There is	Number	5	12	17
		Estimated number	4.6	12.4	17.0
Total		Number	13	35	48
		Estimated number	13.0	35.0	48

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,072[a]	1	,788		
Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,072	1	,789		
Fisher's Exact Test				1,000	,522
Linear-by-Linear Association	,071	1	,790		
N of Valid Cases	48				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.60.

b. Computed only for a 2x2 table

Table 19. P-value = 0.135 > α = 0.05

Result: With p-value = 0.135 > α = 0.05, there are no grounds to reject the null hypothesis (H_0) that the two variables are statistically independent in favour of the alternative hypothesis (H_1) that the two variables are statistically dependent. The two variables are independent, and there is no correlation between them. NB: In one of the cells, the expected frequency is less than 5. The analysis cannot be conducted, and no conclusions can be drawn from its application due to the small sample size.

Conclusion: From the studied group of patients with existing manifestations of diabetic neuropathy (G63.2), it is not clear whether correcting vitamin B_{12} deficiency will lead to a reduction in diabetic polyneuropathy symptoms.

However, it must be considered that, to the extent that vitamin B_{12} deficiency definitely plays a role in the pathogenesis of diabetic neuropathy, prevention of such a deficiency may play a role in delaying the development of clinical manifestations of the disease. Nevertheless, we cannot prove this in our study due to the small number of patients included in the study.

Recommendations for searching and monitoring (screening and monitoring) micronutrient status in patients with type 2 diabetes

Based on the findings for the micronutrient status of type 2 diabetes patients and the subsequent statistical analysis, the following recommendations can be derived.

Type 2 diabetes patients on metformin treatment have a high frequency of micronutrient deficiencies, particularly vitamin D, vitamin B_{12}, and magnesium, and to a lesser extent – folic acid.

The widespread vitamin D deficiency in society and specifically among type 2 diabetes patients on metformin necessitates targeted detection and appropriate correction.

The recommended doses of 2000 IU of vitamin D for deficiency correction may prove insufficient, so a repeat examination after 3 months and dose adjustment are necessary.

The frequency of vitamin B_{12} deficiency in type 2 diabetes patients on metformin is high.

It is necessary to test the baseline vitamin B_{12} level for each newly diagnosed diabetic patient for whom metformin therapy is expected to be prescribed.

Detecting vitamin B_{12} values close to the lower limit (300 pg/ml) before or at the beginning of metformin treatment can be interpreted as a sure sign that the patient will develop vitamin B_{12} deficiency in the future, so such a deficiency needs to be tested, identified, and compensated.

Detecting a high mean corpuscular volume (MCV > 96.0 fL) when examining a type 2 diabetes patient on metformin should be interpreted as a probable marker

of vitamin B_{12} deficiency. In such cases, testing should be appointed, deficiency should be established and corrected.

Patients who regularly consume meat (but not meat products and fish) have a lower likelihood of developing vitamin B_{12} deficiency.

Compensation with a standard oral dose of 1000 μg Cyanocobalamin leads to correction in half of the patients. For the remaining group of patients, continuation of the therapy is necessary. Considering that the transport system for vitamin B_{12} becomes saturated, increasing the oral dose is pointless. In case of persistent deficiency, transition to parenteral supplementation or simply continuing oral supplementation for a longer time is necessary.

During ongoing vitamin B_{12} supplementation, indirect markers (MCV, patient record on meat consumption) are not sufficient grounds to assume that the deficiency is restored. Repeated testing of vitamin B_{12} levels are necessary.

Diabetic neuropathy may occur with vitamin B_{12} deficiency, but such a deficiency is not mandatory for neuropathy manifestations. Vitamin B_{12} deficiency can manifest with numerous other symptoms, including mental symptoms – emotional lability, mood changes, and memory disorders.

With existing diabetic neuropathy manifestations (G63.2), it is unclear whether correcting vitamin B_{12} deficiency will reduce symptoms. However, since vitamin B_{12} deficiency definitely plays a role in diabetic neuropathy pathogenesis, preventing such a deficiency may help delay the development of clinical disease manifestations.

According to literature data and in our experience, correction of individual deficiencies is more appropriate than prescribing combined preparations containing a combination of vitamins.

In the patient group we studied, folic acid deficiency occurs 2 times more frequently than described in literature sources. Even after correction, a small percentage of patients continue to have folic acid deficiency, which must be compensated, including using its methylated form.

Magnesium deficiency in type 2 diabetes patients on metformin is an independent risk factor for cardiovascular diseases. In this patient group, the deficiency occurs in over 50% of cases. Detecting serum magnesium levels < 0.80 mmol/L in type 2 diabetes patients is a reliable indicator of a serious deficiency that requires correction. When a deficiency is detected, it must be corrected with a sufficiently high magnesium dose (3x2 magnesium orotate tablets), after which a maintenance dose of 1–2 tablets daily should be continued. If the correction dose is insufficient, the likelihood of not achieving correction is high.

Regular consumption of legumes in our sample of type 2 diabetes patients on metformin is associated with higher magnesium levels.

Discussion

Metformin is one of the most widely used medications for blood glucose control in type 2 diabetes patients. Its benefits include good glycemic control, improved insulin sensitivity, low risk of hypoglycaemic episodes, protection from cardiovascular diseases, and body weight reduction. In the patients we studied, there was no statistically significant reduction in body weight, body mass index (BMI), and waist circumference. This result particularly emphasises the crucial role of developing a correct, convenient, and easy-to-follow diet for T2DM patients. Despite good laboratory control of blood glucose and glycated haemoglobin, the presence of excessive fat mass and visceral adipose tissue represents an independent risk factor for the deterioration of patients' health condition, that should be highlighted during routine medical check-ups.

In the studied patient group, statistically significant micronutrient deficiencies were found: vitamin B_{12} deficiency (68.75%), vitamin D deficiency (58%), magnesium deficiency (66.67%), and folic acid deficiency (16.7%). Iron deficiency was also found, but with lower frequency (14.59%). In this regard, the usual clinical and laboratory indicators for monitoring diabetes patients (body weight, anthropometry, lipid profile, glycated haemoglobin) prove to be insufficient for a good clinical assessment of the type 2 diabetes patient.

The most well-known micronutrient deficiency in patients treated with metformin is vitamin B_{12} deficiency. According to literature data, the frequency of this deficiency is between 6% and 30%. According to the results of some studies, vitamin B_{12} levels are inversely proportional to the duration of metformin therapy [**Beulens JW et al., 2015**].

In our study, the frequency of vitamin B_{12} deficiency is almost twice as high as reported in literature, namely 68.75%. The small group of study participants did

not allow us to investigate and establish whether there is or there isn't a statistical correlation between metformin dose, duration of metformin treatment, and the degree of vitamin B_{12} deficiency. There are no clear recommendations in the literature about the frequency of control testing for B_{12} levels. It is established that reduced absorption and vitamin B_{12} levels after metformin use typically begin as early as the 4th month after initiating treatment with this medication [**Wulffele M, et al., 2003**]. Therefore, within the first six months after the start of metformin treatment, it is recommended to order the first test of its level.

According to the results of our study, vitamin B_{12} deficiency can be suspected by an increase in erythrocyte volume (MCV) during a routine blood count testing for other reasons. Our study showed a statistically significant increase in the mean corpuscular volume (MCV) in patients with vitamin B_{12} deficiency. This finding can be used as a reference for prescribing a vitamin B_{12} level test. However, MCV cannot be used as a reliable marker for potential deficiency correction.

In our study, prescribing a standard oral dose of 1000 μg Cyanocobalamin leads to correction in half of the patients. Considering that the transport system for vitamin B_{12} becomes saturated, we believe that increasing the oral dose in case of insufficient deficiency correction is pointless. In this case, switching to parenteral supplementation or simply continuing oral supplementation for a longer time is justified.

The duration of supplementation remains a matter of debate without a clear resolution.

In one double-blind, placebo-controlled study on diabetic neuropathy, vitamin B_{12} supplementation continued for one year. Only after maintaining normal vitamin B_{12} levels for a year, according to the study results, improvements were noted in neuropsychiatric parameters, quality of life, sudomotor function and pain symptoms related to diabetic neuropathy [**Didangelos T. et al. 2021**]. In our

study, specifically for diabetic polyneuropathy, we did not observe symptom improvement with vitamin B_{12} deficiency correction, but the number of patients studied was small, and the observation period was much shorter.

Our research does not offer a definitive conclusion regarding the duration for which vitamin B12 supplementation should be maintained and at what intervals patient follow-up should be performed. After the study's completion, most patients were followed up every 6 months for two years, and their follow-up continues. A pattern emerges where, after restoring vitamin B_{12} levels, stopping oral supplementation after about 6 months leads to a recurring vitamin B_{12} deficiency.

Another interesting question can be raised here: is there a connection between vitamin D deficiency and the rate of vitamin B_{12} deficiency development?

It turns out that B_{12} absorption through the small intestine mucosa is calcium-dependent, and metformin antagonizes the physiological action of calcium ions on the small intestine mucosa. In this context, data from a study on an animal model for the application of vitamin D, calcium, and metformin in treating non-alcoholic liver steatosis are interesting [**Shojaei Z. et al. 2021**]. According to the study results, adding vitamin D and calcium to metformin treatment leads to improvement of high-lipid and fructose-rich liver steatosis through a mechanism independent of insulin secretion and adenosine monophosphate-activated protein kinase (AMPK).

Regarding vitamin D deficiency levels in Bulgaria, we can refer to an epidemiological study from 2013 covering 2 032 people, of whom 1 076 were women and 956 were men [**Borisova A-M. et al.., 2013**]. According to its results, "deficiency" of vitamin D was found in 21.3% of those studied, and "insufficient amount" in 54.5% of those studied. There is no literature data suggesting that metformin treatment itself leads to a reduction in vitamin D levels. Of the

medicinal causes of vitamin D deficiency, treatment with corticosteroids, anti-epileptic drugs, orlistat, and cholestyramine are highlighted. However, numerous studies confirm that vitamin D deficiency is widespread among diabetics in a greater extent than among the general population.

Deficiency compensation requires patience and dose monitoring. Doses of 2000 IU Cholecalciferol, equivalent to 50 μg, were able to compensate only half of the patients; 5000 IU Cholecalciferol, equivalent to 125 μg, did not lead to manifestations of vitamin D hypervitaminosis or calcium metabolism disorders in any of the patients.

The "sufficiency level" of vitamin D in type 2 diabetes patients must also be discussed. Worldwide, studies are being conducted on vitamin D target values for diabetes prevention. According to a randomised clinical study involving 2 362 participants over 2.5 years, to make prevention efforts effective in order to halt the advancement of prediabetes into diabetes, vitamin D levels > 40 ng/ml must be maintained [**Chatterjee R. et al. 2023**].

In our study, we find that supplementation with 2000 IU of vitamin D_3 is sufficient for only ½ of the patients with established deficiency (levels < 20 ng/ml or 50 nmol/l). For the others, a personalized approach with level monitoring and supplementation dose correction is necessary.

Patient observation and repeated tests show that attempts to correct the 2000 IU daily doses recommended by the Bulgarian Society of Endocrinology do not lead to levels above 20 ng/ml in almost half of T2DM patients. According to EFSA data, a quantity of 4000 IU (100 μg) can be used daily without problems by adults, elderly people, and children over 11 years old. For mature individuals and elderly people with obesity, the highest average daily vitamin D intake that does not pose a risk of side effects is 10 000 IU (1 250 μg). In our study, half of the highest suggested dosage was applied without observing side effects of overdosing.

According to literature data, low vitamin D levels in diabetics are associated with poorer metabolic syndrome control and worse metabolic control. Therefore, patients can have poor glycemic control and low vitamin D levels, but good glycemic control does not exclude vitamin D deficiency, as cholecalciferol status is just one of many factors affecting the progression and course of T2DM.

Metformin directly reduces folic acid levels, and as the applied metformin dose increases, the likelihood of folate deficiency increases. Metformin treatment for more than 6 weeks leads to a decrease in folate levels in 7.02% of patients [**Mustafa Sahin et al., 2007**].

In our study, we found twice higher frequency of folic acid deficiency (16.67%). Although the frequency of this deficiency is much lower than the deficiency of vitamin B_{12}, magnesium, and vitamin D, the problem should not be underestimated. Adding folic acid for at least 12 weeks significantly reduces fasting insulin levels ($p = 0.002$), with the effect being better at higher doses (at dose ≥ 5 mg/d; $p = 0.001$). A dose-dependent positive effect was also found regarding HOMA-IR ($p < 0.001$) [**Asbaghi O et al., 2021**].

In the Bulgarian recommendations for good clinical practice in diabetes, folic acid is mentioned only as a necessary supplement for pregnant women with diabetes at a dose of 400 μg daily. In some, but not all, international guidelines, a high dose of folic acid (5 mg daily) is recommended for all women in the preconception period with a history of previous diabetes. The recommendation is based on a consensus opinion and reflects the increased risk of neural tube defects in pregnant women with previous diabetes [**Perera N et al., 2023**].

In our study, supplementation with 5 mg of folic acid leads to rapid restoration of its levels. At this dosage, deficiency compensation is achieved in 75% of patients with the BDA-registered medication.

According to literature data, vitamin B_{12} and folic acid deficiencies are associated with high homocysteine levels and, consequently, negative effects on the individual's physical health. We could not confirm nor dismiss this hypothesis as very few patients agreed to test homocysteine levels.

Type 2 diabetes is associated with both extracellular and intracellular magnesium (Mg2+) deficiency. Patients are considered overtly hypomagnesemic at serum Mg2+ concentrations $\leq$ 0.61 mmol/L; concentrations $\leq$ 0.75 mmol/L can be considered preclinical hypomagnesemia. Mg2+ deficiency may be present without hypomagnesemia. According to literature data, between 25 and 38% of diabetics have hypomagnesemia [De Lordes et al. – 230].

In our study, we found magnesium deficiency in 66.7% of patients. The high frequency of magnesium deficiency may be related to the higher age of the patients we observed. Intestinal magnesium absorption is related to vitamin D plasma content. Therefore, the higher its value, the greater the absorption of this mineral. Very high calcium ion and phosphate content can lead to decreased magnesium absorption. On the other hand, all enzymes that metabolize vitamin D seem to require magnesium, which acts as a cofactor in enzymatic reactions in the liver and kidneys. Therefore, it is important to maintain an optimal magnesium level to obtain the optimal benefits of vitamin D.

Diagnosing and correcting magnesium deficiency in diabetic patients is clinically justified. The increased risk of developing impaired glucose tolerance and/or overt type 2 diabetes in people with magnesium deficiency suggests potential benefits of adding magnesium in T2DM patients.

In more than half of the participants in our study (56.25%), compensation of Mg2+ deficiency is achieved with supplementation of 1 tablet of magnesium orotate (500 mg, or equivalent to 32.8 mg of magnesium). In 43.75% of patients with deficiency, it is not compensated with one tablet daily, and the dose must be

titrated to two to three tablets daily. No magnesium toxicity was observed in any of the patients.

Iron deficiency (values < 6.0 μmol/L) was found in 14.59% of study participants. According to the MASTEMIND study, metformin use is associated with an early risk of anaemia in individuals with T2DM. In our study, iron deficiency is quickly overcome with supplementation, but during continued patient monitoring, new patients with iron deficiency are identified. The small number of participants does not allow us to claim that iron deficiency is specifically related to metformin.

No statistically significant deviations from reference values were found for serum potassium, sodium, calcium, and phosphorus, regardless of the presence or absence of diuretic therapy.

According to our study data, patients' micronutrient status does not statistically significantly affect participants' lipid profiles. The therapeutic regimen, including metformin and diet, does not lead to changes in lipid parameters during the observed period.

The number of participants in the study was insufficient to statistically significantly confirm the deterioration of glycemic control (HbA1c) with vitamin D deficiency and improvement of glycemic control with correction of this deficiency. However, at a significance level of $\alpha=0.10$, the null hypothesis (H_0) has grounds to be rejected, and the correlation coefficient is statistically significant. This means that if the same study is conducted with a larger group of patients, it is possible to confirm the alternative hypothesis.

Adequate nutrition and appropriate physical activity for the specific individual are key components in the control and treatment of type 2 diabetes.

The diabetes diet must be individualised, realistic, accessible, liberalised under control depending on clinical and laboratory results, flexible, and motivated. The patient must be educated. The nutritional regimen should limit the intake of proven harmful foods for the patient (foods with high glycemic index, atherogenic foods), reduce glycemic load, and provide sufficient protein and vegetables.

Research findings indicate that consuming sufficient dietary protein as a percentage of caloric intake is an important factor for good glycemic control [**Gannon, MC et al. 2004**]. It is recommended that vegetables be included in every meal. Adding just 20 grams of additional vegetable portions per day reduces mortality in diabetics by 20%.

From the data obtained by analysing the dietary habits of the studied patients, it can be concluded that those who regularly consume meat have a lower risk of developing vitamin B_{12} deficiency. In cases of already developed deficiency, changes in the dietary habits are not sufficient and pharmacological supplementation is necessary.

Fruit and nut consumption positively correlated with magnesium levels during the first visit (rho = 0.297, p = 0.041 for fruits; rho = 0.328, p = 0.023 for nuts). The result supports the idea that these foods are an important source of magnesium (increased consumption of these foods is associated with increased magnesium levels).

According to the self-assessment of the study participants, they are "sufficiently well-educated" on how to eat while managing diabetes. At the same time, almost half of them think that if their condition is not controlled well enough, they would rather change their medication therapy than change their eating pattern.

Analysis of the dietary diary data and food consumption survey of patients participating in the study showed a deficit in their awareness regarding the

importance of therapeutic nutrition. Despite their confidence in their eating practices, it turns out they are not following a healthy diet.

To overcome their information deficit, three tools were developed: a questionnaire on the awareness of type 2 diabetes patients regarding nutrition, a questionnaire on the dietary habits of type 2 diabetes patients, and a simplified guide to healthy eating for T2DM patients with a checklist.

Conclusions

1. In T2DM patients treated with metformin, vitamin D deficiency is found in 58% of those studied.
2. Supplementation with 2000 UI vitamin D_3 successfully compensates for this deficiency in only half of the patients. For the others, the application of vitamin D_3 in doses of 5000 UI daily is necessary.
3. In T2DM patients treated with metformin, vitamin B_{12} deficiency is found in 68.75% of those studied.
4. Oral supplementation with 1000 µg Cyanocobalamin achieves correction in only half of those studied within three months. For the rest, transition to intramuscular administration of Cyanocobalamin is necessary.
5. In T2DM patients treated with metformin, folate deficiency is found in 16.67% of those studied.
6. Correction with 5 mg of folic acid daily successfully overcomes the deficiency in 75% of patients.
7. In T2DM patients treated with metformin, magnesium deficiency is found in 66.67%.
8. To achieve correction, it is necessary to prescribe 3 tablets of magnesium orotate daily.
9. There is a deficit in the awareness of T2DM patients regarding the importance of therapeutic nutrition.

10. Tools have been developed to improve this awareness and to enhance the efficiency of the general practitioner when dealing with T2DM patients.

Conclusions and Recommendations to the Institutions

To the National Association of General Practitioners in Bulgaria (NAGPBG)

Patients with T2DM treated with metformin have a twofold increased risk of micronutrient deficiencies – vitamin D, vitamin B_{12}, folic acid, and magnesium. These deficiencies can be asymptomatic or oligosymptomatic but have a destructive effect on patients' health in the long term. When initiating metformin treatment for a newly diagnosed T2DM patient, it is justified to inform the patient about the high risk of deficiency in these micronutrients and the need to periodically monitor their levels. When a deficiency is detected, an appropriate dosage needs to be administered without delay to address the issue.

Correction of vitamin D deficiency with the recommended dose of 2000 UI is possible only in half of the patients with detected vitamin D deficiency. After a three-month correction attempt, a control test is recommended. If the target value of 20 ng/ml is not reached, the dose can be increased to 5000 UI of vitamin D_3 daily without the risk of vitamin D hypervitaminosis.

Correction of vitamin B_{12} deficiency is carried out with oral Cyanocobalamin at a dose of 1000 μg daily for patients who have no history of chronic gastrointestinal diseases, systemic intake of proton pump inhibitors and other antacid agents. If no correction of the deficiency is found at a control examination after 3 months, increasing the oral dose is pointless because the transport mechanisms for vitamin B_{12} become saturated. In these cases, parenteral administration is initiated. If the patient has a gastrointestinal disease, correction with the parenteral form of Cyanocobalamin is recommended.

Correction of folic acid deficiency with 5 mg daily (5000 µg) is most often completely sufficient. In rare cases, after three months of correction, the dose may be increased to 10 mg. It should be noted that dietary supplements with folic acid contain only 400 µg per tablet, so it is advisable to prescribe the medication.

Magnesium deficiency is very common in diabetics, with the frequency in Bulgaria being twice as high as described in literature sources. Patients with obesity, improper eating patterns, and those chronically treated with diuretics are especially at risk of magnesium deficiency. Magnesium deficiency is an independent risk factor for cardiovascular complications in T2DM patients treated with metformin.

Correcting magnesium deficiency is best started with magnesium orotate 3x2 tablets to quickly replenish magnesium stores.

It is crucial to implement a dietary intervention for all patients with type 2 diabetes mellitus who are receiving metformin therapy.

To assess the patient's basic knowledge about their disease and the importance of nutrition. A convenient tool for this is the "Questionnaire on Patient Awareness of Dietary Nutrition in Type 2 Diabetes" developed by us and intended for use by general practitioners (GPs).

To assess the patient's readiness to be educated on how to eat properly with T2DM diagnoses. A convenient tool for this is the "Nutrition Habits Survey for T2DM Patients" developed by us.

During each follow-up examination, no more than 1–2 questions from the survey should be discussed and no more than 1–2 achievable recommendations should be provided to the patient. Consistency is more important than overwhelming the patient with information they cannot assimilate. Once it is determined that the

patient is ready to cooperate in order to change their dietary pattern in the right direction, the patient's efforts can be supported with the "Short Guide to Nutrition in Type 2 Diabetes" created by us.

Considering everything mentioned earlier and the findings from our research, we suggest the following:

- To promote the results of this study at the forums of the National Association of General Practitioners (NAGPBG) to inform general practitioners about the risk of micronutrient deficiencies in T2DM patients on metformin treatment.
- To create an educational module on the subject at the NAGPBG website in the Electronic Academy for Continuing Education section.

To the National Health Insurance Fund (NHIF)

T2DM patients on metformin treatment are primarily followed up by general practitioners. In patients starting metformin treatment, deficiencies are found in Vitamin B_{12} (68.75%), Vitamin D (58%), and Magnesium (66.67%). Folic acid deficiency is less common. The tests included in the standard package for follow-up monitoring of patients with Type 2 Diabetes are insufficient for objectively tracking the patients' condition. These micronutrient tests are not included in the package for which GPs can provide referrals (neither for acute conditions nor for follow-up monitoring). Timely detection and correction of these deficiencies can prevent numerous adverse health scenarios for T2DM patients that would be much more expensive for society than the cost of the test itself.

We recommend:

- To include the cost of testing for vitamin B_{12}, vitamin D, folic acid, and magnesium in the package reimbursed by the National Health Insurance Fund (NHIF) for T2DM patients on metformin treatment, once annually.
- If a deficiency is found, the patient should have the right to at least one reimbursed by NHIF test per year for the respective micronutrient.

Taking into account all the details discussed and the conclusions drawn from our study, we propose to the NHIF to include the indicators vitamin B_{12}, vitamin D, folic acid, and magnesium in the package of tests reimbursed by NHIF for T2DM patients on metformin, once annually. If a deficiency is found, the patient should have the right to at least one reimbursed by the NHIF test per year for the respective micronutrient.

Contributions

Original scientific contributions

For the first time in Bulgaria, an assessment of the micronutrient status of patients with Type 2 Diabetes on metformin treatment in the outpatient practice has been made.

Significant differences in the frequency of micronutrient deficiencies were found. The study reveals twice higher frequency of vitamin B_9 deficiency and magnesium and several times higher frequency of vitamin B_{12} in T2DM patients on metformin monotherapy compared to those reported in literature sources.

It was established that the standard supplementation plan is not sufficiently effective for a large number of patients, and a correction scheme for severe deficiencies has been proposed.

Based on the analyses, it was found that for patients on metformin treatment already with a deficiency of vitamins B_9, B_{12}, and magnesium, nutritional intervention expressed in increased consumption of certain food groups is not sufficiently effective, and adequate supplementation in an appropriate dosage is necessary to optimise micronutrient levels.

Confirmatory and practical contributions

The main micronutrient deficiencies in type 2 diabetes patients on metformin monotherapy have been confirmed: vitamin D, vitamin B_9, vitamin B_{12}, and magnesium.

A high frequency of vitamin D (58.3%), vitamin B_{12} (68.75%), vitamin B_9 (16.67%) deficiency, and magnesium (66.67%) deficiency among T2DM patients in the outpatient practice is confirmed. This result provides grounds for active testing and monitoring of micronutrient deficiencies in these patients.

Guidelines for active screening of micronutrient deficiencies in T2DM patients during mandatory follow-up examinations have been proposed.

A recommendation for T2DM patients on metformin treatment is formulated that MCV laboratory marker, associated with vitamin B_{12} levels, should be used as a convenient and accessible marker for probable vitamin B_{12} deficiency.

A "Questionnaire on Patient Awareness of Dietary Nutrition in Type 2 Diabetes" had been developed. It is intended for use by general practitioners (GPs) to optimise their work in outpatient practice.

An educational material with questions and advice has been prepared, aimed at first-line physicians (GPs) to assess nutritional habits in T2DM patients and patients' attitudes towards changing their dietary habits.

A practical "Self-Control Nutrition Guide for Type 2 Diabetes Patients" has been compiled. It is a convenient educational material targeted at T2DM patients in general practitioners' practice, aiming to improve nutrition patterns and blood sugar control.

A proposal has been made to update the package of laboratory tests paid by the NHIF by adding three biochemical markers: vitamin D, vitamin B_{12}, and vitamin B_9- at least once annually for patients on metformin monotherapy.

A proposal has been made to increase awareness of micronutrient deficiencies among GPs, screening and monitoring T2DM patients, directed towards the National Association of General Practitioners in Bulgaria (NAGP)

Publications and Participation in Scientific Forums

Publications

● Mineral Metabolism, Electrolytes and Type 2 Diabetes, Enev I., Endocrinology, vol. XXVIII N 4/2023, pp 162-174

● Role of Fat-Soluble Vitamins in the Course and Control of Type 2 Diabetes, Enev I., Naydenova D., Science Endocrinology, Issue 3, 2023

● Role of Water-Soluble Vitamins in the Course and Control of Type 2 Diabetes, Enev I., Naydenova D., Issue 3, 2023

Participation in Scientific Forums

● Maritime Dietary Days Symposium, March 2023. Poster "Stress and Carbohydrate Nutrition", authors T. Mateva, I. Enev

● Alumni Club and Friends Symposium, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, 2021, Presentation on Vitamin B12 Deficiency in Pediatric Practice, Enev I., Naydenova D., Boykova T.

● Maritime Dietary Days Symposium, March 2023. Presentation "Popular Diets and Alternative Nutrition", Enev I.

● Maritime Dietary Days Symposium, March 2024. Presentation "Homeopathic Support in Insulin Resistance", Enev I.