Chapter 12

# Copper, Zinc and Magnesium in Non-Insulin-Dependent Diabetes Mellitus Treated with Metformin

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Additional information is available at the end of the chapter

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## 1. Introduction

Non-insulin-dependent diabetes mellitus is one of the most widely spread and severe disorder currently, being the fourth mortality reason, globally. The number of patients suffering from diabetes mellitus was reported to be over 200 million people worldwide, a big part of it being NIDDM patients. Divalent cations of macro and trace elements play important roles in human body.

Magnesium (Mg) is an important divalent cation mostly localized intracellular.

Zinc (Zn) is one of the most important trace elements in the body. It is required for over 300 different cellular processes, including enzyme activity, protein synthesis and intracellular signaling [1]. It is involved in homeostasis, in immune responses, in oxidative stress, in apoptosis and in ageing [2].

Copper (Cu) is an essential trace element, capable of fluctuating between the oxidized $Cu^{2+}$ and the reduced $Cu^{+}$ state, being co-factor for many enzymes. This divalent cation is involved in SOD activity. Copper has the capacity to form covalent bounds and it takes part in many redox processes. Copper ions are involved in generation of reactive oxygen species through Fenton reaction, having a pro-oxidant action. Moreover, the deficiencies and the excess of Cu are associated with specific clinical manifestations [3]. Diabetes mellitus is a chronic metabolic disorder associated with the increased free radical production leading to oxidative damage, and many of the pathological effects of copper overload are consistent with an oxidative damage to membranes or macromolecules. Variation in the concentrations of those divalent cations is important to oxidative stress to which the diabetic patient's organism is submitted.

Today there is an extensive range of anti-diabetic drugs for oral intake for type 2 diabetes. The main classes are different in their mechanism of action, safety profiles and tolerability and include: agents that stimulate insulin secretion (sulphonylureas), agents that reduce hepatic glucose production (biguanides), glinides, agents that delay digestion and absorption of carbohydrate (alpha-glucosidase inhibitors) or improve insulin action (thiazolidinediones).

Metformin is one of the most used drug in the treatment of NIDDM . This drug does not promote weight gain and has beneficial effects on several cardiovascular risk factors. Metformin is widely regarded as the drug of choice for most patients with type 2 diabetes. [4] This substance is the drug of choice and should be initiated immediately after diabetes is diagnosed. If monotherapy does not provide satisfactory glucose control, other oral anti-diabetic agents or insulin are added in combination. In the metabolic syndrome, metformin is also the drug of choice. [5] The action of oral anti-diabetics at the level of vascular endothelium and the cardio-protective effect play an increasing role in the choice of anti-diabetic agents.

## 2. The aim of the study

This research was performed to determine the plasmatic, cellular and urinary concentration of certain divalent cations in newly diagnosed NIDDM patients that have never received any kind of oral anti-diabetic drugs or insulin and at the same time to determine the metformin effect on intracellular magnesium concentration, plasma and urinary concentrations of different divalent cations.

Our study surveyed the status of magnesium, copper, zinc, calcium in plasma and urine and erythrocyte magnesium, influenced by metformin administration, in NIDDM adult patients from the moment of diagnosis and during the therapy with oral anti-diabetic medication.

At the same time, other biochemical parameters were determined, necessary to evaluate the carbohydrate metabolism and lipidic profile and different correlations were established between carbohydrate-lipidic metabolic parameters and plasma, urinary and cellular divalent cations concentrations.

## 3. Material and methods

The study was performed on a group of 30 adult patients with NIDDM, 18 males and 12 females, with ages between 30 and 60 years ( interval of 30-40y: 2 patients, 40-50y: 13 patients and 50-60y: 15 patients) that have never received any anti-diabetic medication, and a control group of 17 healthy subjects.

Patients with NIDDM, diagnosed in the Diabetes, Nutrition and Metabolic Diseases Clinic of Clinical County Emergency Hospital Constanta, according to the European Guide for Diabetes [6] were administered metformin (Siofor$^{R}$, Berlin Chemie) 500 mg x 2 times /day, together with a diet list comprising approximately 320 mg/day magnesium.

Subject selection criteria were: NIDDM adult subjects with absence of any previous treatment with oral anti-diabetic agents, insulin or trace elements to follow medical therapy with metformin yet without co-administration of other oral anti-diabetic agents. Non-including criteria were: pregnancy, hepatic cirrhosis, renal failure, psychosis, diuretic therapy, chronic diarrhea. An individual investigation protocol was elaborated containing the principal parameters to be investigated, and the study was performed according to the rules of clinical studies of the European Union, with the approval of the Ethical Committee of the University, and informed written consent was obtained from each subject included into the study.

The measured parameters were: glucose, HbA1c, creatinine, HDL, LDL, cholesterol, triglycerides, magnesium, copper, zinc and calcium in blood plasma; intra-erythrocyte magnesium; magnesium, copper, zinc and calcium concentration inurine/24 hours. Measurements were initially made before the administration of metformin, and after 3 months of therapy.

**Material and methods:** measurements were made by means of a Rx Daytona analyzer, a compact fully automated clinical analyzer, produced by Randox LTD Laboratories, UK, also used for all the other quantitative analyses, except erythrocyte magnesium.

Rx Daytona is an automated clinical chemistry analyzer with specific analyzer software, being an "in vitro diagnosis medical device" in compliance with IVD Directive and the EMC Directive of EU. The analyzer is recommended for general chemistry as photometric assay, immunology (latex reagents), with analysis methods: 1 point, 2 point end, 1 point rate, 2 point rate, and calibration options such as: factor, linear, Log Logit, exponential, spline, point to point; sample types for analysis can be: serum, plasma, urine, CSF, supernatants whole blood.

**Methods of measurements for plasma divalent cations:** venous blood samples from the subjects were collected in the morning after an overnight fast, into special blood collection tubes (vacutainer). There were used blood vacutainers with sodium heparin (green cup) for the measurement of zinc, copper, magnesium in plasma.

Plasma concentrations were determined through spectrophotometric method, using Randox kits, with plasma reference materials and controls, normal and abnormal level. Measurements were made by means of a Rx Daytona analyzer. Atomic absorption spectrophotometry (AAS) is the reference method for the determination of the cations in biological specimens, but it is not a usual method in clinical laboratories. The colorimetric methods used in clinical laboratories, especially for magnesium which is widely used, are fairly accurate and precise with a good correlation (r= 0.986) compared to AAS. Heparinized samples were centrifuged at 1500 g for 10 min to separate plasma from erythrocytes. Plasma was used for estimation of extracellular magnesium (without deproteinization), while trichloroacetic acid was added to precipitate proteins, the supernatant being used for analysis of zinc and copper. The measurements were initially made before the administration of metformin, and after 3 months of therapy.

**Methods of measurements for urinary divalent cations:** urine samples were collected in sterile, chemically clean universal containers, 30 ml, from urine / 24 h, the volume being measured. The samples were prepared in 30 minutes after collection, urine been transferred in test tubes. For estimation of calcium and magnesium the samples were used directly, for zinc and copper trichloroacetic acid was added to precipitate proteins.

The concentration of cations/24 h, was determined through spectrophotometric method, by means of Rx Daytona analyzer.

**Methods of measurements for erythrocyte magnesium:** blood vacutainers with sodium heparin (green cup) were used. Heparinized samples were centrifuged at 1500 g for 10 min to separate plasma from erythrocytes. The determination of erythrocyte magnesium was made using the colorimetric assay with xylidyl blue, a metallochromic dye,[3,4] after the lysis of 100 μl erythrocytes with 1500 μl double-distilled water, and deproteinization with 200 μl 0.3 mol/l Na2WO4 and 200 μl 0.35 mol/l H2SO4. The trade kit used was Human, the blue magnesium xylidyl complex, was measured at 532 nm, using a spectrophotometer (AR-Cromaline, Barcelona, Spain). Standard solutions were used along with blank solutions (1000μl working solution, 160 μl double-distilled water, 20 μl 0.3 mol/l Na2WO4 and 20 μl 0.35 mol/l H2SO4), for every analytic procedure. [7]

**Methods for statistical analysis:** the statistical significance and the correlations between plasmatic concentrations of the divalent cations and erythrocyte magnesium with glycemia, HbA1c, cholesterol, triglycerides, HDL were determined.

The results are expressed as means ± S.D. Differences between groups were examined using the unpaired Student's t-test, and considered statistically significant at $p<0.05$, differences in the group were examined using the paired Student's test, considered statistically significant at $p<0.05$, and to asses possible relationships between different variables, Pearson's correlation coefficient (r) was used. The statistical analyses were done using the SPSS for Windows 12.00.

## 4. Results

**Plasma total magnesium** level is reduced in NIDDM patients before treatment compared to healthy controls **(**1.95 ± 0.19 vs. 2.20 ±0.18 mg/dl, $p< 0.001$) and determination of plasma magnesium concentration after 3 months treatment with metformin revealed that there were not significant differences compared to the initial moment (M = 1.96, SD = 0.10 vs. M = 1.95, SD = 0.19, $p= 0.735$) and when compared with control group there are significant differences (M = 1.96, SD =0.105 vs M = 2.21, SD = 0.193, $p< 0.001$). Fig 1

**Plasma zinc** in NIDDM patients**:** data reveal significant differences in the NIDDM group versus the control group, for plasma zinc (67.56 ± 6.21 vs. 98.41± 20.47 μg/dl, $p<0.001$) and determination after 3 months treatment with metformin revealed that there were not significant differences compared to the initial moment (M = 64.25, SD = 5.59 vs. M = 67.56, SD = 6.21 vs. $p=0.067$) and when compared with control group there are still significant differences (M=64.25, SD = 5.60 vs. M = 101.65, SD = 23.14 , $p< 0,001$). Fig 2

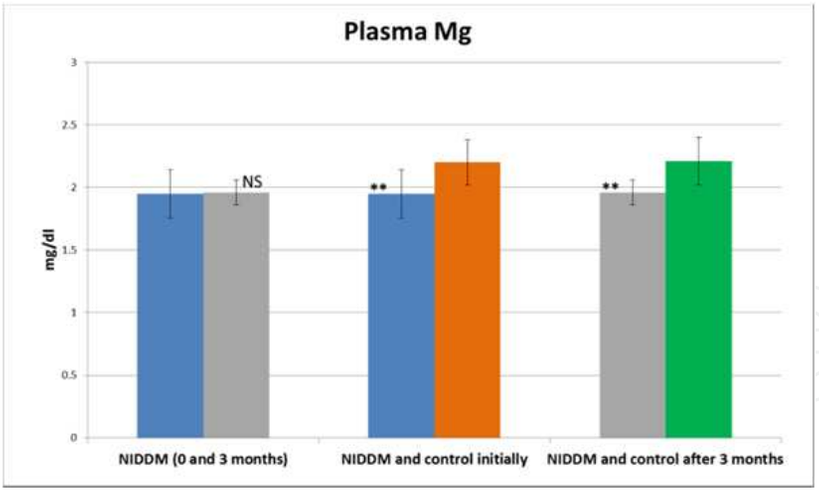


**Figure 1.** Plasma magnesium concentrations (NS – non-significant, * - p<0.05, ** - p<0.01)

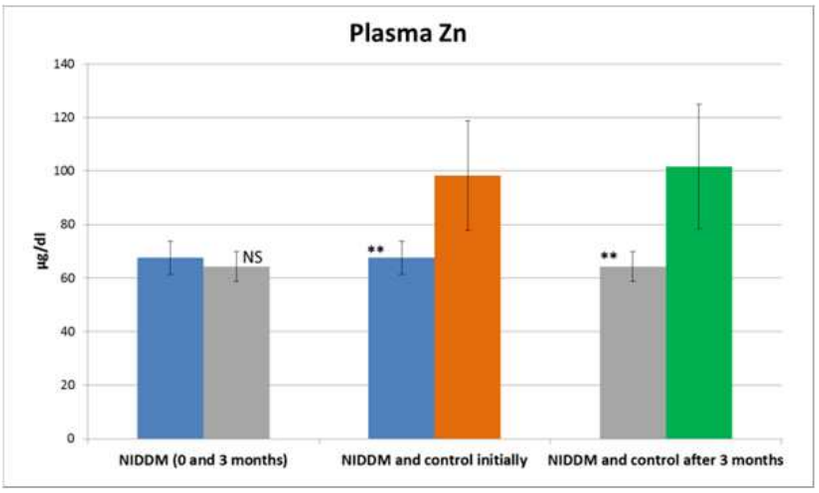


**Figure 2.** Plasma zinc concentrations (NS – non-significant, * - p<0.05, ** - p<0.01)

**Plasma copper** in NIDDM patients is increased when compared to healthy subjects (M=111.91+/-20.98 vs. M= 96.33+/- 8.56 μg/dl, p<0.001) Treatment with metformin did not modify significantly the concentration of this cation (M = 111.91, SD = 20.98 μg/dl vs. M =110.91, SD = 18.61 p= 0.413) and compared to control group there are not significant differences (M =110.08, SD = 18.61 μg/dl vs. M = 101.23, SD =21.73, p=0.147) (Fig 3) but when we compare those levels between the metabolic uncontrolled patients group (8 patients were prescribed another anti-diabetic drug after 3 months therapy with metformin) and the ones with metabolic control of metformin treatment (n=22) we see that there are significant differences (M = 127.22, SD = 22.64 μg/dl vs. M = 103.85, SD = 12.43, p= 0.023). Fig. 4

**Plasma calcium** in NIDDM patients before medication revealed non-significant differences when compared to healthy subjects. (M= 8.93± 0.33 mg/dl vs. 8.87 ± 0.35, p= 0.300) Treatment with metformin did not modify significantly the concentration of this cation (M = 8.987, SD = 0.44 mg/dl vs. M = 8.983, p=0.147) and compared to control group after 3 months of treatment there were not significant differences: (M = 8.98, SD = 0.44 mg/dl vs. M = 8.92, SD = 0.43, p= 0.633).

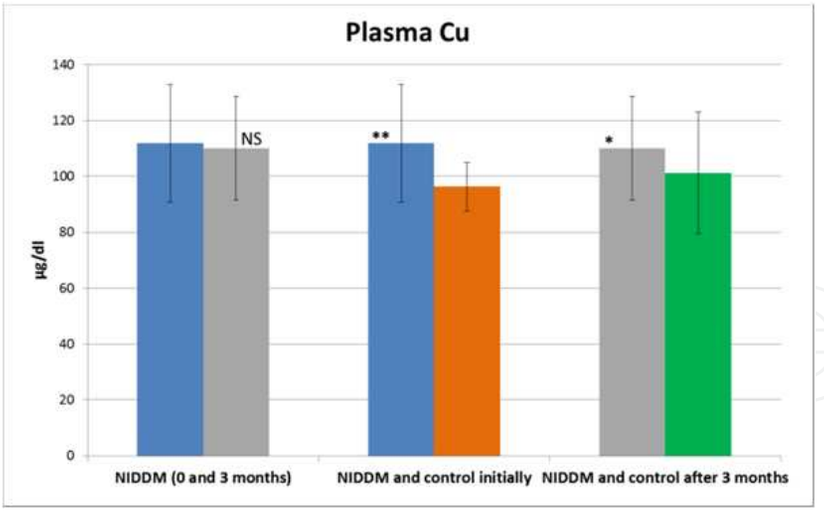


**Figure 3.** Plasma copper concentrations (NS – non-significant, * - p<0.05, ** - p<0.01)

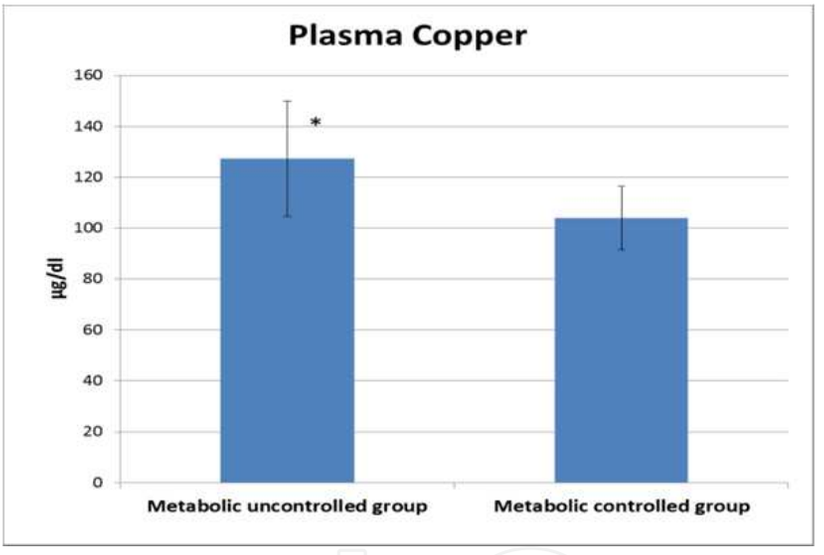


**Figure 4.** Plasma copper concentrations in metabolic uncontrolled patients by metformin vs metabolic controlled patients (NS – non-significant, * - p<0.05, ** - p<0.01)

Data about **intra-erythrocyte total magnesium** concentration in NIDDM patients has revealed a decreased concentration of erythrocyte magnesium in NIDDM patients before medication, when compared to healthy subjects (5.09 ± 0.63 vs. 6.38 ± 0.75 mg/dl, p< 0.001) and treatment with metformin has revealed that there were significant differences compared to the moment before medication: (M = 5.75 SD = 0.61 mg/dl vs. M = 5.09, SD =0.63, p<0.001) but compared to the control group the differences are still significant: (M = 5.75 SD =0.61 vs. M = 6.39 SD = 0.72, p=0.002) Fig 5

**Urinary magnesium** is increased in NIDDM patients before medication, when compared to healthy subjects (237.28±34.51 vs. 126.25±38.82 mg/24h p<0.001) and in NIDDM patients treated with metformin there were significant differences compared to the moment before

medication: (M = 198.27 SD = 27.07 mg/24 h vs. M = 237.28, SD = 34.51 mg/24h p< 0.001), but compared with control group the differences are still significant M = 198.27 SD = 27.07 mg/24h vs. M = 138.39, SD = 41.37 p< 0,001). Fig 6

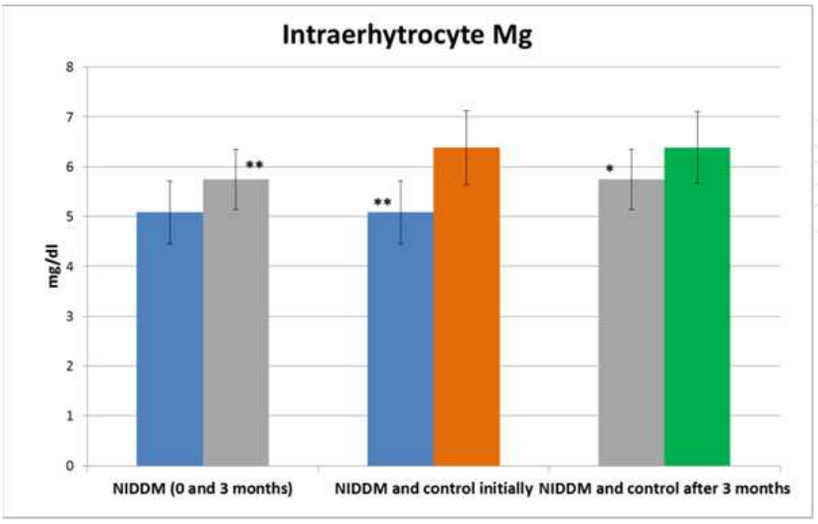


**Figure 5.** Intraerhytrocyte total magnesium concentrations (NS – non-significant, * - p<0.05, ** - p<0.01)

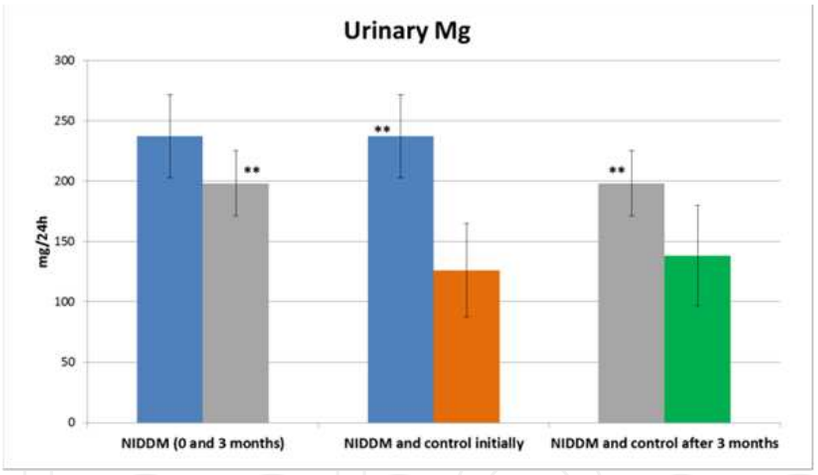


**Figure 6.** Urinary magnesium concentrations (NS – non-significant, * - p<0.05, ** - p<0.01)

**Urinary zinc** in NIDDM patients is increased before medication, when compared to healthy subjects (1347,54 ±158,24 vs. 851,65± 209,75 μg/24 h, p< 0,001) and after 3 months of medication our data has revealed that there were not significant differences compared to the moment before medication: (M = 1339,63 SD= 160,22 μg/24 h vs. M = 1347,54, SD = 158,24, p=0,530 ) and compared to the control group the differences are still significant (M = 1339,63, SD = 160,22 μg/24 h vs. M =880,76,SD = 186,38,p<0,001). Fig 7

**Urinary copper** in NIDDM patients is increased before medication, when compared to healthy subjects (51,70±23,79 vs. 36,00±11,70 μg/24h, p<0,05) and after medication data has revealed that there were not significant differences compared to the moment before medication: (M=51,705 SD = 22,13vs 53,35 μg/24 h , SD = 23,79, p= 0,301) and compared to

the control group there are significant differences (M = 51,70, SD = 22,13 μg/24 h vs. M = 36,00, SD = 11,66 p= 0,009). Fig 8

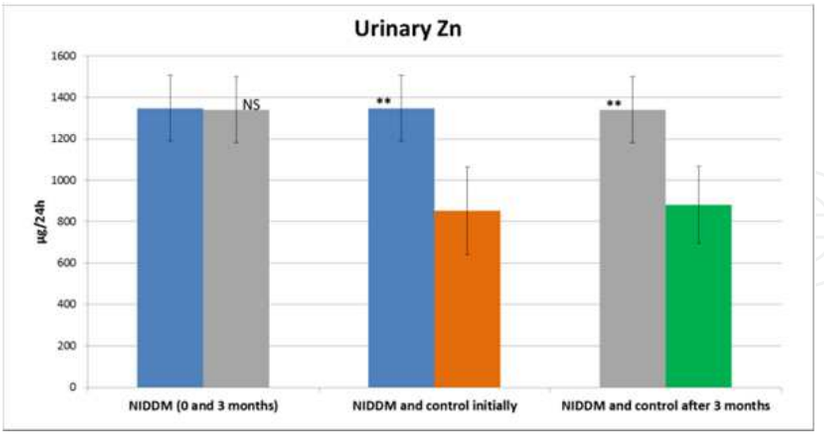


**Figure 7.** Urinary zinc concentrations (NS – non-significant, * - p<0.05, ** - p<0.01)

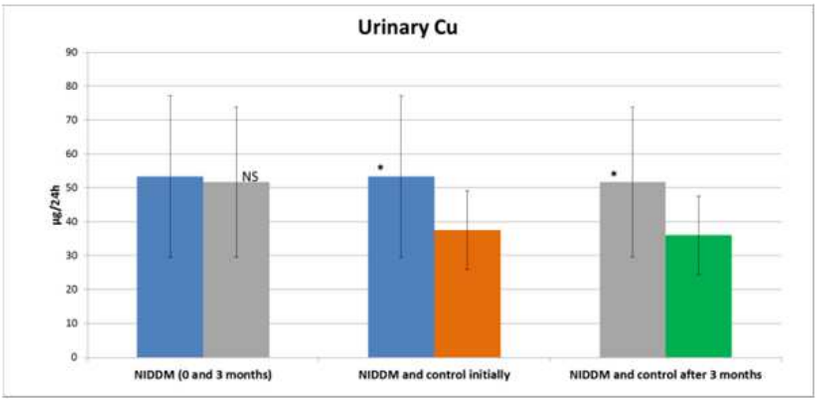


**Figure 8.** Urinary copper concentrations (NS – non-significant, * - p<0.05, ** - p<0.01)

**Urinary calcium** concentration in NIDDM patients is increased before medication, (309,23±58,41 vs. 201,51±62,13 mg/24h, p<0,001) and in NIDDM patients treated with metformin our data has revealed that there were significant differences compared to the moment before medication (M = 287,09 SD = 55,39 mg/24h vs. M = 309,23, SD = 58,41, p< 0,001) and compared to the control group: M = 287,09, SD = 55,39 mg/24h vs. M = 216,9 SD = 57,25 mg/24h, p< 0,001) Fig 9

The following correlations were obvious in metformin treated patients after 3 months: negative correlation between plasma total magnesium and glucose plasma level, positive correlation between plasma total magnesium and HbA1c, positive correlation between plasma $Cu^{2+}$ - glucose plasma level and HbA1c, positive correlation between plasma $Cu^{2+}$ - cholesterol plasma level and triglycerides, positive correlation between plasma $Zn^{2+}$ and glucose plasma level, negative correlation between erythrocyte total magnesium - glucose plasma level and HbA1c, positive correlation between HbA1c and urinary $Zn^{2+}$ concentration.

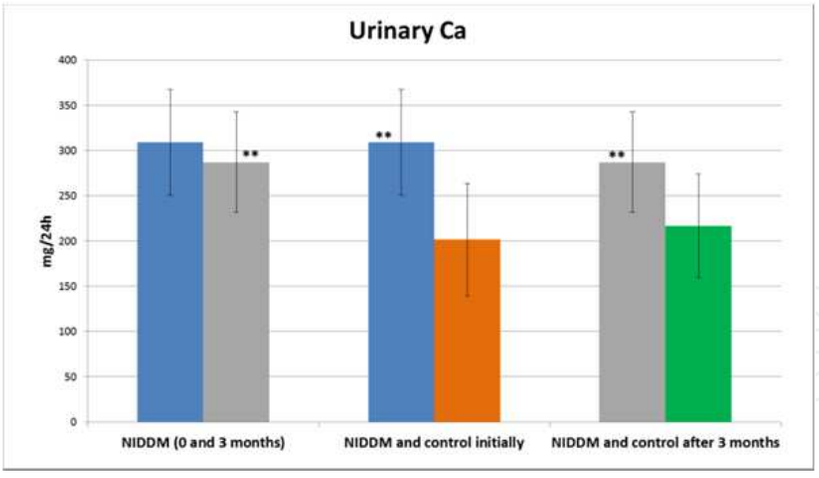


**Figure 9.** Urinary calcium concentrations (NS – non-significant, * - $p<0.05$, ** - $p<0.01$)

## 5. Discussions

The plasma magnesium level is reduced in diabetes mellitus. Our data reveals significant differences in NIDDM group versus the control group, for plasma magnesium (Fig. 1)

In accordance with other authors our data reveals the existence of plasma low total magnesium level in patients with NIDDM when compared to healthy adults. [8] Hypomagnesaemia is also involved in NIDDM pathogenesis and its complications. Both experimental and clinical studies suggest that hypermagnesemia may be the major factor involved in diabetes hypomagnesaemia. A specific renal tubular magnesium defect in diabetes together with the increased osmotic diuresis is responsible for large magnesium losses.[9] There are authors considering that serum magnesium was significantly low in diabetes with complications than in diabetes without complications ($p < 0.001$). In their study, poor glycemic control and the retinopathy were associated with hypomagnesaemia. [10]

Magnesium is cofactor in more than 300 enzymes involved in carbohydrate metabolism, required as energy bound, and it activates many enzymes involved in protein and lipids metabolism. There are data which show that hypomagnesaemia is associated to, and increase insulin resistance and we consider that magnesium can affect cellular action of insulin through: the action on the insulin receptor site, by modifying insulin-receptor binding, by affecting intracellular transduction of biologic signal; some authors [11] consider that Mg could play the role of a second messenger for insulin action by direct action on the entrance of glucose into the cell or by action on certain enzymes sites.

Magnesium deficiency may induce the increase of insulin-resistance in non-diabetic persons. [12] Deficiency of this cation can be considered a factor involved in pathogenesis and complications of type 2 diabetes mellitus.

Our data reveal significant differences for plasma zinc in NIDDM group versus the control group, for plasma zinc. (Fig. 2)

Zinc homeostasis is disturbed in patients with diabetes mellitus. Serum and intracellular zinc were significantly lower in diabetic patients compared to controls and our data are in accordance with other authors. [13] The urinary elimination of zinc is increased and intestinal absorption is decreased in patients with diabetes mellitus. Imbalances in zinc homeostasis with reduced plasmatic levels can determine deficiencies of beta pancreatic cells to produce and secrete insulin. The deficit of this cation can affect phosphorylation/ dephosphorylation of one or more steps in insulin cell signaling. [14]

Zinc ions are involved in the neutralization of free radicals and there is increasing evidence supporting the role of zinc as an antioxidant that could protect insulin and cells from being attacked by free radicals.[15] A possible mechanism of plasma zinc deficiency is due to excessive renal loss. Some authors consider that an impaired gastro-intestinal absorption in diabetics may also be involved. It is presumed that hyperglycemia may affect the tubular transport of zinc. [16] There are some clinical data that showed that zinc deficiency increased zinc occurrence of cataract among people with diabetes mellitus. [17]

Our data reveal an increased plasma level of copper in NIDDM patients when compared to healthy subjects. (Fig. 3) These data are in accordance with other studies [18] There are authors considering that excess copper is involved in type 2 diabetes mellitus pathogenesis and utilization of copper specific carriers may be a prospective therapeutic strategy in NIDDM. [19] Determination of plasma calcium in NIDDM patients, before medication, reveals non-significant differences compared to healthy subjects.

For urinary magnesium in NIDDM patients our data show an increased urinary magnesium level in NIDDM patients, before medication, when compared to healthy subjects. (Fig. 6) Our data are in accordance with other studies [20] that evidenced increased urinary levels of magnesium in newly diagnosed patients with type 2 diabetes mellitus. The possible mechanism involved in increased urinary magnesium elimination may be due to hyperglycemia that induces osmotic diuresis with decreased tubular magnesium reabsorption.

For urinary zinc in NIDDM patients our data reveal an increased urinary zinc level in NIDDM patients, before medication, when compared to healthy subjects. (Fig. 7) Hyperzincuria has been demonstrated in both type 1 and type 2 diabetes mellitus. The urinary elimination of zinc has increased and intestinal absorption has decreased in patients with diabetes mellitus. The possible mechanism involved in increased urinary zinc elimination may be due to hyperglycemia that induces osmotic diuresis. This fact is sustained by data that testified a positive correlation between HbA1c and Zn elimination being presumed that hyperglycemia interferes with the active Zn transport in tubular cells. [20, 21]

For urinary copper in NIDDM patients our data showed an increased level before medication, when compared to healthy subjects but moderate level when compared to Mg and Zn elimination, our patients having no renal disorders. (Fig. 8)

Other studies revealed urinary increased levels of copper in diabetic patients with associate disorders such as infections and neuropathy. [22]

Urinary calcium concentration in NIDDM patients has increased before medication, compared to healthy subjects, (Fig.9), our data being in accordance with other studies that evinced increased urinary levels of calcium in NIDDM patients. [23]

Determination of intra-erythrocyte total magnesium concentration has revealed a decreased concentration of this cation in NIDDM patients, before medication, when compared to healthy subjects, (fig. 5), and this is in accordance with other studies that evinced intracellular hypomagnesaemia in NIDDM patients without medication and dietary control. Insulin resistance in type 2 diabetes mellitus can negatively affect insulin capacity of stimulation for intracellular entry for both magnesium and glucose. [24] Intracellular magnesium has an important role to modulate insulin action, glucose transport and to maintain vascular tonus. Reduced intracellular concentrations of magnesium can determine deficiencies of tyrosine-kinase activity from the receptor site, also affecting intracellular transduction of biologic signal and insulin resistance in type 2 diabetic patients. [25] Magnesium may play the role of second messenger and disturbances in intracellular concentrations of this cation may contribute to insulin resistance.

In NIDDM an inverse correlation between plasma level of magnesium and insulin resistance has been determined. [26] Intracellular deficiency in $Mg^{2+}$ and Mg ATP2 can be a genetic factor that predisposes to type 2 diabetes mellitus. It is not clear if magnesium transporters TPRMs gene expression is affected in NIDDM patients. [27] Treatment with metformin for 3 months did not modify significantly the plasma concentration of magnesium, NIDDM patients being with hypomagnesaemia. (Fig.1) The causes for the lower magnesium levels may be a deficiency in magnesium digestive absorption or an increased elimination. The patients' diet contained approximately 320 mg/day magnesium, while a daily need for adult people is about 350- 400 mg/day. Together with those factors, the increased urinary elimination of this cation, as it appears in diabetic patients, may be a cause.

It was proved that a diet with low Mg content increase the risk of NIDDM and the incidence of metabolic syndrome. [28, 29, 30]

An inefficient NIDDM metabolic control could disturb the plasma magnesium levels. Extracellular hypomagnesaemia is involved in diabetes vascular complications. [31, 32] Magnesium deficiency can increase the risk of vascular complications in diabetes. There are data from previous researches that evinced positive correlations between magnesium deficiency and oxidative stress with reduced plasmatic antioxidant profile and increased lipid oxidation. [33] GSH, a tripeptide containing thiol groups is cofactor of many enzymes as it is glutathione peroxidase , Mg2+ being a mandatory cofactor in GSH synthesis as it is in all processes involving ATPase, and deficiency in Mg may induce alteration in GSH synthesis.

It was proved that oral magnesium supplementation with $MgCl_2$, 2,5 g daily, increases insulin sensitivity and metabolic control in patients with type 2 diabetes mellitus [34] and

patients with chronic complications such as retinopathy have decreased levels than the patients without this complication. It was proved that a low intake in magnesium increases the risk of type 2 diabetes in men and women. Treatment with metformin did not improve the plasmatic concentration of magnesium. Our data are in agreement with other authors who evinced that plasma levels of Mg have not improved at the same time with metabolic control improvement. [35]

Within experimental research, the metformin restored endothelial function and significantly improved NO bioavailability in normal and high-fat fed rats. This supports the concept of metformin as a first-line therapeutic drug to treat diabetic patients in order to protect against endothelial dysfunction associated with type 2 diabetes mellitus. [36, 37]

Metformin improved also the endothelial function in patients with metabolic syndrome. The type 2 diabetes mellitus patients who received metformin has had statistically significant improvement in endothelium-dependent vasodilatation compared to those that received placebo. [38]

Plasma zinc determination in NIDDM patients treated with metformin evinced that there were not significant differences compared to the initial moment and when compared to control group there are still significant differences.( Fig. 2)

Many studies revealed a deficiency in this divalent cation in diabetic patients.

Treatment with metformin did not improve the plasmatic concentration of zinc, patients being with hypozincemia.There are data revealing that diabetes is characterized by intracellular and extracellular imbalances of zinc. Zinc ions are involved in neutralization of ROS and nitrogen species through Cu Zn- SOD enzyme. The deficit of this cation can alter the antioxidant activity of the enzyme Cu Zn- SOD together with the expression and activity of other antioxidant biologic components, moreover, zinc deficiency can increase the fractions with oxidant activity such as ROS and RNA, and as consequence, the oxidant activity on the tissues, with oxidative capacity on the DNA, proteins and lipids. Imbalances in zinc metabolism can be involved in NIMMD pathogenesis and complications. [39]

Recent data revealed that zinc supplementation is associated with a decreased risk of diabetes appearance in women. [40] For short periods, zinc supplementation can improve the plasma glucose level in diabetic patients with increased values of HbA1c and decreased levels of zinc. [41]

A place in type 2 diabetes pathogenesis and complications is assigned to zinc transporter proteins (Zn Ts) and to metallothioneins (MTs). Many authors consider that a change in sub-cellular distribution of those may be more important than the deficiency in zinc, and those should be considered a future therapeutic target in diabetes.[42] There are some clinical data showing that zinc deficiency increased occurrence of cataract among people with diabetes mellitus. [17] Zinc and magnesium concentrations decreased in experimentally induced diabetes in rats [43].

The effect of zinc supplementation in the treatment of diabetes mellitus is controversial. [44] There is no evidence to suggest the use of zinc supplementation in the prevention of type 2 diabetes mellitus [45], but there are studies which suggest that zinc and magnesium supplementation might ameliorate diabetic neuropathy symptoms.[46] Treatment with metformin did not significantly modify the concentration of copper, but when we compare those levels between the metabolic uncontrolled patients and the ones with metabolic control of metformin treatment we see that there are significant differences. There are data revealing that increased levels of copper are not influenced by metabolic control [47]. Diabetes mellitus is a chronic metabolic disorder, which is associated with the increased free radical production leading to oxidative damage: the oxidative stress and copper both have pro-oxidant effects. This cation catalyzes the oxidative modification of LDL, in vitro and in the arterial wall and the presence of copper in high concentration can facilitate the production of free radicals through Fenton reaction [48].

The decrease of serum copper is associated to the decrease of the production of ROS on the animals with experimental diabetes. We believe that the excess of copper is involved in the pathogenesis of some complications of NIDDM. Plasma calcium determination in diabetic patients treated with metformin had showed that there were no significant differences compared to the initial moment. Intra-erythrocyte magnesium in NIDDM patients treated with metformin revealed that there were significant differences compared to the moment before medication. (Fig. 5) The results are in accordance with our previous data [49] and with few other results that revealed an increased tissue levels of magnesium associated with metformin treatment. [50] However, our study revealed there is still a decreased intra-erythrocyte magnesium level when compared to the control group. There are data revealing that hypomagnesaemia is associated to and increases insulin resistance. [26] Low levels of magnesium are associated to the increase of insulin-resistance in non-diabetic subjects as well. Treatment with metformin improved the intracellular concentration of this cation, due probably to the mechanism of action of this biguanide drug that improves the peripheral action of insulin, through an increase of GLUT 4 transporters on the plasmatic membrane, magnesium having insulin-like functions. Metformin is a drug that increases the glucose transport. Within other studies, intracellular magnesium increased after following treatment with rosiglitazone but metformin had no effect on intracellular calcium or magnesium. Ca and Mg were assessed in PBMC from healthy subjects following 72h in vitro treatment with the respective drugs and calcium content increased significantly. [51] Metformin administration improved Na(+)K(+)ATPase activity (0.28±0.08, 0.41±0.07μmol Pi/mg/h) in erythrocyte membrane, but is not clear if this action can influence bivalent cations intracellular concentrations. [52]

Metformin also improved the endothelial function in patients with metabolic syndrome. The type 2 diabetes mellitus patients who received metformin had statistically significant improvement in endothelium-dependent vasodilatation compared to those that received placebo. [53] We think that, in part, the endothelium protective effect of metformin is not produced only by NO way, but also by increasing the magnesium intracellular

concentration. Good magnesium status is associated with reduced diabetes risk. The average glucose-lowering effect of the major classes of oral anti-diabetic agents is broadly similar, but the influence on the various bivalent cations concentrations and on vascular endothelium is not. Our study revealed an improvement of intra-erythrocyte magnesium by metformin medication without an improvement of plasmatic magnesium. Metformin medication improved the urinary elimination of magnesium (Fig. 6), results that are in accordance with other researchers that revealed an improvement of this cation elimination [23] but compared to the control group the diabetic patients still have hypermagnesemia. We suppose that the decreased urinary elimination of magnesium may be due to the decreased in plasma glycemia by use of metformin as well as a possible action of this drug on the renal cation transporters.

Treatment with metformin did not modify zinc elimination, patients with diabetes mellitus type 2 having hyperzincuria. (Fig. 7) The urinary elimination of zinc is increased and intestinal absorption is decreased in patients with diabetes mellitus. There are some research data confirming that patients with type 1 and type 2 diabetes mellitus have increased levels of zinc concentration in urine. [54] Increased urinary levels of this cation can be involved in the plasmatic reduced levels, and the mechanism involved may be due to hyperglycemia that can interfere with the Zn transport back in renal tubular cells, but we cannot exclude other interventions of zinc ions on the zinc transporters.

Metformin administration did not significantly modify the urinary elimination of copper when comparing the metabolic uncontrolled group of metformin administration and the group controlled by medication, there are significant differences. (Fig. 4)

Metformin administration for 3 months did not significantly modify the urinary elimination of calcium but compared to the control group there are still significant differences, diabetic patients being with hypercalciuria. (Fig. 9)

The negative correlation between plasma Mg and plasma glucose level in patients treated 3 months with metformin revealed that the treatment with metformin did not improve the plasma levels of magnesium, but increased the intracellular magnesium concentration.

The negative correlation between plasma magnesium and HbA1c evidenced the fact that the metabolic uncontrolled patients through medication have low levels of magnesium.

Our data are in accordance with other authors that revealed an inverse correlation between the metabolic control of NIDDM and plasma hypomagnesaemia. [32]

There are authors suggesting that the diabetes itself can induce hypomagnesaemia, while others revealed the fact that a supplementation of Mg can reduce the risk of development of type 2 diabetes mellitus and can improve the insulin sensitivity. [55] Our data indicated a low intracellular magnesium concentration in NIMMD patients and an increase of intra-erythrocyte magnesium after metformin treatment. This is an important point in metformin action because magnesium deficiency promotes the development of dyslipidemia and the atherogenesis. [56]

The increased oxidative stress in diabetes mellitus contribute to the development of diabetic macrovascular and microvascular complications through increased lipid oxidation, especially by increasing oxidation of LDL, which is a crucial step in the development of atherosclerosis. Magnesium can reduce the oxidative stress [57] and we consider that this is an important mechanism for antiatherogenic action of certain antidiabetes drugs.

Our results are in accordance with other authors that evinced positive correlations between plasma Cu and glycemia. [58] Once again it is proved that there is a disbalance in the copper ions in diabetes type 2 and once proved the implications of these cations in the pathogenesis and complications of diabetes.

The positive correlation between plasma copper and cholesterol, tryglicerides concentration confirms the fact that increased levels of copper are associated with lipid metabolism disturbances.The involvement of copper ions in plasma lipids metabolism are controversial. Various studies proved inverse correlations between low dietary copper and cholesterol and LDL cholesterol, in both: human and rats, and in rats with low copper diet HDL cholesterol had increased. [59]

Treatment with metformin did not modify the urinary loss nor the plasma decreased levels, all of those implying the hyperglycemia as factor involved. The issue of the correlation between copper, zinc and magnesium status and complications of diabetes mellitus is an open one. In other studies it was reported an increased plasma copper concentration in patients with specific diabetes associated complications (retinopathy, microvascular diseases or arterial hypertension) [60, 61] but no significant differences between control and diabetic patients in erythrocyte copper-zinc superoxide dismutase. The relationship between coronary risk factors in NIDDM patients is also extremely important. The plasma zinc/copper ratio is inversely associated with calculated 10 years coronary risk in non-diabetic patients. [62]

## 6. Conclusions

Our data are in accordance with those of other authors [32] that revealed an inverse correlation between the metabolic control of NIDDM and low plasma magnesium level.

Negative correlation between erythrocyte magnesium and HbA1c evinced that low metabolic control by medication is associated with intracellular deficit of magnesium. In our opinion, the intracellular magnesium concentration plays a more important role in prevention of the development of hypercholesterolemia and atherogenesis than plasma level of this cation.

Metformin did not modify the plasma levels of copper. About the risk for the development of vascular complication of patients with type 2 diabetes, we believe that the ratio between the concentrations of intracellular magnesium +serum zinc/serum copper is a good marker for the risk of diabetes complications development. A higher ratio indicates a more reduced risk and a low ratio an increased risk. In the evaluation of the antidiabetic effect of certain

drugs must be to keep account of their influence on magnesium, zinc and copper plasma and intracellular concentrations.

We consider that magnesium and zinc administration can be of benefit in type 2 diabetes mellitus .The copper chelators could represent also a future medication in diabetes.

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