Serum ionized magnesium in diabetic older persons☆,☆☆

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Objective. Several alterations of magnesium metabolism have been associated with type 2 diabetes pathophysiology, a condition particularly frequent in older persons. We aimed to evaluate serum total (Mg-tot) and serum ionized magnesium (Mg-ion) in older persons with type 2 diabetes in order to explore clinically applicable methods for the detection of magnesium deficit.

Material/Methods. Mg-tot and Mg-ion were measured in 105 fasting subjects with type 2 diabetes (mean age: 71.1 ± 0.8 years; M/F: 45/60) and in 100 age-matched non-diabetic control persons (mean age: 72.2 ± 0.8 years; M/F: 42/58).

Results. Mg-ion concentrations were significantly lower in diabetic persons compared with controls (0.49 ± 0.05 mmol/L vs. 0.55 ± 0.05 mmol/L; p < 0.001). Mg-tot was also slightly but significantly lower in diabetic patients (0.82 ± 0.007 mmol/L vs. 0.84 ± 0.006 mmol/L; p < 0.05). There was an almost complete overlap in the values of Mg-tot in older diabetic patients and controls; conversely, 44.8% of diabetic patients had Mg-ion values below 0.47 mmol/L, while none of the controls did. After adjustment for age, sex, BMI, and triglycerides, Mg-tot was significantly associated with FBG in all the participants (p < 0.05) and Mg-ion was significantly associated with FBG in all the participants (p < 0.01) and with HbA1c in diabetic participants (p < 0.001).

Conclusions. Alterations of magnesium serum concentrations are common in type 2 diabetic older adults; Mg-ion evaluation may help to identify subclinical magnesium depletion (i.e. in patients with normal Mg-tot); the close independent associations of Mg-tot and Mg-ion with FBG and with HbA1c reinforce the possible link between magnesium homeostasis and altered glucose metabolism.

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1. Introduction

There is compelling evidence suggesting that magnesium depletion may play a role in the pathophysiology of insulin-resistance and/or altered glucose homeostasis in type 2 diabetes mellitus [1–3]. Magnesium is the second most abundant intracellular cation after potassium, and it is involved in a number of fundamental biochemical processes, comprising all ATP transfer
reactions. Magnesium ion plays a key role in the regulation of insulin actions, including insulin-mediated glucose uptake [1].

Type 2 diabetes has been associated with extracellular and intracellular magnesium depletion. Epidemiologic studies have found a high prevalence of hypomagnesemia in persons with type 2 diabetes [4–7], especially in those with poorly controlled glycemic values [4,5], and with micro and macrovascular chronic complications [8]. Recently, a close and independent relationship of low serum Mg concentrations and ventricular ectopy in patients with type 2 diabetes has been reported [7]. Hypomagnesemia is currently considered an accurate predictor of death and of progression to ESRD in patients with type 2 diabetic nephropathy [9,10]. Two meta-analyses of prospective studies concluded that magnesium intake is inversely associated with type 2 diabetes [11,12]; magnesium intake has been also strongly and inversely associated with the metabolic syndrome [13,14], while hypomagnesemia has been independently associated with the development of impaired glucose tolerance [15]. A recent study showed a close relationship between the presence of diabetes and the lower levels of magnesium in obese subjects who undergo bariatric metabolic surgery [16].

Although the importance of magnesium homeostasis in glucose metabolism is well appreciated, magnesium metabolism has not become the focus of routine attention for the care of type 2 diabetes patients in the clinical practice. The main reasons for this include the difficulties in obtaining an easily available, accurate, and reproducible measure of magnesium status since the concentrations of total serum magnesium (Mg-tot), commonly used as an estimation of magnesium in the clinical practice, are extremely constant and do not accurately reflect the body magnesium status [1]. Depletion of intracellular as well as of ionized serum magnesium has been reported in the presence of normal levels of Mg-tot [17,18]. Because aging represents a major risk factor for Mg insufficiency [19], it is possible that older diabetic subjects are at further risk of magnesium deficit, which may not always be clinically apparent.

The present study was designed to evaluate magnesium metabolism in older type 2 diabetes patients measuring Mg-tot and the extracellular free levels of magnesium (Mg-ion) with a Mg-specific ion-selective electrode (ISE) in order to explore clinically applicable methods for the detection of magnesium deficit in older persons with type 2 diabetes.

2. Methods

2.1. Subjects

Two hundred and five older persons (aged ≥ 60 years), 105 type 2 diabetic patients (mean age: 71.1 ± 0.8 years; M/F: 45/60) and 100 age-matched non-diabetic controls (mean age: 72.2 ± 0.8 years; M/F: 42/58) were consecutively recruited from the Outpatient Clinic of the Geriatric Unit at the University Hospital of Palermo, Italy. Anthropometric and laboratory data including Mg-tot and Mg-ion were measured (Table 1). All type 2 diabetic persons recruited for the present study were recently being diagnosed with diabetes, treated with diet therapy only and had never been treated before with insulin or hypoglycemic agents. In order to avoid possible interferences with dietary components and physical exercise that may alter serum magnesium concentrations, we advised participants not to modify their dietary and physical activity usual habits during the study period. None of the patients had been on diuretic therapy for at least 1 month before the study and none had significant renal dysfunction, as assessed by serum creatinine levels and calculated glomerular filtration rate (GFR) [20].

No differences in age, sex, race, blood pressure levels, GFR, and body mass index (BMI) were present between the groups (Table 1). It is well known that alcohol abuse may alter magnesium metabolism by means of different mechanisms, i.e. malnutrition, increased urinary magnesium loss, among others [21]. Therefore, we excluded persons with alcohol abuse (intended as alcohol consumption higher or equivalent to more than 1 glass of wine per day) and requested the participants specifically not to change their usual alcohol consumption habits since this could affect magnesium circulating concentrations.

The study was approved by the ethical committee of our Institution and was conducted in accordance with the guidelines of the Declaration of Helsinki for human research. An informed consent was signed by all participants. Exclusion criteria included: not compensated acute disease, such as severe congestive heart failure, severe chronic obstructive pulmonary disease, angina pectoris, acute myocardial infarction or stroke in the previous 6 months of the study, severe uncontrolled hypertension (SBP ≥ 180 mm Hg and/or DBP ≥ 90 mm Hg), moderate to severe renal or hepatic disease, and/or alcohol abuse.

2.2. Magnesium measurements

Blood samples were obtained from participants after they had fasted for 10 h and after they had been in a sitting or supine position for 15 min. Serum Mg-tot concentrations were measured by standard colorimetric techniques with an
automated chemistry analyzer (ISE 900/ISE 1800 modules of Modular Analytics SWA Roche Diagnostics Italia, Monza, Italy). Low magnesium was considered for values below 0.7 mmol/L, as usually accepted [22]. Blood for Mg-ion was drawn into air-evacuated glass tubes containing an inter-cell separating matrix. After clotting and centrifugation, the tubes were inverted and serum was drawn off into a syringe anaerobically, the latter being capped and stored in a freezer (0 °C–4 °C) for further analysis. A magnesium ion-selective electrode (ISE) with a neutral carrier-based membrane (Nova and Stat Profile 8 Ultra analyzers, Waltham, MA, USA) was used to measure serum Mg-ion [18,23].

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**Fig. 1** – Serum ionized magnesium levels (Mg-ion) (Panel A) and serum total Mg (Mg-tot) (Panel B) in normal controls (n = 100) and in type 2 diabetic older persons (n = 105).

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**Fig. 2** – Serum ionized magnesium (Mg-ion) in the presence of normal or low serum total Mg (Mg-tot) concentrations in type 2 diabetic older persons (n = 105).
2.3. Statistical Analyses

Data are expressed as means ± SEM. Analyses of the data were performed using statistical software GraphPad Prism (GraphPad Software, San Diego, CA) and SPSS software package (SPSS, Chicago, IL). Differences between diabetic and non-diabetic participants were assessed with unpaired t tests for continuous variables and with chi-square for categorical variables. Differences in proportions according to magnesium concentration cut-off points were assessed with chi-square (Fig. 2). Pearson’s correlation coefficients were used to analyze the linear correlations between variables (Fig. 3). Multivariate linear regression analysis was used to examine Mg-ion and Mg-tot as predictors of FBG or HbA1c after adjustment for age, sex, BMI, and triglycerides. P values ≤ 0.05 were considered to be statistically significant.

3. Results

Clinical and laboratory data are shown in Table 1. Serum Mg-ion levels were significantly lower in type 2 diabetic participants compared with age-matched non-diabetic control subjects. Serum Mg-ion in diabetic subjects was 0.49 ± 0.05 mmol/L (range 0.32 to 0.65 mmol/L, median 0.49 mmol/L, 25% percentile: 0.44 mmol/L, 75% percentile: 0.53 mmol/L), while in controls it was 0.55 ± 0.05 mmol/L (range 0.47 to 0.73 mmol/L, median 0.54 mmol/L, 25% percentile: 0.51 mmol/L, 75% percentile: 0.58 mmol/L; p < 0.001).

Mg-tot was also slightly but significantly reduced in type 2 diabetes patients (0.82 ± 0.007 mmol/L, range 0.66 to 0.99 mmol/L, median 0.82 mmol/L, 25% percentile 0.78 mmol/L, 75% percentile 0.86 mmol/L) vs. controls (0.84 ± 0.006 mmol/L, range 0.70 to 1.01 mmol/L, median 0.84 mmol/L, 25% percentile 0.80 mmol/L, 75% percentile 0.90 mmol/L, p < 0.05, Fig. 1).

In order to explore the proportion of patients with differences in the measured concentrations of both, Mg-ion and Mg-tot, we divided the groups considering the participants with Mg-tot below or above 0.7 mmol/L (usual conventional cut-off value for considering hypomagnesemia) [22]. We also divided the groups considering participants with Mg-ion concentrations below or above 0.47 mmol/L. We used this cut-off value for Mg-ion because this was the lowest value found in the non-diabetic controls, allowing us to identify the fraction of diabetic patients with lower values than the normal controls, which was in fact the interest of our study. Forty seven type 2 diabetic participants (44.8%) and none of the non-diabetic controls had Mg-ion concentrations below 0.47 mmol/L. Twelve diabetic participants (11.4%) with low Mg-ion had also Mg-tot below 0.7 mmol/L, while a third (n = 35) of all diabetic participants had low Mg-ion and Mg-tot higher than 0.7 mmol/L, which accounts for over half (74.5%) of the diabetic participants with low Mg-ion (Fig. 2). When these proportions were compared to those of the non-diabetic controls, there were significant differences as shown in Fig. 2. Even if the mean Mg-tot was slightly but significantly lower in diabetic participants when compared to non-diabetic controls, the values almost completely overlapped in non-
Mg-tot: Serum total magnesium; SE: standard error; FBG: fasting blood glucose; GFR: glomerular filtration rate.

Model 1: Mg-ion adjusted for age and sex.
Model 2: model 1 plus adjustment for BMI.
Model 3: model 2 plus adjustment for triglycerides.
Model 4: model 3 plus adjustment for GFR.

Mg-ion: serum ionized magnesium. SE: standard error; FBG: fasting blood glucose.

A close relationship was found between serum Mg-ion and fasting blood glucose (FBG) \( r = 0.381, p < 0.001 \) (Fig. 3, panel A) has very modest ability to detect those with low Mg-ion. that Mg-tot is able to identify patients without low Mg-ion but participants was only 25.5% with a specificity of 98.3%. This means (considering Mg-ion as gold standard) in the diabetic participants, the sensitivity of Mg-tot to detect a low concentration of Mg

| Table 2 – Multivariate linear regression models testing the relation of Mg-ion with FBG in all participants and of FBG and HbA1c in diabetic participants. |
|-----------------|-----------------|-----------------|-----------------|
| Fasting blood glucose | HbA1c |
| \( \beta \) | SE (\( \beta \)) | \( p \) | \( \beta \) | SE (\( \beta \)) | \( p \) |
| All participants (n = 205) |
| Model 1 | –290.83 | 50.71 | <0.001 |
| Model 2 | –235.07 | 67.16 | 0.001 |
| Model 3 | –205.33 | 70.62 | 0.004 |
| Model 4 | –196.80 | 82.39 | 0.019 |

Diabetic participants (n = 105) |
| Model 1 | –134.59 | 84.78 | 0.116 |
| Model 2 | –55.22 | 110.38 | 0.619 |
| Model 3 | –44.08 | 108.0 | 0.685 |
| Model 4 | –55.36 | 120.6 | 0.649 |

Multivariate linear regression models testing the relation of Mg-tot with FBG in all participants and of FBG and HbA1c in diabetic participants.

| Table 3 – Multivariate linear regression models testing the relation of Mg-tot with FBG in all participants and of FBG and HbA1c in diabetic participants. |
|-----------------|-----------------|-----------------|
| Fasting blood glucose | HbA1c |
| \( \beta \) | SE (\( \beta \)) | \( p \) | \( \beta \) | SE (\( \beta \)) | \( p \) |
| All participants (n = 205) |
| Model 1 | –108.19 | 46.84 | 0.022 |
| Model 2 | –156.93 | 65.79 | 0.019 |
| Model 3 | –135.92 | 66.89 | 0.044 |
| Model 4 | –144.30 | 80.75 | 0.039 |

Diabetic participants (n = 105) |
| Model 1 | –101.73 | 67.59 | 0.135 |
| Model 2 | –50.99 | 97.56 | 0.603 |
| Model 3 | –31.91 | 95.63 | 0.740 |
| Model 4 | –6.37 | 105.73 | 0.952 |

Mg-tot: Serum total magnesium; SE: standard error; FBG: fasting blood glucose; GFR: glomerular filtration rate.

Model 1: Mg-ion adjusted for age and sex.
Model 2: model 1 plus adjustment for BMI.
Model 3: model 2 plus adjustment for triglycerides.
Model 4: model 3 plus adjustment for GFR.

4. Discussion
Magnesium ion is involved in a wide variety of cellular processes, many of them critical for glucose and insulin metabolism [1–3]. Based on this, our group has previously studied cytosolic free concentrations of magnesium, utilizing non-invasive nuclear magnetic resonance \(^{31}P\) NMR techniques, and has reported that type 2 diabetes is associated with significantly lower intracellular magnesium levels [19,24]. Although spontaneous hypomagnesemia is not an uncommon finding in persons with diabetes and Mg-tot has been extensively utilized in epidemiological studies, it may not be helpful for the detection of subclinical magnesium deficit in an individual basis, while more precise techniques, such as \(^{31}P\) NMR spectroscopy, remain an expensive research-based tool [1].

Resnick et al. have suggested that Mg-ion may be lower in type 2 diabetes in a preliminary study with a small group of 22 diabetic patients, even in the presence of a normal Mg-tot. In that study, intracellular free magnesium measured with \(^{31}P\) NMR spectroscopy was closely correlated with Mg-ion, while it was not significantly related to Mg-tot, suggesting that Mg-tot may not reflect the magnesium status of the intracellular pool [18]. Our present data in a larger population significantly expand previous reports, showing that: a) serum Mg-ion levels and Mg-tot are significantly lower in type 2 diabetic persons compared with age-matched nondiabetic controls (Fig. 1), suggesting that in chronic stable type 2 diabetes, a depletion of extracellular magnesium is present; b) Mg-ion is more accurate in order to identify magnesium depleted patients, while the range of Mg-tot values almost completely overlaps in normal controls and type 2 diabetes patients; c) there is a significant, independent inverse relationship between fasting serum Mg-ion and Mg-tot with glycemic indices (i.e. the lower the Mg-ion and Mg-tot, the higher the FBG and HbA1c (Fig. 2)), which remains highly significant after adjustment for confounders.
Magnesium is a natural physiological calcium blocker [40] and may prevent oxygen radical formation by scavenging free radicals and by inhibiting xanthine-oxidase and NADPH oxidase [41]. Higher levels of magnesium may improve intracellular ATP production and glucose utilization, because magnesium is a cofactor of ATP. Magnesium deficiency may decrease membrane integrity and membrane function, increasing the susceptibility to oxidative stress, and aging-related diseases [42]. Diets with low magnesium content [43,44] as well as low concentrations of serum magnesium [15,45] were associated with an increased risk for the development of glucose intolerance and diabetes. Magnesium intake was found to be inversely correlated to systemic inflammation and insulin resistance [2,46,47].

The present study has diverse strengths including the fact that it studies older diabetics, a population that is rapidly growing worldwide, is generally excluded from clinical studies, and from which a scarce literature is available. The data presented are based on several lines of evidence supporting the alterations of magnesium metabolism in type 2 diabetes, and even after adjustments for several relevant confounders the results remained strongly statistically significant. The study also has some potential limitations. The analyses used cross-sectional data, hence, the confounding of changes in time cannot be evaluated; however, the present results have an important applicability in clinical terms because they show that ionized magnesium was able to identify older diabetic adults with low concentrations of blood magnesium that are not evident with the only measurement of total magnesium. Because the participants were all Caucasian and Italian, the present results may not be applicable for other races and ethnicities, as well as in younger patients. Future studies are needed in order to test the applicability of the present results in populations of diverse races, ethnicity and age.

The translational potential of the present results are relevant because the detection of hypomagnesemia in patients with apparently normal serum magnesium is essential in order to correct the altered magnesium and avoid the multiple negative consequences of the condition, amply discussed above. Our results confirm that diabetic patients are prone to hypomagnesemia; this condition is closely related with glycated hemoglobin even after adjustment for relevant confounders; hence, the detection and correction of altered magnesium may be clinically appropriate. Furthermore, considering all the participants, diabetic and non diabetic, both ionized magnesium and total magnesium were significantly associated with fasting glucose after adjustment for confounders. This supports the importance of magnesium status on glucose homeostasis.

Altogether, our present results in older diabetic patients confirm the importance of studying magnesium homeostasis in patients with type 2 diabetes and suggest that assessment of Mg-ion, together with Mg-tot, may be of help in the clinical practice for the detection of magnesium deficits in patients with type 2 diabetes.

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**Author contributions**

Study concept and design: MB, GDB, LJD; Acquisition of data: VB, PD, DD, AM; Analysis and interpretation of data: MB, LJD, MBe; Drafting of the manuscript: MB, LJD; Critical revision of the manuscript for important intellectual content: MB, GDB, VB, DD, PD, AM, MBe, LJD; Statistical analysis: MB, LJD; Administrative, technical, or material support: GBD, VB, DD, PD, AM; Study supervision: MB, MBe, LJD.
Conflict of interest
None of the authors has any conflict of interest or financial support to disclose.

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