Determinants of hypomagnesemia in patients with type 2 diabetes mellitus

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Abstract

Background: Hypomagnesemia (plasma magnesium (Mg^{2+}) concentration <0.7 mmol/L) has been described in patients with type 2 diabetes. Polypharmacy is inevitable when treating a complex disease such as type 2 diabetes and could explain disturbances in the plasma Mg^{2+} concentration. In this study, we aimed to establish the extent of hypomagnesemia in a cohort of type 2 diabetes patients and to identify the determinants of plasma Mg^{2+} levels.

Methods: Patient data and samples of 395 type 2 diabetes patients were investigated. Plasma Mg^{2+} concentrations were measured using a spectrophotometric assay. Using Pearson correlation analyses, variables were correlated to plasma Mg^{2+} levels. After excluding confounding variables, all parameters correlating ($P < 0.1$) with plasma Mg^{2+} were included in a stepwise backward regression model.

Results: The mean plasma Mg^{2+} concentration in this cohort was 0.74 ± 0.10 mmol/L. In total, 121 patients (30.6%) suffered from hypomagnesemia. Both plasma triglyceride ($r = -0.273$, $P < 0.001$) and actual glucose levels ($r = -0.231$, $P < 0.001$) negatively correlated with the plasma Mg^{2+} concentration. Patients using metformin ($n = 251$, 62%), proton pump inhibitors ($n = 179$, 45%) or β -adrenergic receptor agonists ($n = 31$, 8%) displayed reduced plasma Mg^{2+} levels. Insulin use ($n = 299$, 76%) positively correlated with plasma Mg^{2+} levels. The model predicted (R^2) 20% of all variance in the plasma Mg^{2+} concentration.

Conclusions: Hypomagnesemia is highly prevalent in type 2 diabetes patients. Plasma triglycerides and glucose levels are major determinants of the plasma Mg^{2+} concentration, whereas only a minor part ($<10\%$) of hypomagnesemia can be explained by drug intake, excluding polypharmacy as a major cause for hypomagnesemia in type 2 diabetes.

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Introduction

Over the last decades, evidence is accumulating that hypomagnesemia (plasma Mg^{2+} concentration <0.7 mmol/L) is frequently present in patients with type 2 diabetes (1). Since its first report in the 1940s, hypomagnesemia has been shown in several cohort studies (2, 3, 4). Although plasma Mg^{2+} levels are not regularly monitored in type 2 diabetes patients, the presence of hypomagnesemia is of significant clinical importance (5). Oral Mg^{2+} supplementation has been shown to reduce the progression from pre-diabetes to diabetes and improves insulin sensitivity and glucose handling (3, 6, 7, 8).

Moreover, in type 2 diabetes patients, hypomagnesemia results in a faster renal decline and is associated with a worse disease progression and outcome (9, 10). Mg^{2+} also plays a key role in common comorbidities of type 2 diabetes such as chronic kidney disease, atherosclerosis and hypertension (11, 12, 13, 14).

Hypomagnesemia can have many underlying causes, related or unrelated to type 2 diabetes (1, 15). First, hypomagnesemia can result from mutations in magnesiumotropic genes, which has been extensively reviewed by Viering *et al.* (16). Second, the processing of food leads to

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a marked depletion of Mg^{2+} in the Western diet, resulting in a reduced dietary Mg^{2+} intake (17). Third, hypomagnesemia can be a result of impaired intestinal Mg^{2+} uptake due to diarrhea that could be induced by diabetic autonomic neuropathy or metformin use (18, 19, 20). Fourth, the use of certain medication (i.e. diuretics, immunosuppressive drugs, proton pump inhibitors (PPIs)) has been associated with hypomagnesemia (21). Lastly, metabolic acidosis and insulin resistance can decrease the expression of the renal Mg^{2+} channel transient receptor potential melastatin 6 (TRPM6), increasing urinary Mg^{2+} loss and thereby reducing the plasma Mg^{2+} concentration (22, 23).

The origin of hypomagnesemia in type 2 diabetes is currently unknown. The contributing factors to disturbed Mg^{2+} homeostasis may be multiple and have been poorly studied. Type 2 diabetes patients suffer from a wide range of clinical disturbances including increased plasma glucose concentrations, dyslipidemia (high triglyceride and low HDL cholesterol) and insulin resistance (24).

To maintain proper glucose levels and blood pressure, polypharmacy is an inevitable consequence of effectively treating type 2 diabetes. However, it also constitutes a growing risk factor as each drug carries its own side effects and drug–drug interactions (25). Potentially, the use of medication can contribute to hypomagnesemia. Several regularly prescribed drugs in type 2 diabetes are known to reduce the plasma Mg^{2+} levels, including PPIs and thiazide diuretics (26, 27, 28, 29, 30). However, it is unclear to what extent the hypomagnesemia in type 2 diabetes patients can be explained by the extensive use of medication.

In this study, we aimed to determine the prevalence of hypomagnesemia in a carefully phenotyped cohort of 402 type 2 diabetes patients. Subsequently, the determinants of plasma Mg^{2+} levels were analyzed using laboratory parameters and an extensive screening of drug use.

Subjects and methods

Subjects

Patients data and samples were taken from the Nijmegen-part of the Diabetes Pearl cohort (31). In short, 402 type 2 diabetes patients were included between June 2009 and February 2012 at the Radboud University Medical Center in Nijmegen, The Netherlands. Inclusion criteria were based on the WHO standards: a venous fasting plasma glucose concentration higher than 7.0 mmol/L or a casual venous plasma glucose concentration higher than 11.1 mmol/L (32). Personal information was obtained by dedicated patient questionnaires. This cohort consisted of patients under chronic secondary and tertiary care including many patients at an advanced disease stage.

Body mass index (BMI) was calculated as the weight in kilograms divided by square of the height in meters. Blood pressure and heart rate were measured in triplicate, and the average value was used in subsequent analyses. Waist circumference was measured in duplicate after normal exhalation and if the difference between these two measurements was >1.0 cm, the measurement was repeated for a third time. Plasma and urine samples were

Table 1 Characteristics of type 2 diabetes patients.

Variable	Mean $\pm$ s.d.	Range	Reference value
Demographics			
Gender (m:f, %)	59:41	–	–
BMI (kg/m ²)	32.6 $\pm$ 6.5	17–61	18.5–25
Age (years)	67 $\pm$ 10	34–90	–
Duration diabetes (years)	15.8 $\pm$ 9.5	0–56	–
Waist circumference (m:f, cm)	110 $\pm$ 16	65–152	<102:<88
SBP (mmHg)	143 $\pm$ 20	84–207	90–140
DBP (mmHg)	77 $\pm$ 11	51–117	60–90
Heart rate (beats/min)	73 $\pm$ 12	45–116	60–100
Alcohol consumption (no:yes, %)	52:48	–	–
Laboratory analyses			
Total cholesterol (mmol/L)	4.5 $\pm$ 1.5	2.0–20.0	<5.2
Triglycerides (mmol/L)	2.5 $\pm$ 4.1	0.4–50.7	<1.7
HbA1c (mmol/mol)	63.1 $\pm$ 14.2	33.3–118.6	<42
Glucose (mmol/L)	9.4 $\pm$ 3.4	2.3–24.9	3.9–5.5
LDL (mmol/L)	2.3 $\pm$ 0.8	0.6–4.9	<2.6
HDL (m:f, mmol/L)	1.1 $\pm$ 0.3	0.5–2.2	>1.1:>1.3

BMI, body mass index; DBP, diastolic blood pressure; f, female; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; m, male; SBP, systolic blood pressure.

Table 2 Medication use of type 2 diabetes patients.

Medication	Number of patients (n=395)	Percentage
ACE inhibitor	187	47.3
ANGIIIR antagonist	95	24.1
β -Adrenergic agonist	31	7.8
β -Adrenergic antagonist	209	52.9
Calcium channel blocker	88	22.3
Insulin	299	75.7
K ⁺ -saving diuretic	48	12.2
Loop diuretic	75	19.0
Metformin	251	63.5
Nitrate	54	13.7
PPI	179	45.3
Statin	293	74.2
Sulfonylureum	77	19.5
Thiazide diuretic	128	32.4

ACE, angiotensin-converting enzyme; ANGIIIR, angiotensin II receptor; PPI, proton pump inhibitor.

taken after an overnight fasting period. The samples were analyzed for laboratory parameters (glycated hemoglobin (HbA1c), plasma glucose, creatinine, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides) and immediately stored at -80°C for further analyses. A detailed description of the patient characteristics is reported in Table 1.

Participants were requested to bring all their medication on the day of the visit or lists from pharmacists to score their medication use accurately. Medication was classed into groups according to the Anatomical Therapeutic Chemical Classification System (ATC) for statistical analysis (Table 2). The study was performed according to the Declaration of Helsinki. All patients provided written informed consent. All study investigators had access to the study data, and they reviewed and approved the final manuscript.

Measures

This study was designed as an observational cohort study using samples from the existing Diabetes Pearl cohort biobank (31). Laboratory values (fasting) and use of medication of 402 patients were examined at the inclusion date. Seven patients were excluded because of insufficient sample availability. Not only Mg^{2+} but also sodium (Na^+) and potassium (K^+) concentrations were measured in the stored plasma and urine samples from the biobank. Fasting plasma Mg^{2+} concentrations were determined using a spectrophotometric assay (Roche/Hitachi) and measured at 600 nm on a Bio-Rad Benchmark plus microplate spectrophotometer (Bio-Rad Laboratories),

Figure 1

Hypomagnesemia in type 2 diabetes patients. The plasma Mg^{2+} concentration was determined in 395 type 2 diabetes patients. Hypomagnesemia was observed in 30.6% of the patients. Bars represent the number of patients. Dotted vertical lines indicate the normal range (0.7–1.1 mmol/L).

according to the manufacturer's protocol. Fasting plasma Na^+ , K^+ and creatinine and urinary Mg^{2+} , Na^+ , K^+ and creatinine concentrations were measured at the clinical chemistry department using standardized methods. The fractional excretion of Mg^{2+} (FEMg) was calculated according to the formula $(U_{\text{Mg}} \times S_{\text{crea}}) / (U_{\text{crea}} \times S_{\text{Mg}}) \times 70$ (33). GFR was estimated (eGFR) using the 'Modification of Diet in Renal Disease' (MDRD) formula: $186 \times \text{plasma creatinine}^{-1.154} \times \text{age}^{-0.203} \times 1.210$ (if black) $\times 0.742$ (if female) (34). Patients with an eGFR $<30 \text{ mL/min}$ were excluded from subsequent urinary analyses.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (s.d.). Pearson's correlation tests were performed to determine the association between plasma Mg^{2+} concentration and clinical and anthropomorphic parameters. HbA1c and plasma values of triglycerides, glucose, LDL, HDL and total cholesterol were $\log_{10}$ transformed before use in statistical analyses. All variables with a P value <0.1 in the Pearson's correlation analyses, not corrected for multiple testing, were checked for confounding by performing partial regression analyses. Variables that correlated to plasma Mg^{2+} concentration with $P < 0.1$, after correcting for confounding, were included in the stepwise multivariate backward elimination model. In this model, variables

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Table 3 Univariate analyses for correlation of patient characteristics on plasma Mg^{2+} concentration.

Variable	Pearson's correlation coefficient	P value	n
Demographics			
Gender	0.007	0.897	395
BMI (kg/m^2)	-0.162	0.001	394
Age (years)	0.089	0.076	395
Duration diabetes (years)	0.139	0.008	365
Waist circumference (cm)	-0.158	0.002	392
SBP (mmHg)	-0.056	0.269	395
DBP (mmHg)	-0.144	0.004	395
Heart rate (beats/min)	-0.104	0.039	395
Alcohol consumption (no/yes)	-0.004	0.680	390
Laboratory analyses			
eGFR (mL/min)	-0.168	0.001	371
Log ₁₀ HDL (mmol/L)	0.156	0.002	387
Log ₁₀ total cholesterol (mmol/L)	-0.059	0.247	388
Log ₁₀ HbA1c (mmol/mol)	-0.123	0.015	391
Log ₁₀ triglycerides (mmol/L)	-0.273	<0.001	387
Log ₁₀ glucose (mmol/L)	-0.231	<0.001	383
Log ₁₀ LDL (mmol/L)	0.077	0.145	365
Plasma Na ⁺ (mmol/L)	0.108	0.032	391
Plasma K ⁺ (mmol/L)	0.081	0.110	394
Medication			
ACE inhibitor	-0.094	0.063	
ANGIIR antagonist	0.037	0.469	
β -Adrenergic agonist	-0.103	0.041	
β -Blocker	-0.056	0.270	
Calcium channel blocker	-0.072	0.155	
Insulin	0.109	0.030	
K ⁺ -saving diuretic	-0.023	0.685	
Loop diuretic	0.072	0.153	
Metformin	-0.268	<0.001	
Nitrate	-0.043	0.389	
PPI	-0.084	0.094	
Statin	0.002	0.962	
Sulfonylureum	-0.044	0.382	
Thiazide diuretic	-0.084	0.104	

ACE, angiotensin-converting-enzyme; ANGIIR, angiotensin II receptor; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low density lipoprotein; PPI, proton pump inhibitor; SBP, systolic blood pressure.

with $P > 0.1$ were eliminated. All statistical analyses were performed using SPSS for Windows (V22.0.0.1 IBM). A P value of <0.05 was considered statistically significant.

Results

Study population

In total, 395 type 2 diabetes patients were included in the study cohort. Clinical characteristics and laboratory results are provided in Table 1. This is a group of patients with longstanding diabetes, mostly on insulin treatment with an extensive use of medication (Table 2). The average plasma Mg^{2+} concentration in the cohort was

0.74 ± 0.10 mmol/L, and a total of 121 patients (30.6%) had levels below 0.70 mmol/L, indicating hypomagnesemia (Fig. 1). Hypermagnesemia was found in 1 patient. Plasma Na⁺ and K⁺ levels were normally distributed in the reference range (Supplementary Fig. 1, see section on supplementary data given at the end of this article).

Determinants of the plasma Mg^{2+} concentration

Using univariate regression analyses, a correlation between plasma Mg^{2+} concentration and several clinical characteristics was demonstrated: BMI ($r = -0.162$, $P = 0.001$), waist circumference ($r = -0.158$, $P = 0.002$), diastolic blood pressure (DBP, $r = -0.144$, $P = 0.004$),

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Table 4 Stepwise backward regression analysis on plasma Mg^{2+} concentration.

Variable	Coefficient	P value	Range
$\log_{10}$ triglycerides (mmol/L)	-0.073	<0.001	-0.113 to -0.033
$\log_{10}$ glucose (mmol/L)	-0.149	<0.001	-0.216 to -0.082
eGFR (mL/min)	0.000	0.022	-0.001 to 0.000
PPI*	-0.023	0.022	-0.043 to -0.003
Metformin*	-0.044	<0.001	-0.065 to -0.023
Insulin*	0.023	0.053	0.000–0.046
β -Adrenergic agonist*	-0.047	0.009	-0.082 to -0.012

All variables with a $P > 0.1$ were excluded from the model.

*No: 0, Yes: 1. eGFR, estimated glomerular filtration rate; PPI, proton pump inhibitor.

heart rate ($r = -0.104$, $P = 0.039$), eGFR ($r = -0.168$, $P = 0.001$), HbA1c ($r = -0.123$, $P = 0.015$) and plasma levels of triglycerides ($r = -0.273$, $P < 0.001$) and glucose ($r = -0.231$, $P < 0.001$) negatively correlated with the plasma Mg^{2+} concentration. In contrast, the duration of diabetes ($r = 0.139$, $P = 0.008$) and the plasma level of HDL ($r = 0.156$, $P = 0.002$) and Na^+ ($r = 0.108$, $P = 0.032$) were correlated with an increased plasma Mg^{2+} concentration (Table 3).

The effect of drug use was specifically analyzed. The use of metformin ($r = -0.268$, $P < 0.001$) or β -adrenergic receptor agonists ($r = -0.103$, $P = 0.041$) negatively correlated with plasma Mg^{2+} , whereas use of insulin ($r = 0.109$, $P = 0.030$) was associated with higher plasma Mg^{2+} levels.

Confounding factors were identified using partial correlation analyses and excluded from subsequent analysis (Supplementary Tables 1, 2, 3). After correction for confounding, all parameters correlating ($P < 0.1$) with plasma Mg^{2+} univariately, were included in the stepwise backward regression analysis model (Table 4). In this model, parameters with a P value > 0.1 were eliminated (BMI, DBP and angiotensin-converting enzyme (ACE) inhibitors). In the final model, plasma levels of glucose and triglycerides, as well as the use of PPIs, metformin or β -adrenergic receptor agonists negatively predicted plasma Mg^{2+} levels. Interestingly, patients on insulin had a trend ($P = 0.053$) toward having higher plasma Mg^{2+} levels than people not on insulin. Altogether, the model predicted (R^2) 20% of the plasma Mg^{2+} concentration.

Increased urinary Mg^{2+} excretion

To investigate whether the hypomagnesemia is explained by renal Mg^{2+} loss, the fractional excretion of Mg^{2+} (FEMg) was determined. The mean FEMg in the cohort was $3.9 \pm 2.7\%$. In total, 148 patients (40.8%) suffered from renal Mg^{2+} wasting, defined as FEMg $> 4\%$. Nevertheless,

FEMg was not significantly different ($P = 0.75$) between normomagnesemic patients ($3.9 \pm 2.8\%$) and hypomagnesemic patients ($3.9 \pm 2.5\%$) (Fig. 2).

Figure 2

Increased FEMg in type 2 diabetes patients. The fractional excretion of Mg^{2+} (FEMg) was determined in 369 type 2 diabetes patients, of which 259 were normomagnesemic (A) and 110 were hypomagnesemic (B). Bars represent the percentage of the population. Dotted vertical lines indicate the threshold for urinary Mg^{2+} wasting (FEMg $> 4\%$).

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Discussion

This study further substantiates the high prevalence of hypomagnesemia in type 2 diabetes. A negative correlation between plasma Mg^{2+} concentration and the plasma glucose and triglycerides levels was demonstrated. In addition, polypharmacy could be excluded as the main cause of hypomagnesemia in type 2 diabetes patients as only less than ten percent of changes in plasma Mg^{2+} could be attributed to medication use. These findings suggest that hypomagnesemia in type 2 diabetes patients is intrinsic to the disease.

Hypomagnesemia was observed in 30.6% of the patients, corresponding to incidence numbers of 13.5–47.7% observed in previous cohort studies (3, 4, 35, 36). In comparison, in a large study of 5179 subjects aged 55 and older, the incidence of hypomagnesemia was only 2%, highlighting the extensive amount of hypomagnesemia diabetes mellitus type 2 patients (10). Given that hypomagnesemia is related to a faster disease progression and an increased risk of diabetes-related complications, such as renal failure and cardiovascular disease, it is of great clinical importance to identify the factors that determine plasma Mg^{2+} concentrations (1, 9, 13, 37). Therefore, we constructed a statistical model that includes the factors influencing plasma Mg^{2+} levels. Twenty percent of the variation in plasma Mg^{2+} concentration is explained by our model containing eGFR, the plasma concentrations of glucose and triglycerides and the use of PPIs, insulin, metformin and β -adrenergic receptor agonists. Although this is only a modest part of the total variation, it is comparable to the effect of dietary Mg^{2+} intake and higher than current genetic models, which explain 25–30% and 1–5% of changes in the plasma Mg^{2+} concentration respectively (38, 39, 40). The finding that in our model most of the variance in plasma Mg^{2+} can be explained by factors that determine metabolic control underlines the importance of glucose and lipid homeostasis in the regulation of plasma Mg^{2+} levels.

Plasma glucose and triglyceride levels were the main determinants of plasma Mg^{2+} concentrations in our model. This correlation was independent of obesity-related factors such as waist circumference, BMI and cholesterol. Previous studies investigating metabolic syndrome have shown an association between triglycerides and Mg^{2+} levels (41, 42). However, these studies did not investigate the collinearity between triglycerides and HDL or were not based on the plasma Mg^{2+} concentration. Mg^{2+} supplementation is generally considered to improve the lipid profile in patients;

however, studies addressing the effect of Mg^{2+} on plasma triglyceride concentrations are inconsistent (43, 44). As Mg^{2+} increases the affinity of the insulin receptor tyrosine kinase for ATP, the decreased plasma Mg^{2+} levels could exacerbate insulin resistance in type 2 diabetes patients, and thereby increase plasma glucose and triglyceride concentrations (45, 46).

Medication could only explain a minor part of the changes in plasma Mg^{2+} concentration in type 2 diabetes patients (<10%), showing that polypharmacy is not the primary cause of hypomagnesemia in type 2 diabetes patients. Known hypomagnesemia-causing drugs, β -adrenergic receptor agonists and PPIs negatively correlated with the plasma Mg^{2+} concentration, although with minor effect sizes (<2%) (30, 47, 48, 49). Of all drugs, the use of metformin was most strongly correlated ($r=-0.268$, $P<0.001$) with the plasma Mg^{2+} concentration, irrespective of eGFR or fasting glucose levels. Our study is the first large cohort study to identify the association between the use of metformin and plasma Mg^{2+} levels. A few studies with limited patient numbers from the 70s and 80s suggested that treating patients with metformin reduces plasma Mg^{2+} levels (4, 50). How metformin affects Mg^{2+} handling remains to be elucidated. In contrast, patients taking insulin had a trend ($P=0.053$) toward higher plasma Mg^{2+} levels than those who did not require insulin treatment. This is in concordance with experimental studies showing that insulin stimulates the renal Mg^{2+} channel TRPM6, resulting in increased renal Mg^{2+} reabsorption (23). Therefore, despite their worse glycemic control, patients on insulin treatment have slightly better plasma Mg^{2+} values than metformin-treated patients. These results suggest that the positive renal effect of insulin on the reabsorption of Mg^{2+} overrides the negative effect of poor glycemic control of insulin-dependent patients on their Mg^{2+} levels.

We observed that high renal Mg^{2+} excretion is prevalent among type 2 diabetes patients, with 41% of the patients in the cohort having a FEMg >4%. Although these findings suggest that impaired renal Mg^{2+} reabsorption contributes to hypomagnesemia in type 2 diabetes patients, renal Mg^{2+} wasting was equally frequent in hypomagnesemic and normomagnesemic patients. Hypomagnesemia only arises when body Mg^{2+} stores, such as bone, are depleted (51). Therefore, hypomagnesemia may only develop after several years, despite increased renal Mg^{2+} excretion. Our findings of overall high Mg^{2+} excretion may thus indicate that the complete diabetic population is at risk to develop hypomagnesemia. FEMg was not related to fasting glucose

levels (data not shown), showing that diabetes severity does not influence the amount of urinary Mg^{2+} loss.

The strength of our study is the thorough and extensive phenotyping of the diabetes patients, allowing systematic investigation of the contribution of polypharmacy and metabolism-related parameters to changes in the plasma Mg^{2+} concentration of type 2 diabetes patients. By carefully collecting data on drug use, this is the first cohort study that determined the contribution of medication usage to changes in plasma Mg^{2+} concentrations. The availability of urine samples enabled the determination of the FEMg in a large cohort of severe type 2 diabetes patients for the first time.

However, several limitations have to be considered. First, data on the dietary habits of the participants was not collected, excluding the potential effects of diet from our analyses. However, the influence of diet on plasma Mg^{2+} levels will be minor, as samples were collected after an overnight fast. Secondly, all the samples were collected at a single point, and no follow-up data are available. Therefore, the observed correlations will not provide information about causality. A final limitation is the fact that the extent of insulin resistance was not directly determined. However, several studies have proposed that the product of fasting glucose and triglycerides can be used as a measure to estimate insulin resistance (52, 53). The strong correlation of plasma Mg^{2+} with glucose and triglycerides in our study therefore provides an indirect link with insulin resistance.

In conclusion, this study shows that hypomagnesemia is a prominent feature of type 2 diabetes, and is supported by excessive urinary Mg^{2+} loss. We excluded polypharmacy as the major cause of changes in plasma Mg^{2+} concentration in type 2 diabetes patients. Given that metabolic factors such as glucose and triglyceride concentrations are main determinants of the plasma Mg^{2+} concentration, Mg^{2+} disturbances should be considered and, if required, corrected in type 2 diabetes patients.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-16-0517>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Data were collected by S. K. and H. B. Statistical analysis was done by S. K. Manuscript was written by S. K., J. B., R. B., C. T. and J. H., and it was supervised by J. B., R. B., C. T. and J. H. All authors have read and approved the final version of the manuscript. J. H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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