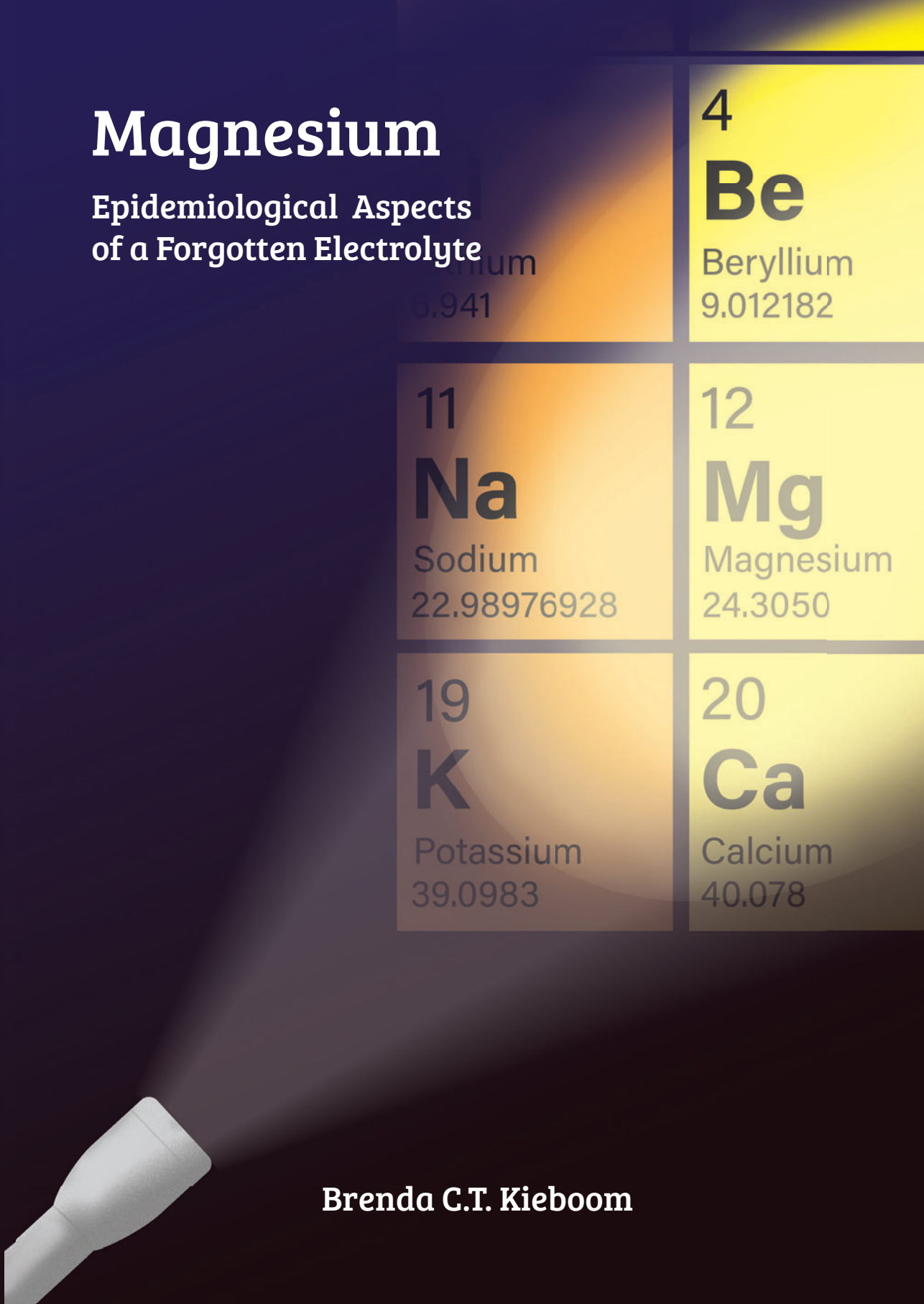
Magnesium

Epidemiological Aspects of a Forgotten Electrolyte



Brenda C.T. Kieboom

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Cover: Remco Wetzels, www.remcowetzels.nl
Printing: Ridderprint BV, www.ridderprint.nl
ISBN: 978-94-6375-162-9

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De epidemiologie van een vergeten electrolyt

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. R.C.M.E. Engels

en volgens het besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

dinsdag 13 november 2018 om 15:30 uur

door

Brenda Cornelia Theodora Kieboom
geboren te Rotterdam

Erasmus University Rotterdam

Promotiecommissie

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The Rotterdam Study is supported by the Erasmus MC and Erasmus University Rotterdam; the Netherlands Organisation for Scientific Research (NOW); the Netherlands Organisation for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); the Netherlands Genomics Initiative (NGI); the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports; the European Commission (DGXII); and the Municipality of Rotterdam.

Financial support by the Department of Research Policy of the Erasmus MC; the Dutch Heart Foundation; and the Dutch Kidney foundation for the publication of this thesis is gratefully acknowledged. Additional support from the Health and Youth Care Inspectorate, and SBOH, employer of GP trainees is gratefully acknowledged.

SBOH
voor artsen in opleiding

Voor Sophie, jij bent mijn motivatie

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Chapter 1

General Introduction and Thesis Outline

Normal serum electrolyte concentrations are crucial for maintaining organ function, especially for the central nervous system, heart, and muscles. Disorders of serum electrolyte concentration negatively contribute to health outcomes and may be the first signs of an underlying disease.(1) Electrolyte disturbances are common in the general population, with approximately 15% of all persons above 55 years having at least one abnormal electrolyte measurement.(2) During the studies reported in this thesis, we encountered a bias within the electrolyte measurements performed within the Rotterdam Study. We explore this bias and the role of automation in this bias in *Chapter 2*. Because of this bias, we were unable to study disorders of serum sodium and potassium concentration, the most common electrolyte disturbances.(2) An electrolyte that was not influenced by this bias and has received little attention, despite its important role in a wide range of cellular functions, is magnesium.(3) The cellular functions of magnesium include a role in DNA replication, RNA transcription, amino acid synthesis and protein formation. In addition, magnesium is a cofactor and regulator for nearly 600 enzymes.(3) Within the body, serum magnesium concentrations are very tightly regulated and are determined by the uptake of magnesium from food through the intestine, storage of magnesium in the bone, and excretion of magnesium by the kidneys.(3)

In the *main part* of this thesis, we study magnesium homeostasis within the general population. To do so, we use data from the Rotterdam Study, a prospective population-based cohort study, ongoing since 1990 in a suburb of Rotterdam, The Netherlands.(4) The routine measurements of serum magnesium levels, extensive data collection on comorbidities, detailed medication history, and long-term follow-up make this cohort suitable to study the role of serum magnesium in healthy participants and to study the effect of pharmaceutical drugs on magnesium homeostasis.

In *Chapter 3*, we study the effect of proton pump inhibitor (PPI) use on serum magnesium levels and the risk of hypomagnesemia. PPIs are the main therapy for gastroesophageal reflux disease, peptic ulcer disease, non-ulcer dyspepsia, and prevention of gastropathy with the use of nonsteroidal anti-inflammatory drugs.(5) PPIs are currently among the most frequently used medicines, although a strict medical indication for their use is not always present.(5) Recent case reports and case series show that PPIs cause hypomagnesemia, however it is unclear if these cases are exceptions or if this effect is also seen in the general population and what underlying factors increase the risk of hypomagnesemia.(6) In *Chapter 4*, we study the effect of loop and thiazide diuretics on serum magnesium levels. Loop and thiazide diuretics are often used for a variety of indications such as heart failure and high blood pressure.(7) They are known to influence serum sodium and potassium levels, however, their effect on serum magnesium levels has been primarily studied in animal models and healthy volunteers. Therefore, it is unknown whether patients using these diuretics are at risk for hypomagnesemia. We study if loop

diuretics and thiazide diuretics cause hypomagnesemia and whether this effect is dependent on dose and duration of use.

In the *Chapters 5, 6, and 7*, we study the consequences of low serum magnesium levels on a variety of diseases, including heart disease, diabetes, and dementia. Low serum magnesium levels have been associated with progression of atherosclerosis and development of arrhythmia's.(8, 9) Therefore, in *Chapter 5*, we explore if low serum magnesium translates into an increased risk of dying from coronary heart disease or sudden cardiac death. Recent estimations indicate that one out of three people will develop diabetes mellitus during their life.(10) In *Chapter 6*, we study if serum magnesium levels are associated with an increased risk of prediabetes, the precursor state of diabetes. In *Chapter 7*, we study if serum magnesium levels could be a potential modifiable risk factor for dementia, as there are currently limited options to prevent or treat dementia and the number of people living with dementia is expected to double in the years to come.(11)

In the *final part* of this thesis we place our main findings with regard to the importance of serum magnesium in the context of current knowledge and discuss implications and suggestions for further research.

Aims of the thesis

1. To analyze the laboratory process of electrolyte measurements in epidemiological studies
2. To analyze whether the use of PPIs is associated with lower serum magnesium levels in the general population.
3. To analyze the effects of diuretics on serum magnesium in the general population.
4. To analyze the association between serum magnesium and disease outcomes, including cardiovascular mortality, diabetes mellitus, and dementia.

PART I
Measurement of Serum Electrolytes

Chapter 2

Standard Process-oriented Workflow Introduces Pre-analytical Error when used in Large Study Sample Batches

The quality of a laboratory measurement can be influenced by various processes within the pre-analytical, analytical and post-analytical phase. Over the past years, advances in automatization and quality control measures have improved the quality and reduced the total number of errors to only 0.31%.(12, 13) When using laboratory measurements for research, even a small bias, which may not be clinically relevant, can dilute or inflate results leading to false results and incorrect interpretation of risk estimates. Knowing the cause of such errors is important to assess the potential impact on study results and ultimately to prevent these errors.
In general, the cause of errors in laboratory measurements used for research does not differ from measurements in routine clinical practice and includes interference with additives in the blood collection tube and the timely processing of samples.(14, 15) However, within the Rotterdam Study, a prospective population-based cohort study(4), We observed a bias in the serum sodium levels measurements not seen in routine clinical work.

From a specific set of snap frozen, stored samples of 9,894 participants, a total of 19 tests was ordered (**Table 2.1**). Measurements were performed using a Cobas 8000 analyzer (Roche Diagnostics, Mannheim, Germany) with intelligent sample routing algorithm.(16) The software controls the flow and logistics of the samples in the system and enables the fastest turnaround time (TAT) for routine clinical samples, maximizing sample throughput based on the real-time workload situation of each module and the entire unit.

Within the samples, the mean serum sodium concentration was 142.1 mmol/L, whereas it was expected to be approximately 140.0 mmol/L given the fact that this study was performed within a general population and serum sodium is regulated within a narrow range (137–142 mmol/L).(17) Serum sodium levels were normally distributed with a standard deviation of 3.1 mmol/L and a range of 124–171 mmol/L. Within 5207 of the 9894 participants, serum sodium had been measured 7 years earlier, showing a mean serum sodium concentration of 140.1 mmol/L, with a standard deviation of 4.1 mmol/L and a range of 124–160 mmol/L. Characteristics of the current study population were compared with the previous round, but no major differences were observed, which could explain the shift toward higher serum sodium levels (data not shown).

Table 2.1 - Roche Cobas 8000 characteristics of measurants within the Rotterdam Study

Analyte	Sample volume (microliter)	Analysis time (minutes)	Module	Unit
Uric acid	3	10	C702	Chemistry
Sodium Potassium Chloride	15	5	ISE	Potentiometry
Calcium	3	10	C702	Chemistry
Magnesium	3	10	C702	Chemistry
Phosphate	2.5	10	C702	Chemistry
IgA	5	10	C502	Chemistry
IgG	5	10	C502	Chemistry
IgM	5	10	C502	Chemistry
C3	10	10	C502	Chemistry
C4	15	10	C502	Chemistry
hsTnT STAT	50	9	E602-1	Immunochemistry
CKMB mass	15	9	E602-1	Immunochemistry
Vitamin B12	15	27	E602-2	Immunochemistry
Folate	25	27	E602-2	Immunochemistry
CEA	10	18	E602-2	Immunochemistry
C-peptide	20	18	E602-1	Immunochemistry
N-MID Osteocalcin	20	18	E602-1	Immunochemistry

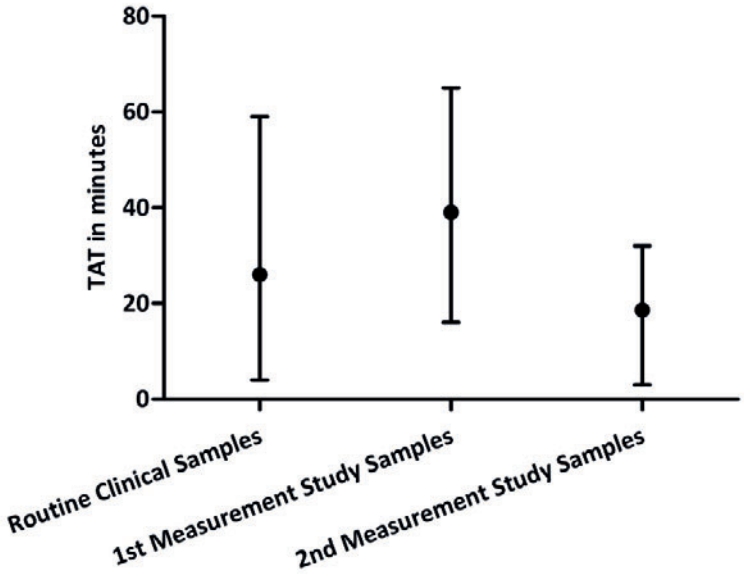
Internal and external quality control reports from that specific time period were examined and showed no systematic deviations (data not shown). Also, no urgent field safety notice had been issued regarding the sodium assay.(18) All routine clinical serum sodium measurements, analyzed within the same time frame as the study samples, were requested and a mean serum sodium concentration of 140 mmol/L was observed within 72,354 measurements (standard deviation of 5.2 mmol/L). Additionally, the validity of the serum sodium measurements in the sample was confirmed by using an alternate method to measure sodium (i.e. flame photometry), ruling out an analytical problem.

In 120 randomly selected participants, we remeasured the electrolytes using a second serum sample processed and stored under the same conditions as the first, and analyzed on the same Cobas 8000 analyzer. This yielded normal results with a mean serum sodium of 140.0 mmol/L and standard deviation of 5.0 mmol/L, ruling out a problem in the pre-analytical phase up until thawing. Comparison of the two measurements showed both a systematic shift of the total distribution of approximately 2 mmol/L, but also a random shift showing a change in ranking of the participants.

Subsequently, we focused on the flow of the study samples through the analyzer and the on-board TAT, defined as the time from sample recognition on the analyzer to the first measured results, for the analyses of sodium. Using a representative sample day, we found a mean TAT for sodium of 36 min (range 16–65 min) in study samples, compared to a mean TAT of 15 min (range 4–59 min) for the same analyte in routine clinical samples (Figure 1). Simulation tools from Roche revealed that this was caused by the large number of samples with a similar predefined test panel offered in bulk to the analyzer, which is different compared to routine clinical work. Because the panel consisted of tests analyzed on all five analyzer units within the Cobas 8000, random trafficking of samples to the different units occurs. Samples were going first to the immunochemistry units, chemistry units or potentiometry unit depending on the work load of the unit. Another difference compared to routine clinical samples was the high number of tests performed within a relatively small sample volume (19 tests per 500 µL vs. 6–8 tests per 4 mL in routine clinical practice). The combination of the prolonged TAT and the low sample volume left in the tube at the end of the analytical run resulted in larger than normal on-board evaporation, affecting the sodium (and potassium and chloride) tests to a greater extent as a large part of the samples were first randomly routed either to the immunochemistry or to the chemistry units, thereby delaying the electrolyte measurement in a large number of samples significantly.

Due to the great variation in on-board time of the sample (range 16–65 min), evaporation did not affect all samples to the same extent, and both a random error as well as a systematic error was observed. The random error made it impossible to calculate a correction factor; therefore, we had to remeasure all samples for serum sodium and other electrolytes, using a new designed handling protocol as the user cannot change the intelligent sample flow algorithm and force the analyzer to measure the analytes in a specific order. In the new protocol, samples were offered in two consecutive rounds of analysis. The samples were first routed to the potentiometry and chemistry unit in a 2-mL tube before transferring the sample to a 500-µL microtainer and routing the sample to the immunochemistry unit, the TAT for analyzing the electrolytes reduced averaging 21 min (range 3–32 min). This brought the average TAT for electrolytes in our study samples back to the range as seen for regular clinical samples (**Figure 2.1**).

Figure 2.1 - Turnaround time of serum sodium measurements



Footnotes

Values represent mean turnaround time and range.

In conclusion, standard process-oriented work flow used for routine clinical samples can introduce bias when used for measurement of study samples, particularly if the ratio diagnostic test to volume is higher than in regular clinical samples and the combination of tests ordered is different from clinical practice. To guarantee the quality of the outcome of clinical and epidemiological studies, we stress that there should be a close collaboration between the researcher, clinicians and laboratory specialists. In case of the setup or large studies, the vendor should be consulted as they can provide insight into the analytical workflow and can predict how the study workflow can influence the results for study samples.

PART II
Drug-induced Hypomagnesemia

Chapter 3

Proton Pump Inhibitors and Hypomagnesemia in the General Population: A Population-Based Cohort Study

ABSTRACT

Background: Proton pump inhibitor (PPI) use has been associated with hypomagnesemia in case reports and hospital-based cohort studies. Our objective was to determine whether PPI use is associated with hypomagnesemia in the general population and whether this is also found in histamine 2 receptor antagonist (H2RA) users.
Study Design: Prospective cohort study.
Setting & Participants: 9,818 individuals from the general population (Rotterdam Study).
Predictor: PPI use and H2RA use compared to no use.
Outcomes & Measurements: Serum magnesium and hypomagnesemia (serum magnesium ≤ 0.72 mmol/L). Analyses were adjusted for age, sex, body mass index, kidney function, comorbid conditions, and alcohol and diuretic use.
Results: Serum magnesium level was 0.011 mmol/L lower in PPI users (n =724; 95% CI, -0.016 to -0.007 mmol/L) versus those with no use. PPI use was associated with increased risk of hypomagnesemia (n = 36; OR, 2.00; 95% CI, 1.36-2.93) compared to no use. Effect modification was found between the use of PPIs and loop diuretics; in participants using loop diuretics (n = 270), PPI use was associated with a further increased risk of hypomagnesemia (n = 5; OR, 7.22; 95% CI, 1.69-30.83) compared to no use. The increased risk with PPIs was only seen after prolonged use (range, 182-2,618 days; OR, 2.99; 95% CI, 1.73-5.15). Including dietary magnesium intake into the model did not alter results (available for 2,504 participants, including 231 PPI users). H2RA users (n = 250) also had a lower serum magnesium level (-0.008 [95% CI, -0.016 to -0.001] mmol/L) and increased risk of hypomagnesemia (n = 12; OR, 2.00; 95% CI, 1.08-3.72) compared to those with no use, but no interaction with loop diuretics.
Limitations: Cross-sectional analysis with single serum magnesium measurement.
Conclusions: PPI use is associated with hypomagnesemia in the general population. Prolonged PPI use and concomitant loop diuretic use are associated with a stronger risk increase. Similar but weaker associations were found in H2RA users, except for interaction with loop diuretics.

Proton pump inhibitors (PPIs) are currently the main therapy for gastroesophageal reflux disease, peptic ulcer disease, non-ulcer dyspepsia, and prevention of gastropathy with the use of nonsteroidal anti-inflammatory drugs.(5) The broad spectrum of indications and the favorable safety profile have made them one of the most frequently used pharmaceuticals.(5, 19) Because of their widespread and often long-term use, the safety of PPIs has received attention since their first introduction. Since 2006, cases of severe hypomagnesemia have been reported in association with the use of PPIs, sometimes accompanied by secondary hypokalemia and hypocalcemia.(20) Severe hypomagnesemia may result in tetany, convulsions, or cardiac arrhythmias.(6) Although mild hypomagnesemia is often asymptomatic, it may still be relevant because population studies have shown that even mild hypomagnesemia is associated with increased risk of diabetes mellitus,(21) osteoporosis,(22) cardiovascular disease,(8, 23) and mortality.(2) Cases of severe hypomagnesemia have not been reported with the use of histamine 2 receptor antagonists (H2RAs), although a recent study showed that their long-term use is also associated with hypomagnesemia.(24) At present, the evidence for the association between PPI use and hypomagnesemia is based on case reports (for review, see (5)), 6 studies in hospitalized patients,(19, 25-29) and 1 study in ambulatory patients.(24) The latter study was important because of its size (95,000 participants) and the suggestion that PPI-induced hypomagnesemia also occurs in the community. Our objective was to analyze the association between PPI use and risk of hypomagnesemia in a population-based cohort with systematic measurements of serum magnesium. We also analyzed whether prolonged duration of PPI use and concomitant diuretic use were associated with increased risk of hypomagnesemia. Finally, we also assessed the association between the use of H2RAs and hypomagnesemia.

METHODS

Study Design, Setting, and Population

This cross-sectional analysis was performed within the Rotterdam Study, a prospective population-based cohort that started in 1990. The first cohort comprised 7,983 persons older than 55 years living in a suburb of the city of Rotterdam, the Netherlands. Starting in 2000, the first cohort was extended with a second cohort of 3,011 persons (aged ≥ 55 years). In 2006, the cohort was extended again with a third cohort of 3,932 persons (aged ≥ 45 years) living in the research area who had not yet been included. Follow-up examinations were conducted periodically. We used the third visit of the first cohort, with 4,797 remaining participants, and baseline visits of the second and third cohorts. These visits were identical in design. All 11,740 eligible participants gave written informed consent to participate in the study and to obtain information from their treating physicians. All participants underwent the same examinations and had identical blood measurements. Detailed information for design, objectives, and methods of the Rotterdam Study are described elsewhere.(30) The Rotterdam Study complies with the

Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Dutch Ministry of Health, Welfare and Sport, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Study Act: Rotterdam Study)."

Measurement of Serum Magnesium

Of 11,740 eligible participants, serum magnesium was available for 9,883, of whom 56 did not give consent to use their pharmacy data, we were unable to retrieve prescription data for 2 participants, and 7 were excluded because of combined use of PPIs and H2RAs. The study population therefore consisted of 9,818 participants. Serum magnesium was measured in all participants at the same time by the Department of Clinical Chemistry of the Erasmus Medical Center using a Roche/Hitachi Cobas c501 analyzer. The cutoff for hypomagnesemia was determined by calculating the mean minus 1.96 standard deviation in participants who did not use acid-suppressive medication, which resulted in a cutoff of 0.72 mmol/L.

Assessment of Medication Use

We used data from visits from 1997 through 2008 because PPI use became more widespread during this period and PPIs were not sold over the counter in this period in the Netherlands. Drug exposure has been monitored continuously since January 1, 1991, through computerized pharmacy records of all outpatient-filled prescriptions within the pharmacies in the district.(31) For each participant, the prescription period was calculated by dividing the total number of dispensed tablets per prescription by the prescribed daily number. Repeat prescriptions that were filled within 7 days after ending a previous one were considered as one single episode of continual use. If the date of serum magnesium measurement was within the prescription period, the participant was classified as being exposed. Cumulative prescription days could also be determined on the basis of this method. Dosage was expressed as standardized "defined daily doses" according to the definition of the World Health Organization.(32) In addition to prescription drugs, we also had interview data for self-reported use of vitamin and mineral supplementation (including magnesium) for 9,282 participants.

Assessment of Dietary Magnesium Intake

Daily magnesium intake was available for a subgroup of 2,504 participants, of whom 2,246 participants did not use acid-suppressive medication, 231 used PPIs, and 27 used H2RAs. Daily magnesium intake was assessed by using an extensive semi-quantitative food frequency questionnaire.(33-35) Dietary energy and magnesium intake were calculated using the Dutch Food Composition Table.(36) Energy-adjusted magnesium intakes were computed as the unstandardized residuals from a linear regression model in which total caloric intake served as the independent variable, and absolute nutrient intake, as the

dependent variable. Because residuals have a mean of zero and thus include negative values, the predicted mean magnesium intake of the study population (300 mg/d) was added to the residuals.(37)

Assessment of Covariables

Assessment of anthropometrics in the Rotterdam Study was described previously.(30) We defined diabetes mellitus as fasting serum glucose level ≥ 7.0 mmol/L, non-fasting serum glucose level ≥ 11.1 mmol/L (only if fasting serum was unavailable), or use of oral blood glucose-lowering drugs or insulin. Body mass index was calculated as weight in kilograms divided by squared height in meters. Information for prevalent stroke and coronary heart disease was determined on the date that blood was drawn from participants and obtained through linkage with general practitioners working in the study area and adjudicated by medical doctors and a neurologist and cardiologist. Blood pressure was measured twice during study visits, and prevalent hypertension was determined on the basis of average systolic blood pressure ≥ 140 mm Hg and/or average diastolic blood pressure ≥ 90 mm Hg or the use of blood pressure–lowering medication. Information for alcohol consumption was obtained during a home interview and categorized as yes or no. Estimated glomerular filtration rate was calculated with calibrated creatinine values using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine equation and was expressed as mL/min/1.73m^2.(38)

Statistical Analysis

Mean ± standard deviation and frequency with percentage were used to report continuous and discrete variables, respectively. One-way ANOVA and Chi-Square tests were used for baseline comparisons. When studying the possible association between PPI use with serum magnesium or hypomagnesemia, H2RA users were excluded from the analysis and vice versa. We used multivariable linear regression to investigate the association with serum magnesium and multivariable logistic regression to investigate the association with hypomagnesemia. All analyses were performed using a crude model and a model adjusted for age; sex; body mass index; estimated glomerular filtration rate; prevalent diabetes mellitus, stroke, coronary heart disease, and hypertension; alcohol use; and use of thiazide or loop diuretics. Effect modification by diuretic use or diabetes mellitus was tested by inclusion of interaction terms. For significant interactions, analysis was stratified on this potential effect modifier. The effect of duration of use was analyzed by defining 3 different exposure categories based on tertiles of duration of use. This was done separately for PPIs and H2RAs, for which no acid-suppression use was used as the reference category. P for trend was calculated across the 3 tertiles and the reference category. To assess confounding by indication, we studied the association between PPIs and serum phosphate level as a proxy of dietary intake (which might be reduced by dyspepsia), as reported previously.(25) In addition, we performed 2 sensitivity analyses to

account for energy-adjusted magnesium intake and magnesium intake through vitamin and mineral supplementation. Missing data for covariables (0%-1.4%) were handled by single imputation using an expectation-maximization algorithm.(39) All analyses were repeated on complete cases to check for potential differences between results based on imputed data and those based on complete cases. With the exception of baseline characteristics, results are reported for imputed data. Data were analyzed using SPSS Statistics (IBM, version 21.0). A 2-sided $P < 0.05$ was considered statistically significant.

RESULTS

Cohort Characteristics

The study population consisted of 9,818 participants, of whom 96.0% were of European ancestry. Population characteristics are shown in **Table 3.1**. A total of 724 participants used PPIs (7.4%), with 1.2 ± 0.6 defined daily doses. Thirty-six PPI users (5.0%) had hypomagnesemia (lowest serum magnesium, 0.34 mmol/L). There were 250 participants who used H2RAs (2.5%), with 0.9 ± 0.4 defined daily doses. Twelve H2RA users (4.8%) had hypomagnesemia (lowest serum magnesium, 0.67 mmol/L). **Figure 3.1** shows how serum magnesium concentrations were distributed between participants without acid-suppressive medication and participants who used PPIs or H2RAs. This figure shows that participants using PPIs or H2RAs more often had a serum magnesium level in the lowest quartile.

Figure 3.1 Serum Magnesium Level in Quartiles

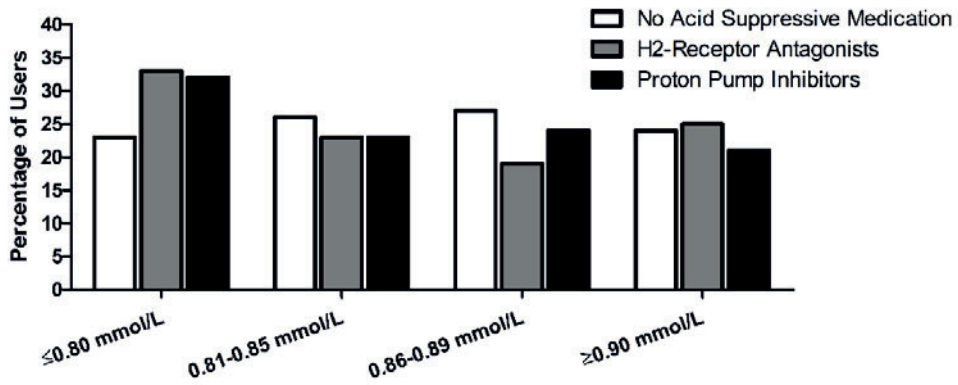


Table 3.1 - Characteristics of the study population

	Total cohort N=9,818	Proton pump inhibitors N=724	H2-receptor antagonists N=250	No acid-suppressive medication N=8,884
Women, N(%)	5570 (56.7)	437 (60.4)*	158 (63.2)‡	4975 (56.3)
Age, years	65.0 (9.9)	65.3 (10.0)†	69.3 (9.3)‡	65.0 (9.9)
Body mass index, kg/m^2	27.3 (4.2)	28.7 (4.5)†*	27.9 (4.1)‡	27.1 (4.2)
Energy-adjusted magnesium intake[a], mg/day	300.0 (77.1)	289.9 (100.2)	297.0 (93.3)	301.1 (74.1)
Diabetes mellitus, N(%)	1024 (10.4)	91 (12.6)*	30 (12.0)	903 (10.2)
Hypertension, N(%)	6091 (62.7)	505 (70.1)*	185 (74.3)‡	5401 (61.8)
Coronary Heart Disease, N(%)	686 (7.0)	71 (9.8)*	35 (14.0)‡	580 (6.6)
History of Stroke, N(%)	340 (3.5)	42 (5.8)*	7 (2.8)	291 (3.3)
eGFR, mL/min/1.73 m^2	79.6 (15.8)	76.6 (16.6)†*	74.0 (16.0)‡	80.0 (15.7)
Hypomagnesemia[b], N(%)	247 (2.5)	36 (5.0)*	12 (4.8)‡	199 (2.3)
Serum magnesium, mmol/L	0.84 (0.06)	0.83 (0.07)*	0.84 (0.07)‡	0.85 (0.06)
Serum calcium, mmol/L	2.43 (0.10)	2.44 (0.11)*	2.43 (0.11)	2.43 (0.10)
Serum potassium, mmol/L	4.35 (0.34)	4.36 (0.39)	4.35 (0.35)	4.35 (0.34)
Thiazide diuretics, N(%)	671 (6.8)	82 (11.3)*	28 (11.2)‡	561 (6.3)
Loop diuretics, N(%)	270 (2.7)	45 (6.2)*	14(5.6)‡	211 (2.4)

Footnotes

Values are counts (percentages) or means (standard deviation).

These data were available for 2504 subjects of whom 231 PPI-users, 27 H2-receptor antagonist users and 2246 no acid-suppression users.

a. Hypomagnesemia was defined as serum magnesium level ≤ 0.72 mmol/L

* $P < 0.05$, when comparing PPI-users with participants who did not use acid-suppressive medication

† $P < 0.05$, when comparing PPI-users with participants who used H2-receptor antagonists

‡ $P < 0.05$, when comparing participants using H2-receptor antagonists with those not using acid-suppressive medication

Relationship of PPI Use to Serum Magnesium

Use of a PPI was associated with significantly lower serum magnesium levels (-0.012 [95% CI, -0.017 to -0.008] mmol/L) compared to no use and this relationship persisted after multivariable adjustment (-0.011 [95% CI, -0.016 to -0.007] mmol/L; **Table 3.2**).

Table 3.2 - Association between proton pump inhibitor use, H2-receptor antagonist use and serum magnesium

	Change in serum magnesium level (mmol/L)			
	β-coefficient	95% confidence interval for β		P-value
		Lower	Upper	
Proton pump inhibitors (n=724)[a]				
Unadjusted model	-0.012	-0.017	-0.008	<0.001
Adjusted model[b]	-0.011	-0.016	-0.007	<0.001
Stratified analysis[c]				
No loop diuretic use (n=679)	-0.010	-0.014	-0.005	<0.001
Loop diuretic use (n=45)	-0.035	-0.060	-0.010	0.007
H2-receptor antagonists (n=250)[a]				
Unadjusted model	-0.008	-0.016	-0.001	0.03
Adjusted model[b]	-0.009	-0.016	-0.001	0.02
Stratified analysis[d]				
No loop diuretics (n=236)	-0.010	-0.017	-0.002	0.01
Loop diuretics (n=14)	0.010	-0.028	0.048	0.6

Footnotes

a. Compared to no acid-suppression users (n=8,844)
b. Model includes: age, gender, BMI, renal function (eGFR), prevalent diabetes mellitus, stroke, coronary heart disease, hypertension, alcohol use, thiazide and loop diuretic use
c. P for interaction: 0.03 between PPIs and loop diuretics
d. P for interaction: 0.09 between H2-receptor antagonists and loop diuretics

We found significant interaction between PPI use and loop diuretic use (P for interaction = 0.03). A greater reduction in serum magnesium levels was observed in participants who concomitantly used PPIs and loop diuretics (-0.035 [95% CI, -0.060 to -0.010] mmol/L) compared to participants who used none of these drugs. No significant interaction between PPI use and thiazide diuretic use or the presence of diabetes mellitus was identified (P for interaction = 0.9 for both). After multivariable adjustment, lower serum

magnesium levels were also observed in participants who used H2RAs (-0.008 [95% CI, -0.016 to -0.001] mmol/L) compared to those with no use. No significant interaction was observed between H2RA use and loop diuretic use (P for interaction=0.09). This also pertained to H2RA use and thiazide diuretic use, as well as the presence of diabetes mellitus (P for interaction=0.09 and 0.5, respectively).

Relationship of PPI Use to Hypomagnesemia

Use of a PPI was associated with higher risk of hypomagnesemia compared to no use, before and after adjustment for potential confounders (odds ratios [ORs] of 2.27 [95% CI, 1.58-3.27] and 2.00 [95% CI,1.36-2.93], respectively; **Table 3.3**).

Table 3.3 - Association between proton pump inhibitor use, H2-receptor antagonist use and hypomagnesemia

	Risk of hypomagnesemia[a]		
	Cases, N(%)	**Odd ratio (95% CI)**	**P-value**
Proton pump inhibitors (n=724)[b]			
Unadjusted model	36 (5.0)	2.27 (1.58 to 3.27)	<0.001
Adjusted model[c]	36 (5.0)	2.00 (1.36 to 2.93)	<0.001
Stratified analysis			
No loop diuretics (n=679)	31 (4.6)	1.79 (1.19 to 2.69)	0.005
Loop diuretics (n=45)	5 (11.1)	7.22 (1.69 to 30.83)	0.008
H2-receptor antagonists (n=250)[b]			
Unadjusted model	12 (4.8)	2.19 (1.21 to 3.98)	0.01
Adjusted model[c]	12 (4.8)	2.00 (1.08 to 3.72)	0.03
Stratified analysis			
No loop diuretics (n=236)	11 (4.7)	1.90 (1.00 to 3.61)	0.05
Loop diuretics (n=14)	1 (7.1)	13.49 (0.68 to 266.03)	0.09

Footnotes

a. Hypomagnesemia was defined as serum magnesium level ≤ 0.72 mmol/L
b. Compared to no acid-suppression use (n=8,844, 199 cases)
c. Model includes: age, gender, BMI, renal function (eGFR), prevalent diabetes mellitus, stroke, coronary heart disease, hypertension, alcohol use, thiazide and loop diuretic use

H2RA use was also associated with increased risk of hypomagnesemia, before and after adjustment for potential confounders (ORs of 2.19 [95% CI, 1.21-3.98] and 2.00 [95% CI, 1.08-3.72], respectively) compared to no use. A duration-of-use analysis showed that the increased risk of hypomagnesemia was mainly present in participants in the highest tertile of duration of PPI use (>182 days; P for trend< 0.001; **Table 3.4**). In these participants, the

risk of hypomagnesemia nearly tripled (OR, 2.99; 95% CI, 1.73-5.15) compared to no use. In H2RA use, the same trend was observed, with increased risk of hypomagnesemia in the highest tertile of duration of use (>111 days; OR, 2.55; 95% CI, 0.99-6.58; P for trend = 0.02) compared to no use.

Table 3.4. Association between duration of proton pump inhibitor use or H2-receptor antagonist use with hypomagnesemia

		Risk of hypomagnesemia[a]	
	Cases, N(%)	**Odds ratio (95%CI)**	**P-value**
Proton pump inhibitors[b,c,d]			
Tertile 1 (1 to 61 days)	9 (3.8)	1.46 (0.72 to 2.97)	0.3
Tertile 2 (62 to 181 days)	10 (4.1)	1.62 (0.83 to 3.18)	0.2
Tertile 3 (182 to 2618 days)	17 (7.0)	2.99 (1.73 to 5.15)	<0.001
H2- receptor antagonists[b,c,e]			
Tertile 1 (1 to 35 days)	3 (3.6)	1.49 (0.45 to 4.89)	0.5
Tertile 2 (36 to 110 days)	4 (4.8)	2.00 (0.70 to 5.67)	0.2
Tertile 3 (111 to 1226 days)	5 (6.0)	2.55 (0.99 to 6.58)	0.05

Footnotes

a. Hypomagnesemia was defined as serum magnesium level ≤ 0.72 mmol/L
b. Compared to no acid-suppression use (n=8,844, 199 cases)
c. Model includes: age, gender, BMI, renal function (eGFR), prevalent diabetes mellitus, stroke, coronary heart disease, hypertension, alcohol use, thiazide and loop diuretic use
d. P for trend < 0.001
e. P for trend = 0.017

Sensitivity Analyses

To analyze whether PPI-induced hypomagnesemia could be explained by confounding by indication, we performed sensitivity analyses with serum phosphate level, dietary magnesium intake, and use of vitamin and mineral supplementation. No significant association was found between PPI use and serum phosphate level (0.001 [95% CI,-0.010 to 0.012] mmol/L) compared to no use. This suggests that the indication for PPIs did not result in poor dietary intake and therefore lower serum magnesium and phosphate levels. Furthermore, the association between PPI use and serum magnesium level was not altered by the addition of energy-adjusted magnesium intake to the adjusted model (before adjustment, -0.015 [95% CI, -0.023 to -0.007] mmol/L; after adjustment, -0.015 [95% CI, -0.022 to -0.007] mmol/L). Similarly, the addition of self-reported use of vitamin and mineral supplementation in the model did not change the association between PPI use and serum magnesium level (-0.011 [95% CI, -0.015 to -0.006] mmol/L before and after adjustment) compared to no use.

DISCUSSION

The 4 key findings of this prospective population-based study were: (1) the demonstration that PPI use is associated with increased risk of hypomagnesemia in the general population, (2) the higher risks with prolonged PPI use, (3) the higher risk with concomitant loop diuretic use, and (4) the identification of similar but weaker associations with the use of H2RAs. In the paragraphs that follow, these findings are discussed in more detail and placed in the context of previous studies of this topic.(24, 25, 29)

In several case reports and case series, it was already noted that PPI-induced hypomagnesemia primarily occurs in patients who use PPIs long term (i.e., .3 months).(5, 6, 40, 41) Our study confirms these observations and is in agreement with the prediction that small deficits in magnesium balance may eventually lead to hypomagnesemia. For example, 40 mg of omeprazole in healthy volunteers reduced magnesium absorption by only 1%,(42) but it was predicted that this could lead to an 80% depletion of magnesium stores over the course of 1 year.(43) The slow depletion of intracellular magnesium stores may place individuals who use PPIs or H2RAs long term at risk for frank hypomagnesemia, especially in combination with additional factors such as diuretics or intercurrent illness.(6)

Although PPI-induced hypomagnesemia is caused by reduced intestinal magnesium absorption,(40, 44, 45) we found that the risk of hypomagnesemia increased with concomitant use of loop diuretics. Loop diuretics may compromise the kidney magnesium reabsorption necessary to compensate for intestinal magnesium loss. A study of critically ill patients also observed that combined use of PPIs and diuretics was associated with a greater decrease in serum magnesium level.(25) In that study, this effect was also primarily seen with loop diuretics, which were used almost twice as frequently as thiazide diuretics.(6) The fact that we only observed effect modification with loop diuretics is surprising. Thiazide diuretics can also cause hypomagnesemia by increased renal magnesium wasting,(46, 47) and thiazide diuretics were used twice as frequently as loop diuretics in our population (providing sufficient power for this analysis). This suggests that quantitatively, magnesium reabsorption in the loop of Henle (where loop diuretics act) is more important than in the distal convoluted tubule (where thiazide diuretics act) to compensate for increased intestinal magnesium loss. Alternatively, this may indicate differences in compensatory mechanisms. It is well known that diuretic use results in a compensatory increase in reabsorption in nephron parts that are not blocked by the diuretic.(48) It was recently shown that PPIs cause a minor but significant decrease in transient receptor potential melastatin type 6 (TRPM6) in the kidney,(49) which is located in the distal convoluted tubule. This could imply that TRPM6 can no longer compensate for increased fecal and urinary magnesium losses induced by the combined use of PPIs and loop diuretics. In contrast, paracellular magnesium reabsorption in the loop of Henle

may remain intact to prevent hypomagnesemia with the combined use of PPIs and thiazide diuretics.

An unexpected result was the association between the use of H2RAs and magnesium level. Although this association was not found for patients admitted to an intensive care unit,(25) a similar association was found in a sensitivity analysis in a large cross-sectional study in a health maintenance organization database in Israel, including more than 95,000 individuals.(24) The effect size in this Israeli study was also smaller than for PPI-induced hypomagnesemia, and the association was only observed for moderate and not for severe hypomagnesemia. The investigators interpreted this finding as residual confounding because H2RA use has not been associated with hypomagnesemia previously. Although we cannot exclude this possibility in our study, we believe that it may reflect a true association that has remained clinically unnoticed due to the smaller effect size (although the risk of hypomagnesemia was similar with the use of PPIs and H2RAs). The lack of effect modification with loop diuretics may explain why no cases of severe hypomagnesemia have been reported in the literature with H2RA use, although publication bias is difficult to exclude.

What are the clinical implications of this study? We expect that few participants had symptomatic hypomagnesemia because only 3 participants had a serum magnesium concentration, 0.50 mmol/L (the level below which symptoms usually occur).(41) A recent study from Canada also showed that the absolute risk of hospitalization for PPI-induced hypomagnesemia is very low (1 excess hospitalization in >70,000 outpatients treated with a PPI for 90 days).(29) That said, our study clearly identified a subgroup of PPI users who are at risk of developing symptomatic hypomagnesemia, including those who use PPIs long term or combine PPIs with loop diuretics. We therefore
believe that the warning issued by the US Food and Drug Administration in 2011 to measure serum magnesium prior to initiation and periodically afterward primarily pertains to this subgroup.(50) Furthermore, although the 0.012 mmol/L lower serum magnesium level in PPI users is not clinically significant in terms of symptoms, it may indicate a total body magnesium deficit that has been associated with adverse outcomes in previous epidemiologic studies.(8, 21, 51)

The strength of this study is that to our knowledge, it is the first performed in a general population using systematically measured serum magnesium concentrations. This minimizes bias associated with clinical settings. However, our study also has limitations. Because we only had 1 serum magnesium measurement per participant, we were not able to study the course of serum magnesium levels after initiation of PPI treatment. We therefore studied the relationship between serum magnesium levels and PPI use over different strata of use, but because this concerns different participants, confounding could

still have had a role. We tried to address this confounding by indication by adjusting for dietary intake, which did not alter our results. Another limitation is the fact that we did not have information for over-the-counter use of PPIs. However, we believe this will have had little impact on our results because our latest inclusion date was before the date that PPIs were allowed to be sold over the counter in the Netherlands. Even if participants were using non-prescribed PPIs, this differential misclassification would have resulted in a dilution of the effect, which would make the association between PPIs and hypomagnesemia even stronger.

In conclusion, this study confirms the association between PPI use and risk of hypomagnesemia in the general population. The risk of hypomagnesemia is further increased when PPI use is prolonged (>6 months) or combined with the use of loop diuretics. Health care professionals should consider monitoring serum magnesium levels periodically in patients expected to be on prolonged treatment or those who combine PPIs with medication that may cause hypomagnesemia, such as diuretics. Although H2RAs had similar effects on serum magnesium level and risk of hypomagnesemia, these effects were weaker and further studies are needed before the precautions for PPIs should also be recommended for H2RAs.

4

Chapter 4

Thiazide, but not Loop Diuretics, are associated with Hypomagnesemia in the General Population

ABSTRACT

Purpose: Hypomagnesaemia has been associated with various adverse outcomes. Loop and thiazide diuretics promote urinary magnesium excretion. However, it is unknown if this links to hypomagnesaemia. We study if loop or thiazide diuretic use affects serum magnesium levels and if it associates with hypomagnesaemia. In addition, we study the effect of combining a potassium-sparing diuretic with a thiazide diuretic on the presence of hypomagnesaemia.

Methods: The study performed a cross-sectional analysis within 9,820 participants from the prospective Rotterdam Study. Hypomagnesaemia was defined as a serum magnesium level ≤0.72 mmol/L. Participants were categorized by defined daily dose (DDD), and all analyses were adjusted for age, sex, BMI, eGFR, serum potassium levels, proton pump inhibitor use, and comorbidities.

Results: Loop diuretic use was associated with higher serum magnesium levels (<1 DDD: 0.004 mmol/L 95% CI: −0.008; 0.017; 1 DDD: 0.023 mmol/L 95% CI: 0.013; 0.032; >1 DDD: 0.043 mmol/L 95% CI: 0.028; 0.057). Thiazide diuretic use was associated with lower serum magnesium levels (<1 DDD: −0.013 mmol/L 95% CI: −0.023; −0.002; ≥1 DDD: −0.018 mmol/L 95% CI: −0.028; −0.010), resulting in an increased odds ratio of hypomagnesaemia of 3.14 (95% CI: 1.67; 5.92) and 2.74
(95% CI: 1.57; 4.77), respectively. These effects were predominantly seen in participants using diuretics for more than 390 days. Combining thiazide diuretics with a potassium-sparing agent was not associated with lower serum magnesium levels or hypomagnesaemia.

Conclusions: Thiazide diuretic use is associated with lower serum magnesium levels and an increased risk of hypomagnesaemia. This increased risk is not seen in participants using a combination of thiazide diuretics with a potassium-sparing agent. The use of loop diuretics is not associated with an increased risk of hypomagnesaemia.

Hypomagnesaemia has been associated with various adverse outcomes including stroke, hypertension, atrial fibrillation, and diabetes mellitus type 2, as well as sudden cardiac death and mortality because of coronary heart disease.(3, 23, 52) The homeostasis of magnesium is maintained by dietary intake, absorption in the intestine, and urinary excretion.1 In the kidney, transport of magnesium is mediated by the thick ascending loop of Henle and the distal convoluted tubule. Loop and thiazide diuretics inhibit sodium transport in these segments and indirectly also affect magnesium reabsorption.(3, 46, 53, 54) Loop diuretics block the Na+-K+-2Cl− cotransporter (NKCC2) in the thick ascending loop of Henle. Because of inhibition of NKCC2, the transepithelial voltage is decreased, thereby losing the driving force for passive paracellular magnesium reabsorption.(3) Thiazide diuretics block the Na+-Cl− cotransporter (NCC) in the distal convoluted tubule. This blockage inhibits magnesium reabsorption either through direct or indirect inhibition of the transient receptor potential melastatin 6 or 7 (TRPM6 or TRPM7) channels.(53, 55-57) The effects of loop or thiazide diuretics on magnesium handling have been studied primarily in animal models and small healthy volunteer studies. Data on the effect of chronic loop or thiazide diuretic use on serum magnesium levels in the general population are lacking.(58) Therefore, it is unclear if reduced magnesium reabsorption by loop and thiazide diuretics results in clinically relevant alterations in serum magnesium levels. Our objective was to study if loop or thiazide diuretic use affects serum magnesium levels and if it associates with the risk of hypomagnesaemia, and if so whether the duration of use or the addition of a potassium-sparing agent to the thiazide diuretic influences the risk of hypomagnesaemia. All analyses were performed within a prospective population-based cohort study.

METHODS

Study design, setting, and population

This study was performed within the Rotterdam Study, a prospective population-based cohort study ongoing since 1990 in a suburb of Rotterdam, the Netherlands. The original cohort was extended with a second cohort in 2000 and a third cohort in 2006, resulting in a total study population of 14,926 participants, aged 45 years and older. The rationale and the design of the Rotterdam Study have been described in more detail elsewhere.(4) The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Dutch Ministry of Health, Welfare and Sport, implementing the “Wet Bevolkingsonderzoek: ERGO (Population Study Act: Rotterdam Study)”. All participants provided written informed consent to participate in the study and to obtain information from their physicians.

Assessment of serum magnesium

Serum magnesium has been measured during the third visit of the first cohort (1997-1999), and the first visits of the second (2000-2001) and third cohorts (2006-2008). These

visits are similar in design and data collection. Serum magnesium was measured by the Department of Clinical Chemistry of the Erasmus Medical Center using a colorimetric endpoint method using the Roche/Hitachi Cobas c501 analyzer (Roche Diagnostics, Indianapolis, IN, USA). Hypomagnesaemia was defined as a serum magnesium level ≤0.72 mmol/L.(59)

Assessment of medication use

Drug exposure is monitored continuously since January 1, 1991, through linkage with the pharmacies within the district which are all using 1 computer network. For each participant, the prescription period was calculated by dividing the total number of dispensed tablets per prescription by the prescribed daily number. Prescriptions that were filled within 7 days after ending a previous one were considered as 1 single episode of continuous use. If the date of serum magnesium measurement was within the prescription period, the participant was classified as being exposed. Using the same method, cumulative prescription days were calculated.(60) Dosage was expressed as standardized "defined daily doses" according to the definition of the World Health Organization.(32) We used the following Anatomical Therapeutic Chemical codes for loop diuretics: C03CA; for thiazide and thiazide-like diuretics (hereafter: thiazide diuretics): C03AA, CO3BA, C07B, and C07D; and for the combination thiazide diuretics and potassium-sparing agents: C03EA.

Assessment of covariates

The assessment of all covariates was done at the same visit as the serum magnesium levels were measured. Assessment of anthropometrics in the Rotterdam Study has been described previously.(4) Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and was expressed as mL/minute/1.73 m^2.(38) Diabetes mellitus was defined as either the use of glucose-lowering drugs, a fasting glucose level ≥7.0 mmol/L, or a non-fasting glucose level ≥11.1 mmol/L when fasting levels were not available.16 The assessment of heart failure was done through active follow-up using general practitioner's records and hospital discharge letters, as described previously.(61) Proton pump inhibitor (PPI) use was assessed using the same method as loop and thiazide diuretic use, using the Anatomical Therapeutic Chemical code: A02BC.

Statistical analyses

Means with standard deviations and counts with valid percentages were used to report continuous and discrete variables, respectively. All participants were categorized on mean defined daily dose (DDD); no use was used as a reference category. For loop diuretics, this resulted in 3 categories (<1 DDD, 1 DDD, and >1 DDD), as a vast majority of participants

used 1 DDD, and for thiazide diuretics and thiazide diuretics in combination with potassium-sparing agents, this resulted in 2 categories (<1 DDD and ≥1 DDD). We used multivariable linear regression models to study the association between serum magnesium levels and current loop diuretic use and current thiazide diuretic use, with or without potassium-sparing agents and compared user to participants using no diuretics. For the association with hypomagnesaemia, we used multivariable logistic regression models. For all analyses, we used 2 models. The first model included age and sex, while the second model additionally adjusted for BMI, eGFR, serum potassium levels, and prevalent diabetes mellitus, heart failure, hypertension, and PPI use. The effect of duration of use was analyzed by defining 3 different exposure categories based on tertiles of use. This was done separately for loop diuretics, thiazide diuretics, and thiazide diuretics with potassium-sparing agents. We performed 2 sensitivity analysis in which we excluded all participants with heart failure, diabetes mellitus, or an eGFR <60 mL/minute/1.73 m^2 and 1 in only participants with hypertension to rule out if confounding by indication influences our results. Missing data for covariables (0 to 9.9%) were handled by single imputation using an expectation-maximization algorithm.(39) All analyses were repeated on complete cases to check for potential differences between results based on imputed data and those based on complete cases. With the exception of baseline characteristics, results are reported for imputed data. A 2-sided P value <0.05 was considered statistically significant. Data were analyzed using SPSS Statistics (IBM, version 21.0).

RESULTS

Population characteristics

Of the 14,926 participants of the Rotterdam Study, 11,740 were eligible for this study as they participated in the selected study rounds. For 1,861 participants, who did participate in the selected study round, no serum magnesium level was available because of technical reasons (i.e., too little serum left for the analysis, erogenous magnesium measurement, or lost sample). Of the remaining 9,879 participants, 59 participants did not provide informed consent to retrieve pharmacy data. In addition, we excluded 6 participants using a combination of loop diuretics, thiazide diuretics, and/or potassium-sparing agents resulting in a total study population of 9,814. The baseline characteristics of this study population are shown in **Table 4.1**.

Table 4.1 - Population characteristics

	Total population N=9,814	Participants without hypomagnesemia N=9,567	Participants with hypomagnesemia N=247	P-value
Age, years	65.1 (9.9)	65.1 (9.9)	67.3 (10.7)	<0.001
Female, N(%)	5569 (56.7)	5424 (56.7)	145 (58.7)	0.529
Caucasian, N(%)	9075 (96.0)	8854 (96.0)	221 (94.4)	0.236
Body Mass Index, kg/m^2	27.3 (4.2)	27.2 (4.2)	29.1 (5.0)	<0.001
Smoking, N(%)				0.394
Never	2990 (30.8)	2925 (30.9)	65 (26.6)	
Past	4546 (46.8)	4421 (46.7)	125 (51.2)	
Current	2173 (22.4)	2119 (22.4)	54 (22.1)	
Serum magnesium, mmol/L	0.84 (0.06)	0.85 (0.05)	0.68 (0.05)	
Serum potassium, mmol/L	4.35 (0.34)	4.36 (0.34)	4.21 (0.40)	<0.001
Estimated glomerular filtration rate (CKD-EPI), ml/min/1.73m^2	79.6 (14.9)	79.6 (14.9)	79.8 (16.6)	0.780
Hypertension, N(%)	6090 (62.8)	5887 (62.2)	203 (83.2)	<0.001
Prevalent diabetes mellitus, N(%)	1021 (10.4)	916 (9.6)	105 (42.5)	<0.001
Prevalent heart failure, N(%)	255 (2.6)	240 (2.5)	15 (6.1)	0.001
Proton pump inhibitor, N(%)	722 (7.4)	690 (7.2)	32 (13.0)	0.001
Loop diuretics, N(%)	297 (3.0)	284 (3.0)	13 (5.3)	0.042
Thiazide diuretics, N(%)	312 (3.2)	281 (2.9)	31 (12.6)	<0.001
Thiazide diuretics and potassium-sparing combination, N(%)	412 (4.2)	386 (4.0)	26 (10.5)	<0.001

Footnotes

Values are counts (valid percentages) or means (standard deviation).

The mean age was 65.1 years, and 56.7% were women. The range of serum magnesium in the total study population was 0.34 to 1.74 mmol/L. Participants with hypomagnesaemia were significantly older, had a higher BMI, more often had diabetes mellitus, and more frequently used PPIs compared to participants without hypomagnesaemia.

Association between diuretic use and serum magnesium levels

The use of loop diuretics was associated with significantly higher serum magnesium levels, showing a dose-dependent relationship (P for trend <0.001). The use of less than 1 DDD was not associated with serum magnesium levels (0.002 mmol/L, 95% CI −0.011 to 0.015). The use of 1 DDD was associated with a 0.021 mmol/L (95% CI: 0.012 to 0.031) higher serum magnesium level, whereas the use of more than 1 DDD with a 0.042 mmol/L (95% CI: 0.027 to 0.056) higher serum magnesium level (**Table 4.2**).

Table 4.2 - Association between loop or thiazide diuretic use and serum magnesium

Dosage	N	Model 1 Beta in mmol/L (95%CI)	Model 2 Beta in mmol/L (95%CI)	P for trend
Current loop diuretic use				
No diuretic use	8,793	0.000 (Reference)	0.000 (Reference)	<0.001
<1 DDD	79	-0.002 (-0.015 to 0.011)	0.002 (-0.011 to 0.015)	
1 DDD	154	0.016 (0.006 to 0.026)	0.021 (0.012 to 0.031)	
>1 DDD	64	0.031 (0.006 to 0.026)	0.042 (0.027 to 0.056)	
Current thiazide diuretic use				
No diuretic use	8,793	0.000 (Reference)	0.000 (Reference)	<0.001
<1 DDD	127	-0.024 (-0.034 to -0.013)	-0.013 (-0.023 to -0.003)	
≥1 DDD	185	-0.029 (-0.038 to -0.021)	-0.018 (-0.027 to -0.010)	
Current thiazide diuretic use + potassium-sparing agents				
No diuretic use	8,793	0.000 (Reference)	0.000 (Reference)	0.342
<1 DDD	111	-0.016 (-0.027 to -0.005)	-0.006 (-0.017 to 0.004)	
≥1 DDD	301	-0.017 (-0.024 to -0.010)	-0.003 (-0.010 to 0.004)	

Footnotes

Model 1 adjusted for age and sex

Model 2 adjusted age, sex, BMI, eGFR (CKD-EPI), prevalent diabetes mellitus, prevalent heart failure, prevalent hypertension, PPI-use and serum potassium level.

Table 4.3 - Effect of duration of use on serum magnesium

Duration of use†	N	Beta in mmol/L (95%CI)	P-value
Current loop diuretic use			
No diuretic use	8,793	0.000 (Reference)	
≤ 420 days	95	0.003 (-0.009 to 0.015)	0.619
420 to 1110 days	102	0.035 (0.023 to 0.046)	<0.001
1110 to 4862 days	100	0.022 (0.010 to 0.034)	<0.001
Current thiazide diuretic use			
No diuretic use	8,793	0.000 (Reference)	
≤ 390 days	103	-0.007 (-0.018 to 0.004)	0.239
390 to 1035 days	106	-0.018 (-0.029 to -0.008)	0.001
1035 to 4215 days	103	-0.023 (-0.034 to -0.012)	<0.001
Current thiazide diuretic use + potassium-sparing agents			
No diuretic use	8,793	0.000 (Reference)	
≤ 824 days	137	0.002 (-0.007 to 0.012)	0.650
824 to 1368 days	137	-0.009 (-0.018 to 0.001)	0.083
1368 to 4390 days	138	-0.005 (-0.015 to 0.005)	0.319

Footnotes

Model 2 adjusted age, sex, BMI, eGFR (CKD-EPI), prevalent diabetes mellitus, prevalent heart failure, prevalent hypertension, PPI-use, and serum potassium level.

† The duration of use of loop and thiazide diuretics, was expressed in tertiles

The effect on serum magnesium level was predominantly seen in participants using loop diuretics for more than 420 days (**Table 4.3**). The use of thiazide diuretics was associated with significantly lower serum magnesium levels, showing a dose-dependent relationship (P for trend <0.001). The use of <1 DDD was associated with a 0.013 mmol/L (95% CI: −0.023 to −0.003) lower serum magnesium level compared to no use, while the use of ≥1 DDD was associated with a 0.018 mmol/L (95% CI: −0.027 to −0.010) lower serum magnesium level, compared to no use (**Table 4.2**). The effect on serum magnesium level was predominantly seen in participants with more than 390 days of thiazide diuretic use (**Table 4.3**). The combination of a thiazide diuretic with a potassium-sparing diuretic was not associated with lower serum magnesium levels.

Table 4.4 - Association between loop or thiazide diuretic use and hypomagnesaemia

Dosage	N (cases)	Model 1 OR (95%CI)	Model 2 OR(95%CI)
Current loop diuretic use			
No diuretic use	8,793 (177)	1.00 (Reference)	1.00 (Reference)
<1 DDD	79 (5)	2.83 (1.11 to 7.19)	1.75 (0.65 to 4.69)
1 DDD	154 (7)	2.02 (0.92 to 4.45)	1.09 (0.47 to 2.55)
>1 DDD	64 (1)	0.65 (0.09 to 4.76)	0.24 (0.03 to 1.86)
Current thiazide diuretic use			
No diuretic use	8,793 (177)	1.00 (Reference)	1.00 (Reference)
<1 DDD	127 (13)	5.65 (3.12 to 10.23)	3.39 (1.78 to 6.46)
≥1 DDD	185 (18)	5.32 (3.19 to 8.85)	3.07 (1.74 to 5.41)
Current thiazide diuretic use + potassium-sparing agents			
No diuretic use	8,793 (177)	1.00 (Reference)	1.00 (Reference)
<1 DDD	111 (5)	2.03 (0.81 to 5.07)	1.25 (0.49 to 3.21)
≥1 DDD	301 (21)	3.31 (2.06 to 5.33)	1.55 (0.92 to 2.61)

Footnotes

Model 1 adjusted for age and sex

Model 2 adjusted age, sex, BMI, eGFR (CKD-EPI), prevalent diabetes mellitus, prevalent heart failure, prevalent hypertension, PPI-use, and serum potassium level.

Association between diuretic use and hypomagnesaemia

Table 4.4 shows the association between diuretic use and hypomagnesaemia. The use of loop diuretics was not associated with an increased odds ratio of hypomagnesaemia. The use of thiazide diuretics was associated with an increased odds ratio for hypomagnesaemia of 3.39 (95% CI: 1.78 to 6.46) when using <1 DDD and 3.07 (95% CI: 1.74 to 5.41) when using ≥1 DDD. The risk of hypomagnesaemia increased with prolonged use of thiazide diuretics (390 to 1035 days: OR: 2.91, 95% CI 1.37 to 6.20; more than 1035 days of use: OR: 4.33, 95% CI 2.32 to 8.07) (**Table 4.5**). The combination of a thiazide diuretic with a potassium-sparing agent was not associated with hypomagnesaemia.

Table 4.5 - Duration of use and risk of hypomagnesaemia

Duration of use†	N (cases)	OR (95%CI)	P-value
Current loop diuretic use			
No diuretic use	8,793 (177)	1.00 (Reference)	
≤ 420 days	95 (4)	1.32 (0.45 to 3.81)	0.614
420 to 1110 days	102 (3)	0.58 (0.17 to 1.98)	0.385
1110 to 4862 days	100 (6)	1.32 (0.51 to 3.43)	0.566
Current thiazide diuretic use			
No diuretic use	8,793 (177)	1.00 (Reference)	
≤ 390 days	103 (6)	2.18 (0.91 to 5.25)	0.082
390 to 1035 days	106 (9)	2.91 (1.37 to 6.20)	0.006
1035 to 4215 days	103 (16)	4.33 (2.32 to 8.07)	<0.001
Current thiazide diuretic use + potassium-sparing agents			
No diuretic use	8,793 (177)	1.00 (Reference)	
≤ 824 days	137 (4)	0.77 (0.27 to 2.19)	0.622
824 to 1368 days	137 (14)	2.66 (1.42 to 4.99)	0.002
1368 to 4390 days	138 (8)	1.18 (0.54 to 2.59)	0.687

Footnotes

Model 2 adjusted age, sex, BMI, eGFR (CKD-EPI), prevalent diabetes mellitus, prevalent heart failure, prevalent hypertension, PPI-use, and serum potassium level.

† The duration of use of loop and thiazide diuretics, was expressed in tertiles

Sensitivity analyses

We repeated our main analyses after excluding participants with heart failure, diabetes, or a eGFR <60 mL/minute/1.73 m^2 and found similar results for the association between loop or thiazide diuretics with serum magnesium levels (**Table 4.6**). In addition, we repeated all of our analysis in only participants with hypertension which yielded similar results to the total study population (data not shown).

Table 4.6 - Sensitivity analysis excluding participants with heart failure, diabetes mellitus or eGFR < 60mL/min/1.73m^2

Dosage	Model 2 Beta in mmol/L (95%CI)
Current loop diuretic use	
No diuretic use	0.000 (Reference)
< 1 DDD	-0.013 (-0.033 to 0.007)
1 DDD	0.016 (0.001 to 0.032)
> 1 DDD	0.035 (0.004 to 0.066)
Current thiazide diuretic use	
No diuretic use	0.000 (Reference)
< 1 DDD	-0.010 (-0.021 to 0.001)
≥ 1 DDD	-0.013 (-0.022 to -0.003)
Current thiazide diuretic use + potassium-sparing agents	
No diuretic use	0.000 (Reference)
< 1 DDD	-0.007 (-0.019 to 0.006)
≥ 1 DDD	-0.001 (-0.009 to 0.008)

Footnotes

Model 2 adjusted age, sex, BMI, eGFR (CKD-EPI), prevalent diabetes mellitus, prevalent heart failure, prevalent hypertension, PPI-use, and serum potassium level.

DISCUSSION

In this large population-based cohort, we found that thiazide but not loop diuretics is associated with lower serum magnesium levels and a higher risk of hypomagnesaemia. In fact, loop diuretic use was associated with significantly higher serum magnesium levels. Both associations are dose-dependent and predominantly seen in participants who have used diuretics for longer periods of time. When combining a thiazide diuretic with a potassium-sparing agent, the effect on serum magnesium levels is attenuated and does not associate with an increased risk of hypomagnesaemia.

Over the last years, small studies have shown that thiazide use impairs the magnesium-retaining ability of the kidney which may result in a magnesium deficiency.(62-65) However, the effect of long-term thiazide use on serum magnesium levels is still unknown. We showed that the use of thiazide diuretics was associated with lower serum magnesium of 0.018 mmol/L. Although this appears to be a minor decrease that may not be clinically relevant, thiazide use was associated with a twofold to threefold increased risk of hypomagnesaemia. The risk of thiazide-induced hypomagnesaemia may be mediated by

the TRPM6 channel in the kidney, which reabsorbs magnesium. In mice, TRPM6 channels are downregulated after administration of hydrochlorothiazide.(46, 66) This suggests that inhibition of NCC by thiazides indirectly inhibits TRPM6. Indeed, mice deficient for NCC also display reduced TRPM6 abundance, increased urinary magnesium excretion, and hypomagnesaemia.(46) However, how NCC inhibition is linked to TRPM6 inhibition is still unknown. Another explanation may be that increased urinary potassium excretion associated with the use of thiazides depolarizes the membrane which in turn reduces the driving force for passive magnesium reabsorption.(67) This possibility is supported by our observation that the combination of thiazide diuretics with a potassium-sparing diuretic does not result in an increased risk of hypomagnesaemia, which is in line with previous observational studies.(65, 68) The recent PATHWAY-3 trial also supports this theory, in which it was found that a combination of hydrochlorothiazide with amiloride prevented glucose intolerance and improved blood pressure control compared to monotherapy of either drug.(69) Both the effect on glucose intolerance and blood pressure is hypothesized to be caused by alterations in serum magnesium levels.(70, 71)

An unexpected result was the association between loop diuretic use and higher serum magnesium levels. Magnesium reabsorption in the thick ascending limb of the loop of Henle is driven by the transepithelial gradient generated by NKCC2 activity.(72) As loop diuretics block NKCC2, and this transepithelial gradient, magnesium reabsorption is decreased and this is predicted to result in magnesium wasting.(73) However, in patients with antenatal Bartter syndrome, the loss of function of NKCC2 does not result in hypomagnesaemia.(72) It is hypothesized that this is because of the compensatory capacity of the distal convoluted tubule, which allows for sufficient reabsorption of magnesium downstream. Indeed, a recent study shows that chronic loop diuretic treatment upregulates TRPM6 in the distal convoluted tubule which might be responsible for this compensation.(58) However, it seems unlikely that such compensatory reabsorption results in higher serum magnesium concentrations. Although a decrease in eGFR could explain higher serum magnesium levels, the association remained after adjusting for eGFR levels. Therefore, some of the other related effects of loop diuretic treatment may explain the higher serum magnesium levels, including increased reabsorption by the proximal tubule or increased TRPM6 activity through metabolic alkalosis.(74)

The main strength of our study is that it is performed in a general population using systematically measured serum magnesium levels, thereby reducing the possibility of information bias. The main limitation of our study is the fact that we only have 1 serum magnesium measurement per participant, and are therefore unable to study changes in serum magnesium levels over time and establish a causal relationship between diuretic use and serum magnesium levels. In theory, participants who use thiazide diuretics could

have other comorbidities resulting in lower serum magnesium levels, thereby confounding the observed relationship. We addressed this potential confounding by adjusting for other known comorbidities influencing serum magnesium level, which had only little impact on the effect estimates. Another limitation is the fact that we were unable to study the effect of magnesium supplementation. Few participants use magnesium supplements prescribed via a health care professional; however, the majority of participants use some form of vitamin or mineral supplement which often contains a small amount of magnesium. Unfortunately, the precise composition of these supplements is often not known, which makes it impossible to calculate a total amount of magnesium ingested via these supplements. In general, however, these supplements only contain a small amount of magnesium which is not likely to influence serum magnesium levels to a great extent.

In conclusion, our study shows that the use of thiazide diuretics is associated with lower serum magnesium levels and an increased presence of hypomagnesaemia. This increased risk is absent in participants using a combination of thiazide diuretics with potassium-sparing agents. The use of loop diuretics is not associated with an increased risk of hypomagnesaemia, and serum magnesium levels even tend to be slightly higher. It may be recommended to check serum magnesium levels regularly in people using thiazide diuretics for longer periods of time as hypomagnesaemia has been associated with an increased risk of many diseases, including hypertension and diabetes mellitus. In addition, in people with low serum magnesium levels, the addition of a potassium-sparing diuretic could be considered.

PART III
Associations of Serum Magnesium with Disease

Chapter 5

Serum Magnesium and the Risk of Death from Coronary Heart Disease and Sudden Cardiac Death

ABSTRACT

Background: Low serum magnesium has been implicated in cardiovascular mortality, but results are conflicting and the pathway is unclear. We studied the association of serum magnesium with coronary heart disease (CHD) mortality and sudden cardiac death (SCD) within the prospective population-based Rotterdam Study, with adjudicated end points and long-term follow-up.

Methods and Results: 9,820 participants (mean age 65.1 years, 56.8% female) were included

with a median follow-up of 8.7 years. We used multivariable Cox proportional hazard models and found that a 0.1 mmol/L increase in serum magnesium level was associated with a lower risk for CHD mortality (hazard ratio: 0.82, 95% CI:0.70–0.96). Furthermore, we divided serum magnesium in quartiles, with the second and third quartile combined as reference group (0.81–0.88 mmol/L).

Low serum magnesium (≤0.80 mmol/L) was associated with an increased risk of CHD mortality (N=431, hazard ratio: 1.36, 95% CI:1.09–1.69) and SCD (N=217, hazard ratio: 1.54, 95% CI:1.12–2.11). Low serum magnesium was associated with accelerated subclinical atherosclerosis (expressed as increased carotid intima-media thickness: +0.013 mm, 95% CI:0.005–0.020) and increased QT-interval, mainly through an effect on heart rate (RR-interval: -7.1 ms, 95% CI :-13.5 to -0.8). Additional adjustments for carotid intima-media thickness and heart rate did not change the associations with CHD mortality and SCD.

Conclusions: Low serum magnesium is associated with an increased risk of CHD mortality and SCD. Although low magnesium was associated with both carotid intima-media thickness and heart rate, this did not explain the relationship between serum magnesium and CHD mortality or SCD. Future studies should focus on why magnesium associates with CHD mortality and SCD and whether intervention reduces these risks.

Magnesium is the second most abundant intracellular cation and it plays a key role in a wide range of cellular functions.(3) Total body magnesium depends on dietary intake and recent studies showed that the vast majority of elderly do not consume the average dietary requirement for magnesium.(8) In addition to a magnesium-insufficient diet, elderly are intracellular cation and it plays a key role in a wide range of cellular functions.(3) Total body magnesium depends on dietary intake and recent studies showed that the vast majority of elderly do not consume the average dietary requirement for magnesium.(8) In addition to a magnesium-insufficient diet, elderly are also at risk for hypomagnesemia due to comorbidities and medication that increase urinary excretion of magnesium.(3) The prevalence of hypomagnesemia in the general population is estimated at 2%,3 but it may be as high as 53% in specific high-risk groups, such as patients with chronic heart failure.(75) Although hypomagnesemia may have acute and chronic complications, serum magnesium is still measured relatively infrequently.(3)

In recent studies, low serum magnesium has been associated with inflammation(76) and disturbances in the regulation of vascular tone and endothelial function.(77-80) These mechanisms are thought to contribute to the development and progression of atherosclerosis, potentially worsening coronary heart disease (CHD).(81, 82) Magnesium is also known for its role in the electrical stability and energy balance of cardiomyocytes.(9) Hypomagnesemia has been associated with atrial and ventricular arrhythmias and low serum magnesium could therefore also be a risk factor for sudden cardiac death (SCD).(8, 75, 83)

Previous studies have addressed the relationship between serum magnesium and CHD(82, 84) and the relationship with SCD,(85, 86) but with conflicting results for both outcomes. In addition, these studies had few cases or lacked adjudicated end points, which could have resulted in misclassification. We therefore aimed to study the association of serum magnesium levels with both CHD mortality and SCD within the Rotterdam Study, a prospective population-based cohort study among middle-aged and elderly persons with adjudicated end points and long-term follow-up. Low serum magnesium has been associated with accelerated atherosclerosis.(87) We therefore hypothesized that this could be the potential mechanism through which serum magnesium increases the risk of CHD mortality. Since carotid intima media thickness (cIMT) is considered a proxy for accelerated atherosclerosis, we explored the association between serum magnesium and cIMT as a potential mediator for the relationship with CHD mortality. QT prolongation is a well-established risk factor for SCD(88) and serum magnesium was shown to influence the QT interval in a clinical setting.(89) We therefore hypothesized that QT prolongation could be the biological pathway through which low serum magnesium is associated with SCD.

METHODS

Study Design, Setting, and Population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study, ongoing since 1990 in a suburb of the city of Rotterdam, The Netherlands. The rationale and design of this study have been described in detail elsewhere.(90) In summary, the original cohort consisted of 7,983 unselected inhabitants of the study area, aged 55 years or over. This cohort was extended in 2000 with 3,011 persons who had become 55 years of age or who had moved into the research area, since the start of the study. In 2006, the cohort was extended again with 3,932 persons aged 45 years and older living in the research area who had not yet been invited. This resulted in a total study population of 14,926 individuals, aged 45 years and older. All eligible participants provided written informed consent to participate in the study. Separate consent was asked for follow-up data collection on clinical outcomes and vital status. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Dutch Ministry of Health, Welfare and Sport, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Screening Act: Rotterdam Study)."

Assessment of Serum Magnesium

For our study, we used blood collections from the third visit of the first cohort (1997–1999), and the first visits of the second cohort (2000–2001) and third cohort (2006–2008), as these visits were virtually identical in design. Of the 11,740 eligible participants, serum magnesium was available for 9,882 participants. Of these 9,882 participants, 59 did not provide consent to collect follow-up information from their treating physicians and 3 participants were lost to follow-up at the date of blood collection and therefore did not contribute to our study. This resulted in a study population of 9,820 participants. Magnesium was measured in serum by the Department of Clinical Chemistry of the Erasmus Medical Center using a Roche/Hitachi Cobas c501 analyzer. We plotted multivariate adjusted log relative hazard curves using restricted cubic splines, to examine the association between serum magnesium and CHD mortality. We found this association to be linear and therefore used serum magnesium as a continuous parameter when quantifying the association with CHD mortality. The association with SCD was also modelled using restricted cubic splines and found to be U-shaped. Therefore, we also divided serum magnesium into quartiles and analyzed serum magnesium as a categorical variable in all our analyses. We combined the second and third quartile into a reference group to best fit the observed U-shape, yielding a low, high, and reference group.(91)

Assessment of Covariables

The assessment of anthropometrics in the Rotterdam Study has been described previously.(90) Body mass index was calculated by dividing body weight in kilograms by height in meters squared. Diabetes mellitus was defined as the use of glucose-lowering

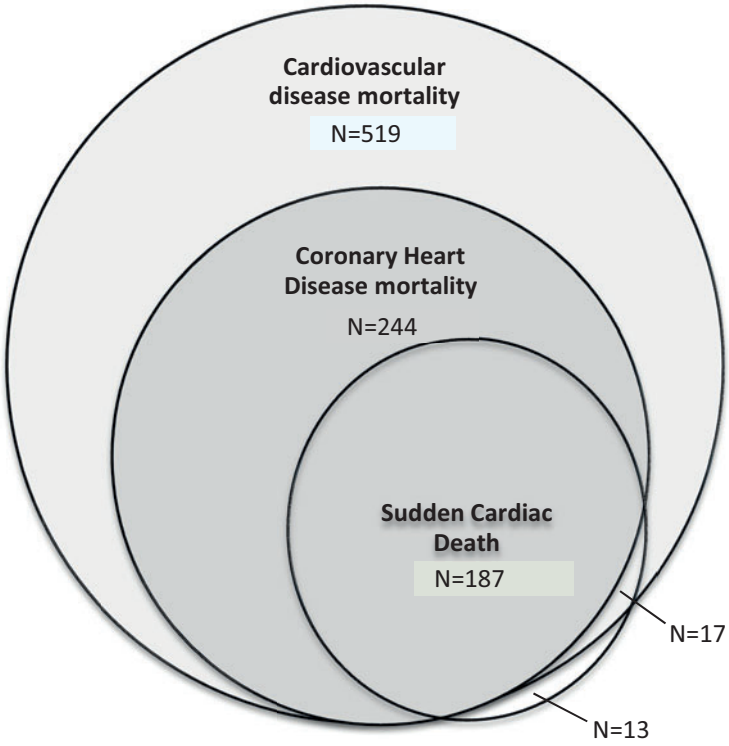
drugs, a fasting glucose level ≥7.0 mmol/L, or a non-fasting glucose level ≥11.1 mmol/L when fasting levels were not available.(92) The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation(38) and was expressed as mL/min per 1.73 m^2 based on serum creatinine values that were measured at the same visit as the serum magnesium levels using an enzymatic assay method. Total cholesterol and high-density lipoprotein cholesterol were measured at the same visit as the serum magnesium levels using the Roche Modular P800. Information on history of stroke, myocardial infarction, and heart failure was obtained through linkage with medical records kept by general practitioners working in the study area, and subsequently adjudicated by 2 research physicians and confirmed by a neurologist or cardiologist.(61) Information on smoking habits and current alcohol consumption was obtained during a home interview. Smoking was categorized into 3 categories: non-smoker, former smoker, and current smoker. Alcohol consumption was categorized into yes or no. Drug exposure has been monitored continuously since January 1, 1991 through linkage with digital pharmacy records of the pharmacies in the study district. The following Anatomical Therapeutic Chemical (ATC) codes were used to retrieve relevant drug exposure for all participants: verapamil (C08DA01), diltiazem (C08DB01), β-blocking agents (C07), digoxin (C01AA05), class I/III antiarrhythmic drugs (C01B), and diuretics (C03).(32) The use of definite QTc-prolonging drugs was determined using the University of Arizona list of QTc-prolonging drugs.(93) Participants were classified as being exposed if the date of the blood draw fell within the prescription period. Physical activity was assessed using a validated adapted version of the Zutphen Physical Activity Questionnaire, and expressed in metabolic equivalent of task hours per week.(94)

Assessment of Outcomes

Follow-up time was calculated from the date that blood was drawn until the date of death, loss to follow-up (n=594), or the end of the study period (January 1, 2012 for CHD mortality and January 1, 2011 for SCD). Methods for outcome data collection and definitions have previously been described in more detail.(61, 95) In short, CHD mortality was defined as definite fatal myocardial infarction, definite fatal CHD, and possible fatal CHD. Information on the vital status of all participants was obtained on a weekly basis from the central registry of the municipality in Rotterdam and through digital linkage with records from general practitioners working in the study area. The cause of death was established by abstracting information from the medical records of the general practitioners or nursing home physicians and hospital discharge letters. SCD was defined as “natural death due to cardiac causes, heralded by abrupt loss of consciousness within 1 hour from onset of acute symptoms; pre-existing heart disease may have been known to be present, but the time and mode of death are unexpected,” according to Myerburgs’ definition endorsed by the European Society of Cardiology(96, 97) Unwitnessed deaths were coded as SCD if death was unexpected in persons found dead, while they were in a

stable medical condition 24 hours before they were found in the absence of evidence of a non-cardiac cause.(95) Each outcome was adjudicated by 2 research physicians who independently classified information on occurrence, certainty, and date using standardized criteria and subsequently validated by an experienced cardiologist.(61, 70) The classification of CHD mortality (as a cause of death) and SCD (as a mode of death) overlap and results on CHD mortality could be driven by the effect on SCD (**Figure 5.1**). Therefore, we also defined a "non-sudden CHD mortality" category including participants who were only classified as death from CHD but not as SCD.

Figure 5.1 - Overlap between the classification of coronary heart disease mortality (as a cause of death) and sudden cardiac death (as a mode of death)



c-IMT and ECG Measurements

cIMT correlates with future cardiovascular events and can therefore be used as a subclinical marker of atherosclerosis.(98) We used longitudinal 2-dimensional ultrasound images of the common carotid artery for a 1-cm length that was proximal to the bulb, obtained with a 7.5 MHz linear array transducer and UltraMark IV (Advanced Technology Laboratories, Bethel, WA), to measure the distance between the lumen intima interface and the media–adventitia interface, which indicates the cIMT. The maximal cIMT, summarized as the mean of the maximal measurements from the near and far walls on both the left and right sides, was used for analysis.(99) Each participant also had a standard 12-lead resting ECG recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz, which was stored digitally. All ECGs were processed by the standardized Modular ECG Analysis System (MEANS) to obtain ECG measurements.(100) The QT interval represents the interval between the start of ventricular depolarization to the end of ventricular repolarization. MEANS determines this interval from the start of the QRS complex until the end of the T-wave. Heart rate modifies this interval; therefore QT-interval measurements are corrected for the individual's RR interval to allow comparison by using Bazett's correction.(101) For all participants, cIMT measurements and ECG measurement were performed during the same visit, during which blood was drawn for serum magnesium measurements.

Statistical Analyses

We used the cmprsk package in "R" to plot cumulative probability curves for CHD mortality, non-sudden CHD mortality, and SCD adjusted for competing risk of death by other causes as proposed by Fine and Grey.(102) We used Cox proportional hazard regression models to examine the relationship between serum magnesium and CHD mortality, non-sudden CHD mortality, and SCD. We analyzed these associations using 2 different models. In the first model we adjusted for age (as a continuous variable) and sex. The second model also included the following continuous variables: eGFR, body mass index, systolic and diastolic blood pressure, total cholesterol/high-density lipoprotein cholesterol ratio, and the following categorical variables: diabetes mellitus, history of myocardial infarction, stroke, or heart failure, smoking status, alcohol consumption, and use of diuretics. We studied the association between serum magnesium and QTc interval in a multivariable linear model using the same covariables and additionally adjusted for drugs that are known to affect the QT and/or RR interval (see above). Because Bazett's formula is known to overestimate at short RR intervals and underestimate at long RR intervals, we also additionally adjusted for RR interval.(103) The association between serum magnesium and heart rate (expressed as RR interval) was also studied using the same covariables. We tested for potential pathways by additionally adjusting the second model for QT and RR interval to account for a potential arrhythmogenic pathway and by including cIMT as marker for subclinical atherosclerosis. The proportional hazard

assumption was tested by plotting log-minus-log survival versus log of survival time curves and visually examining the curves, which did not indicate that the assumption was violated.(104) Results are presented as hazard ratios (HRs) with 95% CIs. Missing data in covariables (0–9.1%) were handled by single imputation using an expectation-maximization algorithm.(39) All analyses were repeated on complete cases to check for potential differences between results based on imputed data and those based on complete cases. With the exception of the baseline characteristics, results are reported for imputed data. We considered a 2-sided $P<0.05$ as statistically significant. Data were analyzed using SPSS Statistics (IBM, version 21.0) and R (The R Foundation for Statistical Computing, version 3.1.2).

Sensitivity Analyses

To further distinguish between an effect of serum magnesium on accelerated atherosclerosis or serum magnesium having an anti-arrhythmogenic effect, we studied the association between serum magnesium and incident myocardial infarction (MI). If the effect of serum magnesium on CHD mortality is mediated through accelerated atherosclerosis, an effect on incident MI would also be expected. We studied the association with incident MI only with serum magnesium as a continuous parameter, as there were only a limited number of MI events and we expected the shape of the association to be similar to CHD mortality. In a previous study, hypertension and cholesterol levels were found to be intermediates in the association between plasma magnesium and fatal CHD.(105) To explore the effect of these factors on our effect estimates, we excluded systolic blood pressure, diastolic blood pressure, and total/high-density lipoprotein cholesterol ratio from our second model. We then compared effect estimates of the full model to the model without blood pressure and cholesterol to check whether our results had been altered by inclusion of this potential mediator. To test the robustness of our findings, we also performed 2 additional analyses where we restricted our analysis to participants free of diabetes mellitus and with normal kidney function (defined as eGFR >60 mL/min per 1.73 m^2) in order to explore potential effect modification by diabetes mellitus or chronic kidney disease. Furthermore, we additionally adjusted for serum sodium, potassium, and calcium levels to test whether the association of magnesium with any of the outcomes could be explained by a concomitant electrolyte disorder. We expected physical activity to be a potential confounder of the association between serum magnesium and CHD mortality. However, data on physical activity were only available for a subset of 6,385 participants. We therefore performed a sensitivity analysis on this subset to check whether inclusion of physical activity would have influenced our results.

RESULTS

Baseline Characteristics

Baseline characteristics of our study population are shown in **Table 5.1.** Among the 9820 participants, the mean age was 65.1 years, 56.8% were women, and 96% were of European descent. The mean serum magnesium level was 0.84 mmol/L (±0.06 mmol/L), with a range of 0.34 to 1.74 mmol/L. Participants in the low magnesium group (N=2,351) more often used diuretics, and more often had diabetes mellitus. During a median follow-up of 8.7 years (80,750 person-years in total), 2,303 of the 9,820 participants died (23.5%). In 2,213 of the 2,303 participants who died, a cause of death could be adjudicated. Among these participants, 780 died of cardiovascular disease, of which 431 were classified as death from CHD. Of the 2,303 participants who died, 217 were classified as SCD. **Figure 5.1** shows how the classifications CHD mortality and SCD relate.

Magnesium and Mortality

Figure 5.2 shows the cumulative probability curves for CHD mortality, non-sudden CHD mortality, and SCD between the different groups of serum magnesium, taking competing risk into account. **Table 5.2** shows the associations between the 3 magnesium groups and mortality. Low serum magnesium was associated with an increased risk for CHD mortality (HR 1.36, 95% CI 1.09–1.69) and an increased risk for SCD (HR 1.54, 95% CI 1.12–2.11). A marginally insignificant association was found with high serum magnesium levels and SCD (HR 1.35, 95% CI 0.96–1.89). Excluding SCD cases from the CHD outcome resulted in an inverse linear association with serum magnesium levels. The risk in participants with low serum magnesium was attenuated (HR 1.27, 95% CI 0.95–1.70) and an association with lower risk was observed in participants with high serum magnesium levels (HR 0.69, 95% CI 0.48–0.98). We tested for a linear association with CHD mortality and non-sudden CHD mortality and found that a 0.1 mmol/L increase in serum magnesium was associated with a decreased risk of CHD mortality (HR 0.82, 95% CI 0.70–0.96), and non-sudden CHD mortality (HR 0.72, 95% CI 0.58–0.88). We found no evidence of effect modification by sex (P for interaction=0.436), and therefore did not stratify by sex.

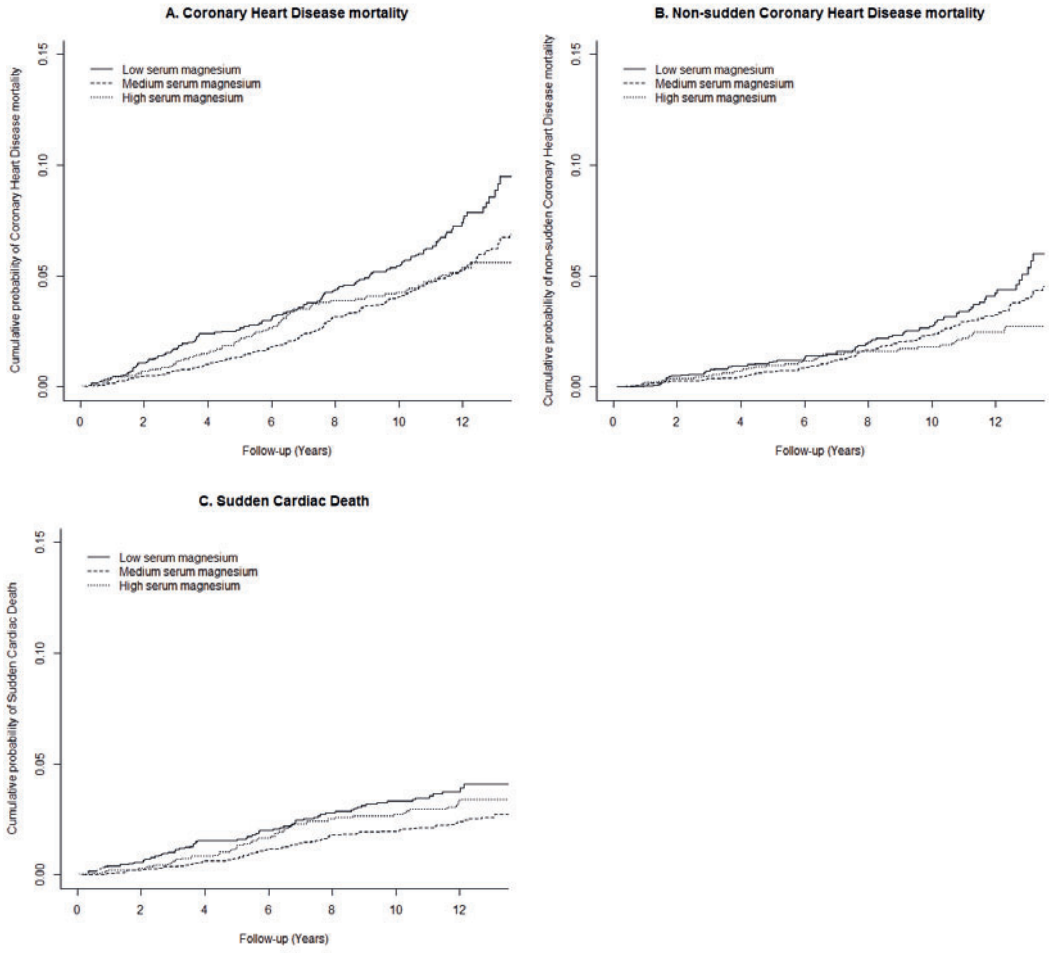
Table 5.1 - Characteristics of the study population

	Total N=9,820	Low (≤0.80 mmol/L) N=2,351	Reference (0.81 to 0.88 mmol/L) N=5,170	High (≥0.89 mmol/L) N=2,299
Age, years	65.1 (9.9)	65.1 (10.2)	64.9 (9.7)	65.6 (10.0)
Women, N(%)	5575 (56.8)	1368 (58.2)	2908 (56.2)	1299 (56.5)
Body mass index, kg/m^2	27.3 (4.2)	27.8 (4.6)	27.2 (4.1)	26.8 (4.0)
Systolic blood pressure, mmHg	140(21)	141 (21)	139 (21)	140 (22)
Diastolic blood pressure, mmHg	79 (12)	79 (12)	79 (11)	79 (12)
Smoking, N(%)				
Never	2991 (30.8)	661 (28.4)	1600 (31.3)	730 (32.2)
former	4549 (46.8)	1094 (47.0)	2406 (47.0)	1049 (46.3)
current	2175 (22.4)	574 (24.6)	1112 (21.7)	489 (21.6)
Alcohol use, N(%)	8274 (85.2)	1919 (82.5)	4430 (86.6)	1925 (84.9)
History of diabetes mellitus, N(%)	1021 (10.4)	466 (19.9)	433 (8.4)	122 (5.3)
History of myocardial infarction, N(%)	494 (5.0)	134 (5.7)	237 (4.6)	123 (5.4)
History of stroke, N(%)	340 (3.5)	92 (3.9)	160 (3.1)	88 (3.8)
History of heart failure, N(%)	257 (2.6)	69 (2.9)	118 (2.3)	70 (3.1)
eGFR, mL/min/1.73m^2	81.5 (17.3)	83.2 (20.0)	81.8 (16.0)	79.0 (17.0)
Serum magnesium, mmol/L	0.84 (0.06)	0.77 (0.04)	0.85 (0.02)	0.92 (0.04)
Total cholesterol, mmol/L	5.7 (1.0)	5.6 (1.0)	5.7 (1.0)	5.8 (1.1)
HDL cholesterol, mmol/L	1.40 (0.41)	1.38 (0.42)	1.41 (0.40)	1.40 (0.41)
Diuretic use, N(%)	1017 (10.7)	325 (14.2)	418 (8.1)	233 (10.6)

Footnotes

Data are shown for non-imputed data, values are counts (valid percentages) or means (standard deviation).

Figure 5.2 - Estimated cumulative probability curves for coronary heart disease mortality, non-sudden coronary heart disease mortality, and sudden cardiac death



IMT, QT-Interval, and Heart Rate

Table 5.3 shows the associations between the different magnesium groups and markers of potential pathways that may play a role in the association between serum magnesium and CHD mortality and SCD. Low serum magnesium was associated with a significantly higher cIMT (+0.013 mm, 95% CI 0.005–0.020); no association was found in participants with high serum magnesium. Both low serum magnesium and high serum magnesium were associated with a significantly longer QTc interval (+1.8 ms 95% CI 0.7–2.9 and +2.2 ms 95% CI 1.1–3.3, respectively).

Table 5.2 - Association between magnesium groups and different types of mortality

Serum magnesium groups	Number of events	Crude incidence rate per 1000 person-years (95%CI)	Model 1 Hazard Ratio (95%CI)*	Model 2 Hazard Ratio (95%CI) †
Coronary Heart Disease mortality				
Low	134	7.1(6.0 to 8.4)	1.53 (1.23 to 1.90)	1.36 (1.09 to 1.69)
Reference	206	4.8 (4.1 to 5.5)	1.00 (reference)	1.00 (reference)
High	91	4.9 (3.9 to 6.0)	0.97 (0.76 to 1.24)	0.94 (0.73 to 1.21)
Non-sudden Coronary Heart Disease mortality ‡				
Low	74	3.9 (3.1 to 4.9)	1.39 (1.04 to 1.85)	1.27 (0.95 to 1.70)
Reference	127	2.9 (2.4 to 3.5)	1.00 (reference)	1.00 (reference)
High	43	2.3 (1.7 to 3.1)	0.73 (0.52 to 1.04)	0.69 (0.48 to 0.98)
Sudden Cardiac Death				
Low	70	4.2 (3.2 to 5.3)	1.76 (1.29 to 2.40)	1.54 (1.12 to 2.11)
Reference	92	2.4 (1.9 to 2.9)	1.00 (reference)	1.00 (reference)
High	55	3.3 (2.5 to 4.3)	1.32 (0.94 to 1.84)	1.35 (0.96 to 1.89)

Footnotes

Low magnesium group ≤0.80 mmol/L, reference group 0.81mmol/L to 0.88mmol/L, high magnesium group ≥0.89mmol/L

* adjusted for age and sex

† Model 1 additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use

‡ This category includes participants who died from coronary heart disease, but who were not classified as sudden cardiac deaths

Table 5.3 - Association between different magnesium groups and markers of potential pathways

Serum magnesium groups	Model 1 Mean difference (95%CI)*	Model 2 Mean difference (95%CI) †
Carotid Intima-Media Thickness (in millimeters)		
Low	0.023 (0.015 to 0.030)	0.013 (0.005 to 0.020)
Reference	Reference	Reference
High	-0.005 (-0.013 to 0.003)	-0.003 (-0.010 to 0.004)
QTc interval (in milliseconds) ‡		
Low	2.7 (1.6 to 3.9)	1.8 (0.7 to 2.9)
Reference	Reference	Reference
High	2.0 (0.9 to 3.1)	2.2 (1.1 to 3.3)
QTc interval (in milliseconds), additionally adjusted for RR interval ‡		
Low	2.0 (1.0 to 3.0)	1.3 (0.3 to 2.3)
Reference	Reference	Reference
High	1.5 (0.5 to 2.5)	1.6 (0.6 to 2.6)
RR interval (in milliseconds) ‡		
Low	-10.1 (-16.7 to -3.5)	-7.1 (-13.5 to -0.8)
Reference	Reference	Reference
High	-7.1 (-13.8 to -0.4)	-7.8 (-14.1 to -1.4)

Footnotes

Low magnesium group ≤0.80 mmol/L, reference group 0.81mmol/L to 0.88mmol/L, high magnesium group ≥0.89mmol/L

* adjusted for age and sex

† Model 1 additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use

‡ In the analysis on ECG parameters, model 2 also includes adjustment for verapamil use, diltiazem use, beta-blocker use, digoxin use, anti-arrhythmic drug use, QT prolonging drug use

When we additionally adjusted this analysis on QTc interval for heart rate, we found that the association remained significant, but the effect sizes became smaller. Therefore, we analyzed the effect of serum magnesium on heart rate by studying the association between serum magnesium and RR interval. We found that both low serum magnesium and high serum magnesium were significantly associated with RR interval (-7.1 ms, 95% CI -13.5 to -0.7 and 7.8 ms, 95% CI -14.1 to -1.4, respectively). Due to the significant associations between serum magnesium and cIMT, QT interval, and heart rate, we

hypothesized that cIMT could be a potential mediator for the association with CHD mortality and non-sudden CHD mortality, and QT interval or heart rate could be a mediator in the association with SCD. **Table 5.4** shows the result of additionally adjusting for these potential mediators.

Table 5.4 - Influence of potential pathway markers on the association between magnesium groups and cardiovascular outcomes

Serum magnesium groups	Model 2 Hazard Ratio (95%CI)*	Model 3 Hazard Ratio (95%CI) †	Model 4 Hazard Ratio (95%CI) ‡
Coronary Heart Disease mortality			
Low	1.36 (1.09 to 1.69)	1.34 (1.08 to 1.68)	1.35 (1.08 to 1.68)
Reference	1.00 (reference)	1.00 (reference)	1.00 (reference)
High	0.94 (0.73 to 1.21)	0.94 (0.73 to 1.20)	0.95 (0.74 to 1.22)
Non-sudden Coronary Heart Disease mortality			
Low	1.27 (0.95 to 1.70)	1.27 (0.95 to 1.71)	1.26 (0.94 to 1.69)
Reference	1.00 (reference)	1.00 (reference)	1.00 (reference)
High	0.69 (0.48 to 0.98)	0.69 (0.48 to 0.97)	0.70 (0.49 to 0.99)
Sudden Cardiac Death			
Low	1.54 (1.12 to 2.11)	1.50 (1.09 to 2.06)	1.53 (1.11 to 2.10)
Reference	1.00 (reference)	1.00 (reference)	1.00 (reference)
High	1.35 (0.96 to 1.89)	1.32 (0.94 to 1.85)	1.35 (0.96 to 1.89)

Footnotes

* Equal to model 2 from Table 2; adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use

† Model 2 + adjustment for QT interval and RR interval

‡ Model 2 + adjustment for carotid intima-media thickness

We found that additional adjustment for cIMT did not change the association between low serum magnesium and CHD mortality (before adjustment HR 1.36, 95% CI 1.09–1.69; after adjustment HR 1.35, 95% CI 1.08–1.68). The same was true for the association with non-sudden CHD (before adjustment HR 1.27, 95% CI 0.95–1.70; after adjustment HR 1.26, 95% CI 0.94–1.69). Additional adjustment for QT interval and heart rate did not change the association between low serum magnesium and SCD (before adjustment HR 1.54, 95% CI 1.12–2.11; after adjustment HR 1.50, 95% CI 1.09–2.06).

Sensitivity Analyses

When studying the association between serum magnesium and incident MI, we found that in our age- and sex-adjusted model a 0.1 mmol/L increase in serum magnesium level was associated with a decreased risk of MI (HR 0.84, 95% CI 0.72–0.99). However, this association was no longer statistically significant after further adjustment for potential confounders (HR 0.90, 95% CI 0.76–1.06, data not shown). To study whether blood pressure and cholesterol could be potential intermediates in the association between serum magnesium and CHD mortality, we excluded systolic blood pressure, diastolic blood pressure, and total/high-density lipoprotein cholesterol ratio from our model. We found that by removing these variables, our analysis was not appreciably altered. When restricting all analyses to participants free of diabetes mellitus and chronic kidney disease (eGFR >60 mL/min/1.73m^2), the relationship found with CHD mortality and SCD was not altered (**Table 5.5**). Additional adjustments for serum sodium, potassium, and calcium to investigate whether the effect of serum magnesium on mortality is mediated through other electrolytes did not alter the point estimates. In the subset of 6,385 participants where data on physical activity was available, the inclusion of physical activity to the full model did not change the association between serum magnesium and CHD mortality, non-sudden CHD mortality, and SCD (data not shown).

DISCUSSION

In this prospective population-based cohort study among 9,820 participants with a median follow-up of 8.7 years, we found that low serum magnesium was associated with an increased risk of CHD mortality and SCD. When we excluded SCD from the CHD mortality end point, we found that higher serum magnesium levels were associated with a lower risk of non-sudden CHD mortality. Even though serum magnesium was associated with QTc, heart rate, and cIMT, this did not explain the relationship between serum magnesium and CHD mortality or SCD.

Table 5.5 - Sensitivity analysis on subset of participants free of diabetes mellitus and with normal kidney function and analysis on the influence of other electrolytes.

Serum magnesium groups	Model 2 Hazard Ratio (95%CI)* N=9,280	Subset of participants free of diabetes mellitus and with normal kidney function Hazard Ratio (95%CI)† N=7,843	Including electrolytes Hazard Ratio (95%CI)‡ N=9,280
Coronary Heart Disease mortality			
Low	1.36 (1.09 to 1.69)	1.44 (1.06 to 1.95)	1.33 (1.06 to 1.66)
Reference	1.00 (reference)	1.00 (reference)	1.00 (reference)
High	0.94 (0.73 to 1.21)	0.93 (0.67 to 1.29)	0.97 (0.76 to 1.25)
Sudden Cardiac Death			
Low	1.54 (1.12 to 2.11)	1.67 (1.11 to 2.51)	1.56(1.13 to 2.14)
Reference	1.00 (reference)	1.00 (reference)	1.00 (reference)
High	1.35 (0.96 to 1.89)	1.19 (0.77 to 1.85)	1.35 (0.96 to 1.90)

Footnotes

Low magnesium group ≤0.80 mmol/L, reference group 0.81mmol/L to 0.88mmol/L, high magnesium group ≥0.89mmol/L

* Equal to model 2 from Table 2; adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, history of diabetes mellitus, history of myocardial infarction, history of stroke, history of heart failure, smoking status, alcohol consumption, total/HDL cholesterol ratio and diuretic use

† Model 2 in subset of participants free of diabetes mellitus and with normal kidney function (defined as an eGFR > 60 mL/min/1.73m^2)

‡ Model 2 additionally adjusted for serum sodium, serum potassium and serum calcium in all participants

We observed a 36% increased risk of CHD mortality in participants with low serum magnesium. The association between serum magnesium and CHD mortality has been studied previously in a cohort of Finnish men but no significant association was found, possibly because of a low number of cases (n=230).(84) The National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (NHEFS) did find a significant association, but only when comparing the third quartile of serum magnesium to the first quartile.(9) The Nurses' Health Study analyzed the association between dietary magnesium and CHD mortality.(105) A significant association was identified when comparing the highest with the lowest quintile of dietary magnesium intake, but they were unable to study the association between serum magnesium and fatal CHD due to a lack of cases.(106) A meta-analysis on the association between low serum magnesium and fatal CHD, which included the 2 studies discussed above,(82, 84) found a nonsignificant trend towards an increased risk.(80) However, this meta-analysis also included 2 studies with SCD as outcome of interest which, judging from the Forest plot, were mainly responsible for this observed inverse effect.(8)

We analyzed SCD independently of CHD and found a significantly elevated risk for SCD in participants with low serum magnesium levels. Investigators from the Framingham Heart Study did not observe an association between low serum magnesium and SCD.(86) However, this study included only 29 cases of SCD (compared to 217 cases in our study), and may therefore have lacked power. Investigators from the Atherosclerosis Risk in Communities Study (ARIC) and from the Nurses' Health Study also analyzed the association between serum magnesium and SCD and both reported a lower risk for SCD when comparing the highest magnesium quartile with the lowest magnesium quartile.(85, 106) The difference in risk in participants with high serum magnesium between our study and the other 2 studies could be the result of differences in kidney function. In comparison to baseline kidney function from the ARIC Study and the Nurses' Health Study, participants within the Rotterdam Study had on average a 10 mL/min per 1.73m^2 lower eGFR at baseline than the participants from the ARIC Study and Nurses' Health Study. The significantly elevated risk of SCD in the high serum magnesium category could therefore still be the result of residual confounding by kidney function, assuming that a single eGFR measurement does not capture all changes associated with a decreased kidney function. We considered QT prolongation as a potential pathway through which serum magnesium may affect SCD risk, since prolongation and shortening of the QT interval is associated with SCD.(88, 107) However, adjustments for QT and RR interval did not fully explain the observed associations. An explanation for this limited change in effect estimate may be that a single measurement of QT interval does not fully capture all the electrophysiological changes caused by low serum magnesium. Another possibility could be that the effect of serum magnesium on QT interval is too small to have impact on the risk of SCD, as it is

currently assumed that a minimal increase of 5 ms is necessary to increase the risk of torsade de pointes.(108)

A substantial overlap between participants classified as CHD mortality and SCD exists. CHD mortality is a mixture of modes of death ranging from ischemia-induced ventricular arrhythmia to chronic ischemic heart failure. SCD is a particular mode of death that is predominantly caused by arrhythmias.(97) When we limited our outcome to non-sudden CHD deaths, we still observed a trend toward increased risk in the low magnesium group. We also observed a potential protective effect of high serum magnesium on non-sudden CHD mortality, which has been observed previously in NHEFS.(82)11 Although this observation combined with the significant effect on cIMT supports a role for magnesium in atherosclerosis, it does not appear to be the main pathway since the association between serum magnesium and non-sudden CHD death remained after adjustment for cIMT. This could be due to the small effect size of serum magnesium on cIMT. In previous studies it was shown that per 0.17 mm increase in cIMT, the risk for acute MI increased by 17%.(109) However, in our study, we only found an increase of 0.013 mm when comparing participants with low serum magnesium with the reference group. It is therefore unlikely that this small increase would have a large influence on our outcome. Furthermore, no association was found between serum magnesium and incident MI, which would have been expected if the main effect of serum magnesium is through accelerated atherosclerosis. This lack of association with incident ischemic heart disease has been observed previously in the National Health and Nutrition Examination Survey study.(82) The discrepancy between an effect on CHD mortality but not on incident MI argues against a direct role of serum magnesium on accelerated atherosclerosis, making an (acute) arrhythmogenic effect of serum magnesium more plausible.

Our study has several strengths. First, we did not use total cardiovascular mortality but studied CHD mortality, non-sudden CHD mortality, and SCD separately. The classification of cardiovascular mortality is heterogeneic and often also includes non-atherosclerotic outcomes such as hemorrhagic stroke. This heterogeneity makes it difficult to study associations, since there is also heterogeneity in the underlying pathophysiology of the various types of cardiovascular disease. Another strength of our study is the high case load, and, more importantly, the availability of adjudicated end points that were blinded from the laboratory results, reducing potential bias due to differential misclassification.

The main limitation of our study was the fact that we only have a single serum magnesium measurement at baseline and no follow-up serum magnesium measurements. We therefore cannot adjust for intra-individual variability over time. However, a recent study showed that there is a strong correlation between serum magnesium concentrations measured 1 year apart,(76) suggesting the possibility of an individual set-point for serum

magnesium, similar to what has been shown for serum sodium.(109, 110) In observational studies, reversed causality remains a limitation. However, the long-term follow-up after the serum magnesium measurement decreases the probability of reversed causality in our study. Another limitation of our study is the potential heterogeneity of SCD cases, which is inherent to the definition of SCD.(111) Generalization to other ethnic groups is also a limitation, since the participants in the Rotterdam Study are predominantly of European descent (96%). Finally, residual confounding can always play a role in observational studies. We tried to address this by adjusting for a large number of potential confounders and performing various sensitivity analyses, which did not indicate substantial amounts of confounding. A potential confounder for which we were unable to adjust is diet. Higher dietary magnesium intake could be a proxy for a healthy diet, therefore resulting in a lower risk of CHD mortality. We were unable to adjust for this confounder, as dietary assessment was available for only 2,504 participants. Since there is only a weak correlation between serum magnesium and dietary magnesium (R=0.06), it is unlikely that confounding by dietary magnesium intake could explain the observed associations.

In conclusion, low serum magnesium is associated with an increased risk of CHD mortality and SCD. Although low magnesium has a significant effect on subclinical atherosclerosis and heart rate, this did not explain the observed associations with CHD mortality or SCD. The results from this and previous studies may provide a rationale to design intervention studies to analyze whether magnesium supplementation could prove to be effective in lowering the burden of CHD mortality and SCD.

6

Chapter 6

Serum Magnesium and the Risk of Prediabetes: a Population-Based Cohort Study

ABSTRACT

Aims: Previous studies have found an association between serum magnesium and incident diabetes; however, this association may be due to reverse causation, whereby diabetes may induce urinary magnesium loss. In contrast, in prediabetes (defined as impaired fasting glucose), serum glucose levels are below the threshold for urinary magnesium wasting and, hence, unlikely to influence serum magnesium levels. Thus, to study the directionality of the association between serum magnesium levels and diabetes, we investigated its association with prediabetes. We also investigated whether magnesium-regulating genes influence diabetes risk through serum magnesium levels. Additionally, we quantified the effect of insulin resistance in the association between serum magnesium levels and diabetes risk.

Methods: Within the population-based Rotterdam Study, we used Cox models, adjusted for age, sex, lifestyle factors, comorbidities, kidney function, serum levels of electrolytes and diuretic use, to study the association between serum magnesium and prediabetes/diabetes. In addition, we performed two mediation analyses: (1) to study if common genetic variation in eight magnesium-regulating genes influence diabetes risk through serum magnesium levels; and (2) to quantify the proportion of the effect of serum magnesium levels on diabetes that is mediated through insulin resistance (quantified by HOMA-IR).

Results: A total of 8555 participants (mean age, 64.7 years; median follow-up, 5.7 years) with normal glucose levels (mean ± SD: 5.46 ± 0.58 mmol/L) at baseline were included. A 0.1mmol/l decrease in serum magnesium level was associated with an increase in diabetes risk (HR 1.18 [95% CI 1.04, 1.33]), confirming findings from previous studies. Of interest, a similar association was found between serum magnesium levels and prediabetes risk (HR 1.12 [95% CI 1.01, 1.25]). Genetic variation in CLDN19, CNNM2, FXYD2, SLC41A2, and TRPM6 significantly influenced diabetes risk ($p<0.05$), and for CNNM2, FXYD2, SLC41A2 and TRPM6 this risk was completely mediated by serum magnesium levels. We found that 29.1% of the effect of serum magnesium levels on diabetes was mediated through insulin resistance, whereas for prediabetes 13.4% was mediated through insulin resistance.

Conclusions: Low serum magnesium levels are associated with an increased risk of prediabetes and this increased risk is similar to that of diabetes. Furthermore, common variants in magnesium regulating genes modify diabetes risk through serum magnesium levels. Both findings support a potential causal role of magnesium in the development of diabetes, where the hypothesized pathway is partly mediated through insulin resistance.

The prevalence of diabetes mellitus is increasing worldwide and recent estimates indicate that one out of three people will develop diabetes during their life.(10) This underlines the importance of prevention and, therefore, the need to identify modifiable risk factors.(61) One potential modifiable risk factor that has emerged recently is magnesium. Magnesium is a co-factor in several pathways, including glucose transport, insulin sensitivity and insulin secretion,(112-114) providing a molecular basis for its involvement in the pathogenesis of diabetes mellitus.

Several population-based cohort studies have identified associations between magnesium intake and incident diabetes, incident prediabetes (defined as impaired fasting glucose) and progression of prediabetes to diabetes.(115-119) However, the association between magnesium intake and diabetes may be explained by an overall healthier eating pattern, since healthy foods, such as green leafy vegetables, are usually magnesium rich. Therefore, magnesium intake could be a proxy for a healthy diet rather than an independent risk factor.(120, 121) These limitations may be partially addressed by using serum magnesium levels instead of magnesium intake as a determinant for association analyses. Indeed, lower serum magnesium levels have also been associated with diabetes.(21, 122)

A remaining issue regarding the association between serum magnesium levels and diabetes is the possibility of reverse causality. Patients with diabetes show increased urinary magnesium loss, caused by hyperglycemia, hyperfiltration or a direct effect of insulin on magnesium channels in the kidney.(123) In contrast, in prediabetes, serum glucose levels are below the threshold for urinary magnesium wasting and, hence, unlikely to influence serum magnesium levels.

In the current study, we aimed to explore the directionality of the relationship between serum magnesium levels and diabetes in a large population-based cohort with adjudicated endpoints and long-term follow-up. To do so, we studied the relationship between serum magnesium levels and prediabetes. An association between serum magnesium levels and this precursor stage of diabetes is not expected if low serum magnesium is caused by long-standing diabetes. To further address the directionality of the association between serum magnesium and diabetes, we investigated whether magnesium-regulating genes are associated with diabetes risk. Finally, we quantified the role of insulin resistance as a potential pathway by which serum magnesium levels influence diabetes risk.

METHODS

Study design, setting and population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study, ongoing since 1990 in a suburb of Rotterdam, the Netherlands. The rationale and design of this study have been described elsewhere.(90) In summary, the original cohort was extended with a second cohort in 2000 and a third cohort in 2006, resulting in a total study population of 14,926 participants, aged 45 years and older. Participants are asked to participate in follow-up examinations every 4–5 years. The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Dutch Ministry of Health, Welfare and Sport, implementing the Wet Bevolkingsonderzoek: ERGO
(Population Study Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their physicians.

Measurements

Magnesium was measured within blood collections from the third visit of the first cohort (1997–1999), and the baseline visits of the second (2000–2001) and the third (2006–2008) cohort. These visits are similar in design and methods for data collection. Magnesium (mmol/L) was measured in serum by the Department of Clinical Chemistry of the Erasmus Medical Center (Rotterdam, the Netherlands). All measurements were carried out using a colorimetric endpoint method and the Roche/Hitachi Cobas c501 Analyzer (Roche Diagnostics, Indianapolis, IN, USA). The CV for repeatability was 0.8% and the CV for intermediate precision was 1.4–1.7%. Serum calcium, serum potassium, total cholesterol, HDL-cholesterol and serum creatinine were measured at the same visit as serum magnesium (Roche Diagnostics, Indianapolis, IN, USA). Serum calcium was measured using a photometric endpoint method, with a CV for repeatability of 0.4–2.0% and a CV for intermediate precision of 0.9–2.5%. Serum potassium was measured quantitatively with ion-selective electrodes, with a CV for repeatability of 0.3–0.6% and a CV for intermediate precision of 0.7–1.6%. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.(38) Total cholesterol and HDL-cholesterol were measured using an enzymatic colorimetric approach. Measurement and assessment of anthropometrics has been described previously.(90) Briefly, blood pressure was measured twice during study visits and the average was calculated. Information on smoking habits and alcohol consumption was obtained during a home interview; smoking habits were categorized as non-smoker, former smoker and current smoker. Alcohol use was categorized into two categories: yes or no.(42) Information on prevalent stroke and coronary heart disease was determined on the date that blood was drawn from the participants via linkage with general practitioners working in the study area, and adjudicated by two medical doctors and a neurologist or cardiologist in case of disagreement.(61) Information regarding the use of diuretics was derived from linkage to

pharmacy dispensing records.(104) Serum insulin was measured using an immunoassay (Roche Diagnostics) and for the majority of all participants (96.8%) fasting insulin levels were available. Insulin resistance was assessed using HOMA-IR values.(124) We used the natural logarithm (log_e) of HOMA-IR levels +1 to account for non-normal distribution.

Genetic analysis

We used a candidate gene approach to select 11 magnesium-regulating genes, which were hypothesized to play a role in the development of diabetes (**Table 6.1**). Lead single nucleotide polymorphisms (SNPs) in these magnesium regulating genes were identified from the literature.(109, 125, 126) Our goal was to select common genetic variations for our analysis; therefore, we only included SNPs with a minor allele frequency >10%, resulting in the following genes and corresponding SNPs being selected: TRPM7 (rs8042919), TRPM6 (rs2274924), SLC41A1 (rs823154), SLC41A2 (rs2463021), CNNM2 (rs3740393), CLDN19 (rs719676), CLDN16 (rs9990270) and FXYD2 (rs948100). Genotyping was carried out using the Illumina 550 duo and Illumina 610 quad BeadChip (Illumina, San Diego, CA, USA). For all participants that were included in this study, SNPs passed genotyping quality control, had a call rate >98% and had a Hardy–Weinberg p value >1× 10−6. Data were imputed to the 1000 Genomes reference panel (phase 1, version 3) using MACH version 1.0.15/1.0.16.(127, 128) Imputation quality for all SNPs was high (>0.97). Assessment of outcomes In this study, we excluded prevalent cases of diabetes. Incident cases of prediabetes and type 2 diabetes were ascertained through active follow-up using general practitioners' records, hospital discharge letters and glucose measurements from Rotterdam Study visits.(10) Diabetes and prediabetes were defined according to the recent WHO guidelines.(92) Briefly, diabetes was defined as fasting blood glucose ≥7.0 mmol/L, a non-fasting blood glucose ≥11.1 mmol/L (in the absence of fasting samples) or the use of blood glucose lowering medication (including oral blood glucose lowering medication such as metformin). Prediabetes was defined as a fasting blood glucose between 6.0 and 7.0 mmol/L or a non-fasting blood glucose between 7.7 and 11.1 mmol/L (in the absence of fasting samples). For the majority of all participants (96.8%) fasting blood glucose samples were available and for the remaining, a random non-fasting blood glucose level was used. Information regarding the use of blood glucose lowering medication was derived from both structured home interviews and linkage to pharmacy dispensing records.(90) All outcomes of prediabetes and diabetes were adjudicated by two independent study physicians. In case of disagreement, consensus was sought with an endocrinologist. Follow-up data was complete until 1 January 2012.

Table 6.1 - Minor allele frequency distribution among diabetes cases and controls, among selected Single Nucleotide Polymorphisms in magnesium regulating genes.

Locus	SNP	Location	Functional consequence	Major/Minor Allele	Minor Allele Frequency	
					Cases	Controls
CLDN16	9990270	Chromosome 3	Non-encoding region	G/C	0.43	0.45
CLDN19	719676	Chromosome 1	Intron	A/G	0.22	0.25
CNNM1	6584273	Chromosome 10	Intron	G/A	0.03	0.03
CNNM2	3740393	Chromosome 10	Intron	G/C	0.13	0.14
FXYD2	948100	Chromosome 11	Intron	C/G	0.10	0.11
KCNJ11	1800467	Chromosome 11	Intron	G/C	0.03	0.03
MRS2	7738943	Chromosome 6	Intron	G/C	0.07	0.06
SCL41A1	823154	Chromosome 1	Intron	C/T	0.39	0.41
SCL41A2	2463021	Chromosome 12	Non-encoding region	A/T	0.10	0.09
TRPM6	2274924	Chromosome 9	Missense	T/C	0.16	0.17
TRPM7	8042919	Chromosome 15	Missense	G/A	0.11	0.10

Footnotes

refSNP numbers, location, functional consequence and major allele was obtained from the Single Nucleotide Polymorphism Database (dbSNP).

Statistical analyses

Means, SD and percentages with frequency were used to report continuous and discrete variables. The relationship between serum magnesium levels and prediabetes was modelled using restricted cubic splines, using three knots, and was found to be linear. Therefore, we used serum magnesium as a continuous variable in multivariate Cox proportional hazards regression models to examine the relationship of serum magnesium levels with incident diabetes and prediabetes. Additionally, we studied the relationship between hypomagnesaemia (defined as serum magnesium ≤0.72 mmol/L) and incident diabetes and prediabetes. Follow-up time was calculated from the date that blood was drawn until the date of death, loss to follow-up or end of the study. The proportional hazards assumption was tested by plotting the $\log_{10}$ minus $\log_{10}$ survival curve and visually examining the curves, with no evidence that the assumption was violated.(104)

We performed two mediation analyses: (1) quantifying the role of insulin resistance in the association of serum magnesium levels with diabetes and prediabetes; (2) to study if genetic variation in magnesium regulating genes associates with diabetes and prediabetes through serum magnesium levels. To do so, we used the logistic model as reported by Baron and Kenny.(129) We calculated the standardized indirect effect, which is a measure for the degree of mediation through the mediator, and tested for significance using bootstrapping procedures (n=1000).(130) For all analyses on diabetes we used a study population including all participants without diabetes at baseline; for the analyses on prediabetes we included all participants with normal glucose levels at baseline.

Missing data in covariables (present in 0.0–1.4%) were handled by single imputation using an expectation–maximization algorithm.(39) All analyses were repeated on complete cases to check for potential differences. With the exception of the baseline characteristics, results are reported for imputed data. We considered a two-sided p value <0.05 as statistically significant. Data were analyzed using SPSS Statistics version 21.0 (IBM, Armonk, NY, USA) and R version 3.1.2. (The R Foundation for Statistical Computing, Vienna, Austria).

Sensitivity analyses

To test the robustness of our findings, we performed several sensitivity analyses. First, we repeated our analysis on prediabetes using electrolytes other than magnesium (sodium, potassium, calcium and phosphate), to rule out that the observed association between serum magnesium levels and prediabetes is caused by a coexisting electrolyte disorder. In the second sensitivity analyses, we studied whether participants without a separate diagnosis date of prediabetes could have influenced our effect estimates. In these individuals, both the diagnosis of prediabetes and diabetes was regarded as being made on the same day, as no separate date of prediabetes diagnosis could be determined.

Therefore, where the date of prediabetes diagnosis was missing, we imputed this date and repeated the analysis on prediabetes using these new dates. In the third sensitivity analysis, we excluded all participants with an eGFR below 60 mL/min/1.73 m^2 to exclude the possibility that impaired kidney function could have impacted our results. In the fourth sensitivity analysis, we excluded all participants with hypomagnesaemia and hypermagnesemia to confirm that our results were not driven by these extremes. In the final sensitivity analysis, we studied if the association between serum magnesium and diabetes risk could have been explained by proton pump inhibitor use as proton pump inhibitors have been previously associated with hypomagnesaemia.(59)

RESULTS

Baseline characteristics

Our total study population comprised 8,555 participants (**Figure 6.1**). **Table 6.2** shows the baseline characteristics of our study population. The mean age was 64.7 years and 57.8% were women. Participants with prevalent prediabetes more often had a history of smoking, hypertension, coronary heart disease, and used diuretics more often. The range of serum magnesium in the total population was 0.34–1.17 mmol/L. For participants with normal glucose levels at baseline, this range was 0.46–1.17 mmol/L, whilst for participants with prediabetes at baseline, this range was 0.34–1.20 mmol/L.

Association between serum magnesium levels and incident diabetes

Among the 8,555 participants without diabetes at baseline, 806 cases of incident diabetes were identified over a median follow-up of 6.7 years. **Table 6.3** shows the association between serum magnesium levels and incident diabetes. We found that a 0.1 mmol/L decrease in serum magnesium levels was associated with an increase in diabetes risk (HR 1.21[95% CI 1.07, 1.37]). After adjustment for lifestyle factors, comorbidities and other electrolytes this association persisted, but was slightly attenuated (HR 1.18 [95% CI 1.04, 1.33]). Participants with hypomagnesaemia had an increased diabetes risk before and after adjustment for confounders (HR 2.12 [95% CI 1.38, 3.28] and HR 1.79 [95% CI 1.16, 2.77], respectively). We found no evidence of effect modification by sex (p for interaction = 0.36).

Figure 6.1 - Flowchart of the study population

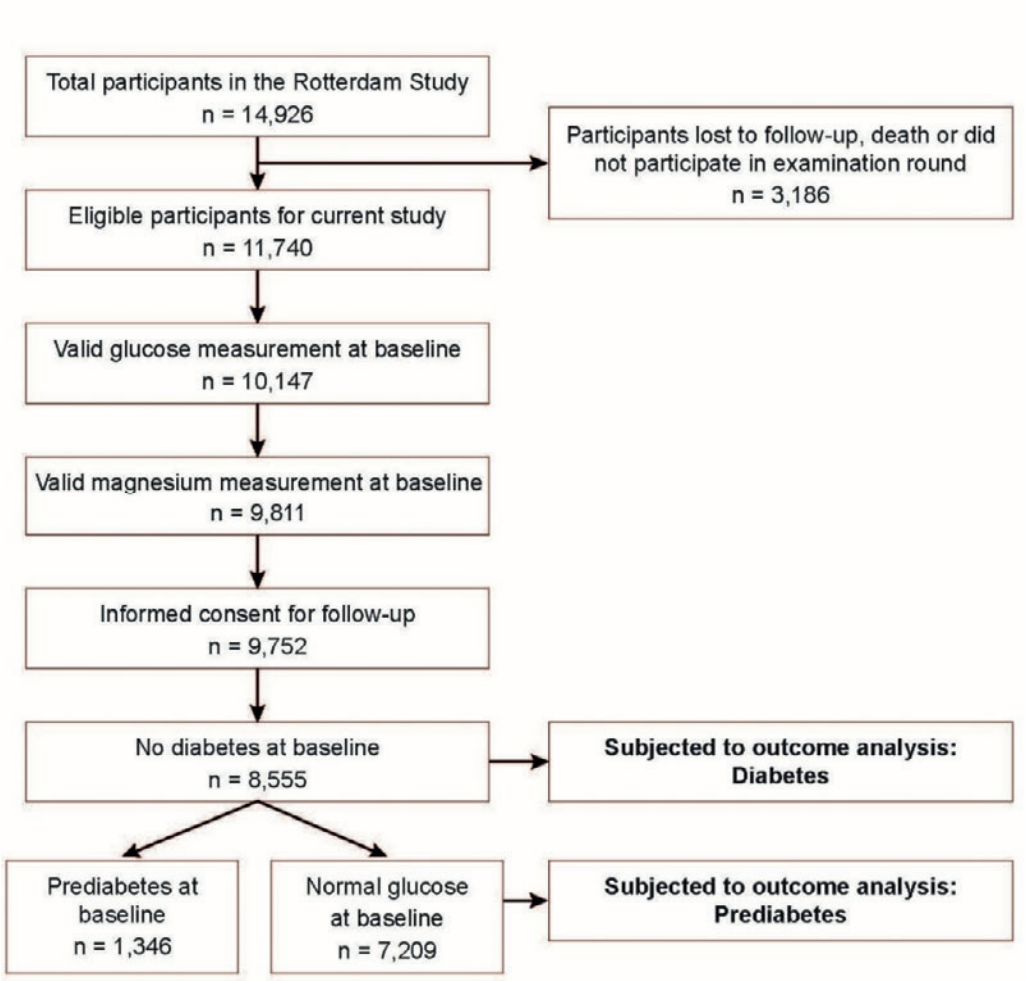


Table 6.2 - Baseline characteristics of the study population

	Total population N=8,555	Prevalent normoglycaemia N=7,209	Prevalent prediabetes N =1,346
Age, years	64.7 (9.7)	64.3 (9.7)	66.6 (9.4)
Women, N(%)	4949 (57.8)	4271 (59.2)	678 (50.4)
Body mass index, kg/m^2	27.0 (4.0)	26.7 (3.9)	28.5 (4.4)
Smoking, N(%) Never	2647 (30.9)	2279 (31.6)	368 (27.3)
Former	3933 (46.0)	3272 (45.4)	661 (49.1)
Current	1914 (22.4)	1602 (22.2)	312 (23.2)
Alcohol use, N(%)	7297 (85.3)	6136 (85.1)	1161 (86.3)
Total cholesterol, mmol/L	5.76 (1.01)	5.76 (1.01)	5.74 (1.02)
HDL cholesterol, mmol/L	1.43 (0.41)	1.44 (0.41)	1.33 (0.40)
History of hypertension, N(%)	5078 (59.4)	4072 (56.5)	1006 (74.7)
History of stroke, N(%)	245 (2.9)	202 (2.8)	43 (3.2)
History of CHD, N(%)	516 (6.0)	409 (5.7)	107 (7.9)
eGFR (CKD-EPI), mL/min/1.73m^2	79.7 (14.6)	80.0 (14.4)	78.3 (15.2)
Serum calcium, mmol/L	2.43 (0.10)	2.43 (0.10)	2.44 (0.10)
Serum potassium, mmol/L	4.35 (0.34)	4.36 (0.33)	4.34 (0.36)
Use of diuretics, N(%)	703 (8.2)	528 (7.3)	175 (13.0)
Serum glucose, mmol/L	5.46 (0.58)	5.30(0.45)	6.32 (0.47)
Serum magnesium, mmol/L	0.85 (0.06)	0.85 (0.06)	0.84 (0.06)
Hypomagnesaemia (≤0.72 mmol/L), N(%)	131 (1.5)	92 (1.3)	39 (2.9)
Hypermagnesaemia (≥0.97 mmol/L), N(%)	185 (2.2)	155 (2.2)	30 (2.2)

Footnotes

Data are shown for non-imputed data. Values are counts (valid percentages) or means (SD)

Table 6.3 - Association between serum magnesium levels and incident diabetes and prediabetes

Variable	At risk (N)	Cases (N)	Follow-up (person-years)	HR (95% CI) Model 1	Model 2	Model 3
Diabetes						
Per 0.1 mmol/L decrease	8,555	806	67,296	1.21 (1.07 to 1.37)	1.17 (1.04 to 1.32)	1.18 (1.04 to 1.33)
No hypomagnesaemia	8,424	785	66,421	1.00 (reference)	1.00 (reference)	1.00 (reference)
Hypomagnesaemia	131	21	875	2.12 (1.38 to 3.28)	1.80 (1.17 to 2.78)	1.79 (1.16 to 2.77)
Prediabetes						
Per 0.1 mmol/L decrease	7,209	1120	54,243	1.14 (1.02 to 1.27)	1.13 (1.01 to 1.25)	1.12 (1.01 to 1.25)
No hypomagnesaemia	7,117	1101	53,667	1.00 (reference)	1.00 (reference)	1.00 (reference)
Hypomagnesaemia	92	19	576	1.72 (1.09 to 2.71)	1.51 (0.96 to 2.37)	1.44 (0.91 to 2.27)

Footnotes

Model 1: adjusted for age, age^2, sex

Model 2: model 1 + BMI, smoking status, alcohol use and total cholesterol:HDL-cholesterol ratio, history of hypertension, history of stroke and history of coronary heart disease

Model 3: model 2 + eGFR (CKD-EPI), serum calcium, serum potassium and use of diuretics

Association between serum magnesium levels and incident prediabetes

Among the 7,209 participants with blood glucose levels within the normal range (fasting blood glucose <6.0 mmol/L or non-fasting blood glucose <7.7 mmol/L) at baseline, 1,120 cases of incident prediabetes were identified over a median follow-up of 5.7 years. **Table 6.3** shows the association between serum magnesium levels and incident prediabetes. We found that a 0.1 mmol/L decrease in serum magnesium levels was associated with an increased risk of prediabetes before adjustment (HR 1.14 [95% CI 1.02,1.27]) and after adjustment (HR 1.12 [95% CI 1.01, 1.25]) for confounders. Participants with hypomagnesaemia had an increased diabetes risk before adjustment for confounders (HR 1.72 [95% CI 1.09, 2.71]). After adjustment for confounders, this risk was attenuated and was no longer statistically significant (HR 1.44 [95% CI 0.91, 2.27]). We found no evidence of effect modification by sex (p for interaction =0.18).

Genetic risk factors

The selected common genetic variants were mainly located in introns (**Table 6.1**). For five SNPs (rs719676, rs3740393, rs948100, rs2463021 and rs2274924), we found a significant association with serum magnesium levels (**Table 6.4**). For four SNPs (rs3740393, rs948100, rs2463021 and rs2274924), we found an indirect effect on diabetes risk. For rs719676, we found a significant direct effect on diabetes risk (OR 0.84 [95% CI 0.74, 0.96]), but no evidence that this effect was mediated through serum magnesium levels. The same analysis was repeated on prediabetes risk, but only a significant direct effect of rs719676 on prediabetes was observed (OR 0.88 [95% CI 0.78, 0.98]). The other selected gene variants were not significantly associated with prediabetes.

Association between serum magnesium levels and insulin resistance

The association between serum magnesium levels, $\log_e$ HOMA-IR levels and diabetes is shown in **Figure 6.2.A**. We confirmed that serum magnesium levels were significantly associated with $\log^e$ HOMA-IR levels and also that $\log^e$ HOMA-IR levels were significantly associated with the risk of diabetes. When studying the direct effect of serum magnesium levels on the risk of diabetes adjusted for $\log_e$ HOMA-IR levels, the effect estimates were attenuated and the association was no longer statistically significant. The indirect effect of serum magnesium levels on diabetes risk, as mediated by $\log_e$ HOMA-IR levels, was significant with an odds ratio of 1.05 per 0.1 mmol/l decrease in serum magnesium levels. The calculated percentage of mediation was 29.1%.

Table 6.4 - Mediation analysis for magnesium-regulating genes

			Diabetes N=7,428		Prediabetes N=6,267	
Gene	**SNP**	**Effect on serum magnesium levels[a] β, mmol/l (95% CI)**	**Direct effect[b] OR (95% CI)**	**Indirect effect[c] OR (95% CI)**	**Direct effect[d] OR (95% CI)**	**Indirect effect[e] OR (95% CI)**
CLDN16	rs9990270	0.001 (−0.001 to 0.002)	0.92 (0.83 to 1.03)	1.00 (1.00 to 1.00)	0.99 (0.90 to 1.09)	1.00 (1.00 to 1.00)
CLDN19	rs719676	0.002 (0.000 to 0.005)*	0.84 (0.74 to 0.96)*	1.00 (0.99 to 1.00)	0.88 (0.78 to 0.98)*	1.00 (0.99 to 1.00)
CNNM2	rs3740393	0.005 (0.003 to 0.008)*	0.97 (0.82 to 1.15)	0.99 (0.98 to 1.00)*	1.07 (0.94 to 1.23)	1.00 (0.99 to 1.00)
FXYD2	rs948100	−0.004 (−0.006 to −0.001)*	1.12 (0.94 to 1.33)	1.01 (1.00 to 1.02)*	1.14 (0.98 to 1.32)	1.00 (1.00 to 1.01)
SLC41A1	rs823154	0.000 (−0.001 to 0.002)	0.94 (0.84 to 1.05)	1.00 (1.00 to 1.00)	0.95 (0.86 to 1.04)	1.00 (1.00 to, 1.00)
SLC41A2	rs2463021	−0.004 (−0.007 to 0.000)*	1.00 (0.82 to 1.22)	1.01 (1.00 to 1.02)*	0.96 (0.80 to 1.14)	1.00 (1.00 to, 1.01)
TRPM6	rs2274924	−0.004 (−0.006 to −0.001)*	1.01 (0.87 to 1.16)	1.01 (1.00 to 1.02)*	1.07 (0.94 to 1.21)	1.00 (1.00 to 1.01)
TRPM7	rs8042919	0.002 (−0.001 to 0.005)	0.95 (0.78 to 1.15)	1.00 (0.99 to 1.00)	1.05 (0.90 to 1.23)	1.00 (0.99 to 1.00)

Footnotes

[a]Effect of gene on serum magnesium level (mmol/l), per allele increase

[b]Direct effect (OR) of gene on diabetes, adjusted for serum magnesium levels

[c]Indirect effect (OR) of gene on diabetes, mediated by serum magnesium levels

[d]Direct effect (OR) of gene on prediabetes, adjusted for serum magnesium levels

[e]Indirect effect (OR) of gene on prediabetes, mediated by serum magnesium levels

* $p<0.05$

Figure 6.2 - The role of insulin sensitivity in the association between serum magnesium levels and prediabetes/diabetes

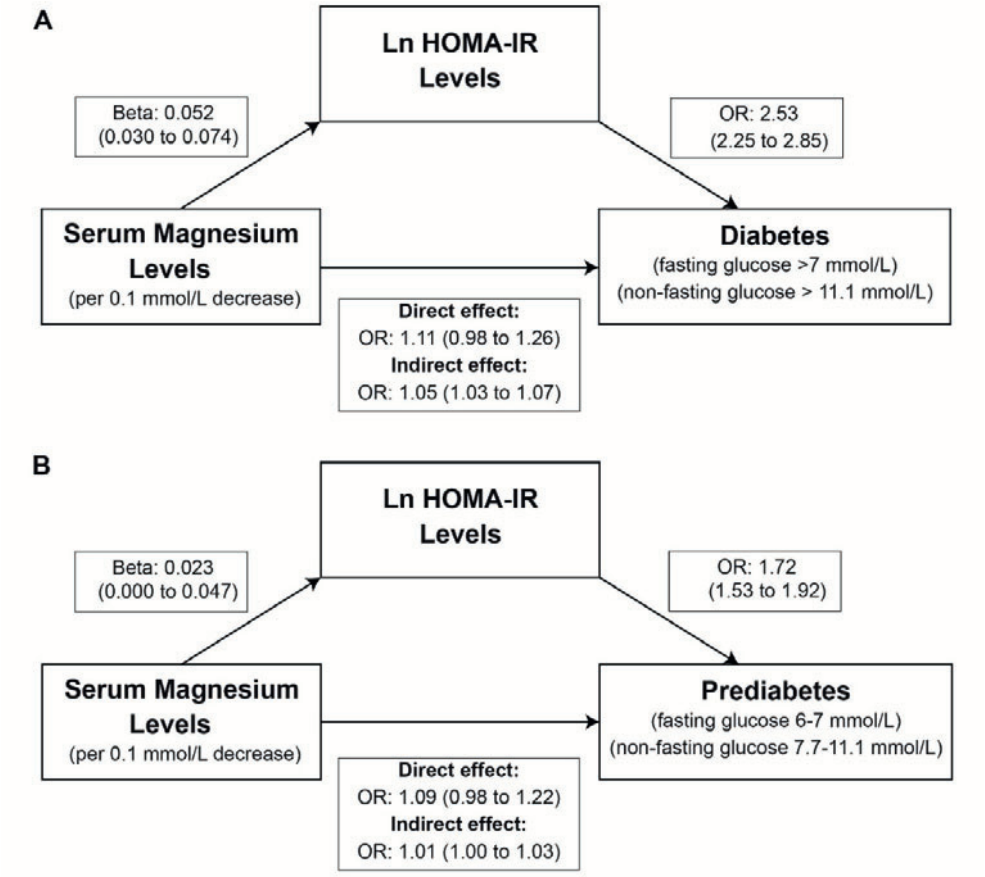


Figure 6.2.B shows the association between serum magnesium levels, $\log_e$ HOMA-IR levels and prediabetes. We found that serum magnesium levels were significantly associated with $\log_e$ HOMA-IR levels, within this group and that $\log_e$ HOMA-IR levels were also significantly associated with the risk of prediabetes, a finding similar to that of the association with diabetes. For the association with prediabetes, the calculated percentage of mediation was 13.4%.

Table 6.5 - Sensitivity analysis using magnesium and other electrolytes as determinants

	Normal glucose to prediabetes	
	HR (95% CI)	***p* value**
Serum magnesium (per 0.1 mmol/l decrease)	1.12 (1.01 to 1.25)	0.034
Serum sodium (per 1 mmol/l decrease)	1.01 (0.99 to 1.03)	0.297
Serum potassium (per 0.1 mmol/l decrease)	1.00 (0.98 to 1.02)	0.821
Serum calcium (per 0.1 mmol/l decrease)	1.04 (0.98 to 1.11)	0.218
Serum phosphate (per 0.1 mmol/l decrease)	1.03 (0.98 to 1.07)	0.216

Footnotes

Model 3 (adjusted for age, age^2, sex, BMI, smoking status, alcohol use, total cholesterol:HDL-cholesterol ratio, history of hypertension, history of stroke, history of coronary heart disease, eGFR

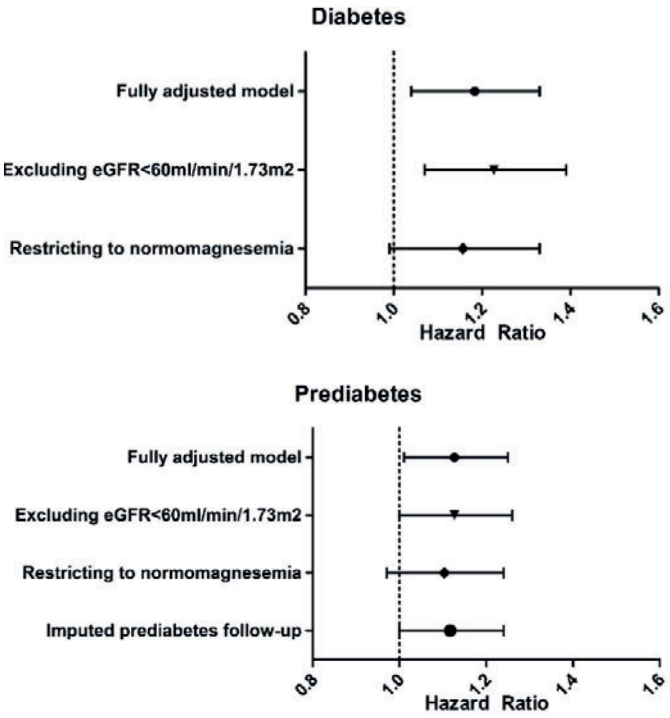
Sensitivity analyses

We found that, besides magnesium, no other electrolyte was associated with the risk of prediabetes (**Table 6.5**). For 235 participants, we did not have a separate date for prediabetes diagnosis, therefore this date was imputed. For 25 participants, data were excluded from this sensitivity analysis as the imputed prediabetes diagnosis date was before serum magnesium measurement. The analysis on imputed prediabetes diagnosis date yielded similar results compared with that using the diabetes diagnosis date (**Figure 6.3**). In the subsequent two sensitivity analyses we excluded participants with an eGFR below 60 mL/min/1.73m^2 and participants with hypomagnesaemia or hypermagnesemia. These analyses yielded similar results as in our main analyses. In the final analysis, we found that the addition of proton pump inhibitor use to our analysis on diabetes and prediabetes did not alter the association (data not shown).

DISCUSSION

In this large population-based cohort, we found that over a median follow-up of almost 6 years, low serum magnesium levels are associated with an increased risk of prediabetes, with comparable risk estimates to that of diabetes. Furthermore, we found that common genetic variants in magnesium-regulating genes influence diabetes risk and that this risk is mediated through serum magnesium levels.

Figure 6.3 - Sensitivity analysis



One strength of our study is that we thoroughly investigated the directionality of the association between serum magnesium and diabetes. Serum magnesium has already been associated with diabetes.(21, 122) However, increased renal magnesium wasting as a result of uncontrolled diabetes could lead to low serum magnesium levels.(131) This reversed causation may explain the observed association between serum magnesium and diabetes. If indeed low serum magnesium levels are only the result of uncontrolled diabetes, rather than a potential cause, one would not expect to find an effect of magnesium supplementation on diabetes risk and there would be no need to design a randomized controlled trial. Our study provides more evidence that magnesium may indeed influence diabetes risk, as we found similar associations for prediabetes as diabetes. The association with prediabetes is unlikely to be caused by reversed causation as glucose levels are not high enough to cause increased urinary magnesium wasting. Additionally, we found that magnesium-regulating genes associate with diabetes risk, further demonstrating that reverse causation is not likely to play a role, since diabetes status cannot alter genetic makeup. Another strength of our study is the detailed classification of diabetes and prediabetes, using a combination of prospectively gathered data that included the medical records of hospitals and general practitioners, electronic

linkage with pharmacy dispensing records in the study area and standardized blood glucose measurements during visits to the study center. This comprehensive assessment reduces potential bias resulting from misclassification. Furthermore, our study used a mediation analysis; this type of analysis goes beyond traditional analyses as it allows for a more causal interpretation of data, when certain assumptions are met.(132) The limitation of this type of analysis is directly linked to these assumptions, one of which is that there is no unmeasured confounding in the relationship between the mediator and the outcome. This assumption is difficult to check as unmeasured confounding can always play a role in observational data. However, we have adjusted our analysis for many potential confounders, which only slightly attenuated the effect estimates. Therefore, it is unlikely that the association between serum magnesium and diabetes risk is due to residual confounding, as this unknown confounder must be stronger and more imbalanced than all the confounders used in the current study. A second limitation of our study is that participants of the Rotterdam Study are predominantly of European descent. Previous studies demonstrated a lack of association between serum magnesium levels and diabetes risk in black participants,(21) but we were unable to stratify on ethnicity. Another limitation of our study is the use of a single measurement of serum magnesium. Consequently, we were unable to study if changes in serum magnesium levels over time might influence diabetes risk. However, in a previous study serum magnesium concentrations measured 1 year apart were found to strongly correlate.(76)

In combination with association directionality, we also studied the pathway by which serum magnesium levels may influence prediabetes and diabetes risk and found evidence of a dominant role of insulin resistance. A previous study demonstrated an effect of serum magnesium levels on insulin resistance.(112) However, the mediating effect of serum magnesium levels on diabetes through insulin resistance was not quantified. We found that approximately 29% of the effect of serum magnesium levels on diabetes and approximately 13% of the effect on prediabetes is mediated through insulin resistance. This partial mediation confirms the results of the Atherosclerosis Risk in Communities (ARIC) study, which found that the effect of serum magnesium levels on diabetes risk remained after adjusting for fasting glucose levels.(21) The remaining effect of serum magnesium levels on diabetes and prediabetes risk could occur via insulin secretion or through its impact on insulin signaling.(133) The effect of magnesium on insulin metabolism and glycemic control, in both patients with diabetes and without diabetes, has been studied in several randomized clinical trials. A meta-analysis of 370 patients with type 2 diabetes found that oral magnesium supplementation of 360 mg/day could influence serum magnesium levels, although not linearly, after a median of 12 weeks. This oral magnesium supplementation also significantly lowered fasting glucose levels, but did not influence long-term glycemic control.(134) In another trial,

the effect of 16 weeks of 2.5 g magnesium chloride administration daily was studied in type 2 diabetes patients with decreased serum magnesium levels at baseline. Magnesium supplementation significantly increased serum magnesium concentration and significantly reduced fasting glucose levels, fasting insulin levels and HbA1c levels compared with the control group.(135) Furthermore, in obese patients without hypomagnesaemia but with decreased insulin sensitivity at baseline, a positive effect of supplementation with 365 mg magnesium per day was observed. However, this supplementation only increased ionized magnesium levels and not total serum magnesium levels.(136) The effect of magnesium supplementation on insulin sensitivity was less pronounced in patients with normomagnesemia. However, a positive effect could still be observed in these patients, supporting our finding of a linear association between serum magnesium levels and prediabetes risk.

We found that five previously identified magnesium regulating genes were significantly associated with serum magnesium levels and that these genes were also associated with diabetes risk. We, hereby, replicate the findings from previous studies on the effect of TRPM6, SLC41A2 and CLDN19 on diabetes risk.(125, 126) The effect of genetic variation in TRPM6 and SLC41A2 was found to be mediated through serum magnesium levels. Variation in TRPM6 can alter serum magnesium levels, as it has an essential role in magnesium reabsorption within the distal convoluted tubule(137) and SLC41A2 is important for mediated magnesium transport across the plasma membrane.(138) CLDN19 was found to associate with diabetes risk independently from serum magnesium levels. CLDN19 is highly expressed in renal tubules where it influences paracellular magnesium transport. However, CLDN19 has also been found in extra-renal tissues, including peripheral neurons where it was found to influence the pore selectivity of tight junctions.(139) Therefore, a magnesium-independent role for CLDN19 in diabetes risk could be proposed since alterations in tight junctions have previously been linked with diabetes.(140) Besides confirming the role of these three genes in the association between magnesium and diabetes, we also identified new associations between two other magnesium regulating genes (CNNM2 and FXYD2) and diabetes risk, which were also mediated through serum magnesium levels. CNNM2 is thought to play a role in intracellular magnesium sensing. However, it is still unclear how this may lead to alterations in magnesium transport and, thus, alterations in serum magnesium levels.(137) FXYD2 encodes the γ-subunit of the Na+–K+-ATPase on the basolateral membrane of the distal convoluted tubule, where it drives paracellular magnesium transport through maintenance of the membrane potential.(141) At present, these findings have little impact on clinical or population health but they do provide more grounds for the role of magnesium in the pathogenesis of diabetes since slight alterations in serum magnesium levels, caused by common genetic variation in magnesium-handling genes, were found to impact diabetes risk. As expected with common genetic variants, the observed ORs were

small. As the effect of magnesium on prediabetes is less pronounced than its effect on diabetes, we also expected smaller effects of magnesium regulating genes on prediabetes risk. A post hoc power calculation showed that we had little power (2.9–17.2%) to detect significant differences in the mediation analysis on prediabetes; therefore, it is not surprising that we did not find significant associations with prediabetes for all magnesium regulating genes.

In conclusion, we found that low serum magnesium levels are associated with an increased risk of prediabetes, with similar effect estimates as compared with diabetes. The effect of serum magnesium on prediabetes and diabetes risk is partly mediated through insulin resistance. Furthermore, common genetic variation in magnesium regulating genes TRPM6, CLDN19, SLC41A2, CNNM2 and FXYD2 significantly modify the risk of diabetes through serum magnesium levels. Both findings support a potential causal role of magnesium in the development of diabetes and warrant future randomized controlled trials to study the effect of long-term magnesium supplementation on diabetes risk.

Chapter 7

Serum Magnesium is associated with the Risk of Dementia

ABSTRACT

Objective: To determine if serum magnesium levels are associated with the risk of all-cause dementia and Alzheimer disease.

Methods: Within the prospective population-based Rotterdam Study, we measured serum magnesium levels in 9,569 participants, free from dementia at baseline (1997–2008). Participants were subsequently followed up for incident dementia, determined according to the DSM-III-R criteria, until January 1, 2015. We used Cox proportional hazard regression models to associate quintiles of serum magnesium with incident all-cause dementia. We used the third quintile s as a reference group and adjusted for age, sex, Rotterdam Study cohort, educational level, cardiovascular risk factors, kidney function, comorbidities, other electrolytes, and diuretic use.

Results: Our study population had a mean age of 64.9 years and 56.6% were women. During a median follow-up of 7.8 years, 823 participants were diagnosed with all-cause dementia. Both low serum magnesium levels (≤0.79 mmol/L) and high serum magnesium levels (≥0.90 mmol/L) were associated with an increased risk of dementia (hazard ratio [HR] 1.32, 95% confidence interval [CI] 1.02–1.69, and HR 1.30, 95% CI 1.02–1.67, respectively).

Conclusions: Both low and high serum magnesium levels are associated with an increased risk of all-cause dementia. Our results warrant replication in other population-based studies.

Current treatment or preventive options for dementia are limited; there is an urgent need to identify novel, potentially modifiable, risk factors. Several vascular factors are established as risk factors for dementia, as are lifestyle factors.(142) Electrolytes have also emerged as interesting candidates, as electrolyte disturbances are associated with a variety of neurologic manifestations.(143) In recent years, magnesium gained specific interest as low serum magnesium levels associate with an increased risk of migraine, depression, and epilepsy and potentially dementia.(3) Most evidence for a role of magnesium in dementia comes from animal models. For instance, in both rat models for dementia as in healthy rats, magnesium was found to have a protective effect on learning.(144, 145) In humans, the evidence for a role of magnesium in dementia is limited and restricted to 4 case-control studies showing contrasting results, and a small randomized trial showing that a magnesium analogue improved executive functioning and working memory in participants with mild cognitive impairment.(146) We studied the association of serum magnesium levels with the risk of dementia in a large prospective population-based cohort with long-term follow-up.

METHODS

Study design, setting, and population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study, ongoing since 1990 in a suburb of the city of Rotterdam, the Netherlands. The rationale and design of this study have been described in detail elsewhere.(90) The original cohort comprised 7,983 persons older than 55 years and living in the study area. This cohort was extended in 2000 with an additional cohort of 3,011 persons who had become 55 years or older or who had moved into the study area. In 2005, the cohort was extended again with 3,932 persons aged 45 years and older living in the research area who had not yet been included. All participants are asked to participate in follow-up examinations every 4 to 5 years.

Standard protocol approvals, registrations, and patient consents

All eligible participants provided written informed consent to participate in the study and separate consent for follow-up data collection. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Dutch Ministry of Health, Welfare and Sport, implementing the Wet Bevolkingsonderzoek: ERGO (Population Study Act: Rotterdam Study).

Assessment of magnesium

For this study, we used blood samples collected at the third visit of the first cohort (1997–1999) and the first visits of the second (2000–2001) and the third cohort (2006–2008), as these visits are similar in design and data collection with regard to serum magnesium measurements. This time point was therefore also considered the baseline for the current

analysis. Magnesium was measured in serum using a colorimetric endpoint method with a coefficient of variation (CV) for repeatability of 0.8% and a CV for intermediate precision of 1.4%–1.7%. Measurements were performed by the Department of Clinical Chemistry of the Erasmus Medical Center using a Roche/Hitachi Cobas c501 analyzer (Roche Diagnostics, Indianapolis, IN).

Ascertainment of incident dementia

The method for dementia screening and diagnosis in the Rotterdam Study has been described in more detail previously.(142) In brief, participants were screened for all-cause dementia (which includes Alzheimer disease [AD], vascular dementia, and Parkinson disease dementia) using a 3-step protocol. In the first step, participants underwent a Mini-Mental State Examination (MMSE) and Geriatric Mental Schedule (GMS) at baseline and during follow-up examinations. Screen-positive participants (MMSE <26 or GMSE>0) were invited for the second step, which consisted of a physician interview using the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX).9 Besides these examinations, all participants are continuously monitored for dementia using digital linkage of the study database with medical records from general practitioners in the area and the Regional Institute for Outpatient Mental Health Care. Final diagnosis is made by a consensus panel, led by a neurologist, according to the standard criteria for dementia (DSM-III-R) and AD (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association [NINCDS-ADRDA]).(147) Follow-up for incident dementia was near complete (98.0% of potential person-years) until January 1, 2015.

Assessment of covariates

Body mass index was calculated as weight in kilograms divided by squared height in meters. Information on education, smoking habits, and alcohol consumption was obtained during a home interview.(90) Educational level was categorized as primary education, lower or intermediate education or lower vocational education, intermediate vocational or higher general education, or higher vocational education or university. Smoking was categorized into 3 categories: non-smoker, former smoker, and current smoker. Alcohol use was categorized into 2 categories: yes or no. Serum sodium, calcium, potassium, total cholesterol, high-density lipoprotein cholesterol, and serum creatinine were measured at the same visit as the serum magnesium levels. The estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology
Collaboration equation and was expressed as mL/min/1.73 m^2.(38) APOE genotype was determined using PCR on coded DNA samples.(148, 149) The assessment of diabetes mellitus, stroke, and heart failure was done through active follow-up, as described previously, using general practitioner records and hospital discharge letters.(10, 61, 150) Information regarding the use of diuretics was derived from linkage to pharmacy dispensing records, as described previously.(90)

Statistical analyses

Means with SDs and counts with valid percentages were used to report continuous and discrete variables, respectively. We first explored the relationship between serum magnesium and incident dementia with restricted cubic splines and found it to be U-shaped. Therefore, to best approximate this U-shape, in all subsequent analyses we categorized serum magnesium in quintiles and used the third quintile as reference group. We used Cox proportional hazards regression models and calculated follow-up time from the date that blood was drawn until date of dementia diagnosis, death, loss to follow-up, or the end of the study, whichever came first. Participants were censored in case of death, loss to follow-up, or at the end of the study. The proportional hazard assumption was tested by plotting the log minus log survival curve and visually examining the curves, with no evidence that the assumption was violated. For all analyses we constructed 2 models. The first model was adjusted for age, sex, Rotterdam Study cohort, and educational level, whereas the second model was additionally adjusted for body mass index, systolic and diastolic blood pressure, smoking status, alcohol use, serum sodium, potassium, and calcium levels, total cholesterol/high-density lipoprotein ratio, kidney function, APOE genotype, prevalent diabetes mellitus, stroke, heart failure, and use of diuretics. To test the robustness of our findings, we performed several sensitivity analyses. First, we excluded all cases of incident dementia within the first 4 years after serum magnesium measurement, to minimize the risk of reverse causality. Second, we excluded all participants with an estimated glomerular filtration rate below 60 mL/min/1.73 m^2 to study if residual confounding by kidney function could have influenced our results. Third, we excluded all participants with prevalent diabetes, to study if the observed association is driven by the association between serum magnesium and diabetes risk. Fourth, we repeated the analysis on all-cause dementia and censored participants if they had a stroke during follow-up. Finally, we studied the effect of serum magnesium on incident AD, as this is the most common clinical subtype of dementia. Missing data on covariables (0%–4.2%) were handled by single imputation using an expectation-maximization algorithm.(39) With the exception of the baseline characteristics, results are reported for imputed data. Data were analyzed using SPSS Statistics, version 21.0 (IBM, Armonk, NY), and R, version 3.1.2 (The R Foundation for Statistical Computing). We considered a 2-sided p value <0.05 as statistically significant.

RESULTS

Baseline characteristics

The flowchart of our study population is shown in **Figure 7.1**. Of the 11,740 eligible participants for this study, 9,883 participants had a serum magnesium measurement available at baseline. We excluded 59 participants as no informed consent was obtained to retrieve follow-up information and 255 participants were excluded due to prevalent dementia. This resulted in a total study population of 9,569 participants. Baseline characteristics of the total study population are presented in **Table 7.1**.

Figure 7.1 – Flowchart of the study population

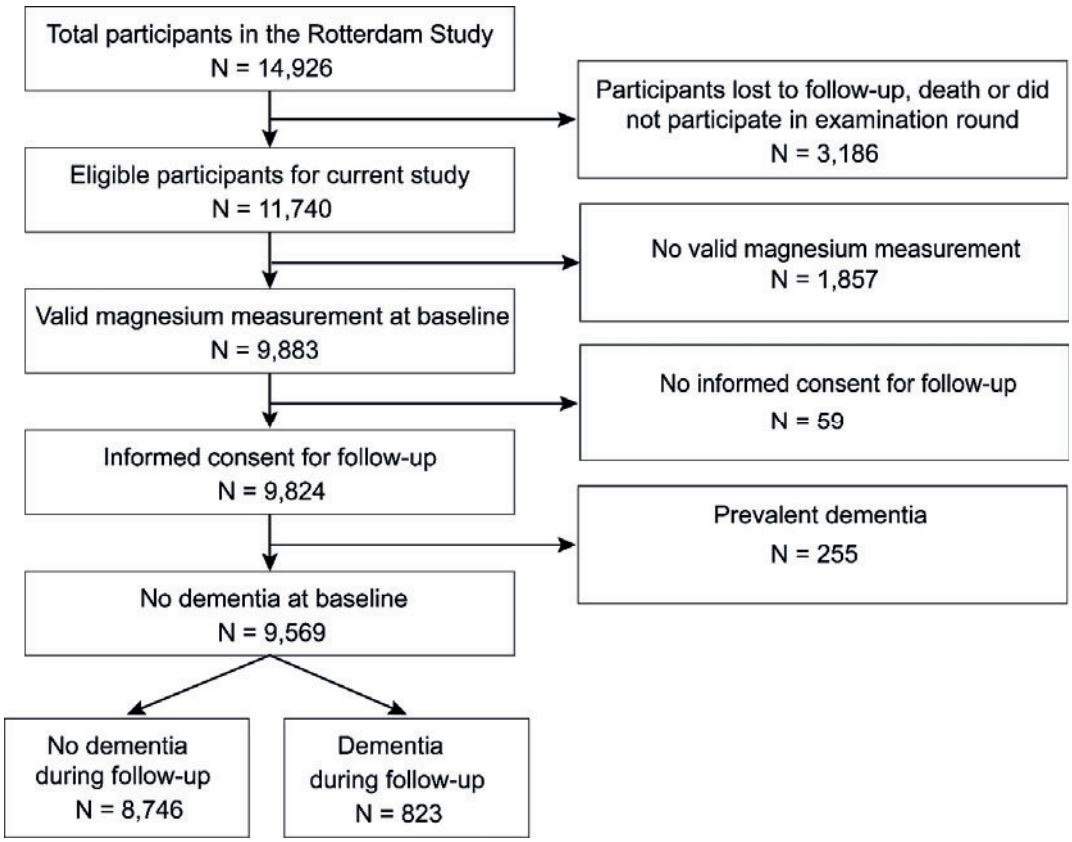


Table 7.1 - Baseline characteristics of the study population

	Total population (N=9,569)
Age, years	64.9 (9.7)
Women, N(%)	5420 (56.6)
Body mass index, kg/m^2	27.3 (4.2)
Systolic blood pressure, mmHg	139.6 (21.1)
Diastolic blood pressure, mmHg	78.9 (11.5)
Educational level, N(%)	
Primary education	1134 (12.0)
Lower, intermediate, or lower vocational education	3853 (40.7)
Intermediate vocational or higher general education	2771 (29.3)
Higher vocational education or university	1699 (18.0)
Smoking, N(%)	
Never	2924 (30.8)
Former	4472 (47.0)
Current	2115 (22.2)
Alcohol use, N(%)	8131 (85.5)
Serum magnesium, mmol/L	0.84 (0.06)
Serum sodium, mmol/L	142.1 (3.1)
Serum calcium, mmol/L	2.43 (0.10)
Serum potassium, mmol/L	4.35 (0.34)
Total cholesterol, mmol/L	5.72 (1.02)
High-density lipoprotein cholesterol, mmol/L	1.40 (0.41)
Estimated glomerular filtration rate (CKD-EPI), mL/min/1.73m^2	79.8 (14.8)
APOE genotype, N(%)	
22	53 (0.6)
23	1173 (12.8)
24	250 (2.7)
33	5347 (58.3)
34	2127 (23.2)
44	198 (2.2)
History of diabetes mellitus, N(%)	977 (10.2)
History of stroke, N(%)	305 (3.2)
History of heart failure, N(%)	240 (2.5)
Use of diuretics, N(%)	875 (9.1)

Footnotes

Values are counts (valid percentages) or means (standard deviation). Data are shown for non-imputed data.

The mean age of the study population was 64.9 years with 56.6% being women. In our population,serum magnesium levels ranged from 0.34 to 1.17 mmol/L, with 108 participants having hypomagnesemia (defined as a serum magnesium level <0.70 mmol/L) and 2 participants having hypermagnesemia (defined as a serum magnesium level >1.10 mmol/L). Participants within the first quintile of magnesium more often had diabetes mellitus, had a lower estimated glomerular filtration rate, and more often used diuretics. Furthermore, serum sodium and potassium levels were lower within this quintile, as compared to other quintiles.

Association between serum magnesium and incident dementia.
During a median follow-up of 7.8 years (interquartile range 5.3–14.0 years), 823 participants were diagnosed with dementia, 662 of them with AD. **Table 7.2** shows the results of the analysis on incident dementia. In the first model, we found an increased hazard for all-cause dementia (hazard ratio [HR] 1.33, 95% confidence interval [CI] 1.04–1.71) in the first quintile compared to the reference group. This association was similar in the fully adjusted model (HR 1.32, 95% CI 1.02–1.69). The fifth quintile of magnesium was also associated with a significantly increased hazard for all-cause dementia in the fully adjusted model (HR 1.30, 95% CI 1.02–1.67).

Sensitivity analysis
In **Figure 7.2**, the results of the sensitivity analyses are shown. Censoring dementia cases diagnosed in the first 4 years after serum magnesium measurement, excluding participants with declined kidney function, excluding participants with prevalent diabetes, or censoring participants for stroke yielded similar results. The association between serum magnesium and AD was similar to that of all-cause dementia, although the risk estimates did not reach statistical significance (HR 1.28, 95% CI 0.97–1.69; HR 1.21, 95% CI 0.92–1.58, respectively).

Figure 7.2 - Sensitivity analysis

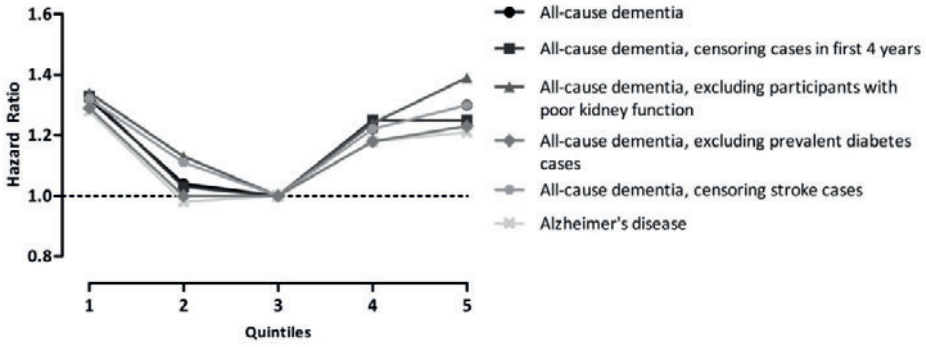


Table 7.2 - Association between serum magnesium and incident dementia

	First Quintile ≤0.79 mmol/L	Second Quintile 0.80-0.83 mmol/L	Third Quintile 0.84-0.85 mmol/L	Fourth Quintile 0.86-0.89 mmol/L	Fifth Quintile ≥0.90 mmol/L
Number of participants (events)	1,771 (160)	2,348 (184)	1,387 (102)	2,315 (198)	1,748 (179)
Incidence rate per 1000 person-years (95%CI)	10.2 (8.7 to 11.9)	8.2 (7.1 to 9.5)	7.8 (6.4 to 9.5)	9.3 (8.0 to 10.6)	11.4 (9.8 to 13.2)
Model 1 HR (95%CI)	1.33 (1.04 to 1.71)	1.06 (0.83 to 1.34)	1.00 (Reference)	1.15 (0.90 to 1.46)	1.24 (0.97 to 1.58)
Model 2 HR (95%CI)	1.32 (1.02 to 1.69)	1.04 (0.82 to 1.32)	1.00 (Reference)	1.22 (0.96 to 1.55)	1.30 (1.02 to 1.67)

Footnotes

Model 1: adjusted for age, sex, Rotterdam study cohort and educational level

Model 2: same as model 1 and additionally adjusted for body mass index, systolic and diastolic blood pressure, smoking status, alcohol use, serum sodium, potassium, and calcium levels, total/high-density lipoprotein cholesterol ratio, estimated glomerular filtration rate, Apolipoprotein E genotype and history of diabetes mellitus, stroke, or heart failure, and use of diuretics.

DISCUSSION

In this large population-based cohort, we found that both low and high serum magnesium levels were associated with an increased risk of all-cause dementia over a median follow-up of almost 8 years. Furthermore, similar associations were found for the risk of AD.

Our findings support the results from the 2 case-control studies showing higher serum magnesium levels in patients with AD and results from 2 other case-control studies showing lower serum magnesium levels.(151-154) A previous meta-analysis concluded that these contradicting findings might be due to the use of different diagnosis criteria (either DSM or NINCDS-ADRDA), resulting in a different case definition, or the fact that the studies came from different part of the world (either Europe/America or Asia), where ethnicity could have a modifying effect on the association.(155) In our study, we use both criteria to diagnose all-cause dementia and AD and find that the association between serum magnesium and the risk of dementia is more likely to be U-shaped rather than linear. In addition, by using a prospective cohort design, we minimize the possibility of information bias or selection bias influencing our results. Another difference between our study and the case-control studies is the range of serum magnesium levels. In our study, serum magnesium quintiles were largely within the normal range as compared to the case-control studies, which reported on serum magnesium levels that showed overlap with the more extreme levels within our study (e.g., the first and fifth quintiles).

Our results are similar to results found in a population-based cohort study in Japan, in which higher self-reported dietary intake of magnesium was found to be associated with a decreased risk of all-cause dementia.(156) Different from our results is that this previous study found a linear rather than a U-shaped association. A limitation of using dietary magnesium intake is that this association could also be the result of other nutrients within the diet or an overall healthier eating pattern, as foods like green leafy vegetables are often magnesium-rich.(121) In addition, dietary magnesium intake only weakly correlates with serum magnesium levels, most likely due to the fact that magnesium absorption, from the diet into serum, strongly depends on other dietary contents and body stores of magnesium.(40, 59)

In the current literature, there are 2 hypotheses for the role of magnesium in dementia. One is a direct effect of neuronal magnesium on regulation of the NMDA receptor.26 The NMDA receptor plays an important role in learning and memory and magnesium modulates the calcium influx through this receptor, which is essential for its functioning.(157, 158) An important assumption within this hypothesis is the possibility of serum magnesium to influence neuronal levels of magnesium, which is still under debate.(159) A second hypothesized pathway, through which serum magnesium can influence dementia risk, is oxidative stress. Magnesium deficiency has been found to

stimulate secretion of inflammatory mediators like interleukins, tumor necrosis factor–a, and nitric oxide.(3) These mediators are thought to stimulate atherosclerosis and thereby increase the risk of
dementia.(160)

Several limitations of our study should be noted. First, we only have a single measurement of serum magnesium. Although serum magnesium levels are relatively stable over time, we cannot rule out that changes in serum magnesium levels over time have influenced our results.(76) Second, serum magnesium levels in our study were virtually all within the clinically defined normal range; therefore, we are unable to study the effect of hypomagnesemia. However, we expect the effect estimates of hypomagnesemia or hypermagnesemia on dementia to be higher than our observed effect estimates. Third, serum magnesium levels do not necessarily represent total body magnesium. There can still be a magnesium deficiency if serum magnesium levels are normal; therefore, misclassification could have occurred.(3) This misclassification, however, would have occurred in both directions, thereby attenuating our risk estimates. Finally, as we are using observational data, it is difficult to infer causality from this data. We have tried to address this by performing several sensitivity analyses, including an analysis in which we censored all dementia cases occurring in the first 4 years after serum magnesium measurement, which yielded similar results. These findings strengthen the possibility of a causal relationship. Strengths of this study include the prospective population-based design, reducing information bias, the long follow-up period, and the comprehensive assessment of dementia status. Furthermore, the detailed assessment of potential confounders and the fact that adjusting for these factors did not alter our effect estimates also strengthens the possibility of a true relationship between serum magnesium levels and dementia, rather than it being the result of other confounders or intermediates. As we are the first to study this association, our results warrant replication in other population-based studies.

PART IV
General Discussion and Summary

D

General Discussion

Disorders of serum sodium and potassium are the most commonly occurring electrolyte disorders and, therefore, are subject of many studies. (2) Disorders of serum magnesium, however, are also common but often not identified as serum magnesium is not measured routinely.(161) The reason that this electrolyte is often 'forgotten' can be largely explained by the knowledge gap between magnesium physiology and its role in health and disease.(162) In recent years, magnesium has gained interest and more information has become available of specific channels important for magnesium homeostasis.(3)

In this thesis, we aim to further extend this knowledge by studying the effect of frequently used pharmaceutical drugs on magnesium homeostasis and the role of magnesium in the development of several major diseases. In this discussion, we place the results of our research into perspective and discuss a number of methodological considerations related to our work.

The effect of pharmaceuticals drugs on magnesium homeostasis

Mild hypomagnesemia is associated with nonspecific symptoms, such as fatigue and muscle weakness. Severe hypomagnesemia will cause a more distinct clinical presentation with tetany and seizures.(163) This presentation was observed with the use of proton pump inhibitors (PPIs) in 2006, and several case series followed subsequently.(6) However, as these reports emerged nearly 20 years after their introduction on the market, it was unclear whether this was a general adverse event of PPI use or if it was a previously unrecognized idiosyncratic reaction to the pharmaceutical drug.(164) We addressed this question within a general population cohort, by using systematically measured serum magnesium levels. This approach allowed us to study the association without the limitation of confounding by indication, which could have been a problem in previous studies.(19, 24, 27) Previous studies were performed within clinical settings, in which serum magnesium is measured for cause. In addition, recent awareness of the possibility that PPI use is a risk factor for hypomagnesemia could trigger physicians to order a serum magnesium measurement. By ordering a serum magnesium measurement more often in patients on PPI than in patients without PPI use, a bias is introduced making it difficult to study the true relationship. By measuring serum magnesium levels in all participants at the same point in time, irrespective of disease or drug use status, we overcome this problem of confounding by indication. Using this approach, we found that there is a relation between prolonged PPI use and hypomagnesemia. In addition we found that the concomitant use of diuretics increased the risk of hypomagnesemia even further. This interaction between PPI use and diuretic use had not been described before but can be explained by the hypothesized pharmacological effect of PPIs and diuretics on magnesium homeostasis. The intended pharmacological effect of PPIs is the inhibition of the H^+-K^+ ATPase pump in the stomach to reduce gastric acid production.(164) It has been hypothesized that this reduction in acid causes a decrease in activity of the transient

receptor potential melastatin 6 (TRPM6) channel in the gastrointestinal tract.(49) By reducing the activity of this magnesium channel, less magnesium will be absorbed from the gastrointestinal tract.(165) This reduced uptake can potentially cause hypomagnesemia, however, the body has two ways to prevent this. The first is via the passive paracellular route, which is independent from the TRPM6 channel. Ongoing studies explore the effect of adding inulin fibers to the diet which enhances the solubility of magnesium, resulting in a higher magnesium gradient across the membrane which stimulates intestinal absorption.(166, 167) The second is increased renal magnesium reabsorption to compensate for the reduced intestinal uptake.(6) In this regard, the thick ascending limb of the loop of Henle and the distal convoluted tubule in the kidney are the most crucial nephron segments for magnesium homeostasis. The loop of Henle is important as 50-70% of all magnesium is reabsorbed in this part of the nephron. Magnesium reabsorption in this part is driven by the transepithelial gradient generated by $Na^+K^+2Cl^-$ cotransporter (NKCC2) activity.(72) The distal convoluted tubule is responsible for only 10% of magnesium reabsorption but does play a crucial role in magnesium homeostasis. This is related to the presence of TRMP6 channels, which can be upregulated or downregulated through a variety of regulating factors, which makes it possible to actively fine-tune magnesium reabsorption in this part of the kidney.

Loop diuretics and thiazide diuretics act on the loop of Henle and distal convoluted tubule and the, respectively. Both classes of diuretics have been linked to disorders in sodium and potassium balance, however, their effect on magnesium homeostasis is less clear. We studied the effect of prolonged thiazide and loop diuretic use on serum magnesium levels and found that thiazide diuretic users have a lower serum magnesium level and an increased risk of hypomagnesemia. In previous animal studies, TRPM6 channels were found to be downregulated in the distal convoluted tubule during thiazide use, suggesting a link between the sodium chloride cotransporter (NCC), which is inhibited by thiazide diuretics, and TRPM6. However, the exact mechanism is still unknown.(46, 67)

We hypothesized that loop diuretics would be associated with lower serum magnesium levels, but found that the use of loop diuretics was associated with a slight increase in serum magnesium levels. The combination of loop diuretics with PPIs did, however, associate with an increased risk of hypomagnesemia. Loop diuretics inhibit NKCC2 in the loop of Henle, thereby blocking the transepithelial gradient which is crucial for magnesium uptake. To compensate for this reduced uptake, TRPM6 channels are upregulated during the chronic use of loop diuretics.(58, 74) The interaction between loop diuretic and PPI use could suggest that this upregulation of TRPM6 channels during chronic loop diuretic use is insufficient to compensate for the reduced magnesium uptake in the intestine in combination with the excessive magnesium loss in the loop of Henle. Interestingly, TRPM6

channels in the kidney were downregulated during PPI use, which might explain why the combination with loop diuretics will increase the risk of hypomagnesemia.(168)

The role of magnesium in the development of major diseases

On a population level, the previously mentioned pharmaceutical drugs cause only minor changes in serum magnesium levels and one might question whether these changes are clinically relevant because only severe hypomagnesemia causes clinical symptoms. In this thesis, we studied the association between serum magnesium levels, within the normal range, and several diseases which are amongst the top 10 causes of death globally as reported by the World Health Organization.(169) We found that low serum magnesium levels were associated with an increased risk of diabetes mellitus, dementia, and cardiovascular mortality. In addition, for dementia and sudden cardiac death, we found that high serum magnesium levels were also associated with an increased risk. Several theories exist regarding the potential pathways underlying these associations, which we will discuss in more detail below.

First, a common mechanism underlying both diabetes mellitus and cardiovascular disease, is inflammation. Both low serum magnesium and hypomagnesemia were found to promote an inflammatory response characterized by increased levels of CRP, interleukin-6, TNF-alpha, and fibrinogen.(170) In turn, these stimuli may trigger oxidative stress, which is an important contributor to the development of cardiovascular disease and diabetes mellitus. (170-175)

Second, magnesium may directly affect vascular tone. This effect is caused by magnesium's effect on both endothelial and vascular smooth muscle cells and likely mediated through prostacyclin and nitric oxide.(80) Hypomagnesemia promotes vasoconstriction, which is unfavorable in the presence of endothelial damage, caused by the inflammatory response. The combination of vasoconstriction and endothelial damage will reduce blood flow in the coronary arteries, hereby negatively contributing to cardiovascular disease.(78-80, 176-178)

Atherosclerosis is also hypothesized to play a role in dementia, including not only vascular dementia but also Alzheimer's disease.(179) Therefore, the same pathways as for cardiovascular disease may be operative in dementia. In addition, a separate theory exists in which magnesium can directly influence the NMDA receptor, a receptor crucial for learning.(157, 158) The latter theory, however, assumes that changes in serum magnesium also affect magnesium concentration in the cerebrospinal fluid, which is unclear.(159)

Methodological considerations

Due to the design of the Rotterdam Study several methodological aspects should be considered when interpreting the results presented in this thesis.

The Rotterdam Study is an observational cohort, which poses a challenge when addressing questions of causality. Confounding is an important issue in the analysis of observational data. Even though we collect many participant characteristics and morbidities within the Rotterdam Study, there is always the possibility of an unknown variable explaining the observed association. An example is confounding by indication, which can be a problem in drug-related research when a certain drug is prescribed because of symptoms associated with the determinant of study. In our study on PPIs and diuretics confounding by indication is less likely to have played a role, because disturbances in magnesium homeostasis are not the indication for prescribing PPIs or diuretics. Instead, hypomagnesemia is an adverse event of these drugs, which can be studied in an observational cohort. However, there is also the possibility of confounding by indication when a drug is prescribed for complaints associated with the determinant under study. In this case confounding by indication would have been an issue if PPIs or diuretics would have been prescribed for symptoms associated with hypomagnesemia. As stated previously, symptoms only occur in case of severe hypomagnesemia. As our study is performed in the general population, none of the participants had a sufficiently low serum magnesium level to expect specific symptoms, but even if this would have been the case, PPIs or diuretics are unlikely to be prescribed for these symptoms.

Another problem with interpreting data from observational studies is reverse causality, which not only plays a role in cross-sectional studies but also in longitudinal studies. Novel epidemiological methods have become available to infer causality and we have used two different methods in our study on diabetes mellitus, namely mediation analysis and Mendelian randomization. (180, 181) The first approach uses complex statistical methods to assess the importance of pathways and mechanism underlying a certain association. Mediation analysis allows for a more causal interpretation of the data when there is a biological plausible relationship between the determinant, mediator, and outcome, and the mediator is found to be of significant importance in the association under study.(182) This type of analysis, however, only allows causal interpretation when certain assumptions are met.(132) One of these assumptions is the absence of an unmeasured confounder between the mediator and the outcome. As mentioned previously, confounding can always play a role in observational data and, therefore, this assumption is difficult to meet. Newer statistical techniques currently under development do not require this assumption, but do require high computational power, which is not always available. Another way to infer causality is with Mendelian randomization. Using this method we analyzed serum magnesium levels as our determinant but genetic variants in magnesium

regulating genes as a proxy for serum magnesium levels.(183) As genes cannot be altered by disease status, this provides more insight into the directionality of the association. This type of analysis comes closest to randomized clinical trials in establishing causal relationships.(184) Randomized clinical trials in the field of magnesium research are scarce and this can partly be explained by the fact that magnesium is a supplement rather than a drug and therefore without involvement of the pharmaceutical industry. Furthermore, the effect size of magnesium on disease risk is often relatively small, and therefore, many patients are needed to study the effect of magnesium supplementation and magnesium supplementation will be needed from long periods of time, which is often not feasible.

Besides bias and potential reverse causality another limitation of our research is the single measurement of serum magnesium levels. In our cohort, serum magnesium levels were often measured many years before the diagnosis of the disease. With a single measurement, we have to assume that levels remain constant over time and hereby influence disease risk. Serum magnesium levels are the result of, among others, the composition of the diet, genetic variants in magnesium regulating genes, and pharmaceutical drugs. Genetic variations are not likely to change over time, but the composition of the diet and drug use are. Furthermore, the development of disease, for example kidney disease, might cause a sudden change in magnesium levels, which could have major impact on the risk of the disease under study.

Repeated serum magnesium levels would therefore provide more insight into the effect of magnesium on disease risk. But even in the case of repeated measurements, there is still an ongoing discussion if serum magnesium is the preferred approach for estimating total body magnesium balance. (185-187) Serum magnesium reflects only 1% of the total body magnesium, while the remaining magnesium is stored within bones, muscles, and soft tissues.(3) As it is difficult to measure magnesium in these compartments, we often use serum magnesium, as it can be easily measured, and extrapolate these findings to total body magnesium. However, by doing so, we assume that normal serum magnesium levels correlate with a normal total body magnesium level. This is not always the case and we may therefore underestimate magnesium deficiency.(3) Besides measuring magnesium in serum, it is also possible to assess magnesium intake through dietary questionnaires and use this as a proxy for magnesium status. However, in the study on PPIs we compared magnesium intake with serum magnesium levels and found only a very weak correlation. This can have various explanations of which one is the method of measuring intake via questionnaires. Questionnaires are prone to recall bias, which can influence the data to a great extent. Even if the total amount of magnesium has been correctly estimated, there is not a one-to-one relationship between dietary intake and serum levels of magnesium.

As mentioned before, passive uptake of magnesium from the intestine depends largely on the amount of magnesium available in the food as compared to the concentration of magnesium on the basal side of the intestinal cells, as this gradient drives passive magnesium transport.(3) In addition, there is a major role for TRPM6 in the active transport of magnesium in the intestine. Common genetic polymorphisms influence the activity of this channel and can thereby influence the serum magnesium level.(109) These mutations also influence magnesium excretion by the kidney as TRPM6 is also located in the distal convoluted tubule. Besides TRPM6 channels, an altered glomerular filtration rate, as seen in kidney disease, can influence magnesium excretion. All these steps result in an individual set-point for magnesium and explain why there is no one-to-one ratio between dietary magnesium intake and serum magnesium levels.(110)

Future research

In this thesis, the role of magnesium in a variety of diseases was explored and novel insights into the possible mechanisms underlying these associations were obtained. However, there are still major steps to be taken before we can recommend patients to take magnesium supplements to reduce certain disease risks.

The first step is to establish whether associations between magnesium and disease risk are causal relationships. There is still a possibility that magnesium is an innocent bystander, rather than the active player. Randomized controlled trials would be the gold standard to establish causality, however, due to the small effect size one would need to follow many patients over a prolonged period of time, which is not always feasible. However, new epidemiological methods are being developed and may help us to gain more insight in causality and directionality of the observed associations. There is also a role for close collaboration between epidemiological and experimental research. Data from observational cohorts can help identifying the most promising targets for magnesium research, which experimental research can build upon.

The final step after establishing causal relationships is whether or not disease risk can be altered by actively supplementing magnesium and which form is most efficient. Studies found conflicting results whether or not serum magnesium levels could be increased by the use of magnesium supplements, and this is crucial in the process of altering disease risk. In this regard, adverse effects are also an important issue, especially when magnesium supplementation should be taken chronically. Oral magnesium supplementation can cause diarrhea, which can have a major impact on quality of life and will make it difficult to adhere to the therapy. Finally, there is still a remaining question whether there is a level of magnesium that is too high, because in our studies on dementia and sudden cardiac death, we observed a U-shaped curve for risk.

Summary

The starting point of research is high quality data, as statistical analysis cannot overcome bias introduced by the study design or errors introduced during data collection. In *Chapter 2* of this thesis, we describe how laboratory measurements within the Rotterdam Study were biased by using the same laboratory workflow for study samples as for routine clinical samples. The algorithm underlying this workflow is optimized to maximize the number of routine clinical samples. However, study samples differ significantly from clinical samples as they have a generally more elaborate test panel. In combination with a high test-to-volume ratio (volume of study samples 500µL versus 4mL in routine clinical samples), this causes a delay on the analyzer leading to significant on-board evaporation. This evaporation causes both a systematic and random error of measurements at the end of the analytical run, in our case these measurement were sodium and potassium. To overcome this issue, we have designed a new handling protocol and remeasured all serum sodium and potassium levels.

Serum magnesium levels were unaffected by these processes, as magnesium is measured independently from sodium and potassium and was not measured at the end of the analytical run. We focused on this 'forgotten' electrolyte as it plays a role in a wide range of cellular functions and it has been linked to many diseases. In this thesis, we studied the effect of proton pump inhibitor (PPI) use and diuretic use on serum magnesium levels, and investigate the role of magnesium in cardiovascular mortality, diabetes, and dementia. The Rotterdam Study provides an unique opportunity to study disturbances in serum magnesium levels as it is an unselected population-based cohort in which serum magnesium levels were measured routinely in all participants, irrespective of their health status or drug use.

In *Chapter 3*, we found that PPI users have significantly lower serum magnesium levels as compared to participants who do not use PPI's. This was associated with a two- to three-fold higher risk of hypomagnesemia, defined as a serum magnesium level < 0.70 mmol/L. The increased risk was primarily seen in participants using PPI's for more than 182 days. We also found an interaction between PPI use and diuretic use, where the concomitant use of PPIs and loop diuretics increased the risk of hypomagnesemia seven-fold. We explored the effect of diuretic use on serum magnesium levels further in *Chapter 4*. We found that the use of loop diuretics alone was not associated with hypomagnesemia. However, the prolonged use of thiazide diuretics increased the risk of hypomagnesemia by four-fold. Interestingly, the combination of thiazide diuretics with a potassium-sparing agent was not associated with hypomagnesemia, suggesting that this combination may prevent hypomagnesemia.

In *Chapters 5, 6, and 7* we study the effect of serum magnesium levels in relation to the occurrence of common diseases. In *Chapter 5*, we found that low serum magnesium levels

were associated with an increased risk of coronary heart disease mortality and sudden cardiac death. The hypothesized pathways of these associations are that lower serum magnesium accelerates atherosclerosis or increases the QT-interval. Although we found that these potential intermediates were associated with serum magnesium levels, they did not completely explain the observed associations with coronary heart disease mortality and sudden cardiac death.

In *Chapter 6*, we look into the relationship between serum magnesium levels and diabetes risk. This relationship had been studied before, but it remains unclear whether a low serum magnesium level contributes to the development of diabetes mellitus or low serum magnesium levels are the result of uncontrolled diabetes mellitus. We used several approaches to study this directionality. We found that low serum magnesium levels were associated with prediabetes, the precursor stage of diabetes. This precursor stage is unlikely to influence serum magnesium levels, therefore supporting a role of magnesium in the development of diabetes mellitus. In addition, we found that genes encoding magnesium regulating channels also associate with the risk of diabetes mellitus. An example of one of these genes is the transient receptor potential melastatin 6 (TRPM6) channel. This channel is important for reabsorption of magnesium in the kidney, variation will cause more renal magnesium wasting, hereby lowering serum magnesium levels.

In *Chapter 7*, we focus on the relatively understudied association between serum magnesium levels and dementia. Up until now, only four case-control studies studied this association, but results were conflicting. We found that both low and high serum magnesium levels were associated with an increased risk of dementia, supporting the observations from the case-control studies. There are two hypothesized pathways for this association. First, a direct pathway where magnesium directly influences the N-methyl-D-aspartate (NMDA) receptor in the brain which is important for learning. Second, an indirect pathway where magnesium influences dementia risk through its effect on atherosclerosis and oxidative stress.

In the *general discussion*, we elaborate on the potential pathways underlying the associations studied within this thesis, some of which overlap. We focus on the effect of magnesium on atherosclerosis, as the key underlying cause of cardiovascular mortality and a risk factor for the development of dementia. In addition, we explore the role of magnesium in oxidative stress, as this is also important in the development of diabetes mellitus. In the general discussion, we also focus on the TRPM6 channel. This magnesium channel is expressed in the kidney, but also in the intestine. TRPM6 in the kidney and intestine appears to be involved in PPI-induced hypomagnesemia, as TRPM6 channels are upregulated during PPI use in both organs. Within the kidney, TRPM6 plays an important role in the fine-tuning of magnesium reabsorption. This is especially important during loop

diuretic use as downstream magnesium reabsorption through TRPM6 might prevent urinary magnesium wasting. In the remaining part of the discussion we address the methodological considerations associated with this thesis. The most important consideration is causality, as we used observational data in all of our studies. Reverse causality or confounding are major issues to consider in this regard, but novel statistical techniques can help us establishing causal relationships. Furthermore, collaborations between epidemiological and experimental researchers will help us in the future to establish the role of magnesium in health and disease.

Samenvatting

Om wetenschappelijk onderzoek te kunnen doen heeft men onderzoeksgegevens nodig zonder verstoring in de meting, ook wel bias genoemd. In *Hoofdstuk 2* van dit proefschrift beschrijven wij een bias in de laboratoriumbepalingen binnen ERGO (het Erasmus Rotterdam Gezondheid Onderzoek), ontstaan door het gebruik van identieke werkprocessen voor bloedmonsters voor patiëntenzorg en wetenschappelijk onderzoek. Deze werkprocessen zijn geoptimaliseerd om zoveel mogelijk klinische bloedmonsters tegelijkertijd te verwerken. Echter, er worden meestal meer en andere type bepalingen verricht in bloedmonsters voor wetenschappelijk onderzoek dan in bloedmonsters voor patiëntenzorg . Dit zorgt voor vertraging in het apparaat waardoor verdamping optreedt in de bloedmonsters. Deze verdamping heeft een significant effect op uitslagen van bloedmonsters voor wetenschappelijk onderzoek omdat deze een kleinere volume hebben dan bloedmonsters voor patiëntenzorg (500µL versus 4mL) en het aantal bepalingen in dit kleine volume hoger ligt (19 versus 8), waardoor er relatief weinig residu is. De verdamping resulteerde in een systematische en willekeurige bias in de bepalingen aan het einde van de analyse, in dit geval serum natrium en kalium. Door middel van een nieuw werkproces kon de vertraging op het analyse apparaat voorkomen worden, waarna alle serum natrium en kalium waarden opnieuw bepaald konden worden voor het verkrijgen van valide onderzoeksgegevens.

Dit proefschrift richt zich op serum magnesiumwaarden. Deze bepaling was niet aangedaan door de hiervoor genoemde bias, doordat magnesium los van natrium en kalium bepaald wordt en niet aan het einde van de analyse. Magnesium is betrokken bij diverse cruciale celfuncties en de hypothese is dat verstoring van magnesium daarom een belangrijke rol speelt in het ontstaan van diverse ziektebeelden. ERGO is wereldwijd het grootste cohort waarin serum magnesiumwaarden routinematig bepaald worden in alle deelnemers, onafhankelijk van eventuele comorbiditeit of medicatiegebruik. Dit maakt ERGO uniek en leent het zich om onderzoek te doen naar stoornissen in serum magnesiumwaarden. Het doel van dit proefschrift is het bestuderen van het effect van protonpompremmers (“maagzuurremmers”) en diuretica (“plaspillen”) op serum magnesiumwaarden en daarnaast om de rol te bestuderen van serum magnesiumwaarden bij het overlijden ten gevolge van hart- en vaatziekten, het ontstaan van diabetes mellitus, en het ontstaan van dementie.

In *Hoofdstuk 3* vonden wij dat gebruikers van protonpompremmers (PPI) significant lagere serum magnesiumwaarden hebben in vergelijking met mensen die deze medicijnen niet gebruiken. Hierdoor hadden zij een twee tot drievoudig verhoogd risico op het krijgen van hypomagnesiëmie, gedefinieerd als een serum magnesiumwaarde onder de 0.70 mmol/L. Dit verhoogde risico werd met name gezien bij mensen die langdurig PPI’s gebruikten. Daarnaast vonden wij dat mensen die een combinatie van PPI’s en lisdiuretica gebruikten zelfs een zeven keer verhoogd risico hadden op hypomagnesiëmie. In *Hoofdstuk 4* gaan

wij dieper in op het effect van diuretica op serum magnesiumwaarden. Wij vonden dat lisdiuretica de serum magnesiumwaarde niet verlaagde, echter thiazidediuretica deden dit wel. Langdurig gebruik van thiazidediuretica was geassocieerd met een vier keer verhoogd risico op het krijgen van hypomagnesiëmie., De combinatie van een thiazidediureticum met een kaliumsparend diureticum veroorzaakte geen hypomagnesiëmie hetgeen suggereert dat deze combinatie hypomagnesiëmie kan voorkomen.

In *Hoofdstukken 5, 6 en7* bestuderen wij het effect van serum magnesiumwaarden op het ontwikkelen van diverse ziektebeelden. In *Hoofdstuk 5* laten wij zien dat een lage serum magnesiumwaarde geassocieerd is met een verhoogd risico om te overlijden aan hart- en vaatziekten of aan plotse hartdood. Gedacht wordt dat dit effect verloopt via, respectievelijk, versnelde atherosclerose (aderverkalking) en een verlenging van het QT-interval. In onze studie vonden wij inderdaad een relatie tussen deze twee processen en serum magnesiumwaarden. Echter deze effecten waren onvoldoende groot om de volledige associatie tussen serum magnesiumwaarden en beide eindpunten te verklaren.

In *Hoofdstuk 6* bestuderen wij de relatie tussen serum magnesiumwaarden en het risico op diabetes mellitus. Deze relatie was reeds bekend, maar een onbeantwoorde vraag is de richting van deze associatie. Onvoldoende gereguleerde diabetes mellitus kan namelijk ook een verlaagde serum magnesiumwaarde veroorzaken door verhoogde magnesiumuitscheiding in de urine. Wij bestudeerden de richting van deze associatie op verschillende manieren. Ten eerste keken we naar de relatie tussen serum magnesium en prediabetes. Bij dit voorstadium van diabetes zijn de suikerwaarden onvoldoende hoog om een verhoogde uitscheiding van magnesium in de urine te veroorzaken. Wij vonden een vergelijkbare relatie tussen serum magnesiumwaarden met prediabetes als met diabetes. Daarnaast vonden wij dat mutaties in magnesiumregulerende genen een invloed hebben op het ontstaan van diabetes mellitus. Beide bevindingen ondersteunen de gedachte dat serum magnesiumwaarden een rol spelen in het ontstaan van diabetes mellitus.

In *Hoofdstuk 7* hebben wij de associatie bestudeerd tussen serum magnesium en dementie. Deze associatie was tot nu toe alleen beschreven in vier case-controle studies, waarbij de resultaten tegenstrijdig waren. Wij vonden dat zowel lage als hoge serum magnesiumwaarden een risicofactor is voor het ontstaan van dementie, wat de resultaten uit alle 4 de cohort studies ondersteunt. Er zijn meerdere theorieën over deze associatie, waaronder een directe theorie waarbij gedacht wordt dat serum magnesiumwaarden een effect hebben op het NMDA kanaal in het brein, welke van belang is bij het leren. Daarnaast is er ook een indirecte theorie waarbij serum magnesiumwaarden invloed hebben op het ontstaan van dementie door het effect op atherosclerose en oxidatieve stress.

In de *algemene discussie* gaan wij dieper in op de onderliggende theorieën van de hiervoor genoemde associaties. Naast een rol in dementie, heeft atherosclerose een cruciale rol in het ontstaan van hart- en vaatziekten en de daaraan gekoppelde sterfte. Daarnaast is oxidatieve stress ook gekopppeld aan het ontstaan van diabetes mellitus. In de discussie richten wij ons verder op het *transient receptor potential melastatin 6* (TRPM6) kanaal. Dit magnesiumkanaal bevindt zich zowel in de nier als in de darm. PPI geïnduceerde hypomagnesiëmie zou kunnen ontstaan omdat PPIs een effect hebben op TRPM6 in de darm, waardoor er minder magnesium opgenomen wordt uit de voeding. In de nier speelt TRPM6 ook een belangrijke rol omdat het hier de magnesium reabsorptie beïnvloed wat cruciaal is bij verminderd opname in darm, maar ook bij een verhoogde uitscheiding van magnesium in de urine ten gevolge van diuretica gebruik. In het laatste gedeelte van de discussie bespreken wij enkele methodologische overwegingen bij dit proefschrift. Gezien het feit dat wij observationele gegevens gebruiken in al onze studies is bewijs van causaliteit één van de belangrijkste uitdagingen. Nieuwe statistische technieken kunnen ons in de toekomst helpen om te bepalen of er sprake is van een causaal verband. Daarnaast is er ook ruimte voor samenwerkingsverbanden met onderzoekers uit het experimentele veld, waarbij hypothesen uit observationeel onderzoek getest kunnen worden in een experimentele setting.

PART V
Appendices

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List of Publications

1. **Kieboom BCT**, Zietse R, Ikram MA, Hoorn EJ, Stricker BH. Thiazide but not loop diuretics is associated with hypomagnesaemia in the general population. *Pharmacoepidemiol Drug Saf. 2018 Aug 10*
2. Trajanoska K, Schoufour JD, de Jonge EAL, **Kieboom BCT**, Mulder M, Stricker BH, Voortman T, Uitterlinden AG, Oei EHG, Arfan Ikram M, Carola Zillikens M, Rivadeneira F, Oei L. Fracture incidence and secular trends between 1989 and 2013 in a population based cohort: The Rotterdam Study. *Bone. 2018 Sep;114:116-124*
3. **Kieboom BCT**, Hoorn EJ, Ramakers C, van Rooij FJA, Ikram MA, Hofman A, van Duijn CM, Heeringa J, Zietse R, Peeters RP, Stricker BH, de Rijke YB. Standard process-oriented workflow introduces pre-analytical error when used in large study sample batches. *Clin Chem Lab Med. 2018 Jun 2*
4. **Kieboom BCT**, Licher S, Wolters FJ, Ikram MK, Hoorn EJ, Zietse R, Stricker BH, Ikram MA. Serum magnesium is associated with the risk of dementia. *Neurology. 2017 Oct 17;89(16):1716-1722*
5. **Kieboom BCT**, Ligthart S, Dehghan A, Kurstjens S, de Baaij JHF, Franco OH, Hofman A, Zietse R, Stricker BH, Hoorn EJ. Serum magnesium and the risk of prediabetes: a population-based cohort study. *Diabetologia. 2017 May;60(5):843-853*
6. de Jonge EA, Kiefte-de Jong JC, Hofman A, Uitterlinden AG, **Kieboom BCT**, Voortman T, Franco OH, Rivadeneira F. Dietary patterns explaining differences in bone mineral density and hip structure in the elderly: the Rotterdam Study. *Am J Clin Nutr. 2017 Jan;105(1):203-211*
7. **Kieboom BCT**, Stricker BH. Low serum magnesium is associated with hypertension. *J Pediatr. 2016 Jul;174:279-80*
8. **Kieboom BCT**, Niemeijer MN, Leening MJG, van den Berg ME, Franco OH, Deckers JW, Hofman A, Zietse R, Stricker BH, Hoorn EJ. Serum magnesium and the risk of death from coronary heart disease and sudden cardiac death. *J Am Heart Assoc. 2016 Jan 22;5(1)*
9. **Kieboom BCT**, Kiefte-de Jonge JC, Eijgelsheim M, Franco OH, Kuipers EJ, Hofman A, Zietse R, Stricker BH, Hoorn EJ. Proton pump inhibitors and hypomagnesemia in the general population: a population-based cohort study. *Am J Kidney Dis. 2015 Nov;66(5):775-82*
10. Schepers T, **Kieboom BCT**, Bessems GH, Vogels LM, van Lieshout EM, Patka P. Subtalar versus triple arthrodesis after intra-articular calcaneal fractures. *Strategies Trauma Limb Reconstr. 2010 Aug;5(2):97-103*
11. Schepers T, **Kieboom BCT**, van Diggele P, Patka P, van Lieshout EM. Pedobarographic analysis and quality of life after Lisfranc fracture dislocation. *Foot Ankle Int. 2010 Oct;31(1):858-64*

EMC PhD portfolio

Name PhD student	Brenda C.T. Kieboom
Departments	Epidemiology, Erasmus MC – University Medical Center Rotterdam
	Internal Medicine, Erasmus MC - University Medical Center Rotterdam
Research school	Netherlands Institute for Health Sciences (NIHES)
PhD period	2013-2017
Promotors	Prof. dr. B.H.Ch. Stricker
	Prof. dr. E.J. Hoorn

Training	**ECTS**	**Year**
MSc in Clinical Epidemiology	40.9	2013-2015
NIHES, Erasmus MC, Rotterdam, NL		
Harvard T.H. Chan School of Public Health, Boston, MA, U.S.		
Courses and seminars		
Causal Mediation Analysis, NIHES	1.4	2016
Scientific English Writing Course, Erasmus MC	3.0	2016
Scientific Integrity Course, Erasmus MC	0.3	2016
Systematic Literature Retrieval and Endnote, Erasmus MC	1.0	2015
WGIKD Course - Renal fluids and electrolytes, Radboud Universiteit Nijmegen	0.6	2015
Rotterdam Course in Electrolyte and Acid-Base disorders, Erasmus MC	0.3	2014
Research Impact and Relevance seminar, Erasmus Universiteit Rotterdam	0.1	2014
Conferences		
Dutch Epidemiology Conference (WEON), Wageningen, NL [a.]	0.6	2016
American Heart Association Scientific Session, Orlando, FL, U.S.	1.2	2015
American Society of Nephrology Kidney Week, San Diego, CA, U.S. [b.]	1.2	2015
BENELUX Kidney Meeting, Eindhoven, NL [b.]	0.3	2015
Dutch Epidemiology Conference (WEON), Maastricht, NL [b.]	0.3	2015
Nefrologiedagen, Veldhoven, NL [a.]	0.6	2015
American Society of Nephrology Kidney Week, Philadelphia, PA, U.S. [b.]	1.2	2014

a. Oral presentation b. Poster presentation

Teaching tasks	**ECTS**	**Year**
Pharmacoepidemiology and Drug safety (NIHES)	1.0	2015-2016
Principle of Research in Medicine and Epidemiology (NIHES)	0.5	2015
Supervising MSc/DSc students		
Edis Sevo	1.0	2014
Pichaya Tantiyavarong	2.0	2016-2018
Coaching medical students		
Sven Goetstouwers	1.0	2015-2018
Anouk Gruter		
Marieke van der Kamp		
Machteld van der Linde		
Rafael de Windt		
Miscellaneous		
Peer review various medical journals	1.0	2015-2018
Rotterdam Study management team	2.0	2016-2017
Rotterdam Study Data Wiki Panel	1.0	2015-2017
NIHES Student Panel	0.5	2015

D

Dankwoord

Een promotietraject is meer dan alleen onderzoek doen. Het is een periode waarin je jezelf ontwikkelt tot onafhankelijke wetenschapper, waarin je leert kritisch naar andermans werk te kijken, maar nog kritischer naar je eigen werk. Het is een traject waarin er een beroep wordt gedaan op je creativiteit, assertiviteit en doorzettingsvermogen. Maar bovenal is het een traject waarin je jezelf als persoon leert kennen en ontwikkelen. Ik ben bevoorrecht met veel lieve mensen om mij heen die mij in de afgelopen jaren hierin onvoorwaardelijk hebben gesteund. Daarnaast heb ik in de afgelopen 5 jaar de mogelijkheid gekregen om met diverse inspirerende mensen te mogen samenwerken, in dit dankwoord wil ik enkele in het bijzonder bedanken.

Prof. dr. E.J. Hoorn, Beste Ewout, bedankt dat jij mij vanaf het eerste moment de ruimte hebt gegeven om mijzelf te ontdekken als onderzoeker. Ondanks dat magnesium niet jouw favoriete electrolyt is heb ik onnoemelijk veel van jou geleerd. Ik bewonder jouw oog voor detail en positivisme en ik ben dankbaar dat ik jij mijn (co)promotor bent.

Prof. dr. B.H.Ch. Stricker, Beste Bruno, een excuus is op zijn plaats voor het feit dat er in dit proefschrift geen paper over natrium en thiazide diuretica te vinden is. Bedankt dat ik in de afgelopen jaren altijd mijn eigen ding heb kunnen doen, ook al is het misschien niet helemaal in lijn met wat jij initieel bedacht had.

Prof. dr. R. Zietse, Beste Bob, 5 jaar na ons eerste gesprek over een promotieplek moet ik concluderen dat ik iets compleet anders heb gedaan dan wat wij toen bedacht hadden. Ik ben geen proefdier tegen gekomen, en ik heb al mijn dagen gespendeerd achter de computer turend naar een statistiek programma. Ondanks dat jij niet meer officieel mijn promotor bent, ben ik je veel dank verschuldigd. Jouw grenzeloze enthousiasme heeft mij keer op keer geïnspireerd om het beste uit mijzelf te halen.

Prof. dr. M.A. Ikram, Beste Arfan, dank voor het plaatsnemen in mijn kleine commissie. Bedankt voor alle mogelijkheden die jij mij hebt gegeven en voor jouw grenzeloze vertrouwen in mijn kunnen.

Prof. dr. Y.B. de Rijke, Prof. dr. J.G.J. Hoenderop en Prof.dr. J.W. Deckers, bedankt voor de samenwerking in de afgelopen jaren en jullie bereidwilligheid om plaats te nemen in mijn promotiecommissie

Beste Ronald Jansen, bedankt voor de ruimte die jij mij hebt geboden om een baan bij de IGZ als farmacovigilantie inspecteur te combineren met een promotietraject. Jouw manier van leiding geven is nog steeds een bron van inspiratie voor mij.

Beste Jolande, jij bent voor mij één van de belangrijkste personen binnen ERGO. Jij regelt alles en als jij het niet kan regelen weet jij altijd iemand die dat wel kan. Jouw optimisme is aanstekelijk en ik ben je ontzettend dankbaar voor alles wat jij voor mij regelt en doet.

Beste Anneke, het gezicht van ERGO, mijn steun en toeverlaat. Bedankt voor je vertrouwen, jouw luisterend oor, en voor alles wat jij voor mij wegvangt waar ik geen weet van heb.

Beste ERGO dames, bedankt voor het eindeloze geduld dat jullie met mij hebben en voor jullie grenzeloze inzet voor de deelnemers. Jullie zijn de reden dat alle deelnemers jaar in jaar uit weer terug komen en dat dit mooie onderzoek nog steeds loopt.

Lieve Emmely, ik bewonder jouw positieve blik op de wereld. Bedankt voor alle momenten waarop ik mocht spuien of het nu op werk of persoonlijk vlak was. Bedankt voor al je knuffels, motiverende smsjes en voor jouw onuitputtelijke bron van gezelligheid.

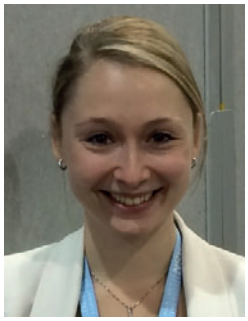
Lieve Natalie, na één jaar een kamer met 2 mannen te hebben gedeeld, gaf jij een hele nieuwe invulling aan het concept kamergenoot. Mijn beker was vanaf dat moment altijd gevuld met thee, er was altijd eten, maar bovenal bracht jij een oneindige hoeveelheid liefde mee. Jij bent een enorm sterke vrouw en een geweldige moeder en ik bewonder jou enorm. Ik vind het een eer dat jij mijn paranimf wilt zijn.

Lieve Jacqueline, al 14 jaar ben jij onmisbaar voor mij. Van de eerste dagen van onze studie in de metro, naar de balie van de spoedeisende hulp, en nu als mijn paranimf. Onze koffie dates waren het hoogtepunt van mijn week en zorgde voor de motivatie om door te gaan. Bedankt dat jij er altijd voor mij bent en ik hoop dat er nog veel koffie/cocktail/ eetdates mogen volgen.

Lieve ouders, bedankt voor jullie onvoorwaardelijke liefde en steun in alles wat ik doe. Het is voor jullie misschien niet altijd duidelijk waar ik mee bezig ben, maar jullie zijn altijd trots op mij en ik ben dankbaar dat jullie mijn ouders zijn. Bedankt dat jullie deur altijd open staat en dat ik altijd op jullie kan bouwen.

Lieve Maarten, jij haalt het beste uit mij naar boven. Jij was mijn motivatie om te gaan promoveren, en jij bent de reden dat ik dit traject succesvol heb afgesloten door al jouw hulp bij het uitvoeren van statistische analyses en het schrijven van manuscripten. Je bent een ongelofelijk goede wetenschapper, maar bovenal ben jij een geweldige vader en mijn maatje. Bedankt voor alles, ik weet niet wat ik zonder jou zou moeten.

About the Author



Brenda Cornelia Theodora Kieboom was born on 15th September 1986 in Rotterdam, the Netherlands. When she was two years old she moved to a suburb of Rotterdam, only 3.8 kilometers apart from the Rotterdam Study research center in Ommoord.

In 2004, she finished high school at the Emmauscollege in Rotterdam and began medical school at the Erasmus University Rotterdam. She dedicated one year to the Medical Faculty Students Association Rotterdam (MFVR), where she managed all finances as the treasurer. After this year she remained actively involved in various committees representing medical students and medical interns. In November 2011, she obtained her medical degree and started working in the IJsselland Hospital in Capelle aan den IJssel, as a resident in Internal Medicine. During this time, she became interested in pursuing a PhD as she failed to have any significant input in scientific discussions at work or at home.

In 2013, she started her PhD at the department of Epidemiology and Internal Medicine under the supervision of Prof. Ewout J. Hoorn, Prof. Robert Zietse, and Prof. Bruno H. Stricker. Simultaneously, she started working as a pharmacovigilance inspector at the Health Care Inspectorate in Utrecht. In 2015, she obtained her master degree in Clinical Epidemiology at the Netherlands Institute of Health Sciences (NIHES). During this master she was given the opportunity to attend several courses at the Harvard Th. Chan School of Public Health. In the meanwhile, she experienced several life events as she married the love of her life in August of 2015, bought her first house in 2016, and gave birth to a baby girl in June of 2017.

In November 2017, she returned back to work to finish her PhD and to start a new job as the medical study coordinator of the Rotterdam Study. As of March 2018, she combines this challenging job with specialty training to become a general practitioner.