Clinical Chemistry 67:6
899–911 (2021)

Plasma Concentrations of Magnesium and Risk of Dementia: A General Population Study of 102 648 Individuals

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BACKGROUND: Low and high concentrations of plasma magnesium are associated with increased risk of future all-cause dementia; however, the underlying reasons remain elusive. The magnesium ion is an important electrolyte serving as a cofactor in many enzymatic processes in the human organism. Magnesium affects both neuronal and vascular functions. We investigated the associations of plasma concentrations of magnesium associate with common subtypes of dementia as Alzheimer dementia and non-Alzheimer dementia, and potential pathways by which magnesium may affect risk of dementia.

METHODS: Plasma concentrations of magnesium were measured in 102 648 individuals from the Copenhagen General Population Study. Cox regression and natural effects mediation analyses evaluated associations with either Alzheimer dementia or non-Alzheimer dementia.

RESULTS: Multifactorially adjusted hazard ratios for non-Alzheimer dementia were 1.50(95% confidence interval (CI):1.21–1.87) for the lowest and 1.34(1.07–1.69) for the highest vs the fourth quintile (reference) of plasma magnesium concentrations. Diabetes, cumulated smoking, stroke, and systolic blood pressure mediated 10.4%(3.1–22.8%), 6.8%(1.2–14.0%), 1.3%(0.1–3.6%), and 1.0%(0.2–2.6%), respectively, in the lowest quintile, whereas stroke mediated 3.2%(0.4–11.9%) in the highest quintile. No associations were observed for Alzheimer dementia.

CONCLUSIONS: Low and high plasma magnesium concentrations were associated with high risk of vascular-related non-Alzheimer dementia, with the lowest risk observed at a concentration of 2.07 mg/dL (0.85 mmol/L). No association was observed for Alzheimer dementia. Mediation analysis suggested that diabetes may be in the causal pathway between low plasma magnesium concentrations and high risk of non-Alzheimer dementia, while cumulated smoking, stroke, and systolic blood pressure played minor mediating roles.

Introduction

Half of the US population has an inadequate intake of magnesium, leading to clinical magnesium deficiency. The magnesium ion is an important electrolyte serving as a cofactor in more than 300 enzymatic processes in the human organism (1, 2). Magnesium affects both neuronal and vascular functions (3), and low plasma concentrations of magnesium have been reported to be associated with risk of stroke (4, 5), dementia pathology (3), and all-cause dementia (6). Additionally, magnesium is a popular over-the-counter laxative, commonly used among the elderly, and may lead to mild to moderate hypermagnesemia (7). Thus, studies of the impact of plasma magnesium for age-related common diseases as dementia and stroke, as well as potential mediating pathways, are warranted.

Magnesium influences the cardiovascular system through several pathways including vascular tone, blood pressure, endothelial function, platelet aggregation and coagulation, glucose and insulin metabolism, as well as through cardiac arrythmias (8–13). Furthermore, observational and Mendelian randomization studies have shown that low plasma magnesium concentrations or low magnesium intake are associated with higher risk of stroke (4, 5). In postmortem brain examinations of Alzheimer dementia patients, low concentrations of magnesium were observed across a wide range of fluids and tissues (3). Moreover, magnesium supplements

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Received December 14, 2020; accepted February 18, 2021.
DOI: 10.1093/clinchem/hvab041

have been suggested as a treatment for Alzheimer dementia, due to the ability of magnesium supplementation to protect against neuronal excitotoxicity mediated by N-methyl-D-aspartate receptors (3). This treatment is supported by studies showing that magnesium supplements can improve memory (14). How magnesium associates with dementias with vascular etiology remains unknown.

Therefore, we tested whether plasma concentrations of magnesium are associated with vascular-related non-Alzheimer dementia and Alzheimer dementia, and applied mediation analysis to unravel by what pathways magnesium may affect risk of dementia. For this purpose, we used the Copenhagen General Population Study, a prospective cohort study representative of the Danish general population aged 20–100+ years and including 102 648 individuals.

Materials and Methods

The study was approved by institutional review boards and Danish ethical committees, and was conducted according to the Declaration of Helsinki, with written consent from participants. All individuals were white and of Danish descent.

PARTICIPANTS

We included participants from the Copenhagen General Population Study (CGPS), a prospective study of the Danish general population initiated in 2003 with the first enrollment period from 2003–2015, and with follow-up examinations ongoing (15). Individuals were randomly selected from the national Danish Civil Registration System to reflect the adult white population aged 20–100+. The Danish Civil Registration System records all births, immigrations, emigrations, and deaths in Denmark by means of civil registration numbers, which uniquely identify all inhabitants in Denmark and include information regarding age, sex, ethnicity, and civil status. The Danish Civil Registration System is 100% complete and no persons are lost to follow-up. Follow-up began at the time of blood sampling in 2003–2015, and ended at occurrence of a dementia event, death, emigration, or on December 7, 2018 (last update of the registries), whichever came first. Data collected included a questionnaire, a physical examination, and blood sampling for biochemical and DNA analyses.

ENDPOINTS

Diagnoses of dementia were obtained from the national Danish Patient Registry, including data on all patient contacts from all clinical hospital departments in Denmark since 1977 and emergency wards and outpatient clinics since 1995, and from the national Danish Causes of Death Registry with data on causes of all deaths in Denmark, as reported by hospitals and general practitioners since 1977. Alzheimer dementia was International Classification of Disease (ICD) 8 code 290.10 and ICD10 codes F00 and G30. Non-Alzheimer dementia was ICD8 code 290.8 and ICD10 codes F01 and F03. Individuals were included in the Alzheimer dementia endpoint, if they had the relevant ICD codes during follow-up. Individuals included in the non-Alzheimer dementia endpoint had the relevant ICD codes and no Alzheimer dementia diagnosis during follow-up.

BIOCHEMICAL ANALYSES

Plasma concentrations of magnesium, calcium, sodium, potassium, and creatinine were measured using standard hospital assays; Taqman-based assays were used to genotype apolipoprotein E (*APOE*) for p. Cys130Arg (rs429358) defining the ε4 allele and p. Arg176Cys (rs7412) defining the ε2 allele.

COVARIATES, MEDIATORS, AND CONFOUNDERS

The roles of the different covariates in the analyses were determined through a thorough examination (Supplemental Fig. 1). First, we scrutinized published literature for evidence (randomized clinical trials or Mendelian randomization studies) of whether a covariate should be considered as a confounder or as a mediator. Second, we investigated in our dataset whether the covariate had similar associations as reported in the literature, ensuring that no dubious association structures would bias the results. If evidence from both the literature and from the current study were present, the covariate was used as a confounder or mediator. Additionally, some covariates were only used in auxiliary functions in the mediation analysis (detailed in the Statistical analyses section below). To be classified as a confounder, there needs to be a substantiated relation in the literature and in the dataset between the confounder and independent variable (plasma magnesium concentrations) and between the confounder and the dependent variable (non-Alzheimer dementia). The direction of a literature-supported assumed causal association must be from the confounder to plasma magnesium. To be classified as a mediator, a similar association as for the confounder must be present, but the direction of the literature-supported assumed causal association must be from the independent variable (plasma magnesium) to the mediator. To be a covariate in auxiliary models, a covariate needs to be a risk factor for the mediator (16). The rationale for using covariates as confounders or mediators are detailed in Supplemental Table 1.

Confounders were sex, age, education, body mass index, *APOE* genotype, estimated glomerular filtration rate (eGFR), and cations (plasma concentrations of calcium, sodium, and potassium). Age was the age at examination. Low education was defined as self-reported <8 years of education. Body mass index was measured weight (kg) divided by measured height (cm) squared. eGFR was calculated based on the CKD-EPI creatinine formula.

Mediators were diabetes, cumulated smoking, stroke, and systolic blood pressure. Diabetes was defined as self-reported disease, use of insulin or oral hypoglycemic agents, or a nonfasting plasma glucose concentration of ≥11 mmol/L or a registry diagnosis (ICD8: 249–250, ICD10: E10, E11, E13, E14) before the day of enrollment. Cumulated smoking was estimated based on self-reported use. Stroke was defined as a registry diagnosis (ICD8: 430–431,433–435, ICD10: I60, I61, I63, I64, G45) of any stroke before day of enrollment. Systolic blood pressure was measured at examination. Covariates used in auxiliary functions (described below in Statistical analyses) were high alcohol consumption, physical inactivity, smoking status, income, lipid lowering therapy, chronic kidney disease, atrial fibrillation, hypertension, and ischemic heart disease. High alcohol consumption was defined as >14/21 units per week for women/men (1 unit = 12 g alcohol). Physical inactivity was defined as ≤ 4 h per week of light physical activity in leisure time. Smoking status, income, and lipid lowering therapy was self-reported. Chronic kidney disease was defined as eGFR <60 mL/min/L.73 m^2 at time of examination. Atrial fibrillation was defined as a registry diagnosis (ICD8 427.93—94 and ICD10 I48.0–I48.9) before the day of enrollment. Hypertension was defined as self-reported use of blood pressure-lowering drugs or measured systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at examination or a registry diagnosis (ICD8 401-404; ICD10 I10-I13, I15) before the day of enrollment. Ischemic heart disease was defined as a registry diagnosis (ICD8: 410-414, ICD10: I20-I25) before the day of enrollment.

STATISTICAL ANALYSES

We used Stata SE 14.2 (Stata Corp.) and the Medflex package (v. 0.6-6) in R (v. 3.5.2) (natural effects mediation analyses) for statistical analyses. Cubic spline cause-specific Cox regression models were used to evaluate hazard ratios (HRs) between plasma magnesium concentrations and risk of non-Alzheimer dementia and Alzheimer dementia. Cause-specific Cox regression was used to estimate hazard ratios for quintiles (5 equally sized age and sex adjusted groups) or reference interval groups (<1.73; ≥ 1.73 to <1.90; ≥ 1.90 to <2.07; ≥ 2.07 to <2.28; ≥ 2.28 mg/dL [<0.71; ≥ 0.71to <0.78; ≥ 0.78 to <0.85; ≥ 0.85 to <0.94; ≥0.94 mmol/L]) of plasma magnesium concentrations and dementia. The proportional hazards assumption was examined by visual inspection of log(-log[survival]) plots. No major violations were observed. Cox regression models were adjusted for confounders: age (time scale), sex, body mass index, and educational level. Models were also adjusted for *APOE* genotype to test whether the observed associations between plasma concentrations of magnesium and non-Alzheimer dementia were independent of this strong genetic risk factor (17). Further adjustments were performed for eGFR, chronic kidney disease, and plasma concentrations of calcium, sodium, and potassium, as these factors may interact with magnesium biology. Whether reverse causation was an issue, was investigated by sensitivity analyses omitting individuals with endpoints less than 5 years from baseline.

Natural effects mediation was used to estimate the direct effect from plasma magnesium and the indirect effect through the mediator (16). The mediation design is described in detail in Supplemental Fig. 1. For each mediator an auxiliary model using either logistic regression or linear regression was used to link plasma magnesium concentrations to the mediator adjusting for relevant risk factors. Auxiliary models are illustrated in Supplemental Fig. 2 and detailed in Supplemental Table 1. Subsequently, we used the auxiliary models to calculate weights for use in a natural effects Cox model to estimate direct and indirect effect on the HR scale, as well as mediated proportions. The natural effects Cox model was adjusted for the same confounders as in the cause-specific Cox model in the first part of the article. 95% confidence intervals (CI) were calculated based on bootstrapping with 10 000 repeats for quintiles and 3000 repeats for reference level groups.

DATA AVAILABILITY

The data underlying this article will be shared upon reasonable request to the corresponding author.

Results

Baseline characteristics of the 102 648 individuals are shown in Table 1. The individuals were followed for up to 15 years (956 530 person years, median follow-up time 9.5 years) during which 855 individuals were diagnosed with non-Alzheimer dementia, and 1600 individuals were diagnosed with Alzheimer dementia. The median concentration of plasma magnesium was 1.97 mg/dL (0.81 mmol/L) and 25% and 75% percentiles were 1.87 mg/dL (0.77 mmol/L) and 2.09 mg/dL (0.86 mmol/L).

Table 1. Baseline characteristics in the general population in age and sex-adjusted P-magnesium quintiles.

	P-Magnesium					
Characteristic	**1–20% quintile**	**21–40% quintile**	**41–60% quintile**	**61–80% quintile**	**81–100% quintile**	***P*** [a]
Individuals, N (%)	21,645 (21)	20,992 (21)	20,210 (20)	20,089 (20)	19,712 (19)	…
P-Magnesium, median (IRQ), mg/dL	1.75 (1.70–1.80)	1.90 (1.87–1.92)	1.97 (1.97–1.99)	2.07 (2.04–2.09)	2.19 (2.14–2.24)	…
P-Magnesium, median (IQR), mmol/L	0.72 (0.70–0.74)	0.78 (0.77–0.79)	0.81 (0.81–0.82)	0.85 (0.84–0.86)	0.90 (0.88–0.92)	…
Confounders						
Sex–women, N (%)	11,784 (54)	11,767 (56)	11,152 (55)	11,114 (55)	10,738 (54)	0.005
Age, median (IQR), years	58 (48–67)	58 (48–67)	58 (48–67)	58 (48–67)	58 (48–67)	0.331
Education < 8 years, N (%)	2,432 (11.2)	2,105 (10,0)	1,973 (9.8)	1,840 (9.2)	1,725 (8.8)	< 0.001
Body mass index, kg/m^2, N (%)						
BMI < 18.5	192 (0.9)	184 (0.9)	161 (0.8)	165 (0.8)	171 (0.9)	< 0.001
18.5≤BMI < 25	8,867 (41)	8,918 (42)	8,796 (44)	8,972 (45)	8,815 (45)	
25≤BMI < 30	8,463 (40)	8,462 (40)	8,096 (40)	7,941 (40)	7,920 (40)	
30≥BMI	4,123 (19)	3,428 (16)	3,157 (16)	3,011 (15)	2,806 (14)	
APOE genotype, N (%)						
ε22	141 (0.8)	147 (0.7)	135 (0.7)	156 (0.8)	113 (0.6)	0.336
ε32	2,626 (13)	2,592 (13)	2,392 (12)	2,375 (12)	2,410 (13)	
ε33	11,667 (56)	11,242 (55)	10,919 (56)	10,848 (56)	10,563 (55)	
ε42	603 (3)	553 (3)	571 (3)	573 (3)	578 (3)	
ε43	5,282 (25)	5,181 (25)	5,053 (26)	4,953 (25)	4,849 (25)	
ε44	567 (3)	625 (3)	544 (3)	579 (3)	562 (3)	
eGFR, median (IQR), ml/min/1.73m^2	88 (77–99)	87 (76–97)	87 (76–97)	86 (75–96)	85 (74–96)	< 0.001
P-Calcium ion, median (IQR), mmol/L	1.21 (1.18–1.25)	1.21 (1.18–1.24)	1.21 (1.18–1.24)	1.21 (1.18–1.24)	1.21 (1.18–1.24)	< 0.001
P-Sodium, median (IQR), mmol/L	140 (138–141)	140 (138–141)	140 (138–142)	140 (139–142)	140 (139–142)	< 0.001
P-Potassium, median (IQR), mmol/L	4.1 (3.9–4.3)	4.1 (3.9–4.3)	4.1 (3.9–4.3)	4.1 (4.0–4.3)	4.2 (4.0–4.4)	< 0.001
Mediators						
Diabetes mellitus, N (%)	1,742 (8.1)	807 (3.8)	659 (3.3)	554 (2.8)	510 (2.6)	< 0.001
Cumulated smoking, median (IQR), pack years	18 (7–33)	16 (6–30)	15 (6–30)	15 (6–29)	15 (6–30)	< 0.001
Stroke, N (%)	751 (3.5)	631 (2.9)	593 (2.9)	575 (2.9)	660 (3.4)	< 0.001
Systolic blood pressure, median (IQR), mm Hg	140 (126–154)	140 (126–154)	140 (126–154)	140 (126–155)	140 (126–155)	0.001
Covariates used in sensitivity analyses						
High alcohol consumption, N (%)	4,203 (19,4)	3,641 (17,3)	3,380 (16.7)	3,172 (15.8)	3,209 (16.3)	< 0.001
Physical inactivity, N (%)	10,692 (50)	9,989 (48)	9,680 (48)	9,446 (47)	9,540 (49)	< 0.001
Smoking status, N (%)						
Never smoker	8,528 (39.4)	8,657 (41.2)	8,528 (42.2)	8,678 (43.2)	8,608 (43.7)	< 0.001
Current smoker	4,301 (19.9)	3,800 (18.1)	3,427 (17.0)	3,172 (39.4)	3,068 (15.6)	
Ex smoker	8.756 (40.5)	8.474 (40.4)	8,194 (40.5)	8,173 (40.68)	7,971 (40.4)	
Unknown	60 (0.2)	61 (0.3)	61 (0.3)	66 (0.3)	65 (0.3)	
Income						
<200,000 DKK	3,112 (14,6)	2,619 (12.6)	2,426 (12.2)	2,367 (11.9)	2,265 (11.6)	< 0.001
200,000–400,000 DKK	5,299 (24.8)	4,772 (23.0)	4,657 (23.3)	4,531 (22.9)	4.456 (22.8)	
400,000–800,000 DKK	8,046 (37.7)	8.151 (39.3)	7,888 (39.5)	7,709 (38.9)	7,688 (39.4)	
>800,000 DKK	4,912 (23.0)	5,191 (25.0)	4,999 (25.0)	5,215 (26,3)	5.100 (26.1)	
Lipid lowering therapy, N (%)	2,908 (13.5)	2,346 (11.2)	2,225 (11.0)	2,285 (11.4)	2,271 (11.6)	< 0.001
Chronic kidney disease, N (%)	990 (4.6)	924 (4.4)	950 (4.7)	980 (4.9)	1,220 (6.2)	< 0.001

Continued

Table 1. (continued)

	P-Magnesium					
Characteristic	1-20% quintile	21-40% quintile	41-60% quintile	61-80% quintile	81-100% quintile	*P* [a]
Atrial fibrillation, N (%)	742 (3.4)	562 (2.7)	566 (2.8)	465 (2.3)	483 (2.5)	< 0.001
Hypertension, N (%)	13,207 (61.0)	12,476 (59.4)	12,057 (60.0)	12,079 (60.1)	11,927 (60.2)	0.006
Ischemic heart disease, N (%)	1,318 (6.1)	1,168 (5.6)	1,094 (5.4)	1,106 (5.5)	1,098 (5.6)	0.024

Values are number (percentage), mean ±standard error or median (inter quartile range, IQR) and are from the day of enrollment (2003-2014).
[a]P for differences by Kruskal-Wallis equality-of-populations rank test or by Pearson's chi square test as appropriate. *APOE*, apolipoprotein E gene; DKK, Danish crowns.

PLASMA CONCENTRATIONS OF MAGNESIUM AND NON-ALZHEIMER AND ALZHEIMER DEMENTIA

Restricted cubic spline Cox regression models evaluated risk of non-Alzheimer and Alzheimer dementia by plasma magnesium concentrations, age, and sex adjusted (Fig. 1, upper panels), multifactorially adjusted (Fig. 1, middle panels), and multifactorially and eGFR and cations adjusted (Fig. 1, lower panels). In all 3 models, low plasma concentrations of magnesium were associated with high risk of non-Alzheimer dementia relative to the reference of 2.07 mg/dL (0.85 mmol/L), which defined the lowest overall risk of non-Alzheimer dementia. For high plasma magnesium concentrations, 95% confidence intervals for the spline curves do overlap 1.0; however the tendency was towards high risk of non-Alzheimer dementia. No associations between plasma concentrations of magnesium and risk of Alzheimer dementia were observed. Results for the combined endpoint (all-cause dementia) are shown in Supplemental Fig. 3.

For the lowest vs the 4th quintile (reference) of plasma concentrations of magnesium, multifactorially adjusted HRs were 1.50 (95% confidence interval (CI): 1.21–1.87) for non-Alzheimer dementia and 1.03 (0.88–1.20) for Alzheimer dementia (Fig. 2, middle panel). For the highest vs the 4th (reference) quintile multifactorially adjusted HRs were 1.34 (1.07–1.69) and 1.01 (0.86–1.19) for non-Alzheimer and Alzheimer dementia (Fig. 2, middle panel). When comparing the group of individuals with plasma concentrations of magnesium below the reference interval, used in routine clinical practice, with the group at the median, multifactorially adjusted HRs were 1.64 (1.32–2.04) for non-Alzheimer dementia and 1.06 (0.86–1.27) for Alzheimer dementia (Supplemental Fig. 4). Estimates for analyses of the combined endpoint, all-cause dementia, are shown in Fig. 3 and Supplemental Fig. 5. Estimates from sensitivity analyses omitting individuals with endpoints less than 5 years from baseline showed similar results (Fig. 4).

MEDIATED EFFECT OF PLASMA CONCENTRATIONS OF MAGNESIUM ON RISK OF NON-ALZHEIMER DEMENTIA

Mediation analysis was performed for non-Alzheimer dementia with diabetes, cumulated smoking, stroke, and systolic blood pressure as mediators. The estimated direct and indirect effects for the investigated mediators are shown in Fig. 5. Diabetes, cumulated smoking, and systolic blood pressure show an increasing indirect effect with decreasing plasma concentrations of magnesium, whereas stroke displays indirect effect with both low and high plasma concentrations of magnesium. The mediated proportion in the lowest quintile was estimated to 10.4% (3.1–22.8%), 6.8% (1.2–14.0%), 1.3% (0.1–3.6%), and 1.0% (0.2–2.6%) for diabetes, cumulated smoking, stroke, and systolic blood pressure, respectively (Fig. 5). Corresponding results for the lowest group according to reference intervals were 12.3% (4.4–24.8%), 5.8% (2.3–12.9%), 1.5% (0.2–3.7%), and 0.5% (0.0–1.6%) (Supplemental Fig. 6). In the highest quintile the estimated mediated proportion was 3.2% (0.4–11.9%) for stroke (Fig. 5). The corresponding result for the highest group according to reference intervals did not reach statistical significance (Supplemental Fig. 6).

Discussion

In this study of 102 648 individuals from the general population, the association between plasma concentrations of magnesium and risk of vascular-related non-Alzheimer dementia was U-shaped, where both low and high concentrations conferred high risk. A magnesium concentration of 2.07 mg/dL (0.85 mmol/L) was associated with the lowest risk. No association was observed for Alzheimer dementia. Diabetes mediated a substantial proportion of the association between low plasma magnesium concentrations and high risk of non-Alzheimer dementia, whereas cumulated smoking, stroke, and systolic blood pressure played minor mediating roles. These findings are novel.

The mechanisms behind our findings on diabetes, cumulated smoking, stroke, and systolic blood pressure as mediators are unknown. Underlying explanations are however likely to be found, at least partly, within the vascular system. Non-Alzheimer dementia harbors strong vascular components, as evident from large-scale sources (18, 19), and vascular risk factors as diabetes,

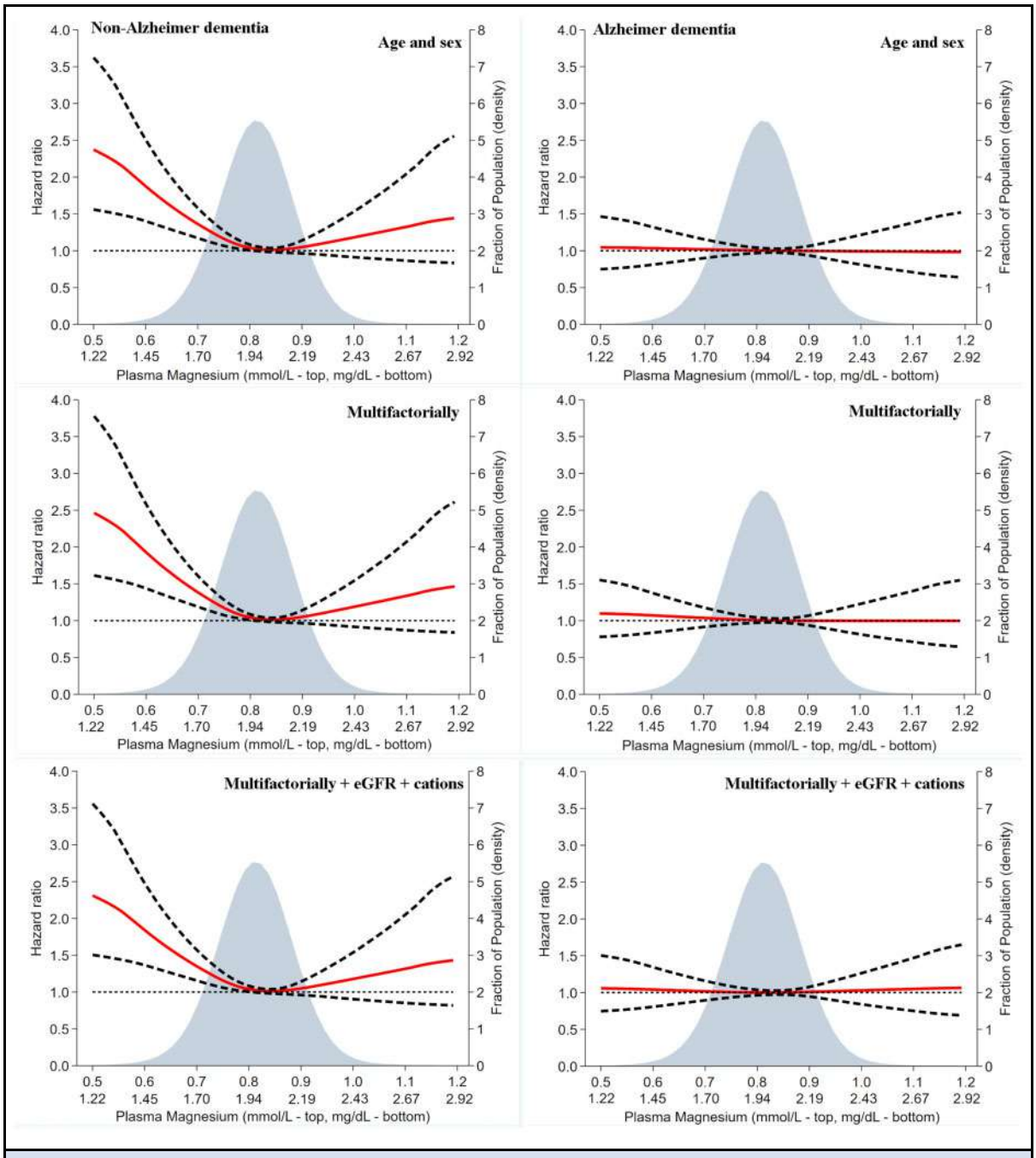

Fig. 1. Spline plots of risk of non-Alzheimer and Alzheimer dementia as a function of plasma magnesium concentrations. Restricted cubic spline Cox regression models were adjusted for age (time scale) and sex (upper panels), age, sex, educational level, body mass index and *APOE* genotype (middle panels), and finally for age, sex, educational level, body mass index, *APOE* genotype, eGFR, and plasma concentrations of calcium, sodium, and potassium (lower panels). Red lines are hazard ratios and dashed lines indicate 95% confidence intervals derived from restricted cubic splines with 3 knots and with the reference defined as the plasma magnesium level with lowest risk. Blue indicates density plots of plasma magnesium concentrations. *APOE* genotype, apolipoprotein E ε2/3/4 genotype; CI, 95% confidence interval; eGFR, estimated glomerular filtration rate.

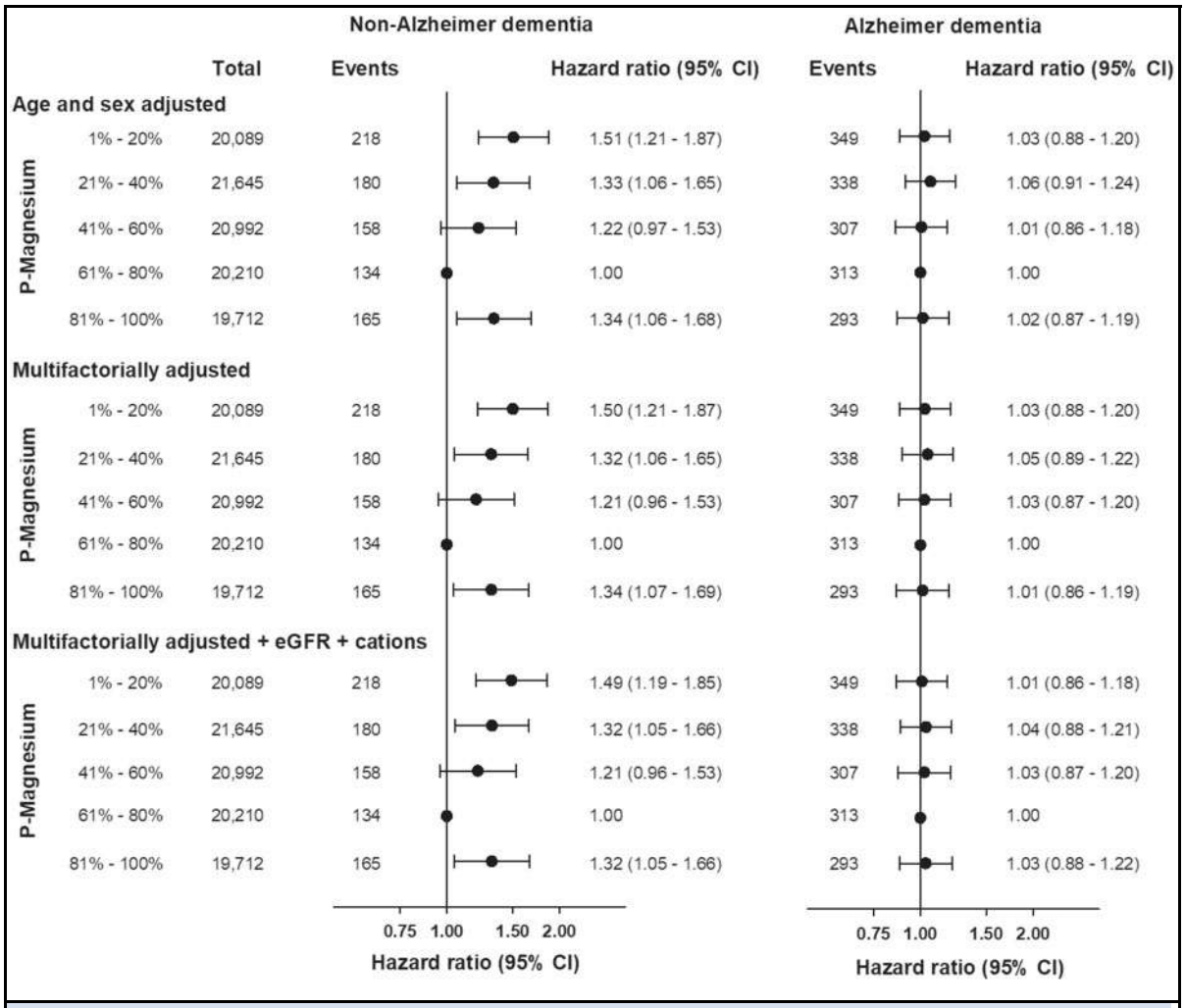

Fig. 2. Risk of non-Alzheimer and Alzheimer dementia as a function of plasma magnesium quintiles. Cox regression models were adjusted for age (time scale) and sex (upper panels), age, sex, educational level, body mass index and *APOE* genotype (middle panels), and finally for age, sex, educational level, body mass index, *APOE* genotype, eGFR, and plasma concentrations of calcium, sodium and potassium (lower panels). *APOE* genotype, apolipoprotein E ε2/3/4 genotype; CI, 95% confidence interval; eGFR, estimated glomerular filtration rate.

smoking, stroke, and systolic blood pressure all mediate risk at low concentrations of magnesium. Low plasma concentrations of magnesium are reported to be associated with increased risk of insulin resistance and type 2 diabetes (11–13). Diabetes increases risk of micro- and macrovascular disease through atherosclerosis and excess glucose concentrations, increases risk of lacunar infarcts, general cerebral atrophy, and ultimately all-cause dementia and vascular dementia (20, 21). Interestingly, low concentrations of magnesium are suggested to modify the cerebral dopaminergic reward system, leading to increased smoking quantity and higher risk of smoking. Smoking is associated with atherosclerosis, hypertension, inflammation, and damage to vessel walls—components all related to micro- or macrovascular damage in the brain (22, 23), although the mediated proportion only accounted for a minor part of the total risk. Systolic blood pressure is an established risk factor for cerebral infarction and white matter lesions (24, 25). The association between low concentrations of magnesium and higher systolic blood pressure, however, was weak in the present study, leading to hardly any mediated risk of non-Alzheimer dementia due to systolic blood pressure. High diastolic blood pressure is more strongly associated with low plasma magnesium concentrations (10, 26), but was not associated with risk of

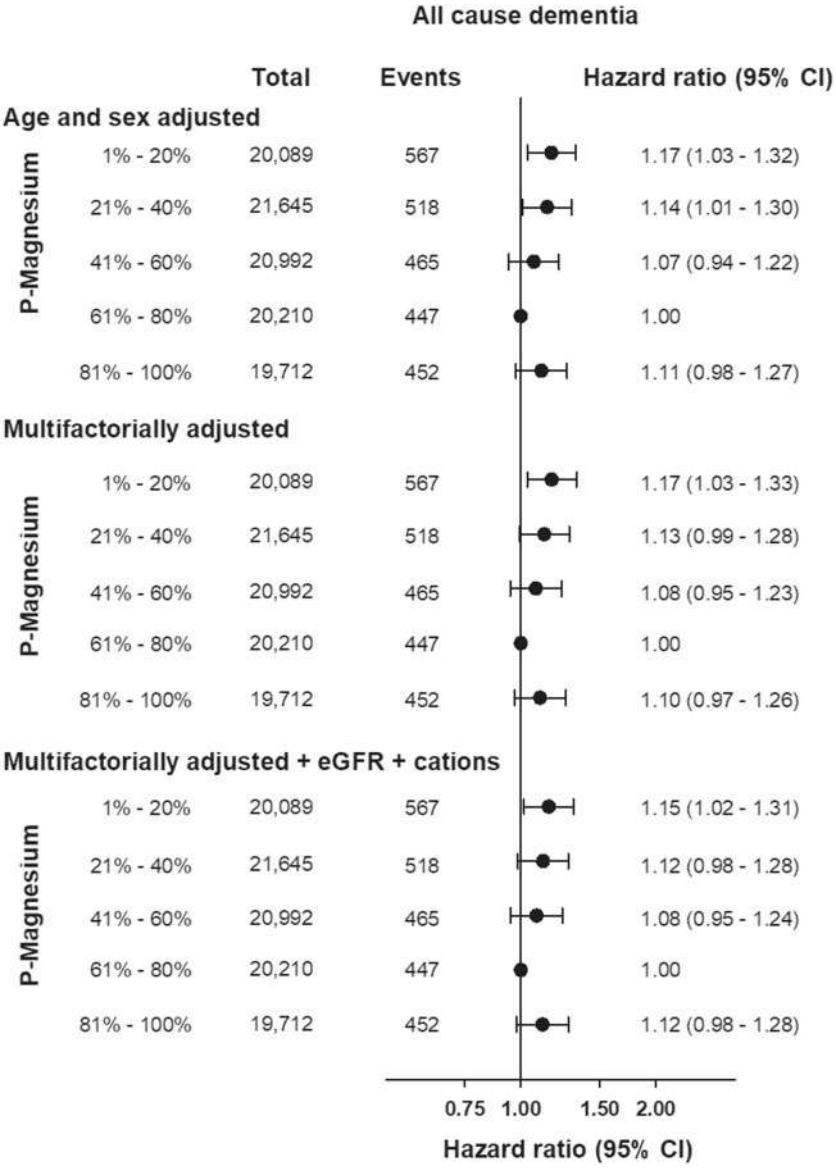

Fig. 3. Risk of all-cause dementia as a function of plasma magnesium quintiles. Cox regression models were adjusted for age (time scale) and sex (upper panel), age, sex, educational level, body mass index and *APOE* genotype (middle panel), and finally for age, sex, educational level, body mass index, *APOE* genotype, eGFR, and plasma concentrations of calcium, sodium and potassium (lower panel). *APOE* genotype, apolipoprotein E ε2/3/4 genotype; CI, 95% confidence interval; eGFR, estimated glomerular filtration rate.

non-Alzheimer dementia in our study. Taken together, plasma magnesium concentrations were not important as mediators of the association between blood pressure and non-Alzheimer dementia in our study.

Stroke is a well-known cause of vascular dementia, and low plasma magnesium concentrations were recently suggested to be causally related to ischemic stroke (5). Since subcortical vascular disease and white matter lesions, and not necessarily overt stroke, account for a large proportion of vascular dementia (27), this may explain why stroke only mediated a minor fraction of the association between low plasma magnesium and non-Alzheimer dementia. Stroke may also be a mediator of the association between high plasma magnesium

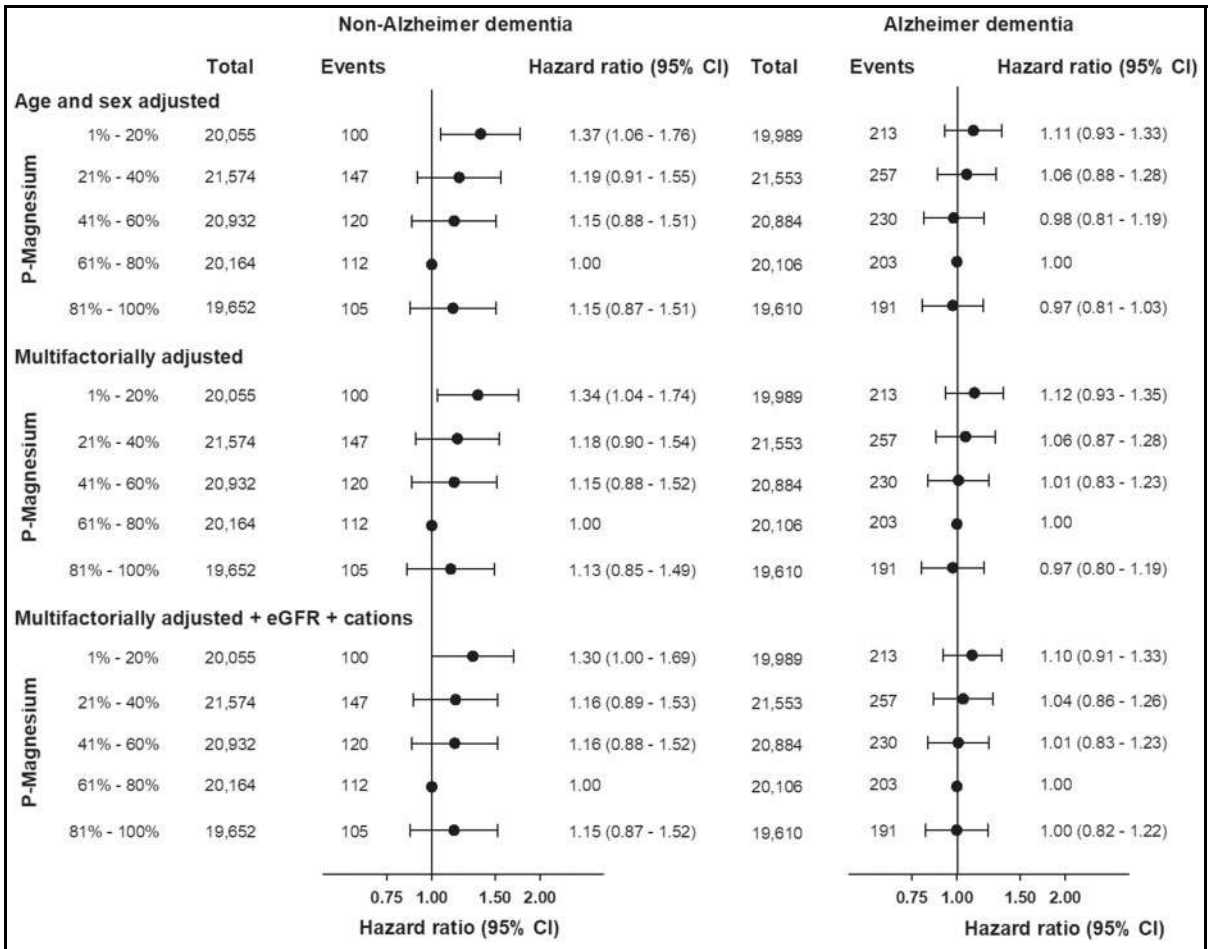

Fig. 4. Risk of non-Alzheimer and Alzheimer dementia as a function of plasma magnesium quintiles omitting individuals with endpoints less than 5 years from baseline. Cox regression models were adjusted for age (time scale) and sex (upper panels), age, sex, educational level, body mass index, and *APOE* genotype (middle panels), and finally for age, sex, educational level, body mass index, *APOE* genotype, eGFR, and plasma concentrations of calcium, sodium, and potassium (lower panels). *APOE* genotype, apolipoprotein E ε2/3/4 genotype; CI, 95% confidence interval; eGFR, estimated glomerular filtration rate.

concentrations and high risk of non-Alzheimer dementia, although the present evidence for a role of high plasma magnesium concentrations is weak and may seem counterintuitive. A potential biological explanation may be that hypermagnesemia can cause neuromuscular toxicity leading to respiratory depression and apnea (7).

Additionally, hypermagnesemia is associated with cardiovascular effects (hypotension, conduction defects and bradycardia (7, 28)) and hypocalcemia (7)—conditions that are well-known potent risk factors for stroke (29–32). The present findings on high plasma magnesium concentrations were supported by the Rotterdam study (6) for all-cause dementia and were indirectly supported by the Atherosclerosis Risk in Communities Study for all-cause stroke (4). The study of high magnesium is however limited by the fact that only a few individuals have high magnesium concentrations, due to tight regulation by the kidneys, and that individuals with high magnesium concentrations often have underlying morbidities acting as strong confounders. Only a few studies have been sufficiently powered to study high concentrations of magnesium; however these studies still displayed wide confidence intervals at high magnesium concentrations (4, 6).

Our results are supported by recent findings of plasma magnesium concentrations and all-cause dementia (6, 33, 34). We have shown, however, for the first time to our knowledge, that the associations are restricted to non-Alzheimer dementia, the subtype of

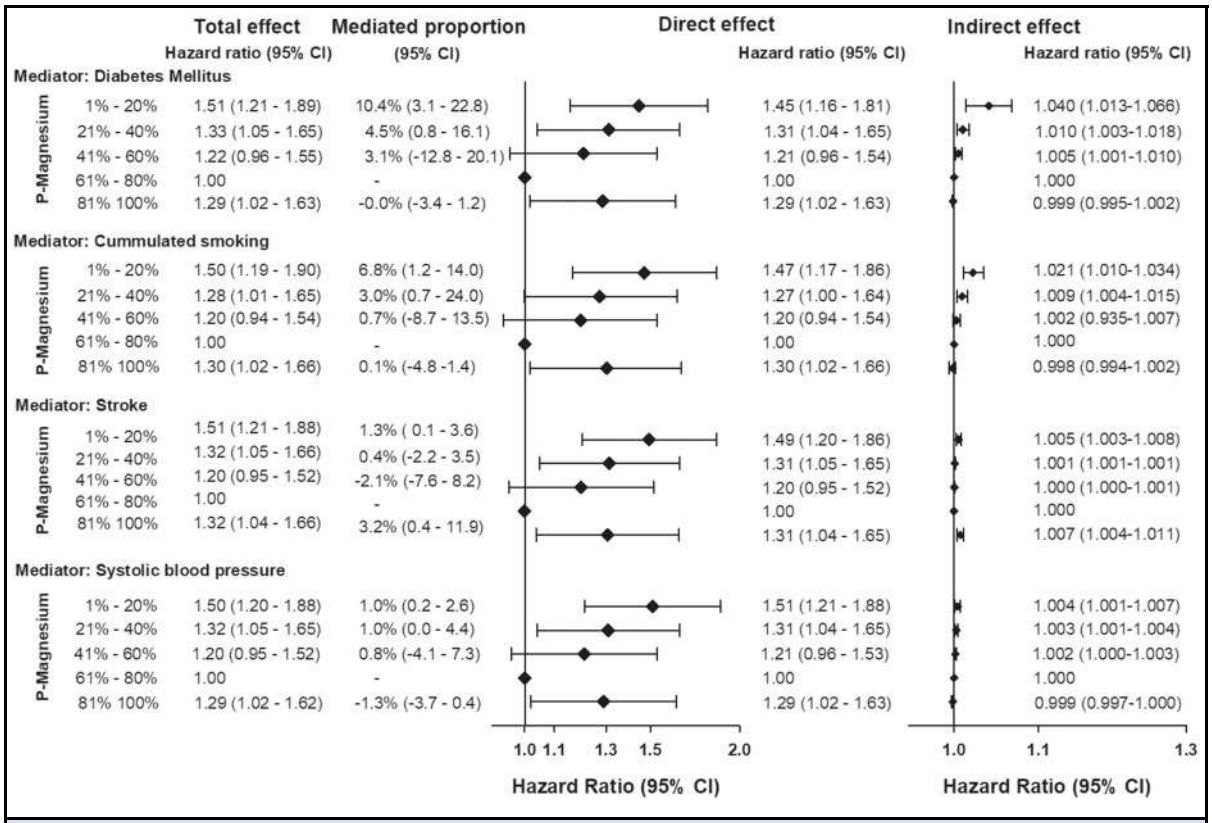

Fig. 5. Mediation proportion of risk of non-Alzheimer dementia as a function of plasma magnesium quintiles. Total effect, mediated proportion, direct effect, and indirect effect of the mediators diabetes, cumulated smoking, stroke, and systolic blood pressure by 5 quintiles of plasma magnesium concentrations. The total effect is the combined effect of the direct effect and indirect effect and the mediated proportion is the ratio between the indirect effect and total effect. CI, confidence intervals.

dementia enriched in vascular components. We further extended these observational findings with novel causative directions from mediation analyses, where especially diabetes is suggested to explain a causal fraction of the association between low plasma magnesium concentrations and high risk of non-Alzheimer dementia. Because diabetes is associated with macro-and/or microvascular pathologies also in the brain (20, 35), this may indicate a potential causal role of diabetes on risk of dementia with the most obvious vascular pathologies as in the non-Alzheimer dementia subtype including vascular and mixed dementia. These suggestions are also highly supported by a recent report on a potential causal effect of high plasma glucose concentrations and risk of unspecified dementia (36). Further, our data suggest stroke to explain a causal fraction of the association between both low and high plasma magnesium concentrations and high risk of non-Alzheimer disease. This is supported by the observations that low plasma magnesium was causally associated with stroke in a Mendelian randomization study (5), and that stroke is a causal risk factor for dementia with vascular pathologies. The genetic instruments used were however not strong, and this has so far kept us from performing a Mendelian randomization study of plasma magnesium concentrations and dementia in the CGPS, to avoid a false negative result. When robust genetic information on magnesium-related variants emerges, we will pursue this strategy. Also, a 2-sample Mendelian randomization strategy using summary level data from genetic consortia is currently not possible to perform, because no publicly available genetic consortia data yet exist for non-Alzheimer dementia.

A major strength of our study is the large prospective general population design with no losses to follow-up. That is, every single individual could be followed to end of follow-up, death, emigration, or dementia diagnosis. Due to the large sample size, we were able to perform mediation analyses on a set of age and sex adjusted quintiles of plasma magnesium concentrations with adjustment for known confounders. Interestingly, our findings between plasma magnesium and non-Alzheimer

dementia remained after omitting individuals with events within 5 years from baseline, suggesting that reverse causation is probably not important, and lending support to a direct causal association or an association mediated through other causal factors.

A potential limitation concerns the completeness and accuracy of the diagnostic information. The national Danish Patient Registry and the national Danish Causes of Death Registry include, respectively, all hospital and outpatient visits and all death certificates, and all Danish individuals can be tracked in these systems by their personal identification number. This ensures that there are no losses to follow-up of participants, because these registries contain information on all individuals living in Denmark. Even though the national Danish registries are regarded among the best of their kind (37, 38), the use of registry-based diagnoses may suffer from potential underdiagnosis, which in this case is anticipated to result in underestimation of the true effect sizes. The all-cause dementia and Alzheimer dementia diagnoses have good diagnostic validity (39) and are indirectly validated by similar *APOE* genotype effect sizes in the present cohort (15, 40) as observed generally in the literature. Further, we ensured, by scrutinizing department codes for all events, that 91%–93% of all-cause dementia diagnoses were obtained from dementia clinics, other neurological outpatient clinics, departments of neurology and neurosurgery, in- and outpatient clinics at departments of geriatrics, and departments of internal medicine (38). The ability to sort our Alzheimer dementia from non-Alzheimer dementia seemed reasonable due to especially 2 reasons: 1) Because all diagnoses are from 2003 or later, appropriate brain imaging studies have been performed in most patients. According to guidelines (41), an Alzheimer diagnosis should not be given if vascular-related brain pathology is present on brain imaging; 2) Other conditions such as hydrocephalus or alcoholic delirium have distinct ICD-10 codes and are exclusion criteria for a dementia diagnosis and, thus, will not likely confound the dementia diagnoses used in the present article. Further, annual statistics from the Danish Dementia Quality Database show that most of the non-Alzheimer dementia fraction has a clear vascular component (vascular dementia or mixed Alzheimer- and vascular dementia) (19). Similarly, previous studies have shown that the diagnose codes underlying non-Alzheimer dementia associate with vascular-related diseases such as stroke, myocardial infarction, heart failure, and diabetes (42–45).

A limitation of the mediation analysis method is that it cannot take circular causal structures into account (eg, evidence of A causing B and evidence of B causing A). Therefore, it is necessary to take an informed choice based on present knowledge (the literature) on the direction of the causal direction. Based on evidence from the literature we chose to model that plasma magnesium concentrations affect cumulated smoking, omitting the less likely possibility that smoking may affect plasma magnesium concentrations, for example, due to a diet rich in magnesium. Also, based on the literature we chose to model that plasma magnesium concentrations affect risk of diabetes, omitting the less likely possibility that individuals with diabetes may have lower plasma magnesium concentrations due to increased plasma glucose concentrations. This issue is an inherent limitation of the presently applied statistical mediation analysis.

Another limitation is the sparse amount of evidence regarding the relationship between low plasma magnesium concentrations and increased cumulated smoking in pack-years. The association has been described in a few observational studies and the suggested explanation for the association is the diet in smokers. One randomized clinical trial showed that magnesium supplements caused heavy smokers to smoke less (46). There is a growing number of animal studies suggesting that magnesium may modulate the dopaminergic response (47, 48). In support of this potential mechanism, we observed the strongest association between plasma magnesium concentrations and smoking status by using cumulated smoking. When we developed the auxiliary function for the mediation analysis, the association between plasma magnesium concentrations and cumulated smoking remained strong, even when adding adjustment for educational level, income, body mass index, low physical activity, and high alcohol consumption. These covariates associate with both cumulated smoking and diet; hence the association between plasma magnesium and cumulated smoking should be attenuated if diet was the cause of the association. Collectively, observational studies, animal studies, and one randomized clinical trial suggest that the association between plasma magnesium concentrations and cumulated smoking may have causal aspects. The evidence is, however, sparse and unmeasured confounding may explain the observed association.

In conclusion, both low and high plasma magnesium concentrations were associated with high risk of non-Alzheimer dementia, with the lowest risk observed at a concentration of 2,07 mg/dL (0.85 mmol/L). No association was observed for Alzheimer dementia. Mediation analysis suggested that diabetes may be in the causal pathway between low plasma magnesium concentrations and high risk of non-Alzheimer dementia, whereas cumulated smoking, stroke, and systolic blood pressure played minor mediating roles. Robust Mendelian randomization studies with strong genetic instruments for plasma magnesium concentrations will be needed to

probe causality between magnesium concentrations and risk of dementia in the general population.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: BMI, body mass index; CGPS, the Copenhagen General Population Study; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; ICD, International Classification of Diseases

Human Gene: *APOE*, apolipoprotein E.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

J.Q. Thomassen, statistical analysis; B.G. Nordestgaard, financial support, provision of study material or patients; A. Tybjærg-Hansen, provision of study material or patients; R. Frikke-Schmidt, financial support, administrative support, provision of study material or patients.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: This work was supported by the Research Council at Rigshospitalet and the Lundbeck Foundation, both to R. Frikke-Schmidt.
Expert Testimony: None declared.
Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: We thank staff and participants of the CGPS for their important contributions.

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