Aus dem
Helmholtz-Zentrum München
Institut für Epidemiologie (EPI)


# Magnesium and Cardiovascular-Kidney-Metabolic Syndrome: An Epidemiological Perspective

Dissertation
zum Erwerb des Doctor of Philosophy (Ph.D.) an der Medizinischen Fakultät der Ludwig-Maximilians-Universität München

vorgelegt von
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aus
Sanaa / Jemen

Jahr
2025

Mit Genehmigung der Medizinischen Fakultät der
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# List of abbreviations

| | |
|---|---|
| 24-H-FL | 24-hour food lists |
| 25-OHD | Serum vitamin D |
| AAC | Aortic Artery Calcification |
| AF | Atrial fibrillation |
| BMI | Body mass index |
| CHD | Coronary heart disease |
| CKD | Chronic kidney disease |
| CKM | Cardiovascular-Kidney-Metabolic |
| CT | Computed tomography |
| CVD | Cardiovascular disease |
| DEXA | Dual-energy x-ray absorptiometry |
| EGTA | Ethylene glycol tetraacetic acid |
| EDTA | Ethylenediaminetetraacetic acid |
| eGFR | Estimated glomerular filtration rate |
| FFQ | Food frequency questionnaire |
| GLUT4 | Glucose transporter protein 4 |
| GWAS | Genome-wide association studies |
| HF | Heart failure |
| IVW | Inverse-variance weighting |
| KORA | Cooperative Health Research in the Region of Augsburg |
| LV | Left ventricle |
| MDS | Magnesium depletion score |
| MetS | Metabolic syndrome |
| MRI | Magnetic resonance imaging |
| MTB | Methylthymol blue |
| NAKO | The German National Cohort |

| | |
|---|---|
| NO | Nitric oxide |
| RDA | Recommended daily allowance of magnesium |
| SNPs | Single nucleotide polymorphisms |
| SSFP | Steady-state free precession |
| T2D | Type 2 diabetes |
| TXA2 | Thromboxane A2 |
| UACR | Urine albumin-to-creatinine ratio |

# List of papers included in this thesis

This chapter offers a brief overview of the three papers that form the foundation of this cumulative thesis.

**Paper I**

**Shugaa Addin N**, Niedermayer F, Thorand B, Linseisen J, Seissler J, Peters A, Rospleszcz S. Association of serum magnesium with metabolic syndrome and the role of chronic kidney disease: A population-based cohort study with Mendelian randomization. Diabetes Obes Metab. 2024 May;26(5):1808-1820.

**Paper II**

**Shugaa Addin N**, Schlett CL, Bamberg F, Thorand B, Linseisen J, Seissler J, Peters A, Rospleszcz S. Subclinical Cardiovascular Disease Markers in Relation to Serum and Dietary Magnesium in Individuals from the General Population: The KORA-MRI Study. Nutrients. 2022 Nov 22;14(23):4954.

**Paper III**

**Shugaa Addin N**, Schuppert C, Full PM, Brenner H, Dörr M, Keil T, von Krüchten R, Meinel FG, Niendorf T, Pischon T, Schmidt B, Schulz-Menger J, Schwichtenberg J, Völzke H, Willich SN, Bamberg F, Peters A, Schlett CL, Rospleszcz S. Magnesium Depletion, Metabolic Impairment, and Cardiac Alterations: The NAKO-MRI Study with Mendelian Randomization. J Clin Endocrinol Metab. 2025 Aug 21:dgaf476. doi: 10.1210/clinem/dgaf476. Epub ahead of print. PMID: 40839755.

# Contributions to the included papers

In all three papers included in this thesis, I am the first author and was responsible for developing the overarching research idea, which evolved as a continuation of my master's thesis.

I assumed full responsibility for the integrity of the data and the accuracy of the data analysis. Under the guidance of my doctoral supervisors, Dr. Susanne Rospleszcz and Prof. Dr. Annette Peters, I formulated the research questions and conceptualized the study design. Through comprehensive literature reviews, I gained an in-depth understanding of MRI-derived markers of subclinical cardiovascular disease and explored magnesium literature extensively. This foundation allowed me to refine the research questions and develop clear, methodologically robust statistical analysis plans.

I applied for the necessary data for this thesis and prepared detailed statistical analysis plans for each paper. I conducted all statistical analyses using the R programming language and interpreted the results in collaboration with members of my Thesis Advisory Committee. Additionally, I worked closely with radiologists, physicians, nutritionists, and statisticians to refine the interpretation of the findings. I was responsible for drafting the manuscripts, integrating feedback from co-authors, and critically revising the texts. I managed the submission process, responded to reviewers' comments, and integrated their suggestions into the final version of the paper.

# Introductory summary

## 1. Background

Magnesium is a vital mineral essential for numerous physiological processes, including insulin regulation, glucose metabolism, vascular tone, and cardiac function. As the fourth most abundant mineral and the second most abundant intracellular cation, magnesium serves as a cofactor for over 300 enzyme systems that regulates a wide range of biochemical processes essential for maintaining bodily functions [1]. Magnesium deficiency contributes to pathophysiological mechanisms such as oxidative stress, inflammation, and insulin resistance, increasing the risk of cardiometabolic disorders like dyslipidemia, hypertension, and obesity [2]. These conditions drive the progression of Cardiovascular-Kidney-Metabolic (CKM) syndrome**,** a complex interplay of obesity, type 2 diabetes, cardiovascular disease (CVD), and chronic kidney disease (CKD). CKM syndrome advances through four stages, starting with excess and dysfunctional adiposity and insulin resistance, progressing to metabolic syndrome (MetS) and CKD, followed by subclinical CVD, and ultimately leading to clinical CVD [3]. The development of CVD cannot be entirely attributed to traditional cardiovascular risk factors [4]. Consequently, there is a critical need to identify additional biomarkers of early CVD to develop effective prevention and intervention strategies to mitigate the escalating burden of CVD. Investigating the association of magnesium deficiency with subclinical stages of CVD, including MetS, early cardiac impairment, and preclinical atherosclerosis, holds significant public health importance for risk stratification and the prevention of CVD progression (Figure 1). Moreover, whether these associations are causal remains to be determined.

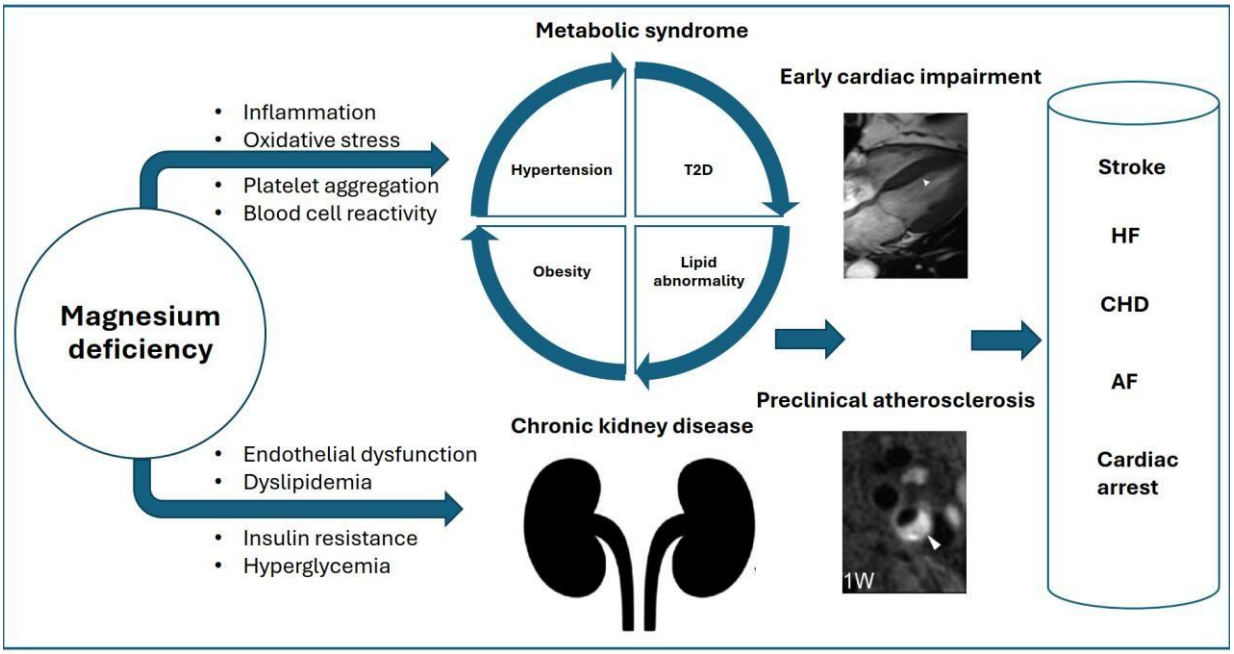


**Figure 1:** Physio-pathological mechanisms of magnesium deficiency in Cardiovascular-Kidney-Metabolic (CKM) syndrome. Figure adapted from [3, 5]; Early cardiac impairment figure sourced from [6]; Preclinical atherosclerosis figure sourced from [7]; Own illustration. T2D, type 2 diabetes; HF, Heart failure; CHD, coronary heart disease; AF, atrial fibrillation.

## 1.1. Magnesium

### 1.1.1. Magnesium hemostasis

The recommended daily allowance (RDA) for magnesium is 350 mg per day for adult men and 300 mg per day for adult women. For children and adolescents, the RDA varies between 170 and 300 mg per day, depending on age and sex. According to the European Food Safety Authority, there is currently no evidence supporting an increased magnesium intake for pregnant or lactating women [8]. Good dietary sources include spinach, chard, pumpkin, banana, nuts, and seeds [9].

Magnesium hemostasis is maintained through the interplay between intestinal absorption, bone exchange, and renal excretion. Magnesium absorption occurs via passive diffusion and active transport, predominantly in the distal small intestine [10]. Approximately half of the body's magnesium is stored in bone, with 30% of it being exchangeable, serving to stabilize serum magnesium levels. The remaining magnesium is primarily in soft tissues and muscles, with less than 1% found in the blood [11]. Renal magnesium excretion involves a complex filtration-reabsorption process. Approximately 80% of magnesium is filtered in the glomerulus, with 20-30% reabsorbed in the proximal tubule and 50-65% in the distal loop of Henle. The renal excretion of magnesium is largely determined by serum magnesium levels, which are tightly regulated to maintain homeostasis, even under conditions of low dietary intake or excessive excretion. While serum magnesium may remain within the normal range, intracellular magnesium stores in bone and soft tissues can become depleted [12].

### 1.1.2. Role of magnesium in the cardiovascular system

Magnesium exerts numerous beneficial effects on the cardiovascular system. It enhances insulin and glucose metabolism by modulating the activity of glucose transporter protein 4 (GLUT4) and improving insulin sensitivity [13]. Magnesium also possesses antiarrhythmic properties by modulating action potential duration and myocardial excitability. Its infusion slows atrioventricular nodal conduction, leading to prolonged PR and QRS intervals [14]. Additionally, magnesium has anticoagulant and antiplatelet effects by inhibiting platelet activation—reducing the production of thromboxane A2 (TXA2) and increasing the release of prostacyclin (PGI2) [15]. It promotes endothelial-dependent vasodilation by stimulating endothelial proliferation, angiogenesis, and upregulating endothelial nitric oxide synthase, thereby increasing nitric oxide (NO) release [16]. Magnesium also has anti-inflammatory effects by modulating nuclear factor-kB and offers antioxidant protection by scavenging oxygen radicals [17].

### 1.1.3. Assessment of magnesium status

The assessment of magnesium status in humans remains challenging due to the lack of a simple, rapid, and accurate test. Serum magnesium concentration is the most commonly used indicator, with levels below 0.75 mmol/L signifying deficiency. However, there is no consensus on the reference range, and some suggest that hypomagnesemia be defined by levels below 0.85 mmol/L. It is important to note that only 1% of the body's magnesium is found in the blood, with just 0.3% in the serum. Additionally, the body's tight regulation of serum magnesium can maintain normal levels even when overall magnesium deficiency is present [18, 19].

Dietary magnesium intake is typically measured using food frequency questionnaires, 24-hour dietary recall, or food diaries, all of which rely on self-reporting and are subjected to recall bias and inaccuracies in portion size estimation [20]. Variations in magnesium content across different food sources and the bioavailability of magnesium can complicate accurate assessment. These methods may not account for magnesium losses through cooking or differences in individual absorption rates, leading to potential underestimation or overestimation of actual intake [21]. Additionally, the use of different dietary assessment tools across studies makes it difficult to compare results and replicate findings. Consequently, while dietary assessments provide valuable insights into chronic magnesium deficiency, they should be complemented with other measures, such as serum magnesium or biomarkers, for a more comprehensive evaluation of magnesium status [22].

The magnesium tolerance test is a robust diagnostic tool for identifying magnesium deficiency. This test involves administering a magnesium load, either orally or intravenously, and assessing retention by measuring urinary excretion. Individuals with hypomagnesemia retain significantly more magnesium compared to those with normal levels, indicating an underlying deficiency [23]. Despite its sensitivity, the test's complexity and time requirements limit its routine use. Furthermore, it is not suitable in individuals with kidney impairment [24].

Consequently, a new scoring method was developed by researchers to better define magnesium deficiency. The Magnesium Depletion Score (MDS) is an emerging tool for identifying individuals with magnesium deficiency, considering factors that influence renal magnesium reabsorption, such as alcohol consumption, estimated glomerular filtration rate (eGFR), and the use of proton pump inhibitors and diuretics. It has been validated to predict magnesium deficiency status more accurately than serum magnesium alone [25]. This approach can aid in guiding precision-based nutritional interventions aimed at reducing inflammation and cardiovascular risk [25].

### 1.1.4. Magnesium deficiency

Magnesium deficiency results from various factors (Figure 2). Environmental factors, including climate change, global warming, and modern agricultural practices, significantly influence magnesium availability in soil and throughout the food chain, contributing to widespread magnesium deficiency. This deficiency often originates at the very beginning of the food chain—plants. Climate-related stressors such as water scarcity, soil waterlogging, elevated $CO_2$ levels, and rising temperatures directly affect plant nutrition and physiology, thereby impairing magnesium uptake. Moreover, the long-term use of chemical fertilizers has progressively reduced the ability of plant roots to absorb magnesium from the soil, further exacerbating the problem.[26]. Alterations in dietary habits, particularly the rising intake of ultra-processed foods and the frequent consumption of overcooked meal, have been associated with reduced magnesium intake. As a result, a substantial proportion of the global population does not meet the recommended daily requirements for magnesium.[26]. Additionally, conditions that impair magnesium absorption, such as inflammatory bowel disease and short bowel syndrome, as well as those that hinder magnesium reabsorption, like chronic kidney disease (CKD) and diuretic use, further exacerbate the deficiency [27].

Consequently, magnesium deficiency contributes to several pathophysiological processes, including chronic low-grade inflammation, oxidative stress, platelet aggregation, endothelial dysfunction, dyslipidemia, insulin resistance, and hyperglycemia. These factors collectively cluster to form various metabolic risk factors, which elevate the likelihood of developing CVD (Figure 1) [2]. Interestingly, magnesium deficiency is often asymptomatic, with symptoms typically emerging only when serum levels drop below 1.2 mg/dL. In cases of severe deficiency, symptoms may include neuromuscular hyperactivity, psychiatric disturbances, cardiac arrhythmias, hypocalcemia, and hypokalemia [28].

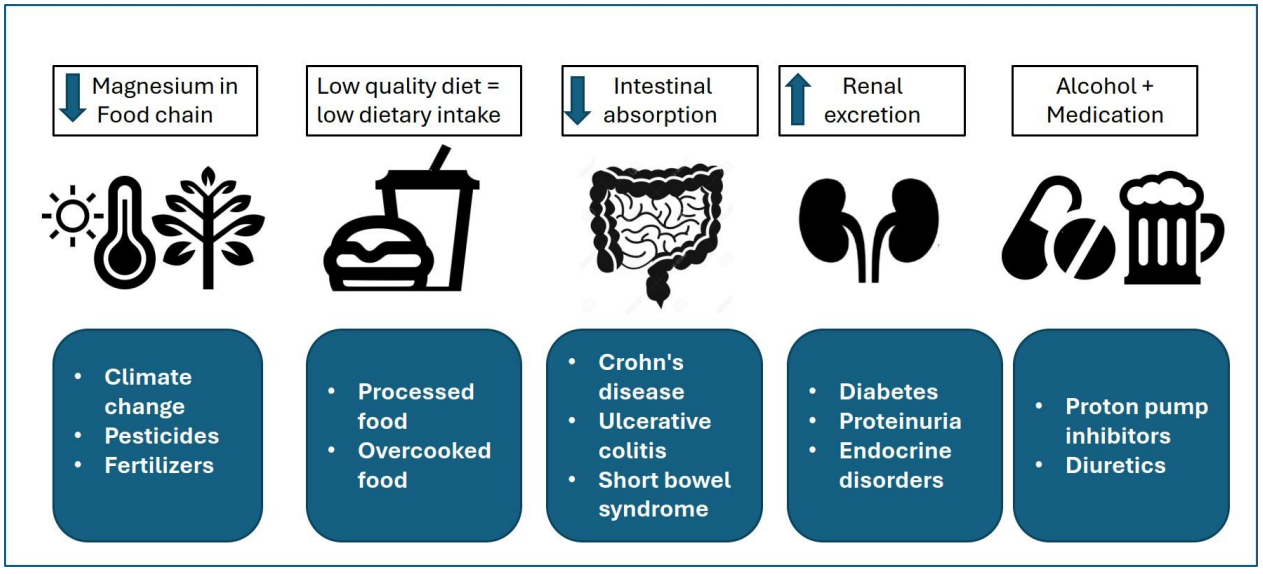


**Figure 2:** Common causes of magnesium deficiency

## 1.2. Cardiovascular-kidney-metabolic syndrome

The CKM syndrome is a newly defined concept by the American Heart Association, representing the complex interplay between metabolic risk factors, chronic kidney disease, and cardiovascular system. This syndrome is characterized by multisystem pathophysiological relationships, including hyperglycemia, insulin resistance, oxidative stress, and chronic inflammation [3]. The syndrome is classified into four stages based on risk factors and clinical signs, with an estimated 25% of the population having at least one CKM condition [29]. CKM syndrome originates from dysfunctional and excess adipose tissue, leading to inflammation and tissue damage. Stage 2 of CKM involves MetS and CKD, both of which are global health challenges with high morbidity and mortality. Stage 3 is characterized by subclinical CVD, including preclinical heart failure and atherosclerosis, while Stage 4 represents overt CVD, such as coronary heart disease and stroke. The American Heart Association emphasizes the importance of screening for both biological factors and social determinants of health in CKM syndrome [3].

### 1.2.1. Metabolic syndrome

MetS, the second stage of CKM syndrome, is a complex disorder characterized by a constellation of metabolic abnormalities, including central obesity, hypertension, insulin resistance, and dyslipidemia. The prevalence of MetS has increased globally, largely due to the obesity epidemic [30]. This syndrome results from the interaction of genetic, hormonal, and lifestyle factors and is associated with an increased risk of developing diabetes and cardiovascular diseases. The underlying pathophysiology involves altered metabolic pathways, particularly in triglyceride metabolism and insulin resistance [31]. Early diagnosis is crucial for implementing lifestyle modifications and risk factor management.

### 1.2.2. Chronic kidney disease

CKD is a widespread global health concern, affecting over 10% of the population worldwide [32]. It is characterized by persistent abnormalities in kidney structure or function for at least three months, typically evaluated through glomerular filtration rate and urinary albumin-to-creatinine ratio [33]. CKD prevalence increases with age and varies across different races and genders [32]. CKD significantly increases the risk of CVD, with CVD being the leading cause of death in CKD patients. The cardiovascular risk in CKD patients is 5-10 times higher than in age-matched controls, with dialysis patients facing a 10-30-fold increased risk [34]. This elevated risk stems from both traditional and non-traditional factors. Traditional risk factors include hypertension, diabetes, dyslipidemia, smoking, and physical inactivity. However, these factors alone do not fully explain the high CVD prevalence in CKD patients [35]. Mineral metabolism disorders are particularly important among these non-traditional risk factors [36].

The complex interaction of these factors underscores the need for enhanced screening, diagnostic, and treatment strategies to address CVD in CKD patients effectively.

### 1.2.3. Subclinical cardiovascular disease

Subclinical CVD, the third stage of CKM syndrome, is an early, often asymptomatic stage of cardiovascular disease that significantly increases the risk of clinical cardiovascular events in older adults [5]. Evidence suggests that subclinical atherosclerosis and cardiac dysfunction begin early in life, driven by a combination of environmental factors and genetic predisposition. [37]. Recent advancements in imaging technologies have significantly enhanced the early detection of subclinical cardiovascular phenotypes—such as left ventricular dysfunction or arterial stiffness—and improved the accuracy of cardiovascular risk stratification in both clinical and population-based settings. A variety of imaging modalities, such as carotid ultrasound, echocardiography, computed tomography (CT), magnetic resonance imaging (MRI), and lateral dual-energy X-ray absorptiometry (DEXA) scans of the thoracolumbar spine, are now available to identifying subclinical CVD markers and risk factors [38]. These imaging devices identify preclinical atherosclerosis, myocardial perfusion abnormalities, coronary artery plaque and calcification, carotid-intima-media thickness, alteration of cardiac morphology and function, and Aortic Artery Calcification (AAC) in clinically asymptomatic individuals [39-41]. Early identification of these markers has the potential to stratify and develop effective preventive strategies and treatments.

Cardiac MRI has emerged as a powerful, non-invasive imaging technique for evaluating subclinical CVD. Cardiac MRI is considered the gold standard for quantifying ventricular volumes, function, and diagnosing various conditions like carotid plaque [42]. Unlike carotid ultrasound, high-resolution MRI provides an accurate information on plaque volume and composition of the carotid arteries, distinguishing various plaque components, including the fibrous cap, necrotic core, hemorrhage, and calcification. Additionally, contrast-enhanced MRI can provide enhanced visualization in the plaque fibrous cap, making it an ideal modality for detecting early atherosclerosis [43]. Similarly, cardiac MRI is a highly reproducible imaging technique that provides exceptional spatial resolution and is less dependent on the operator compared to echocardiography. As a result, it is regarded as the clinical gold standard for evaluating cardiac structure and function, such as cardiac remodeling and ventricular volumes [44]. Furthermore, contrast-enhanced cardiac MRI is an accurate tool for detecting and quantifying myocardial fibrosis in various conditions. For instance, late-gadolinium enhancement imaging can assess the presence, pattern, and size of replacement fibrosis in ischemic cardiomyopathies [45].

## 1.3. Epidemiological evidence linking magnesium deficiency with cardiometabolic outcomes

### 1.3.1. Magnesium and metabolic syndrome

Several studies have explored the relationship between dietary magnesium intake and MetS. A systematic review and meta-analysis found that higher magnesium intake was associated with a reduced prevalence of MetS [46]. Additionally, findings from longitudinal studies suggest a significant inverse relationship between dietary magnesium intake and incident MetS [47, 48]. Magnesium supplementation was effective in improving components of metabolic syndrome, particularly among individuals with low magnesium levels, as demonstrated by a systematic review of randomized, double-blind, controlled trials. [49]. Focusing on serum magnesium, a cross-sectional study involving 1,000 adults revealed that a 1-SD increase in serum magnesium was linked to lower odds of developing MetS [50]. Furthermore, a meta-analysis of 3,487 individuals showed that serum magnesium levels were approximately 0.19 mg/dL lower in those with MetS compared to those without the condition, though notable heterogeneity across studies was observed [47].

Despite these findings, there is limited information on the relationship between serum magnesium and the incident MetS, as well as whether this association is causal. Furthermore, there is scarcity of studies investigating the association between serum magnesium and MetS in high-risk populations, such as men, individuals with obesity, individuals with vitamin d deficiency, and those taking diuretic medications. The evidence on the relationship between MDS and MetS remains limited. Notably, to our knowledge, only one study has reported that a higher MDS, indicative of magnesium depletion, is associated with a greater prevalence of MetS. [51].

### 1.3.2. The role of chronic kidney disease in the association between magnesium and metabolic syndrome

The kidney plays a crucial role in magnesium hemostasis, with hypomagnesaemia being a common electrolyte abnormality in CKD [52]. This results from impaired magnesium reabsorption due to tubular dysfunction, diuretic use, and change in diet. Hypomagnesemia is prevalent in 14.7% of pre-dialysis CKD patients, and proteinuria is identified as a significant risk factor for hypomagnesemia, leading to renal magnesium wasting through tubular injuries [53]. CKD and MetS are closely interrelated, with MetS being associated with CKD development and progression [54]. Based on genetic data, a mendelian randomization study showed an association between hypertension and CKD risk and a causal association between other MetS components and renal function [55]. Recent research has highlighted the potential role of magnesium in this relationship. Higher serum magnesium was associated with lower

risks of all-cause mortality and cardiovascular events in CKD patients [56]. Magnesium's protective effects may extend beyond its role as a calcification inhibitor, potentially influencing MetS components such as hypertension and diabetes [57]. The link between magnesium and MetS has been explored in CKD and post-kidney transplants. In patients with CKD, pre-diabetes, and obesity, magnesium supplementation was found to enhance metabolic status by reducing HbA1c, fasting glucose levels, and HOMA-IR [58]. A prospective study indicated that higher dietary fiber intake, but not serum magnesium, was linked to a lower incidence of MetS one-year post-renal transplantation [59]. However, to our knowledge, the association between magnesium and MetS has not been previously demonstrated in individuals with CKD from the general population. Given the substantial health implications of both MetS and CKD, it is crucial to investigate these associations on a population-based level.

### 1.3.3. Magnesium and subclinical cardiovascular disease

Magnesium plays a crucial role in cardiovascular health and has been linked to heart failure risk. Studies have shown that serum magnesium is inversely associated with incident heart failure in both middle-aged and older men [60, 61]. Additionally, dietary magnesium intake has been linked to heart failure with reduced ejection fraction [62]. Moreover, elevated MDS has been associated with an increased risk of congestive heart failure, particularly in individuals with low magnesium intake [63]. Evidence on the relationship between magnesium and pre-heart failure remains limited. An experimental study indicated that magnesium supplementation improves diastolic function in mice by enhancing mitochondrial function and reducing oxidative stress [64]. In a population-based longitudinal study using echocardiography, low serum magnesium levels were independently associated with increased left ventricular mass [65]. In another study, dietary magnesium intake was inversely associated with Doppler peak mitral E wave velocity, a surrogate for diastolic function, and with tricuspid regurgitation peak velocity, an indicator of pulmonary systolic pressures [66]. Furthermore, the causal relationship between magnesium status and MRI-based cardiac alterations has not yet been established.

Evidence supports the role of magnesium in preclinical atherosclerosis. In animal models, dietary magnesium supplementation has been found to inhibit the development of atherosclerosis in cholesterol-fed rabbits by reducing lipid accumulation in the aortic wall [67]. Furthermore, magnesium deficiency in rats has been associated with increased intima-media thickness and alterations in the mechanical properties of the carotid artery, potentially contributing to the development of atherosclerosis and cardiovascular diseases [68]. Elevated MDS was associated with increased scores of AAC, as measured by DEXA [69]. Research suggests a significant association between serum magnesium and carotid atherosclerosis. Lower serum magnesium was demonstrated to be independently associated with increased

carotid intima-media thickness and higher risk of carotid plaques, even in individuals with normal blood pressure [70]. This relationship was observed in both the general population and in patients with high cardiovascular risk or undergoing hemodialysis [70, 71]. However, a systematic review and meta-analysis of randomized clinical trials demonstrated that magnesium supplementation improves endothelial function but not carotid intima-media thickness [72]. It is important to note that carotid intimal-medial thickness measurements have limitations in predicting atherosclerosis compared to measures of plaque area or volume [73]. To date, no study has explored the relationship between magnesium and carotid plaque using MRI.

# 2. Aims and outline of the thesis

The primary objective of the thesis is to investigate the relationship of magnesium with MetS as well as MRI-derived subclinical CVD markers, including early cardiac impairment and preclinical atherosclerosis, in the overall population and in individuals with CKD. We used three markers for magnesium status assessment: serum magnesium, dietary magnesium, and MDS. The cumulative work included three projects. Figure 3 provides a schematic overview of the three research objectives addressed in the thesis.

In project I, our aims were:

(1) Examine the association of serum magnesium with prevalent and incident MetS and its individual components in a sample from the general population (KORA F4 – FF4).
(2) Assess the effect modification by CKD status.
(3) Investigate the association between serum magnesium and prevalent MetS in high-risk populations, including men, individuals with obesity, individuals with vitamin D deficiency, and individuals taking diuretic medications.
(4) Assess the potential causal association between serum magnesium and MetS using a two-sample Mendelian randomization analysis.

In project II, our aims were:

(1) Examine the correlation between serum and dietary magnesium.
(2) Examine the association of serum and dietary magnesium with MRI-derived right and left ventricular morphology and function, such as ejection fraction, end diastolic volume, and remodeling index in a sample from the general population (KORA-MRI).
(3) Investigate the association of serum and dietary magnesium with MRI-derived carotid plaque.
(4) Explore the mediation effect of serum total cholesterol in the association between serum magnesium and carotid plaque.

In project III, our aims were:

(1) Examine the correlation between serum magnesium and MDS (continuous) and assess the distribution of serum magnesium in MDS categories.
(2) Investigate the association of serum magnesium with MetS and MRI-derived right and left ventricular morphology and function in a sample from the general population (NAKO-MRI).
(3) Investigate the association of MDS with MetS and MRI-derived right and left ventricular morphology and function.

(4) Evaluate the potential mediation of MetS in the relationship of serum magnesium and MDS with MRI-derived right and left ventricular morphology and function.

(5) Assess the potential causal association between serum magnesium and MRI-derived right and left ventricular morphology and function using a two-sample MR analysis.

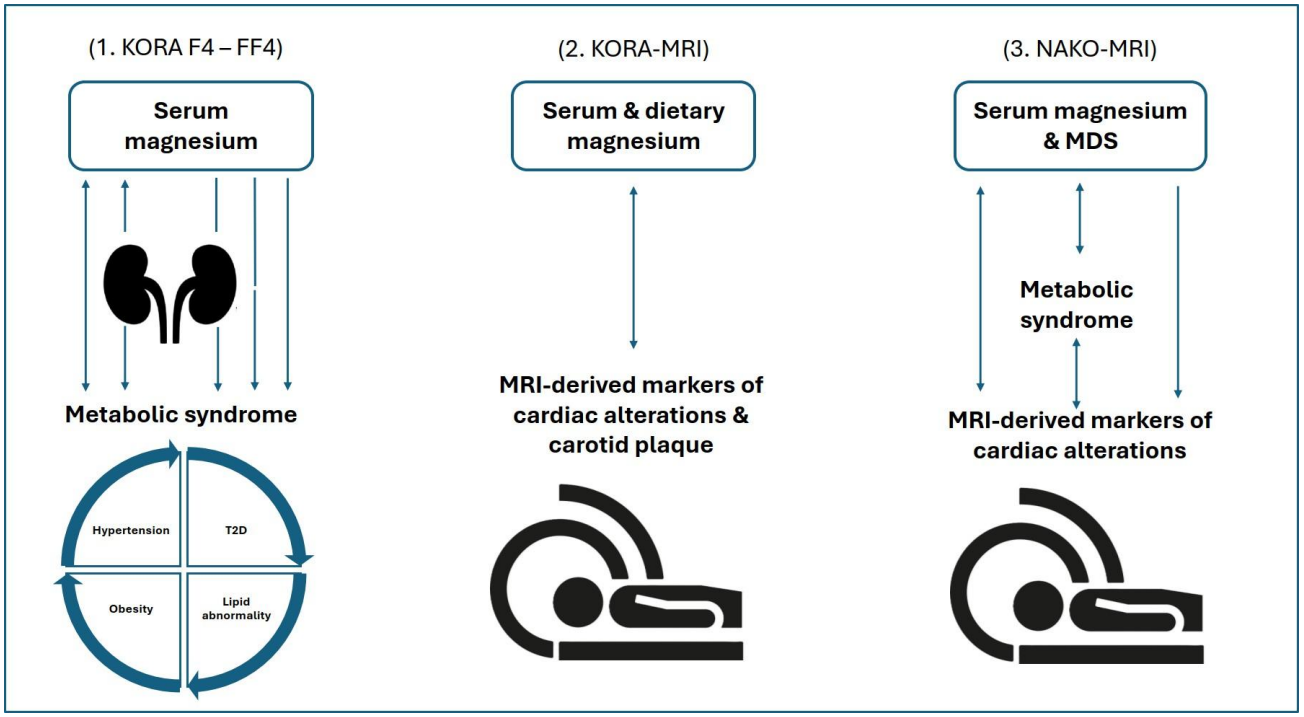


**Figure 3:** Schematic structure of the current thesis: Magnesium and Cariovascular-Kidney-Metabolic (CKM) syndrome. The numbers are corresponding to the three projects included in the thesis. A double-arrowed line represents a prevalenc e association, indicating a relationship without a specific direction. A dashed line signifies an incidence association, reflecting an assumed directional relationship. A line without arrows indicates that only effect modification was explored. A solid line with a single arrow denotes a causal association, examined using two-sample Mendelian randomization. The arrow points toward the outcome. MDS, magnesium depletion score. T2D, type 2 diabetes.

# 3. Methods

## 3.1 Study design and population

The first project of the thesis is based on data from the prospective, population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 and FF4 studies. The second project is based on data from the KORA MRI study, which is a substudy of KORA FF4. For the third project, data were obtained from the MRI baseline examination of the German National Cohort Study (NAKO). For the two-sample Mendelian randomization analysis in project I and project III publicly available genome-wide association studies (GWAS) of European ancestry were used.

### 3.1.1. KORA F4/ FF4 Study

The KORA (Cooperative Health research in the region of Augsburg) framework comprises multiple population-based surveys (S1-4) and their follow-ups, allowing for prospective analyses. KORA F4 (2006–2008) and KORA FF4 (2013-2014) are the first and second follow-up of KORA S4 (1999-2001) [74]. The analysis for the first project was based on the KORA F4/FF4 studies, as serum magnesium was not measured during the baseline S4 survey. The KORA F4 study (n = 3,080, ages 32–81) was used to perform a cross-sectional analysis of prevalent MetS. For the longitudinal analysis of incident MetS, data from KORA F4/FF4, with an average follow-up of 6.5 years, were analyzed.

### 3.1.2. KORA-MRI Substudy

The KORA-MRI study examined subclinical disease in 400 participants from the KORA FF4 study, aged 39–73, who had no previous CVD and were eligible for whole-body MRI [6]. The study's main objective was to evaluate subclinical disease in individuals with prediabetes and diabetes. Using data from KORA MRI in project I, we examined the association of serum and dietary magnesium with MRI-derived CVD markers, including carotid plaque, carotid thickness, and left and right cardiac morphology and function. Due to missing data, the sample sizes for serum and dietary magnesium varied across the respective outcomes.

### 3.1.3. German National Cohort (NAKO) Study

The German National Cohort (NAKO) is Germany's largest population-based cohort study, aimed at investigating disease development, identifying risk factors, and enhancing prevention strategies. From 2014 to 2019, a total of 205,415 participants, aged 19 to 74, were recruited from 18 study centers across the country [75]. Participants underwent comprehensive evaluations, including interviews, questionnaires, biomedical tests, and biosample collection. Additionally, a subgroup of 30,861 participants received whole-body MRI scans [76, 77].

Follow-up examinations are conducted at intervals of 4 to 5 years, with 134,428 participants having completed their second examination by April 2024. Project III utilized data from the MRI sample of the NAKO study's baseline examination to investigate the association between serum magnesium and MDS with MetS and MRI-derived parameters of left and right ventricular morphology and function.

### 3.1.4. Genome Wide Association Studies (GWAS)

For project I and project III, we extracted single nucleotide polymorphisms (SNPs) that reached genome-wide significance ($p < 5 \times 10^{-8}$) from publicly available GWAS data on serum magnesium. These studies were based on a combined analysis of a discovery cohort of 15,366 participants and a replication cohort of 8,463 participants, explaining 1.6% of the variance in serum magnesium levels [78]. The summary statistics for MetS in the first project were derived from the MetS study by Lind et al., which included 291,107 participants [79]. We extracted SNP-outcome associations for the third project from the largest available GWAS on MRI-derived left and right ventricular parameters. For the left ventricle, we used GWAS summary statistics from 16,923 European participants for six measures: end-diastolic volume, end-systolic volume, ejection fraction, left ventricular mass, and remodeling index [80]. Right ventricular measures (end-diastolic volume, end-systolic volume, ejection fraction, and stroke volume) were based on publicly available GWAS data. Analyses included 29,506 UK Biobank participants of European ancestry, free of prior myocardial infarction or heart failure [81].

## 3.2. Assessment of magnesium

Given the limitations of serum magnesium in accurately reflecting total body magnesium status, we employed three distinct biomarkers to enhance the robustness and validity of our analysis. In all projects, we used serum magnesium. Additionally, dietary magnesium was included in Project II, while MDS was used in Project III.

### 3.2.1. Serum magnesium

In all projects, serum magnesium was measured in mmol/L. In KORA F4, a colorimetric assay using the Chlorophosphonazo III method was performed on the Roche Cobas C system, with ethylene glycol tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) added as chelating agents. In KORA FF4 and the NAKO study, serum magnesium was measured using the modified methylthymol blue (MTB) method on the Siemens Dimension Vista system. Calcium interference was minimized using Ba-EGTA, and the magnesium-MTB complex was quantified at wavelengths of 600 and 510 nm.

### 3.2.2. Dietary magnesium

Dietary magnesium intake was measured in mg/day using a combination of three 24-hour food lists (24H-FL) and a food frequency questionnaire (FFQ). The 24H-FL, which included 246 food items, assessed food consumption over the past day, while the FFQ, with 148 items, captured dietary habits over the previous 12 months [82]. The detailed measurement process is outlined in the manuscript to Project II.

### 3.2.3. Magnesium Depletion Score (MDS)

MDS was used to assess magnesium status in the body, calculated by summing the following scores [25]: (1) One point for current use of diuretics; (2) One point for current use of proton pump inhibitors (PPIs); (3) One point for an estimated glomerular filtration rate (eGFR) between 60 mL/min/1.73 m² and 90 mL/min/1.73 m², with two points assigned for an eGFR below 60 mL/min/1.73 m²; (4) One point for heavy alcohol consumption, defined as more than one drink per day for women (14 g/day) and more than two drinks per day for men (28 g/day). The score ranges from 0 to 5. For our analysis in project III, we combined the last three categories, resulting in the following groups: (0, 1, 2, ≥3). Higher scores reflect a greater degree of magnesium deficiency.

## 3.3. Assessment of Cardiovascular-Kidney-Metabolic (CKM) syndrome

This thesis focuses on the second stage, involving MetS and CKD, and the third stage, through utilizing MRI-derived markers of subclinical CVD within the CKM syndrome framework.

### 3.3.1. Metabolic syndrome assessment

In both Project I and Project II, MetS was defined using the harmonized criteria [83], which require the presence of at least three of the following factors: (1) elevated waist circumference (≥94 cm in men or ≥80 cm in women); (2) elevated triglycerides (serum fasting triglycerides ≥150 mg/dL or the use of fibrates); (3) reduced HDL cholesterol (HDL-C <40 mg/dL in men or <50 mg/dL in women, or the use of fibrates); (4) elevated blood pressure (systolic ≥130 mmHg or diastolic ≥85 mmHg, or treatment with antihypertensive medication); (5) elevated fasting glucose (fasting serum glucose ≥100 mg/dL or the use of antidiabetic medication). In Project I, we also employed alternative definitions of MetS, such as the diagnostic criteria established by the Adult Treatment Panel III and the International Diabetes Federation, to demonstrate the robustness of our findings.

### 3.3.2. Chronic kidney disease assessment

In Project I, we investigated the role of CKD in the association of serum magnesium and MetS and its components. CKD was determined by the presence of either an estimated eGFR below 60 mL/min/1.73 m², albuminuria (urine albumin-to-creatinine ratio [UACR] of 30 mg/g or higher), or both. In Projects II and III, however, we were unable to investigate the role of CKD. In the KORA-MRI study (Project II), the use of contrast-enhanced whole-body MRI required participants to have normal kidney function (creatinine < 1 mg/dL), as CKD constituted a contraindication to contrast administration. Similarly, in the NAKO MRI study (Project III), the very low prevalence of CKD (0.8%) limited our ability to explore its potential role in the observed associations.

### 3.3.3. Subclinical cardiovascular disease assessment

Whole-body MRI examinations in the KORA MRI study were conducted using a 3 Tesla Magnetom Skyra (Siemens AG, Healthcare Sector, Erlangen, Germany) equipped with an 18-channel body coil system. All image analyses were performed by independent readers who were blinded to the participants' clinical status. Regarding heart analysis, 4-chamber view steady-state free precession (SSFP) and short-axis stack SSFP sequences were employed. Axial black-blood T1-weighted fat-saturated imaging was used to evaluate carotid plaque. A detailed assessment and information on the markers used in MRI examinations of left and right ventricular morphology and function, as well as carotid plaque, are provided in the manuscript of Project II.

In the NAKO MRI study, whole-body MRI assessment was performed using a standardized protocol that included cardiovascular magnetic resonance imaging on 3T MR scanners (MAGNETOM Skyra, Siemens Healthineers, Erlangen, Germany). The protocol included the acquisition of steady-state free precession (SSFP) CINE sequences in a 2D, multi-slice, short-axis (SAX) orientation, capturing the heart from apex to base throughout the entire cardiac cycle. Clinical cardiac MRI metrics were obtained by segmenting the epicardial and endocardial borders utilizing an enhanced nnU-net framework, with comprehensive quality control measures implemented to assess both image and segmentation accuracy, as well as to identify and address imaging artefacts [84].

In Projects II and III, we analyzed left and right ventricular end-diastolic and end-systolic volumes, stroke volume, ejection fraction, and left ventricular end-diastolic mass. Cardiac volumes and mass were indexed to body surface area (BSA), which was calculated using the Du Bois formula: BSA (m²) = 0.007184 × weight (kg)^0.425 × height (cm)^0.725. Additionally, Project II incorporated carotid plaque markers, including the presence and type of plaque, as well as left and right carotid wall thickness.

## 3.4. Statistical analysis

In project I, we used multivariable logistic regression to demonstrate the association between serum magnesium (per 1 standard deviation) as the exposure and prevalent and incident MetS, as well as its individual dichotomous components, in the overall sample and stratified by CKD status. Three models were employed for adjustment: Model 1 adjusted for age and sex; Model 2 expanded on this by including smoking status, alcohol consumption, and physical activity; and Model 3 further incorporated diuretic medication and serum potassium. The exposure-response relationship between serum magnesium and prevalent MetS and its components was visualized using restricted cubic spline models, adjusted for the variables in Model 3. Additionally, we conducted stratified analyses to explore the cross-sectional association between serum magnesium and MetS across various subgroups, including sex, menopausal status, CKD status, serum vitamin D (25OHD) status, body mass index (BMI) category, and diuretic medication use, employing a logistic regression analysis with a multiplicative interaction approach. Finally, a two-sample MR analysis was performed to investigate the potential causal relationship between serum magnesium and MetS. The inverse-variance weighting (IVW) method was used as the primary MR estimate, supplemented by three robust MR approaches: the weighted-median approach, the weighted mode approach, and the MR-Egger approach.

In Project II, we evaluated the correlation between serum magnesium (and dietary magnesium using Spearman's rho correlation coefficient. Subsequently, we employed multivariable linear regression to investigate the associations of both serum and dietary magnesium (on a continuous scale per 1 SD with MRI markers of left and right ventricular morphology and function, as well as carotid plaque. Categorical MRI markers were analyzed using either logistic regression or ordered logistic regression, where appropriate. The models were adjusted for age, sex, BMI, systolic blood pressure, diabetes status, smoking status, and serum total cholesterol. For analyses involving dietary magnesium intake, models were additionally adjusted for daily caloric intake. Furthermore, we conducted causal mediation analysis to determine whether the relationship between serum magnesium and carotid plaque was mediated by serum total cholesterol.

In project III, Spearman's rho correlation was used to assess the correlation between serum magnesium and MDS (continuous). We investigated the associations of serum magnesium and MDS with MetS and MRI-derived markers of cardiac morphology and function using multivariable logistic and linear regression, respectively. Since MDS inherently encompasses potential confounders such as eGFR and diuretic use, the adjustment strategies differed between the analysis of serum magnesium and MDS. The adjustment strategy is explained in

detail in project III. We assessed the association of MDS with left ventricular concentricity using multivariable logistic regression. Subgroup analyses were performed based on age, sex, MetS, BMI (≥25 vs. <25), elevated waist circumference, hypertension, and type 2 diabetes, and formal tests for multiplicative interaction were conducted. Finally, we performed a two-sample Mendelian randomization analysis to explore the causal association between serum magnesium and MRI-derived markers of cardiac morphology and function.

In all included papers, statistical significance was determined by *p*-value below 0.05. In the third project, we used false discovery rate (FDR, Benjamini–Hochberg method) to correct for multiple testing. All analyses were carried out with R version 4.1.2 (R Core Team, Vienna, Austria).

# 4. Results

**Key findings 1: Serum magnesium was associated with prevalent MetS, with the association being more pronounced in individuals with CKD and those at higher metabolic risk. There was no association between serum magnesium and incident MetS, despite MR analysis suggesting a potential causal relationship.**

At baseline, 1,052 individuals (35%; mean age 62.1 years, 60.2% men) had prevalent MetS, and over 6.5 years of follow-up, 251 individuals (17%; mean age 57.1 years, 54.6% men) developed incident MetS. For the cross-sectional analysis, serum magnesium was inversely associated with MetS after controlling for different cardiovascular risk factors (OR 0.90, 95% CI 0.83–0.98), with a stronger effect in individuals with CKD (OR 0.75, 95% CI 0.59–0.94). The inverse association was more pronounced in men, individuals with obesity, and those taking diuretic medication. Among MetS components, serum magnesium was inversely associated with elevated fasting glucose. Restricted cubic spline analysis indicated a nonlinear relationship, with protective effects plateauing beyond a certain serum magnesium level. In the longitudinal analysis, we did not find an association between serum magnesium and incident MetS or any of its components. MR analyses suggested a causal link between genetically predicted serum magnesium and lower odds of MetS (IVW OR 0.91, 95% CI 0.85–0.97; weighted median OR 0.91, 95% CI 0.84–0.99).

**Key findings 2: Serum and dietary magnesium showed distinct associations with MRI-based markers of subclinical cardiovascular disease**

There was no correlation between serum and dietary magnesium (Spearman's rho = 0.04, p = 0.5), which may partially explain the contradicting directions of results. Serum magnesium was linked to decreased ventricular volumes, particularly right end-diastolic volume (coefficient: -1.21mL/$m^2$; 95% CI -2.39mL/$m^2$ to -0.04mL/ $m^2$), while dietary magnesium was associated with increased ventricular systolic and diastolic volumes, such as left end-diastolic volume (coefficients: 0.06 mL/$m^2$; CI 0.014 mL/$m^2$ to 0.114 mL/ $m^2$). Dietary magnesium intake was associated with decreased remodeling index (coefficients: -0.001 g/mL/$m^2$; CI -0.001 g/mL/$m^2$ to -0.0002 g/mL/$m^2$). Additionally, dietary magnesium intake was inversely associated with severe plaque (OR 0.99; 95% CI 0.98 to 0.99). These associations remained significant after adjusting for sex, age, and cardiovascular risk factors like hypertension and diabetes. The association between serum magnesium and carotid plaque was not mediated by serum total cholesterol (coefficient: 0.003 mg/dl; 95% CI - 0.06 mg/dl to 0.12 mg/dl).

**Key findings 3: Higher MDS was associated with prevalent MetS and unfavorable alterations in cardiac morphology and function, especially markers related to diastolic**

**function, such as decreased end-diastolic volume and increased left ventricular remodeling index. Two-sample Mendelian randomization did not support a causal relationship between serum magnesium and MRI-derived cardiac parameters.**

Out of 9,568 participants (mean age: 45.9 ± 12.2 years; 58.0% men), 30.4% had MetS. Our finding indicated no substantial correlation between serum magnesium and the continuous MDS (Spearman's rho = 0.065; $p < 0.001$). A 1 SD increase in serum magnesium was associated with a lower prevalence of MetS (OR 0.93 [95% CI: 0.88, 0.99]) and a reduction in both left and right systolic and diastolic ventricular volumes. Higher MDS, which indicates magnesium deficiency, was linked to an increased prevalence of MetS (OR 1.32 [95% CI: 1.23, 1.41]) and its individual components. Additionally, higher MDS was associated with unfavorable changes in cardiac structure, including a higher left ventricular remodeling index (Estimate 0.012 g/mL [95% CI: 0.008, 0.017]) and a decrease in left ventricular end-diastolic volume (Estimate -1.132 mL/m² [95% CI: -1.538, -0.727]), markers of concentric hypertrophy. These associations were attenuated to the point of becoming non-significant in individuals with MetS. Furthermore, MDS was positively associated with left ventricular concentricity (OR per 1 unit 1.33 [95% CI: 1.06, 1.65]), particularly in subgroups at higher risk, such as men, those with obesity, and individuals with MetS. Two-sample Mendelian randomization analysis revealed no evidence for a causal relationship between serum magnesium and MRI-derived markers of cardiac alterations.

# 5. Discussion

The findings of this thesis suggest an association between magnesium depletion and stages two and three of CKM syndrome. Our results further emphasize the importance of using multiple methods to evaluate magnesium status. To assess the magnesium-CKM associations, we employed three key markers: serum magnesium, dietary magnesium, and the newly developed and validated MDS.

We have shown that serum magnesium does not correlate well with dietary magnesium and MDS. This could be attributed to several reasons. First, serum magnesium comprises merely 0.3% of the total magnesium in the body, which may not be sufficient to accurately reflect overall magnesium levels in the body [18, 19]. Second, magnesium concentration is maintained through a balance between intestinal absorption and kidney excretion. Conditions that impair intestinal absorption, such as aging, inflammation, bowel disorders, or reduced kidney function, can impact magnesium bioavailability [85]. Most importantly, Serum magnesium is tightly regulated by homeostatic mechanisms, allowing it to remain normal even with low intake by decreasing urinary excretion and mobilizing magnesium from bones, muscles, and internal organs [86].

Our findings indicate that magnesium serves as a marker for both metabolic disorders and subclinical CVD. We observed an inverse relationship between serum magnesium and MetS, with evidence suggesting a potentially causal link, as indicated by MR analysis. This association was demonstrated in high-risk populations, such as men and individuals with obesity. Additionally, both serum and dietary magnesium were associated with various MRI parameters related to cardiac morphology and function, as well as carotid plaque presence. Furthermore, we demonstrated that a higher MDS, indicative of magnesium deficiency, was linked to cardiac alterations, particularly left ventricular remodeling index and concentricity.

Our results emphasize the crucial role of the kidneys in regulating magnesium homeostasis, subsequently contributing to metabolic impairment. It is important to mention that disturbances in magnesium balance are frequently seen in individuals with CKD, mainly due to reduced renal reabsorption, diuretic use, and dietary modifications [87]. Evidence indicates that hypomagnesemia was the most common electrolyte disturbance in a cohort of 2,126 predialysis patients, and proteinuria was found to be an independent risk factor for magnesium loss [53]. We demonstrated that the inverse association between serum magnesium and MetS was stronger in individuals with CKD, and this relationship became weaker when CKD was defined by eGFR without considering proteinuria. In the third project, we utilized MDS, which offers a more comprehensive evaluation of magnesium status. The MDS takes into account factors that hinder renal magnesium reabsorption, such as alcohol consumption, diuretic use,

PPI use, and kidney disease [25]. As a result, it may be a more reliable indicator of magnesium deficiency associated with metabolic dysfunction.

Our findings suggest a link between magnesium and diastolic function of the heart (Figure 4). In Project II, we demonstrated that higher magnesium intake was linked to improved diastolic function, since higher dietary magnesium intake was associated with a larger left ventricular end-diastolic volume and a lower left ventricular remodeling index. Project III supports these results by showing a relationship between magnesium deficiency and diastolic dysfunction through preclinical concentric hypertrophy. A higher MDS, indicating hypomagnesemia, was associated with a smaller left ventricular end-diastolic volume and an increased left ventricular remodeling index. This relationship was notably stronger in people with greater metabolic risk, such as men, individuals with overweight and obesity, and individuals with MetS. This is consistent with experimental findings showing impaired relaxation and a reduced ratio between early and late diastolic velocity of the mitral valve in mice fed a low-magnesium diet, which was reversed following magnesium repletion [64]. A population-based study further supports these findings by showing an inverse association between serum magnesium and left myocardial mass [65].

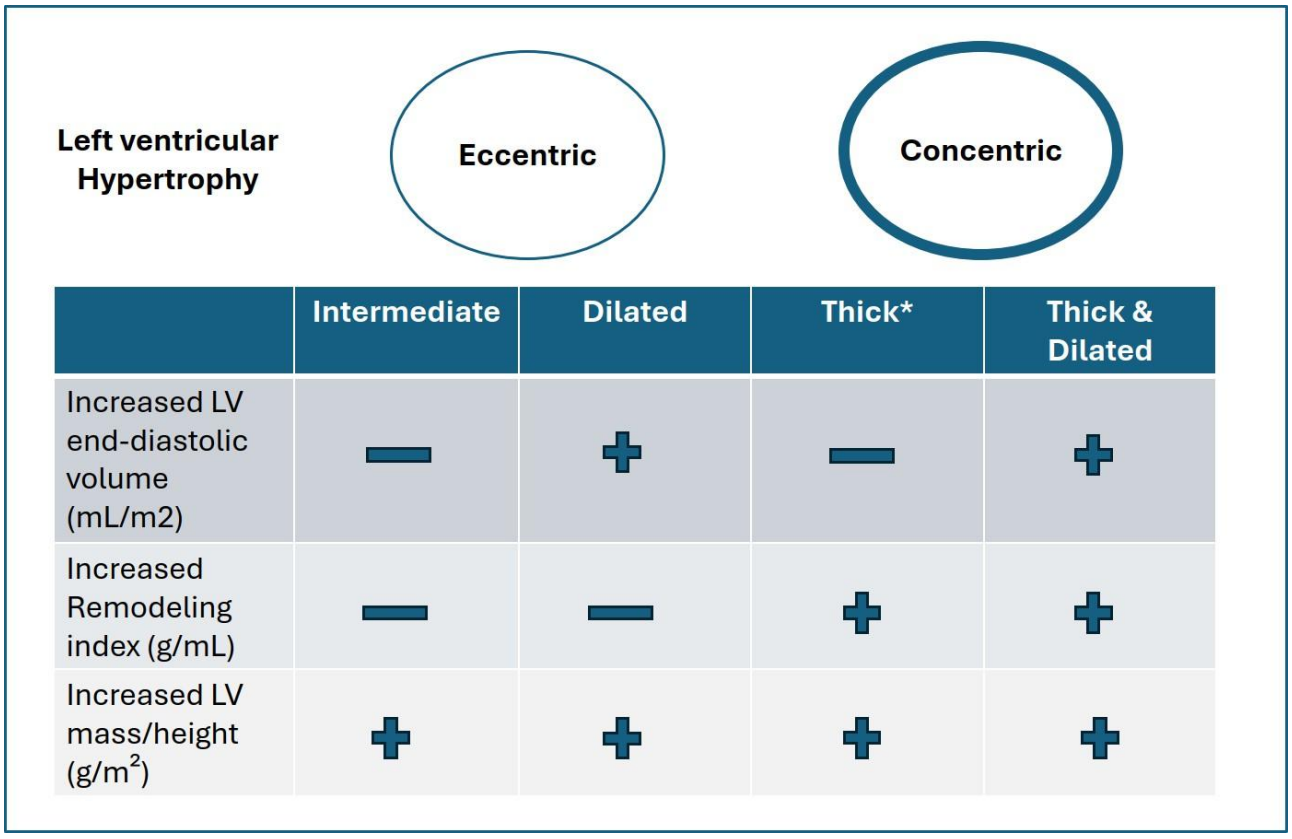


**Figure 4:** Schematic representation of the different classifications of left ventricular hypertrophy. Figure adapted from the 4 Tiered Classification of Left Ventricular Hypertrophy from the Dallas Heart Study [88]. * Results from this thesis indicate that magnesium deficiency is associated with an increased remodeling index and decreased end-diastolic volume, potentially suggesting concentric hypertrophy of the left ventricle. LV, left ventricle.

A more comprehensive discussion of the results and their comparison with existing literature can be found in the respective manuscripts for Projects I–III.

## Strengths and limitations

A key strength of this study was the use of advanced MRI techniques to measure functional and structural cardiac parameters as markers of subclinical CVD burden, in contrast to previous studies that relied on echocardiography and ultrasound. A further strength was the availability of three measurements of magnesium status, namely serum magnesium, dietary magnesium, and MDS Additionally, the studies included in this thesis utilized well-designed, population-based data with comprehensive measurements and thorough assessment of potential confounders. Furthermore, the availability of GWAS data for serum magnesium, MetS, and MRI-based cardiac parameters enabled the use of Mendelian randomization analysis to assess causality.

Despite its strengths, this thesis had several limitations. The sample size in Project II was relatively small, which reduced the study's power to detect associations. Since the study design in both Project II and Project III was cross-sectional, we were unable to establish the temporality or causality of the associations between serum magnesium, MDS, and MRI parameters. A further limitation was the lack of dietary magnesium intake data in the NAKO study, as well as the absence of information on magnesium supplementation and urinary magnesium across the studies included in this thesis. This may have restricted the ability to fully assess magnesium status and its potential benefits. Another limitation in Project III was the absence of GWAS data for MDS, which limited our ability to investigate its genetic determinants and assess potential causal relationships.

# 6. Conclusion and Outlook

The growing burden of CVD, coupled with the fact that traditional risk factors cannot be entirely attributed to this increase, underscores the need to identify new biomarkers that may contribute to this rise. Additionally, the early detection of subclinical CVD, including conditions such as MetS and cardiac impairment, is essential for improved risk stratification and early intervention. Understanding these biomarkers—whether causal, to serve as potential targets for intervention, or diagnostic, to support early detection and prevention—will play a critical role in developing more effective strategies to combat the increasing incidence of CVD.

Magnesium may serve as one of these potential biomarkers, given its well-established cardiac protective functions. A deficiency in magnesium is associated with various pathophysiological processes that contribute to the development of both subclinical and overt cardiovascular disease. However, it is crucial to ensure robust measurement of magnesium status, as traditional methods have inherent limitations. Therefore, we recommend using multiple measurement approaches to validate the results and obtain a more accurate assessment of magnesium levels.

The role of magnesium deficiency as a predictor of cardiometabolic outcomes remains unclear. In this thesis, we were unable to establish a prospective association between serum magnesium and MetS or its individual components. Although MR suggested a potential causal link, the evidence remains weak, as the MR sensitivity analyses were insignificant. Furthermore, due to the cross-sectional nature of our analyses, we could only demonstrate non-directional associations between magnesium deficiency and MRI-based cardiac alterations. Therefore, further prospective studies, including clinical trials, are warranted to investigate the potential causal relationship, particularly examining the effect of magnesium supplementation on improving MetS components and its impact on cardiac parameters.

The kidney plays a crucial role in maintaining magnesium homeostasis, and previous studies have suggested that magnesium deficiency is the most common electrolyte imbalance, with proteinuria contributing to increased magnesium wasting. This underscores the importance of kidney function in magnesium regulation. Our findings in Project III support this, as we observed that using the MDS, which considers factors that impair magnesium reabsorption in the kidneys, showed stronger associations with MetS and cardiac parameters compared to serum magnesium alone. Additionally, in Project I, we found that serum magnesium was inversely associated with MetS in individuals with chronic kidney disease and those taking diuretic medications. Together, this evidence highlights the significant role of the kidney in the relationship between magnesium and cardiometabolic diseases.

The results of this thesis highlight the link between magnesium deficiency and prevalence of subclinical CVD, in the total population and in individuals with high risk, and suggest that magnesium could serve as a diagnostic marker of cardiometabolic health and early cardiac impairment. This finding may potentially guide physicians in identifying high-risk individuals who would benefit from magnesium supplementation.

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# 8. Publications

## Project I

| | |
|---|---|
| Title: | Association of serum magnesium with metabolic syndrome and the role of chronic kidney disease: A population-based cohort study with Mendelian randomization |
| Authors: | Nuha Shugaa Addin, Fiona Niedermayer, Barbara Thorand, Jakob Linseisen, Jochen Seissler, Annette Peters, Susanne Rospleszcz |
| Journal: | Diabetes, Obesity and Metabolism |
| Year: | 2024 |
| Volume: | 26 |
| Issue: | 5 |
| Pages: | 1808 - 1820 |
| DOI: | https://doi.org/10.1111/dom.15497 |
| Impact factor: | 5.4 |
| Rank: | 27/186 in Endocrinology & Metabolism<br>(Journal Citation Reports™, year 2023) |

Received: 13 November 2023 | Revised: 25 January 2024 | Accepted: 31 January 2024

DOI: 10.1111/dom.15497

ORIGINAL ARTICLE

WILEY

# Association of serum magnesium with metabolic syndrome and the role of chronic kidney disease: A population-based cohort study with Mendelian randomization

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Münchner Zentrum für Gesundheitswissenschaften, Ludwig-Maximilians-Universität München; Bundesministerium für Bildung und Forschung (BMBF); Hanns-Seidel-Stiftung

## Abstract

Objectives: To assess the association of serum magnesium with prevalent and incident metabolic syndrome (MetS) and its individual components in the general population and to examine any effect modification by chronic kidney disease (CKD) status.

Methods: We analysed longitudinal data from the population-based KORA F4/FF4 study, including 2996 participants (387 with CKD) for cross-sectional analysis and 1446 participants (88 with CKD) for longitudinal analysis. Associations with MetS, as well as single components of MetS, were assessed by adjusted regression models. Nonlinearity was tested by restricted cubic splines and analyses were stratified by CKD. Causality was evaluated by two-sample Mendelian randomization (MR).

Results: Serum magnesium (1 SD) was inversely associated with prevalent MetS (odds ratio [OR] 0.90, 95% confidence interval [CI] 0.83, 0.98). The association was more pronounced in individuals with CKD (OR 0.75, 95% CI 0.59, 0.94). Among MetS components, serum magnesium was negatively associated with elevated fasting glucose (OR 0.78, 95% CI 0.71, 0.88) and, again, this association was more pronounced in individuals with CKD (OR 0.67, 95% CI 0.53, 0.84). Serum magnesium was not associated with incident MetS or its components. Restricted cubic spline analysis revealed a significant nonlinear inverse relationship of serum magnesium with MetS and elevated fasting glucose. MR analysis suggested an inverse causal effect of serum magnesium on MetS (OR 0.91, 95% CI 0.85, 0.97).

Conclusion: Serum magnesium is associated with prevalent, but not incident MetS, and this effect is stronger in individuals with CKD. MR analysis implies a potential, albeit weak, causal role of magnesium in MetS.

KEYWORDS
chronic kidney disease, Mendelian randomization, metabolic syndrome, population-based cohort, serum magnesium

## 1 | INTRODUCTION

Increased cardiovascular risk is found in individuals with metabolic syndrome (MetS), a condition characterized by a clustering of interrelated risk factors, namely, abdominal obesity, hyperglycaemia, dyslipidaemia, and elevated blood pressure.[1] MetS affects 25% of the adult population and its prevalence continues to rise, aggravated by the obesity pandemic caused by unhealthy eating habits and a sedentary lifestyle within an obesogenic environment.[2,3] While the exact pathogenesis of MetS is still unknown, a complex interaction of genetic, metabolic and environmental factors is suggested.[4]

The prevalence of chronic kidney disease (CKD) is dramatically increasing, affecting over 10% of the general population worldwide.[5] Kidney impairment is considered an independent risk factor for cardiovascular disease development.[6] Evidence demonstrated a significant association between MetS and CKD prevalence and progression, with a 5.34 odds of estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 $m^2$ and a 2.91 odds of albuminuria of 30 mg/g or higher.[7] Consequently, individuals with CKD experience heightened cardiovascular morbidity and mortality, emphasizing the importance of identifying novel biomarkers to improve clinical outcomes.

Magnesium, the fourth most abundant mineral in the body, is a crucial cofactor in numerous enzymatic reactions involved in a multitude of metabolic processes. Magnesium deficiency occurs either due to inadequate intake, decreased absorption, or increased excretion.[8] Because 80% of this mineral is lost during food processing, a significant proportion of the global population fails to meet the minimum daily magnesium requirement.[9]

Hypomagnesaemia, which usually presents as a subclinical, chronic latent deficiency, has been associated with several cardiometabolic diseases.[8] A systematic review and meta-analysis has revealed an inverse association between magnesium and prevalent MetS.[10] However, there is a scarcity of studies that specifically investigate this association in high-risk populations (e.g., men, individuals with obesity, individuals with diuretic medication intake). Furthermore, limited information is available on the association of magnesium with incident MetS and whether any putative association is causal.

The kidney is the chief organ responsible for regulating magnesium homeostasis. There is evidence that hypomagnesaemia is the predominant electrolyte abnormality observed in predialysis CKD patients, and its association with adverse clinical outcomes has been well established.[11,12] The relationship between magnesium and MetS was previously investigated in CKD and post-kidney transplant patients.[13,14] However, to our knowledge, no population-based study has explored this association in individuals with CKD. Given the major public health impact of both MetS and CKD, it is necessary to investigate these associations at a population-based level.

In this study, therefore, we aimed to assess the association of serum magnesium with prevalent and incident MetS and its individual components in the general population and to examine any effect modification by CKD status. We also explored the link between serum magnesium and prevalent MetS in individuals at higher risk. Moreover, we aimed to assess the causality of this putative association using two-sample Mendelian randomization (MR) analysis.

## 2 | METHODS

### 2.1 | Study population

The Cooperative Health Research in the Region of Augsburg (baseline KORA S4, 1999–2001, first-follow-up F4, 2006–2008, second follow-up FF4, 2013–2014) is a population-based cohort study designed to assess the risk factors, prevalence and trajectories of cardiometabolic outcomes. Participants were examined at the study centre, where detailed clinical and demographic information was collected and blood was drawn.[15] Since serum magnesium was not measured in the baseline S4 survey, the present analysis is based on data from KORA F4 and FF4.

KORA F4 ($n$ = 3080, age 32–81 years), was used for cross-sectional analysis of prevalent MetS. KORA F4/FF4 (mean follow-up time 6.5 years) was used for longitudinal analysis of incident MetS. After applying the exclusion criteria listed in Figure S1, the respective sample sizes for cross-sectional and longitudinal analyses were $n$ = 2996, of whom 387 had CKD, and $n$ = 1446, of whom 88 had CKD.

The study was performed in adherence to the declaration of Helsinki, including approval from the ethics committee of the Bavarian Chamber of Physicians (Munich, Germany) and written informed consent from all participants.

### 2.2 | Serum magnesium assessment

Serum magnesium was measured in mmol/L with a colorimetric assay (Chlorophosphonazo III method) with the addition of ethylene glycol tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) as chelating agents on the Roche Cobas C system.[16]

### 2.3 | MetS assessment

According to the harmonized definition,[17] MetS was defined as the presence of at least three of the following criteria: (1) waist circumference ≥ 94 cm in men or ≥ 80 cm in women (elevated waist circumference); (2) serum fasting triglycerides ≥150 mg/dL (1.69 mmol/l) or the use of fibrates (elevated triglycerides); (3) serum high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL (1.03 mmol/l) in men or < 50 mg/dL (1.29 mmol/l) in women or the use of fibrates (reduced HDL-C); (4) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or treatment with antihypertensive medication, being aware of having hypertension (elevated blood pressure); (5) fasting serum glucose level ≥ 100 mg/dL (5.6 mmol/l) or intake of antidiabetic medication (elevated fasting glucose). As a sensitivity analysis, we used alternative definitions of MetS (Table S1).[18]

Incident MetS was determined based on the presence of at least three of the above criteria at follow-up visits after exclusion of prevalent cases at baseline. For example, incident elevated waist circumference was defined as waist circumference < 94 cm in men or < 80 cm in women at baseline and waist circumference ≥ 94 cm in men or ≥ 80 cm in women at the follow-up visit. Due to the exclusion of prevalent cases, the sample sizes differ between the analyses of each individual component (Table 3).

## 2.4 | CKD assessment

Chronic kidney disease was assessed based on the presence of either eGFR < 60 mL/min per 1.73 $m^2$ or albuminuria (urine albumin-creatine ratio [UACR] ≥ 30 mg/g), or both.[19] Glomerular filtration rates were estimated from serum creatinine concentrations according to the Chronic Kidney Disease Epidemiology Collaboration equation.[20] As a sensitivity analysis, we used the CKD definition based on eGFR only.

## 2.5 | Covariates assessment

Physical activity was obtained according to the time spent per week on leisure time sport activities in summer and winter, using a four-category interview question: (1) > 2 h, (2) 1–2 h, (3) <1 h and (4) none. The total physical activity score was calculated by combining the responses for both summer and winter. Individuals with a total score of 5 or higher were categorized as 'physically inactive', otherwise 'physically active'. Smoking status was categorized as never smoker, former smoker, and current smoker. We classified alcohol consumption as none (0 g/day), moderate (0.1–39.9 g/day for men and 0.1–19.9 g/day for women), and excessive (≥40 g/day for men and ≥20 g/day for women).[21] BMI was categorized as underweight (<18.5 kg/$m^2$), normal weight (18.5–25 kg/$m^2$), overweight (25–30 kg/$m^2$), and obesity (≥30 kg/$m^2$). Menopausal status was classified as postmenopausal, premenopausal, and on hormone replacement therapy. Women were considered postmenopausal if they had no menses for more than 12 consecutive months, had hysterectomy with or without bilateral oophorectomy, or were over the age of 60 years.

Laboratory parameters, including total cholesterol, HDL-C, and low-density lipoprotein cholesterol (LDL-C) were measured using standardized methods as described previously.[22] Serum concentrations of 25-hydroxyvitamin D (25OHD) were measured according to an electrochemiluminescence immunoassay method (Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany). We categorized serum 25OHD according to the US Endocrine Society into a deficient group (<20 ng/mL), a suboptimal group (20–29 ng/mL), and a sufficient group (>29 ng/mL).[23]

## 2.6 | Statistical analysis

Participants' baseline characteristics are presented as mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables, whereas categorical variables are reported as counts with corresponding percentages. Differences according to presence/incidence of MetS and CKD were evaluated by *t*-test, Mann–Whitney *U*-test or chi-squared test, where appropriate. Correlations between serum magnesium and cardiovascular risk factors were assessed by Spearman's correlation coefficients.

We used logistic regression to quantify the association between baseline serum magnesium as exposure and prevalent or incident MetS and the five underlying dichotomous components as outcomes. For the longitudinal analysis, we excluded individuals who had pre-existing MetS and its components at baseline. Furthermore, we used linear regression to quantify the association between serum magnesium and continuous variables constituting the underlying components of prevalent MetS (waist circumference, blood pressure, blood glucose, triglycerides, HDL-C) as outcomes. All analyses were conducted in the total population and according to CKD status. Confounder adjustment was as follows: Model 1 was adjusted for age and sex; Model 2 included variables in Model 1 plus smoking status, alcohol consumption, and physical activity; and Model 3 included variables in Model 2 plus diuretic medication and serum potassium. Nonlinearity was visually examined by restricted cubic splines adjusted for Model 3, with three knots (10th, 50th and 90th percentile) as quantified by a likelihood ratio test. Moreover, we performed subgroup analyses according to sex, menopausal status, CKD status, serum 25OHD status, BMI category and diuretic medication intake, and multiplicative interaction was examined.

We carried out several sensitivity analyses. In the cross-sectional analysis of serum magnesium with MetS, in addition to the full adjusted model, we performed mutual adjustment for each individual component of MetS. Furthermore, in the analyses not stratified for CKD, we adjusted for renal function-related indicators, such as serum phosphorus, serum calcium, serum sodium, serum 25OHD, eGFR and UACR. In the longitudinal analysis, we repeated the analysis between serum magnesium and incident MetS using Poisson regression given the relatively small number of MetS events.

Serum magnesium was centred and scaled per SD before regression modelling. Results are provided as odds ratios (ORs), rate ratios and β-coefficients per 1-SD increase, with corresponding 95% confidence intervals (CIs) for logistic, Poisson and linear regression, respectively. All analyses were carried out with R version 4.1.2 (R Core Team, Vienna, Austria) with a level of significance set at 5%.

## 2.7 | MR analysis

Mendelian randomization uses genetic variants as proxies for exposure to infer causality. Three assumptions are required: a strong association between the genetic variant and exposure; no confounding by factors affecting exposure–outcome association; and exclusive influence of the genetic variant on outcome through the exposure.[24]

Using publicly available summary-level data from genome-wide association studies (GWAS), we conducted a two-sample MR study to assess the causal association between serum magnesium and MetS.

We identified and included six single-nucleotide polymorphisms (SNPs) that exhibited strong and independent associations with serum magnesium at genome-wide significance ($p < 5 \times 10^{-8}$). These SNPs were derived from a combined analysis of a discovery cohort ($n$ = 15 366 individuals) and a replication cohort ($n$ = 8463 individuals) which could explain 1.6% of serum magnesium variance.[25] We then extracted the outcome summary statistics from the study on MetS by Lind et al. ($n$ = 291 107 individuals) for the selected instrumental variables.[26] As rs7965584 was not present in the dataset on MetS, we included a proxy rs10858938 in high linkage disequilibrium ($r^2$ = 0.95). Detailed information on the employed SNPs is given in Table S2.

As MR methods, we used inverse variance-weighted (IVW), weighted median, MR-Egger, simple mode, and weighted mode. To evaluate the robustness of our findings, we repeated the MR analysis excluding two SNPs (rs448378 and rs4072037) known to be associated with MetS components, namely, blood pressure and fasting blood sugar.[25] We scaled all ORs per 0.1-mmol/L increase in serum magnesium. The 'TwoSampleMR' R-package was used for analysis.[27]

## 3 | RESULTS

### 3.1 | Characteristics of the study population

At baseline, 1052 individuals (prevalence 35%; mean age: 62.1 ± 11.4 years; 60.2% men) had prevalent MetS and during a follow-up of 6.5 years, 251 individuals (incidence rate 17%; mean age: 57.1 ± 10.8 years; 54.6% men) developed incident MetS (Table 1). Individuals with prevalent and incident MetS had a more unfavourable metabolic and cardiovascular risk profile, such as older age and higher BMI, dyslipidaemia, and vitamin D deficiency. Furthermore, participants with prevalent MetS were more likely to have CKD compared to those without MetS (22.3% vs. 7.8%; $p$ value < 0.001). Individuals with incident MetS had a significantly lower eGFR compared to those without incident MetS (87.5 vs. 93.4; $p$ < 001), although the prevalence of CKD was not significantly different (7.6% vs. 5.8%; $p$ = 0.349). Baseline characteristics of participants with prevalent and incident MetS stratified by CKD status are shown in Tables S3 and S4, respectively.

In both the overall sample and among individuals with CKD, Spearman correlation analysis revealed a significant negative correlation between serum magnesium and fasting glucose, and positive correlations between serum magnesium and serum sodium and potassium, cholesterol, and triglyceride levels (Table S5).

### 3.2 | Association of serum magnesium and prevalent MetS

Logistic regression demonstrated a significant association between a 1-SD increase in serum magnesium and lower odds of prevalent MetS (OR 0.90 [95% CI 0.83, 0.98] in the fully adjusted model; Table 2). This association was more pronounced in individuals with CKD (0.75 [95% CI 0.59, 0.94]), although a formal multiplicative interaction test was not statistically significant ($p$ = 0.096). Restricted cubic spline analysis revealed that the association was nonlinear ($p$ = 0.028) with protective effects reaching a plateau after a certain threshold of serum magnesium (Figure 1).

The inverse association between serum magnesium and prevalent MetS remained robust across various MetS definitions. However, in individuals with CKD, this association was attenuated and lost significance when using alternative MetS criteria (Figure S2). Mutual adjustment for individual components of MetS revealed loss of association between serum magnesium and prevalent MetS when adjusting for elevated fasting glucose (Table S6). In addition, the association between serum magnesium with MetS remained significant even after accounting for renal function-related indicators as illustrated in Table S7.

In stratified analyses, the inverse association between serum magnesium and prevalent MetS was observed in individuals at higher metabolic risk, such as men, individuals with obesity, and individuals taking diuretic medication ( $p$ value multiplicative interaction 0.424, 0.029, 0.070, respectively; Figure S3). No significant association was found according to serum 25OHD status. In women, we found no statistically significant association between serum magnesium and MetS according to menopausal status (Table S8).

### 3.3 | Association of serum magnesium and components of prevalent MetS

Among the MetS components, serum magnesium showed a significant inverse association with elevated fasting glucose (OR 0.78 [95% CI 0.71, 0.85]), again with a stronger effect in individuals with CKD (OR 0.67 [95% CI 0.53, 0.84]; $p$ value interaction = 0.484). The association was nonlinear ( $p$ = 0.013) with a similar shaped association to that with MetS (Figure 1). There was no significant association between serum magnesium and other components of MetS (Table 2), including nonlinear associations (all $p$ > 0.1; Figure 1).

For continuous variables constituting the underlying dichotomous components of MetS, there was a significant association between increased serum magnesium and lower fasting glucose levels (β —3.10 mg/dL per 1-SD in serum magnesium [95% CI —3.72, —2.48]) in the overall sample, again with a stronger effect in individuals with CKD (β —6.11 [95% CI —9.08, —3.13]; Table S9). Moreover, in the overall sample and in individuals without CKD, serum magnesium was also associated with lower waist circumference (Table S9).

When CKD was defined based on eGFR only, the observed association of serum magnesium with MetS and elevated fasting glucose among individuals with CKD was attenuated to the point of becoming nonsignificant (Table S10).

### 3.4 | Association of serum magnesium and incident MetS

In our analysis of incident MetS and its components, we had varying sample sizes due to the exclusion of prevalent cases at baseline: MetS

TABLE 1 Descriptive statistics of the study participants by metabolic syndrome prevalence and incidence.

| | Cross-sectional analysis (N = 2996) | | | Longitudinal analysis (N = 1446) | | |
|---|---|---|---|---|---|---|
| | Prevalent MetS | No MetS | | Incident MetS | No MetS | |
| | (N = 1052) | (N = 1944) | p value | (N = 251) | (N = 1195) | p value |
| Demographics | | | | | | |
| Age, years | 62.1 (11.4) | 53.0 (13.0) | <0.001 | 57.1 (10.8) | 50.5 (11.9) | <0.001 |
| Male, *n* (%) | 633 (60.2) | 814 (41.9) | <0.001 | 137 (54.6) | 476 (39.8) | <0.001 |
| Components of MetS, *n* (%) | | | | | | |
| Elevated waist circumference | 1009 (95.9) | 1036 (53.3) | <0.001 | 68 (27.1) | 202 (16.9) | <0.001 |
| Elevated blood pressure | 897 (85.3) | 566 (29.1) | <0.001 | 91 (36.3) | 93 (7.8) | <0.001 |
| Elevated fasting glucose | 783 (74.4) | 204 (10.5) | <0.001 | 165 (65.7) | 156 (13.1) | <0.001 |
| Elevated triglycerides | 611 (58.1) | 140 (7.2) | <0.001 | 87 (34.7) | 51 (4.3) | <0.001 |
| Reduced HDL-C | 431 (41.0) | 172 (8.8) | <0.001 | 19 (7.6) | 9 (0.8) | <0.001 |
| Anthropometrics | | | | | | |
| Waist circumference, cm | 103.7 (11.9) | 88.5 (12.0) | <0.001 | 95.7 (10.7) | 86.5 (11.4) | <0.001 |
| Waist-to-hip ratio | 0.94 (0.07) | 0.85 (0.08) | <0.001 | 0.90 (0.07) | 0.84 (0.08) | <0.001 |
| BMI, kg/m$^2$ | 30.6 (4.6) | 26.0 (4.1) | <0.001 | 28.4 (4.2) | 25.4 (3.8) | <0.001 |
| BMI category[a], *n* (%) | | | <0.001 | | | <0.001 |
| Normal weight | 70 (6.7) | 863 (44.6) | | 50 (19.9) | 602 (50.8) | |
| Overweight | 472 (44.9) | 783 (40.3) | | 131 (52.2) | 456 (38.2) | |
| Obesity | 510 (48.5) | 288 (14.8) | | 70 (27.9) | 128 (10.7) | |
| Lipid profile | | | | | | |
| Total cholesterol, mmol/L | 5.67 (1.07) | 5.54 (0.99) | 0.001 | 5.75 (1.04) | 5.46 (0.93) | <0.001 |
| LDL-C, mmol/L | 3.63 (0.92) | 3.46 (0.88) | <0.001 | 3.73 (0.91) | 3.39 (0.84) | <0.001 |
| HDL-C, mmol/L | 1.24 (0.31) | 1.56 (0.36) | <0.001 | 1.44 (0.32) | 1.58 (0.36) | <0.001 |
| Triglycerides, mmol/L | 2.00 (1.17) | 1.07 (0.58) | <0.001 | 1.28 (0.62) | 1.00 (0.48) | <0.001 |
| Kidney function | | | | | | |
| eGFR, mL/min/1.73 m$^2$ | 81.0 (17.0) | 91.5 (15.4) | <0.001 | 87.5 (14.9) | 93.4 (14.1) | <0.001 |
| UACR, median (IQR) mg/g | 7.7 (4.3, 16.7) | 5.3 (3.4, 9.4) | <0.001 | 4.9 (3.2, 8.8) | 5.0 (3.4, 8.5) | 0.670 |
| CKD, *n* (%) | 235 (22.3) | 152 (7.8) | <0.001 | 19 (7.6) | 69 (5.8) | 0.349 |
| Diuretic medication, *n* (%) | 358 (34.0) | 172 (8.8) | <0.001 | 42 (16.7) | 58 (4.9) | <0.001 |
| Behavioural risk factors | | | | | | |
| Smoking, *n* (%) | | | <0.001 | | | 0.087 |
| Never | 431 (41.0) | 820 (42.2) | | 93 (37.1) | 528 (44.2) | |
| Former | 481 (45.7) | 736 (37.9) | | 103 (41.0) | 455 (38.1) | |
| Current | 140 (13.3) | 388 (20.0) | | 55 (21.9) | 212 (17.7) | |
| Alcohol consumption, *n* (%) | | | 0.003 | | | 0.745 |
| None | 352 (33.5) | 546 (28.1) | | 71 (28.3) | 320 (26.8) | |
| Moderate | 509 (48.4) | 1062 (54.6) | | 136 (54.2) | 679 (56.8) | |
| Excessive | 191 (18.2) | 336 (17.3) | | 44 (17.5) | 196 (16.4) | |
| Physically active | 498 (47.3) | 1143 (58.8) | <0.001 | 145 (57.8) | 745 (62.3) | 0.200 |
| Blood markers | | | | | | |
| HbA1c, % | 5.9 (0.8) | 5.4 (0.4) | <0.001 | 5.5 (0.3) | 5.3 (0.3) | <0.001 |
| Serum magnesium, mmol/L | 0.906 (0.071) | 0.911 (0.059) | 0.014 | 0.917 (0.057) | 0.911 (0.057) | 0.137 |
| Serum sodium, mmol/L | 139.0 (2.6) | 139.1 (2.5) | 0.172 | 138.9 (2.6) | 139.2 (2.4) | 0.151 |
| Serum potassium, mmol/L | 4.19 (0.31) | 4.18 (0.26) | 0.124 | 4.16 (0.26) | 4.17 (0.25) | 0.545 |
| Serum phosphorus, mmol/L | 1.07 (0.17) | 1.10 (0.16) | <0.001 | 1.09 (0.16) | 1.10 (0.15) | 0.209 |
| Serum calcium, mmol/L | 2.41 (0.12) | 2.39 (0.11) | <0.001 | 2.39 (0.11) | 2.39 (0.11) | 0.575 |

(Continues)

TABLE 1 (Continued)

| | Cross-sectional analysis (N = 2996) | | | Longitudinal analysis (N = 1446) | | |
|---|---|---|---|---|---|---|
| | Prevalent MetS (N = 1052) | No MetS (N = 1944) | p value | Incident MetS (N = 251) | No MetS (N = 1195) | p value |
| Serum 25OHD, ng/mL | 17.4 (8.4) | 20.3 (9.8) | <0.001 | 19.6 (8.1) | 20.7 (9.8) | 0.079 |
| Vitamin D category, *n* (%) | | | <0.001 | | | 0.028 |
| Sufficient | 97 (9.2) | 327 (16.8) | | 30 (12.0) | 214 (17.9) | |
| Suboptimal | 250 (23.8) | 575 (29.6) | | 73 (29.1) | 372 (31.1) | |
| Deficient | 705 (67.0) | 1042 (53.6) | | 148 (59.0) | 609 (51.0) | |

*Note:* Values are reported as mean (SD), unless otherwise indicated. Number of missing values for UACR was 14 and five for cross-sectional and longitudinal datasets, respectively. Elevated waist circumference: waist circumference ≥ 94 cm in men or ≥ 80 cm in women. Elevated blood pressure: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or treatment with antihypertensive medication being aware of having hypertension. Elevated fasting glucose: fasting serum glucose level ≥ 100 mg/dL (5.6 mmol/l) or intake of antidiabetic medication. Elevated triglycerides: serum fasting triglycerides ≥150 mg/dL (1.69 mmol/l) or the use of fibrates. Reduced HDL-C: serum HDL-C < 40 mg/dL (1.03 mmol/l) in men or < 50 mg/dL (1.29 mmol/l) in women or the use of fibrates. Excessive alcohol consumption: Men with an alcohol intake ≥30 g/day and women ≥20 g/day. Vitamin D deficiency: serum 25OHD < 20 ng/mL.

Abbreviations: 25OHD, 25-hydroxyvitamin D; BMI, body mass index; CKD, chronic kidney disease; eGFR, glomerular filtration rate estimated by creatinine; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; UACR, urine albumin-creatinine ratio.

[a]Number of individuals classified as underweight was 10 in the cross-sectional dataset and nine in the longitudinal dataset.

(*n* = 1446), elevated waist circumference (*n* = 736), elevated blood pressure (*n* = 1186), elevated fasting glucose (*n* = 1463), elevated triglycerides (*n* = 1617), and reduced HDL-C (*n* = 1707). There was no significant association between serum magnesium and incident MetS or its components (Tables 3 and S11).

### 3.5 | Mendelian randomization

Genetically predicted serum magnesium was causally associated with lower odds of MetS in both IVW (OR per 0.1-mmol/L increase: 0.91 [95% CI 0.85, 0.97]) and weighted median MR analyses (0.91 [0.84, 0.99]; Table 4). MR-Egger analysis showed no evidence of directional pleiotropy (MR-Egger intercept: 0.006; $p = 0.610$). Exclusion of SNPs rs448378 and rs4072037 that were associated with components of MetS did not change the results in either the IVW or the weighted median analysis (Table 4).

In a single SNP analysis, only rs13146355 reached statistical significance (OR: 0.87 [95% CI 0.77, 0.99]; Figure S4). No significant heterogeneity was observed between the estimates from the individual SNPs ($p$ heterogeneity = 0.975).

## 4 | DISCUSSION

In this comprehensive analysis of data from a population-based study, we have shown that serum magnesium was associated with prevalent MetS. The effect was more prominent in individuals with CKD and in those at higher metabolic risk. Although MR analyses indicated causality, we failed to find an association with incident MetS.

In line with our findings, a recent cross-sectional study including 1000 adults, found that a 1-SD increment of serum magnesium was associated with reduced odds of MetS (OR: 0.70 [95% CI 0.57-0.85]).[28] Additionally, a meta-analysis including 3487 individuals found that serum magnesium was approximately 0.19 mg/dL lower in participants with MetS compared to those without MetS, although significant heterogeneity between studies was observed.[10] Notably, there is currently no evidence for the relationship between serum magnesium and incident MetS. Nevertheless, findings from longitudinal studies on dietary magnesium intake indicated a significant inverse relationship with incident MetS.[29,30] Our MR analysis also provided further evidence for this association, revealing a negative causal link between serum magnesium and MetS. While the exact mechanisms remain inconclusive, the beneficial effect of magnesium on MetS potentially acts through the MetS components. A randomized clinical trial demonstrated that oral magnesium supplementation improved MetS by reducing blood pressure, hyperglycaemia, and hypertriglyceridaemia.[31] In the present study, however, we only found a significant protective effect of serum magnesium on elevated fasting glucose, as reported previously.[32] This observation was further supported by the results of the mutual adjustment, revealing the loss of significance in the association between serum magnesium and prevalent MetS after adjusting for elevated fasting glucose. This underscores the role of elevated fasting glucose as the main driver of this association. Magnesium plays a vital role in insulin action and pancreatic ß-cell function, acting as a critical cofactor for numerous enzymes involved in carbohydrate metabolism.[33] Magnesium deficiency has been observed to decrease glucose utilization in skeletal muscles and fat tissue, resulting in insulin resistance.[33] Furthermore, magnesium deficiency was shown to increase inflammation and oxidative stress, common pathways leading to insulin resistance.[34] Indeed, a population-based prospective study indicated that reduced serum magnesium levels were linked to an increased risk of prediabetes and diabetes, with insulin resistance acting as a mediating factor.[35]

TABLE 2 Results of the multivariable logistic regression for the association between serum magnesium and prevalent metabolic syndrome and its components in the total population and stratified by chronic kidney disease status.

| | Cross-sectional analysis (N = 2996) | | CKD (N = 387) | | No CKD (N = 2609) | |
|---|---|---|---|---|---|---|
| Metabolic syndrome | OR (95% CI) n = 1052 | p value | OR (95% CI) n = 235 | p value | OR (95% CI) n = 817 | p value |
| Model 1 | 0.88 (0.81, 0.95) | 0.002 | 0.72 (0.57, 0.90) | 0.001 | 0.93 (0.85, 1.01) | 0.092 |
| Model 2 | 0.89 (0.82, 0.96) | 0.003 | 0.74 (0.58, 0.92) | 0.008 | 0.93 (0.85, 1.01) | 0.097 |
| Model 3 | 0.90 (0.83, 0.98) | 0.019 | 0.75 (0.59, 0.94) | 0.014 | 0.94 (0.86, 1.03) | 0.200 |
| Elevated waist circumference | *n* = 2045 | | *n* = 312 | | *n* = 1733 | |
| Model 1 | 0.95 (0.87, 1.03) | 0.195 | 0.96 (0.73, 1.25) | 0.742 | 0.94 (0.86, 1.05) | 0.188 |
| Model 2 | 0.95 (0.87, 1.03) | 0.223 | 0.99 (0.75, 1.31) | 0.958 | 0.94 (0.86, 1.03) | 0.181 |
| Model 3 | 0.95 (0.87, 1.04) | 0.239 | 1.01 (0.74, 1.36) | 0.978 | 0.94 (0.86, 1.03) | 0.161 |
| Elevated blood pressure | *n* = 1463 | | *n* = 311 | | *n* = 1440 | |
| Model 1 | 0.95 (0.88, 1.03) | 0.254 | 0.88 (0.68, 1.14) | 0.335 | 0.97 (0.89, 1.06) | 0.507 |
| Model 2 | 0.96 (0.88, 1.04) | 0.276 | 0.93 (0.70, 1.21) | 0.571 | 0.97 (0.89, 1.06) | 0.485 |
| Model 3 | 1.00 (0.91, 1.10) | 0.977 | 0.98 (0.72, 1.32) | 0.907 | 1.00 (0.91, 1.10) | 0.981 |
| Elevated fasting glucose | *n* = 987 | | *n* = 214 | | *n* = 773 | |
| Model 1 | 0.77 (0.71, 0.84) | <0.001 | 0.66 (0.53,0.83) | 0.001 | 0.80 (0.73, 0.87) | <0.001 |
| Model 2 | 0.77 (0.71, 0.84) | <0.001 | 0.66 (0.52, 0.83) | 0.001 | 0.79 (0.72, 0.87) | <0.001 |
| Model 3 | 0.78 (0.71, 0.85) | <0.001 | 0.67 (0.53, 0.84) | 0.001 | 0.80 (0.73, 0.88) | <0.001 |
| Elevated triglycerides | *n* = 751 | | *n* = 143 | | *n* = 608 | |
| Model 1 | 1.01 (0.93, 1.10) | 0.760 | 0.96 (0.78, 1.18) | 0.707 | 1.03 (0.94, 1.14) | 0.472 |
| Model 2 | 1.02 (0.94, 1.11) | 0.654 | 0.97 (0.79, 1.20) | 0.786 | 1.04 (0.94, 1.14) | 0.456 |
| Model 3 | 1.03 (0.94, 1.12) | 0.549 | 0.96 (0.78, 1.19) | 0.726 | 1.04 (0.95, 1.15) | 0.381 |
| Reduced HDL-C | *n* = 603 | | *n* = 115 | | *n* = 488 | |
| Model 1 | 0.93 (0.85, 1.02) | 0.130 | 1.05 (0.84, 1.31) | 0.686 | 0.92 (0.83, 1.01) | 0.082 |
| Model 2 | 0.95 (0.87, 1.04) | 0.245 | 1.09 (0.87, 1.37) | 0.471 | 0.92 (0.83, 1.03) | 0.121 |
| Model 3 | 0.96 (0.88, 1.06) | 0.424 | 1.12 (0.89, 1.41) | 0.334 | 0.93 (0.84, 1.03) | 0.168 |

*Note*: Model 1 was adjusted for age and sex. Model 2: Model 1 + smoking status, physical activity, and alcohol consumption. Model 3: Model 2 + Serum potassium and diuretic medication. Serum magnesium was standardized. The coefficients represent the OR of prevalent metabolic syndrome and its components according to a 1-SD increase of serum magnesium. CKD was defined based on estimated glomerular filtration rate and urine albumin-creatinine ratio. Elevated waist circumference: waist circumference ≥ 94 cm in men or ≥ 80 cm in women. Elevated blood pressure: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or treatment with antihypertensive medication being aware of having hypertension. Elevated fasting glucose: fasting serum glucose level ≥ 100 mg/dL (5.6 mmol/l) or intake of antidiabetic medication. Elevated triglycerides: serum fasting triglycerides ≥150 mg/dL (1.69 mmol/l) or the use of fibrates. Reduced HDL-C: serum HDL-C < 40 mg/dL (1.03 mmol/l) in men or < 50 mg/dL (1.29 mmol/l) in women or the use of fibrates.

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; HDL-C, high-density lipoprotein cholesterol; OR, odds ratio.

Our results suggested an inverse association of serum magnesium with waist circumference when modelled as a continuous variable. Moreover, in stratified analysis, serum magnesium showed a protective effect against MetS in individuals with obesity, as measured by BMI. Randomized clinical trials report inconsistent results on the effects of magnesium supplementation on MetS in individuals with obesity. Some studies indicated that magnesium supplementation improves cardiometabolic risk markers in individuals with overweight or obesity, whereas another study did not support this conclusion.[13,36,37] Interestingly, magnesium supplementation in individuals with overweight was shown to elicit changes in gene expression and proteomic profiling, consistent with favourable effects on various metabolic pathways.[38]

We did not find an association between serum magnesium and elevated lipid profile. Currently, there is no consensus on the effect of magnesium on lipid profile, as reported by a recent extensive systematic review.[39] Despite existing evidence, we failed to find an association between serum magnesium and elevated blood pressure. A systematic review and meta-analysis, including studies on dietary magnesium intake, serum magnesium, or both, revealed an inverse dose–response relationship between dietary magnesium intake and incident hypertension. However, the association was marginal for serum magnesium.[40]

Our study showed an inverse association of serum magnesium with MetS and elevated fasting glucose in individuals with CKD.

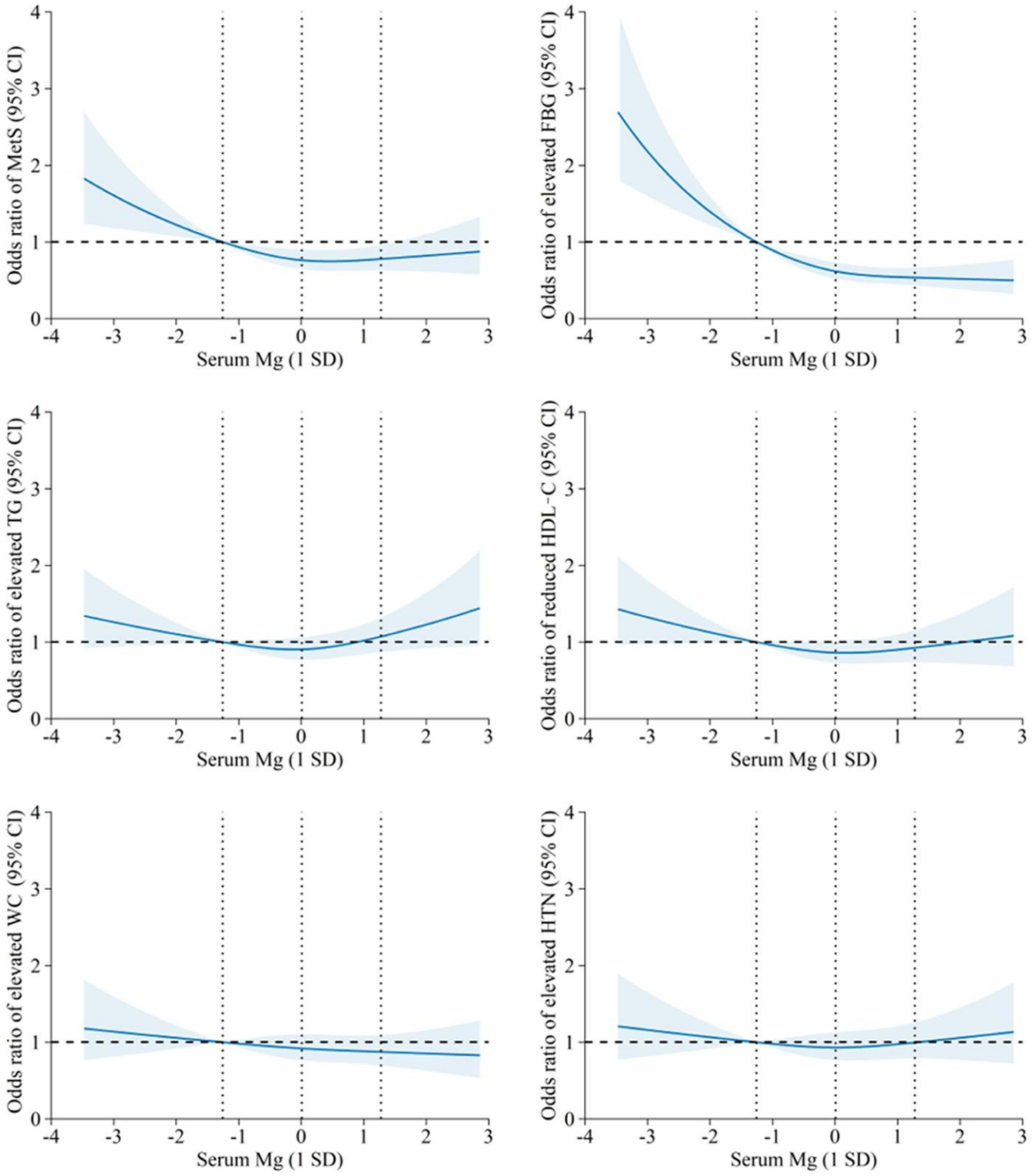


FIGURE 1 Restricted cubic spline showing the exposure–response functions between serum magnesium (1 SD) and prevalent metabolic syndrome (MetS) and its components using logistic regression models adjusted for age, sex, smoking status, physical activity, alcohol consumption, serum potassium and diuretic medication (corresponding to Model 3). Three knots were applied at the 10th, 50th and 90th percentiles corresponding to —1.26, 0.01 and 1.28 of standardized serum magnesium (reference is the 10th percentile). Solid lines indicate odds ratios, and shaded areas indicate 95% confidence intervals (CIs). Elevated waist circumference (WC): WC ≥ 94 cm in men or ≥ 80 cm in women. Elevated blood pressure: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or treatment with antihypertensive medication being aware of having hypertension. Elevated fasting glucose: fasting serum glucose level ≥ 100 mg/dL (5.6 mmol/l) or intake of antidiabetic medication. Elevated triglycerides (TG): serum fasting TG ≥150 mg/dL (1.69 mmol/l) or the use of fibrates. Reduced high-density lipoprotein cholesterol (HDL-C): serum HDL-C < 40 mg/dL (1.03 mmol/l) in men or < 50 mg/dL (1.29 mmol/l) in women, or the use of fibrates. FBG, fasting blood glucose; Mg, magnesium; HTN, hypertension.

TABLE 3 Results of the multivariable logistic regression for the association between serum magnesium and incident metabolic syndrome and its components in the total population and stratified by chronic kidney disease status.

| | OR (95% CI) | p value | OR (95% CI) | p value | OR (95% CI) | p value |
|---|---|---|---|---|---|---|
| | All (n = 1446) | | CKD (n = 88) | | No CKD (n = 1358) | |
| Incident MetS | n = 251 | | n = 19 | | n = 232 | |
| Model 1 | 1.03 (0.90, 1.19) | 0.673 | 0.73 (0.43, 1.25) | 0.262 | 1.06 (0.92, 1.23) | 0.414 |
| Model 2 | 1.02 (0.89, 1.17) | 0.770 | 0.64 (0.33, 1.19) | 0.168 | 1.05 (0.91, 1.21) | 0.527 |
| Model 3 | 1.02 (0.89, 1.18) | 0.778 | 0.65 (0.33, 1.22) | 0.188 | 1.05 (0.90, 1.21) | 0.529 |
| Incident elevated waist circumference | All (*n* = (736) | | CKD (*n* = 46) | | Non-CKD (*n* = 690) | |
| | *n* = 285 | | *n* = 19 | | *n* = 266 | |
| Model 1 | 1.09 (0.94, 1.27) | 0.248 | 1.44 (0.76, 3.00) | 0.285 | 1.07 (0.91, 1.25) | 0.405 |
| Model 2 | 1.09 (0.94, 1.27) | 0.269 | 2.28 (0.81, 8.61) | 0.162 | 1.07 (0.91, 1.25) | 0.406 |
| Model 3 | 1.08 (0.93, 1.26) | 0.308 | n.p. | n.p. | 1.06 (0.91, 1.25) | 0.431 |
| Incident elevated blood pressure | All (*n* = 1186) | | CKD (*n* = 60) | | Non-CKD (*n* = 1126) | |
| | *n* = 228 | | *n* = 20 | | *n* = 208 | |
| Model 1 | 0.98 (0.84, 1.14) | 0.772 | 0.72 (0.37, 1.31) | 0.305 | 0.99 (0.85, 1.16) | 0.898 |
| Model 2 | 0.97 (0.84, 1.13) | 0.739 | 0.64 (0.31, 1.21) | 0.192 | 0.98 (0.84, 1.15) | 0.833 |
| Model 3 | 0.97 (0.84, 1.13) | 0.708 | 0.70 (0.34, 1.38) | 0.317 | 0.99 (0.84, 1.15) | 0.853 |
| Incident elevated fasting glucose | All (*n* = 1463) | | CKD (*n* = 101) | | Non-CKD (*n* = 1362) | |
| | *n* = 401 | | *n* = 31 | | *n* = 370 | |
| Model 1 | 0.92 (0.82, 1.04) | 0.182 | 0.84 (0.53, 1.31) | 0.446 | 0.92 (0.82, 1.05) | 0.250 |
| Model 2 | 0.91 (0.81, 1.03) | 0.133 | 0.80 (0.48, 1.30) | 0.375 | 0.92 (0.81, 1.04) | 0.180 |
| Model 3 | 0.91 (0.81, 1.03) | 0.130 | 0.80 (0.47, 1.31) | 0.373 | 0.92 (0.81, 1.04) | 0.188 |
| Incident elevated triglycerides | All (*n* = 1617) | | CKD (*n* = 126) | | Non-CKD (*n* = 1491) | |
| | *n* = 185 | | *n* = 16 | | *n* = 169 | |
| Model 1 | 1.00 (0.86, 1.17) | 0.989 | 1.09 (0.59, 1.70) | 0.994 | 1.00 (0.85, 1.17) | 0.975 |
| Model 2 | 0.99 (0.85, 1.16) | 0.917 | 0.99 (0.56, 1.71) | 0.957 | 0.99 (0.84, 1.16) | 0.874 |
| Model 3 | 1.00 (0.85, 1.16) | 0.961 | 1.00 (0.57, 1.74) | 0.999 | 0.99 (0.84, 1.17) | 0.929 |
| Incident reduced HDL-C | All (*n* = 1707) | | CKD (*n* = 136) | | Non-CKD (*n* = 1571) | |
| | *n* = 45 | | *n* < 5[a] | | *n* = 41 | |
| Model 1 | 1.13 (0.84, 1.53) | 0.425 | | | 1.19 (0.87, 1.62) | 0.282 |
| Model 2 | 1.12 (0.83, 1.51) | 0.468 | | | 1.18 (0.86, 1.60) | 0.304 |
| Model 3 | 1.09 (0.80, 1.46) | 0.594 | | | 1.14 (0.84, 1.56) | 0.395 |

*Note*: Model 1 was adjusted for age and sex. Model 2: Model1 + Smoking status, physical activity, and alcohol consumption. Model 3: Model 2 + Serum potassium and diuretic medications. Serum magnesium was standardized. The coefficients represent the OR of incident metabolic syndrome and its components according to a 1 SD increase of serum magnesium. CKD was defined based on baseline estimated glomerular filtration rate and urine albumin-creatinine ratio. Incident MetS and its components were modelled after exclusion of prevalent cases at baseline. Incident elevated waist circumference: Waist circumference < 94 cm in men or < 80 cm in women at baseline and waist circumference ≥ 94 cm in men or ≥ 80 cm in women at the follow-up visit. Incident elevated blood pressure: Systolic blood pressure < 130 mmHg or diastolic blood pressure < 85 mmHg or no hypertension medication at baseline and systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or treatment with antihypertensive medication being aware of having hypertension at follow up visit. Incident elevated fasting glucose: Fasting serum glucose level < 100 mg/dL or no diabetic medication at baseline and fasting serum glucose level ≥ 100 mg/dL or intake of antidiabetic medication at follow up visit. Incident elevated triglycerides: Serum fasting triglycerides <150 mg/dL or no fibrate medication at baseline and Serum fasting triglycerides ≥150 mg/dL or the use of fibrates at follow up visit. Incident reduced HDL-C: Serum HDL-C ≥ 40 mg/dL in men or ≥ 50 mg/dL in women or no fibrate intake at baseline and serum HDL-C < 40 mg/dL in men or < 50 mg/dL in women or the use of fibrates at follow up visit. n.p. modelling not possible.

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; HDL-C, high-density lipoprotein cholesterol; MetS, metabolic syndrome; OR, odds ratio.

[a]No modelling was possible due to low sample size.

TABLE 4 Mendelian randomization results showing associations between genetically determined levels of serum magnesium and metabolic syndrome.

| | Six SNPs | | | Four SNPs[a] | | |
|---|---|---|---|---|---|---|
| Method | OR | 95% CI | p value | OR | 95% CI | p value |
| Inverse variance-weighted | 0.91 | (0.85, 0.97) | 0.004 | 0.90 | (0.84, 0.98) | 0.011 |
| MR Egger | 0.85 | (0.67, 1.07) | 0.253 | 0.84 | (0.48, 1.47) | 0.608 |
| Weighted median | 0.91 | (0.84, 0.99) | 0.020 | 0.90 | (0.82, 0.99) | 0.045 |
| Simple mode | 0.92 | (0.82, 1.02) | 0.179 | 0.93 | (0.81, 1.05) | 0.290 |
| Weighted mode | 0.91 | (0.83, 1.00) | 0.083 | 0.91 | (0.82, 1.02) | 0.204 |

Abbreviations: CI, confidence interval; MR, Mendelian randomization; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]rs448378 (associated with systolic blood pressure) and rs4072037 (associated with diastolic blood pressure and fasting glucose) were excluded from analysis. ORs and 95% CIs were scaled per 0.1-mmol/L increase in serum magnesium.

Analogously, studies have demonstrated an inverse association of serum magnesium with various surrogate parameters of cardiovascular disease in CKD patients.[41] However, a randomized clinical trial revealed that 3 months of magnesium supplementation did not improve MetS in patients with CKD that had prediabetes and obesity.[13] Similarly, another study in kidney transplant patients indicated that dietary magnesium intake did not exhibit a significant relationship with the risk of MetS over 1 year of follow-up.[14] These findings may be attributed to the relatively short follow-up duration and the specific selection of participants. Generally, it is important to note that findings relating to dietary magnesium intake are not directly translatable to findings from serum magnesium levels, since these two parameters do not correlate well.[42]

Disturbance in magnesium balance is common in individuals with CKD, primarily due to impaired renal reabsorption, diuretic use, and changes in diet.[43] In fact, Oka et al. reported that hypomagnesaemia was the most frequent electrolyte abnormality among 2126 predialysis patients, and positive proteinuria was identified as an independent risk factor for magnesium wasting. Interestingly, this association was mediated by urinary tubular markers representing tubular dysfunction.[11] This could potentially explain the attenuation and loss of association of serum magnesium with MetS and elevated fasting glucose in our study when CKD was defined based on eGFR alone, without considering proteinuria. It is, therefore, reasonable to consider that magnesium may have a protective effect in individuals susceptible to hypomagnesaemia. Nevertheless, we cannot rule out the hypothesis that hypomagnesaemia could be a consequence of MetS, exacerbated by the presence of other comorbidities, as we were unable to establish a prospective association. Moreover, it should be noted that our definition of CKD was based on biomarkers of renal function only, whereas generally CKD also comprises heterogeneous phenotypes such as nephrotic syndrome or chronic nephritis. Unfortunately, information on these phenotypes was not available in our study.

Vitamin D biosynthesis, activation and transport are dependent on magnesium bioavailability. Similarly, vitamin D is necessary for the intestinal uptake and absorption of magnesium.[44] Dysregulation in any of these nutrients has been connected to MetS,[10] yet research exploring their combined interaction effect on MetS remains limited. Among 126 patients with diabetes, a significant increase in serum magnesium was observed after consumption of vitamin D supplements for 6 months.[45] Among women with obesity, low serum magnesium was significantly modified by vitamin D injection.[46] Our results, however, indicated no effect modification of serum magnesium on MetS by vitamin D status.

We found the inverse association of serum magnesium with MetS and elevated fasting glucose to be nonlinear, indicating that potential protective effects of high magnesium levels remain stable after a certain threshold. In line with this, a prospective cohort study in 5044 participants demonstrated a nonlinear relationship of serum magnesium with diabetes and insulin resistance.[47] On the other hand, Negrea et al. found that serum magnesium <1.9 mg/dL and >2.1 mg/dL was associated with all-cause mortality in a large cohort of patients with CKD.[48]

The observed lack of longitudinal association between serum magnesium and MetS in our study was unexpected, given a—albeit weak—causal relation. However, it could be attributed to several factors, including insufficient statistical power, heterogeneity of the sample, variations in the duration of the disease, and changes in risk factor distribution over time. Notably, within the 6.5-year follow-up period, the determination of the precise timing of MetS development was impossible, posing a challenge to capture the latency period. The dynamic nature of risk factor distribution over time, particularly changes in magnesium levels, further complicated the matter.

While we observed a more pronounced association between serum magnesium and MetS among participants with CKD, a formal test for multiplicative interaction did not reach statistical significance, probably owing to the relatively small sample size of the CKD group. Additionally, this association dissipated when employing alternative MetS definitions. Consequently, further research is warranted to thoroughly explore the potential effect modification by CKD status.

We performed MR using publicly available association data from GWAS for MetS and magnesium. However, it is important to note that the various components of MetS exhibit both common and distinct genetic associations.[49,50] Conducting MR on the individual components and utilizing genetic instruments specific to each underlying trait will offer additional insights into the relationship between serum magnesium and MetS.

In conclusion, we found that higher serum magnesium levels were associated with a lower risk of MetS, particularly in individuals with CKD. MR analysis showed that this association is potentially causal; however, we failed to find associations with incident MetS. Serum magnesium thus has the potential to serve as a diagnostic marker for metabolic impairment. Larger, well-characterized longitudinal cohorts are warranted to further establish its role as a prognostic marker.

## AUTHOR CONTRIBUTIONS

Conceptualization: Nuha Shugaa Addin and Susanne Rospleszcz; Methodology: Nuha Shugaa Addin, Fiona Niedermayer and Susanne Rospleszcz; Formal analysis: Nuha Shugaa Addin; Resources: Barbara Thorand, Jakob Linseisen, Jochen Seissler and Annette Peters; Writing—original draft preparation: Nuha Shugaa Addin and Susanne Rospleszcz; Writing—review and editing: Nuha Shugaa Addin, Fiona Niedermayer, Barbara Thorand, Jakob Linseisen, Jochen Seissler, Annette Peters and Susanne Rospleszcz; Supervision: Jochen Seissler, Annette Peters and Susanne Rospleszcz; Funding acquisition: Barbara Thorand, Jakob Linseisen, Jochen Seissler and Annette Peters. All authors have read and agreed on the final version of the manuscript.

## ACKNOWLEDGEMENTS

We thank all participants for their long-term commitment to the KORA study, the staff for data collection and research data management, and the members of the KORA Study Group (https://www.helmholtz-munich.de/en/epi/cohort/kora) who were responsible for the design and conduct of the study. The KORA study was initiated and financed by the Helmholtz Zentrum München-German Research Center for Environmental Health, which is funded by the Bundesministerium für Bildung und Forschung (BMBF) and by the State of Bavaria. Data collection in the KORA study is carried out in cooperation with the University Hospital of Augsburg. KORA research was supported within the Munich Center of Health Sciences, Ludwig-Maximilians-Universität, as part of LMUinnovativ. Nuha Shugaa Addin is supported by an individual doctoral scholarship of the *Hanns-Seidel-Stiftung*. Open Access funding enabled and organized by Projekt DEAL.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15497.

## DATA AVAILABILITY STATEMENT

The informed consent given by KORA study participants does not cover data posting in public databases. However, data are available upon request from the KORA database by means of a project agreement. Requests should be sent to kora.passt@helmholtz-muenchen.de and are subject to approval by the KORA Board.The GWAS summary data for serum magnesium are available from the GWAS Catalog, study accession GCST000756 (https://www.ebi.ac.uk/gwas/studies/GCST000756). Genome-Wide Association Study of MetS was extracted from the UK Biobank, study accession GCST009602 (https://www.ebi.ac.uk/gwas/studies/GCST009602).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Shugaa Addin N, Niedermayer F, Thorand B, et al. Association of serum magnesium with metabolic syndrome and the role of chronic kidney disease: A population-based cohort study with Mendelian randomization. *Diabetes Obes Metab*. 2024;1-13. doi:10.1111/dom.15497

## Project II

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|---|---|
| Title: | Subclinical Cardiovascular Disease Markers in Relation to Serum and Dietary Magnesium in Individuals from the General Population: The KORA-MRI Study |
| Authors: | Nuha Shugaa Addin, Christopher L. Schlett, Fabian Bamberg, Barbara Thorand, Jakob Linseisen, Jochen Seissler, Annette Peters and Susanne Rospleszcz |
| Journal: | Nutrients |
| Year: | 2022 |
| Volume: | 14 |
| Issue: | 23 |
| Article number: | 4954 |
| DOI: | https://doi.org/10.3390/nu14234954 |
| Impact factor: | 4.8 |
| Rank: | 21/109 in NUTRITION & DIETETICS<br>(Journal Citation Reports™, year 2022) |

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MDPI

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# Subclinical Cardiovascular Disease Markers in Relation to Serum and Dietary Magnesium in Individuals from the General Population: The KORA-MRI Study

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**Citation:** Shugaa Addin, N.; Schlett, C.L.; Bamberg, F.; Thorand, B.; Linseisen, J.; Seissler, J.; Peters, A.; Rospleszcz, S. Subclinical Cardiovascular Disease Markers in Relation to Serum and Dietary Magnesium in Individuals from the General Population: The KORA-MRI Study. *Nutrients* **2022**, *14*, 4954. https://doi.org/10.3390/nu14234954

Academic Editors: Ligia J. Dominguez, Nicola Veronese and Mario Barbagallo

Received: 27 October 2022
Accepted: 18 November 2022
Published: 22 November 2022



**Abstract:** Several studies have implied a role of magnesium in the development of cardiovascular disease (CVD). Thus, magnesium might serve as a potential risk marker for early CVD. Therefore, we investigated the association of serum magnesium and dietary magnesium intake with markers of subclinical CVD in a population-based study. We used cross-sectional data from the sub-study of the Cooperative Health Research in the Region of Augsburg (KORA-FF4). Markers of subclinical CVD, namely, left and right ventricular structure and function and carotid plaque and carotid wall thickness, were derived by magnetic resonance imaging (MRI). Multivariable-adjusted regression models were applied to assess the relationship between serum and dietary magnesium and MRI-derived subclinical CVD markers. Among 396 included participants (mean age: 56.3 ± 9.2 years; 57.8% male), 181 (45.7%) had low serum magnesium levels (<2.07 mg/dL). Among 311 subjects with complete dietary data (mean age: 56.3 ± 9.1 years; 56.3% male), 154 (49.5%) had low dietary magnesium intake (≤155.2 mg/1000 kcal/day). Serum and dietary magnesium were not correlated ($p$-value = 0.5). Serum magnesium was significantly associated with presence of carotid plaque (OR 1.62, $p$-value 0.033). Dietary magnesium was associated with higher left ventricular end-systolic and end-diastolic volume (0.04 mL/m$^2$, 0.06 mL/m$^2$; $p$-value 0.011, 0.013, respectively), and also with a decrease in left ventricular remodeling index and mean diastolic wall thickness (−0.001 g/mL/m$^2$, −0.002 mm/m$^2$; $p$-value 0.004, 0.029, respectively). In summary, there was no consistent association of serum and dietary magnesium with imaging markers of subclinical CVD.

**Keywords:** magnesium; preclinical atherosclerosis; early cardiac impairment; cardiac MRI

## 1. Introduction

Cardiovascular disease (CVD) is a major contributor to reduced quality of life and a leading cause of mortality worldwide [1,2]. The Global Burden of Disease (GBD) Study 2019 showed that the prevalence, mortality, and disability-adjusted life years (DALYs) of CVD have risen significantly since 1990 [2]. Heart failure prevalence is projected to rise by 40% between 2015 and 2035 [3]. The global prevalence of carotid plaque, an indicator of cardiovascular risk, was estimated to be 21.1% in people aged 30–79 years in 2020 [4].

However, the prevalence of CVD and plaque burden cannot be solely explained by the traditional cardiovascular risk factors such as hypertension and diabetes [5,6]. Thus, there is an urgent need to identify additional markers of early CVD in order to implement prevention and treatment strategies to limit the growing burden of CVD.

One potential candidate is magnesium, which has been reported to be implicated in CVD development [7,8]. As the fourth most abundant mineral and the second most abundant intracellular cation, magnesium modulates neuronal excitation, intracellular conduction, and myocardial contraction [9]. Furthermore, magnesium plays a key role in regulating mitochondrial function and energy production [10]. Liu et al. found that magnesium deficiency can induce diastolic cardiomyopathy in mice with a low magnesium diet mainly through ATP depletion, mitochondrial dysfunction, and overproduction of reactive oxygen species [11]. Magnesium deficiency has also been shown to accelerate atherosclerosis by promoting platelet activity and endothelial dysfunction and increasing the production of pro-inflammatory cytokines and neuropeptides [12].

Dietary magnesium is deemed to be one of the shortfall nutrients due to the modern Western diet characterized by a wide use of processed foods, demineralized water, and agricultural practices that use soils deficient in magnesium [13]. A meta-analysis of prospective cohort studies found that increasing dietary intake of magnesium was associated with a 22% reduction in the risk of heart failure [14]. Low magnesium intake was associated with higher heart failure incidence and hospitalization [15,16]. However, to assess if magnesium is a potential target for CVD prevention, it is necessary to study its relationship with early subclinical CVD, which could be assessed with cardiovascular imaging.

Using echocardiography, serum magnesium was inversely associated with left ventricular mass even after adjustment for cardiovascular risk factors [17]. Previous population-based studies have demonstrated an inverse relationship between serum magnesium and carotid artery intima-media thickness [18–20]. On the other hand, a systematic review and meta-analysis of randomized clinical trials showed that magnesium supplementation may improve endothelial function without affecting carotid intima-media thickness [21]. However, intima-media thickness is not a good measure of atherosclerotic plaque [22]. Cardiac magnetic resonance imaging (MRI) is considered a safe, non-invasive, highly accurate, and reproducible method that enables a detailed characterization of cardiac morphology and function and atherosclerotic plaque [23]. We now aim to make use of the detailed cardiac MRI data as measures of subclinical CVD and analyze the association between serum and dietary magnesium with early cardiac impairment and preclinical atherosclerosis.

## 2. Materials and Methods

### *2.1. Study Design and Population*

We used cross-sectional data from a subsample (KORA-MRI, n = 400) of a population-based cohort in southern Germany (KORA-FF4, n = 2279). KORA-FF4 is the second follow-up of the original baseline survey KORA-S4 (N = 4261, enrolled between 1999 and 2001). Details on the study design, sampling method, and data collection of the KORA surveys have been described elsewhere [24]. In the KORA-MRI sub-study, a total of 400 participants aged 39 to 73 years without known cardiovascular disease underwent whole-body MRI. The main aim of the study was to assess subclinical disease in individuals with prediabetes and diabetes. Study setup, imaging protocol, and inclusion and exclusion criteria were described in detail previously [25].

The KORA-FF4 study was approved by the Ethics Committee of the Bavarian Medical Association (Bayerische Landesärztekammer). All investigations were performed in accordance with the Declaration of Helsinki, including written informed consent of all participants. The MRI examination protocol was further approved by the ethics committee of the Ludwig-Maximillian-University Hospital, Munich.

### 2.2. Assessment of Serum and Dietary Magnesium

Plasma magnesium was measured in mmol/L with the Siemens Dimension Vista (Siemens Health Care Diagnostics Inc, Newark, DE) using a modification of the methyl thymol blue (MTB) procedure that forms a blue complex with magnesium [26]. The amount of magnesium-MTB complex formed is proportional to the magnesium concentration and was measured using a biochromatic (600 and 510 nm) endpoint technique. Based on a review of the literature that adopted an evidenced-based reference interval for serum total magnesium concentration, we used a cutoff point of <0.85 mmol/L (2.07 mg/dL) to indicate hypomagnesemia [27].

Habitual dietary intake, including magnesium, was obtained based on a combination of up to three 24 h food lists (24H-FL) and a food frequency questionnaire (FFQ), as described previously [28]. While the 24H-FL, consisting of 246 food items, was used to calculate the consumption of foods over the previous day, the FFQ, consisting of 148 food items, was used to assess the dietary habits in the past 12 months. For each participant, the assessment of the usual dietary intake of each food item on any given day was derived from the multiplication of the calculated consumption probability and consumption amount. Consumption probability was determined by logistic mixed models adjusted for covariates and FFQ information and the consumption amount was estimated based on data from the Bavarian Food Consumption Survey II (BVS II). According to the EPIC-Soft classification scheme, food items were combined into 16 food groups and 21 subgroups for both approaches. Dietary magnesium was measured in mg/day. In order to take the total energy consumption into account, we dichotomized dietary magnesium intake based on the median split of the dietary density for dietary magnesium. A magnesium intake ≤ 155.2 mg/1000 kcal/day was defined as low.

### 2.3. Assessment of Subclinical Cardiovascular Disease Markers by MRI

Whole-body MRI examinations were performed using a 3 Tesla Magnetom Skyra (Siemens AG, Healthcare Sector, Erlangen, Germany) supplied with an 18-channel body coiling system. All participants underwent imaging, within 3 months after their clinical examination at the study center, consisting of sequences covering the entire body. For analysis of the heart, 4-chamber view steady-state free precession (SSFP) and short-axis stack SSFP were used. An axial black-blood T1 weighted fat-saturated (T1w fs ax) was used to assess carotid plaque. All image analyses were performed by independent readers blinded to the clinical covariates of the participants [25]. Missing values in MRI parameters were due to technical malfunction, low image quality, or imaging artifacts.

#### 2.3.1. Left Ventricular Structure and Function

Cine-SSFP sequences were evaluated semi-automatically with cvi42 (Circle Cardiovascular Imaging, Calgary, Canada) software. The derived left ventricular (LV) markers [29] included end-diastolic volume (the phase with the biggest left ventricular volume), end-systolic volume (the phase with the smallest left ventricular volume), stroke volume (end-diastolic volume minus end-systolic volume), ejection fraction ((stroke volume/end-diastolic volume) * 100), left ventricular mass assessed during end diastole, cardiac output (left ventricular stroke volume * heart rate), and left ventricular wall thickness. Presence of late gadolinium enhancement (LGE) was assessed visually on fast low-angle shot inversion recovery sequences. Furthermore, filling and ejection rates were quantified using dedicated in-house software estimating peak gradients during early (passive left ventricular filling) and late (left ventricular filling due to atrial contraction) filling [30].

#### 2.3.2. Right Ventricular Structure and Function

Right ventricular (RV) function was assessed by manual segmentation of the right ventricular endocardial border on axial cine-SSFP sequences using dedicated software (cvi42, Circle Cardiovascular Imaging, Calgary, Canada). Markers included end-systolic volume, end-diastolic volume, stroke volume, cardiac output, and ejection fraction [31].

#### 2.3.3. Carotid Plaque

The presence and composition of carotid plaque was determined on black-blood T1-weighted sequences on 14 slice positions and semiautomatic software (CASCADE; University of Washington Seattle, WA) was used to obtain vessel wall thickness and lumen dimension [32]. According to plaque composition [33], measures were classified as type I, type III, type IV/V, and type VI/VII plaques, and any plaque > type I was defined as having plaque.

### *2.4. Assessment of Covariates*

All participants underwent standardized interviews, comprehensive medical examinations, and a fasting blood draw at the study center. Interviews included information on demographic variables (e.g., age, sex), medication intake (e.g., antihypertensive and antidiabetic medication), and health behavior (e.g., smoking and physical activity). Smoking status was classified as never smoker, ex-smoker, and current smoker. Height, weight, BMI, and waist circumference were assessed by trained staff according to standard protocols using standardized instruments. Systolic and diastolic blood pressure was measured three times on the right arm of seated participants after at least a five-minute resting period. The mean of the second and third BP measurements was used for the analyses. Hypertension was defined as blood pressure greater or equal to 140/90 mmHg or the use of antihypertensive medication under the awareness of having hypertension.

Diabetes status (normoglycemia, prediabetes, or diabetes) was determined by an oral glucose tolerance test (OGTT) according to WHO criteria [34] or as previous diabetes diagnosed by a physician. Laboratory parameters, such as glucose, HbA1c as well as total cholesterol, triglycerides, and low- and high-density lipoprotein cholesterol were assessed by standardized methods as described elsewhere [35].

### *2.5. Statistical Analyses*

Participants' demographics, cardiovascular risk factors, and MRI outcomes are presented as means and standard deviations (SD) for continuous variables and counts and percentages for categorical variables. Description was also stratified by serum and dietary magnesium with cut-off points of 0.85 mmol/L (2.07 mg/dl) and 155.2 mg/1000 kcal/day, as outlined above. Differences in continuous and categorical variables between the groups were examined by a *t*-test and $\chi^2$-test, respectively. We calculated the body surface area (BSA) according to the Du Bois formula (BSA [$m^2$] = weight [kg]$^{0.425}$ × height (cm)$^{0.725}$ × 0.007184) [36] and indexed all measures of cardiac morphology and function by BSA. Correlations between serum and dietary magnesium and correlations with MRI outcomes were determined by Spearman's rho correlation coefficient and corresponding *p*-value.

To assess the association between continuous exposures of serum and dietary magnesium with continuous MRI outcomes, a linear regression model adjusted for age, sex, BMI, systolic blood pressure, and diabetes status was calculated providing β-coefficients with 95% confidence intervals (CIs). Serum magnesium was standardized by subtracting its mean and dividing by its SD. For dietary magnesium intake, models were additionally adjusted for daily caloric intake. Binary MRI outcomes (presence of LGE and presence of plaque) were analyzed by logistic regression adjusted for the same variables. Categorical MRI outcome (plaque-type) was analyzed by ordered logistic regression with a cumulative logit link under the proportional odds assumption. As an additional analysis for serum magnesium, logistic models were additionally adjusted for serum total cholesterol and smoking status. Estimates for all logistic models are reported as odds ratios (ORs) with 95% CIs. To assess if the association of serum magnesium with presence of plaque was mediated by serum total cholesterol, causal mediation analysis was applied. Due to the varying number of missing values in the MRI parameters, we performed complete-case analyses for each exposure and each MRI outcome. $p$ values < 0.05 were considered to indicate statistical significance. All analyses were conducted with R (Version 4.1.2).

## 3. Results

### *3.1. Study Population*

Samples sizes for the analyses of serum and dietary magnesium with the respective MRI outcomes of LV structure and function, RV structure and function, and carotid plaque are presented in Figure 1. Among the 400 participants who underwent whole-body MRI, 396 and 311 subjects were included in the analysis serum and dietary magnesium, respectively. Data on the relationship between serum magnesium and subclinical cardiovascular disease markers were available in 363, 333, and 245 participants for LV, RV, and carotid plaque, respectively. Similarly, the association between dietary magnesium and subclinical cardiovascular disease markers was available in 287, 263, and 188 individuals for LV, RV, and carotid plaque, respectively. Differences in the clinical characteristics of the study population between missing and complete case analysis are presented in Table S1.

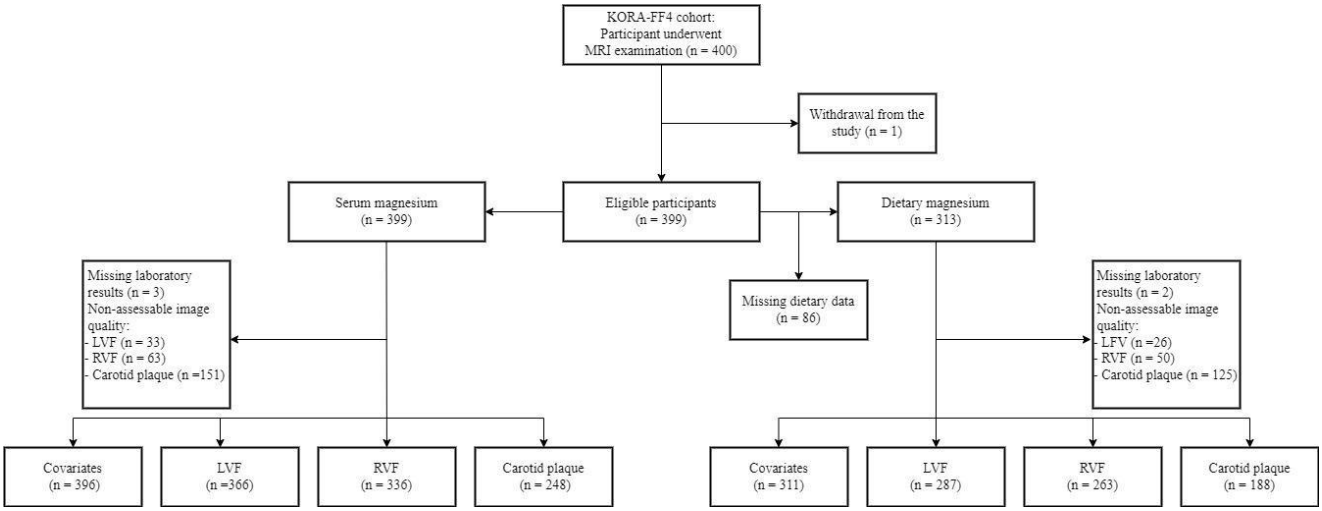


**Figure 1.** Participant flow diagram. LVF, left ventricular function; RVF, right ventricular function.

Table 1a provides an overview of the clinical characteristics of the study population stratified by serum magnesium levels. Out of 396 included participants (mean age: 56.3 ± 9.2 years; 57.8% male), 181 (45.7%) had low serum magnesium levels (mean age: 57.04 ± 9.65 years; 59.7% male). Individuals with hypomagnesemia had higher systolic blood pressure, more often had diabetes, had higher blood glucose levels, with a more frequent use of diabetic medications.

**Table 1.** (**a**): Demographic and cardiovascular risk factors by serum magnesium status; (**b**): demographic and cardiovascular risk factors by dietary magnesium intake.

| | **(a)** | | | |
|---|---|---|---|---|
| | **All** | **Mg ≤ 2.07 mg/dL** | **Mg > 2.07 mg/dL** | ***p* Value** |
| | **N = 396 (99.2%)** | **n = 181 (45.7%)** | **n = 215 (54.3%)** | |
| Age (years) | 56.34 (9.20) | 57.04 (9.65) | 55.75 (8.79) | 0.167 |
| Male sex | 229 (57.8) | 108 (59.7%) | 121 (56.3%) | 0.563 |
| Weight (kg) | 83.00 (16.63) | 83.60 (15.96) | 82.49 (17.19) | 0.508 |
| BMI (kg/m$^2$) | 28.10 (4.91) | 28.21 (4.83) | 28.00 (4.99) | 0.67 |
| Smoking | | | | 0.345 |
| Never smoker | 145 (36.6%) | 67 (37.0%) | 78 (36.3%) | |
| Ex-smoker | 171 (43.2%) | 83 (45.9%) | 88 (40.9%) | |
| Smoker | 80 (20.2%) | 31 (17.1%) | 49 (22.8%) | |

**Table 1.** *Cont.*

| (a) | | | | |
|---|---|---|---|---|
| | **All** | **Mg ≤ 2.07 mg/dL** | **Mg > 2.07 mg/dL** | ***p* Value** |
| | **N = 396 (99.2%)** | **n = 181 (45.7%)** | **n = 215 (54.3%)** | |
| Waist circumference (cm) | 98.56 (14.37) | 99.24 (14.17) | 98.00 (14.55) | 0.394 |
| Systolic BP (mmHg) | 120.54 (16.64) | 122.60 (16.31) | 118.81 (16.75) | 0.024 |
| Diastolic BP (mmHg) | 75.24 (9.99) | 75.93 (9.67) | 74.67 (10.24) | 0.514 |
| Physically active | 237 (59.8%) | 112 (61.9%) | 125 (58.1%) | 0.523 |
| Hypertension | 133 (33.6%) | 65 (35.9%) | 68 (31.6%) | 0.428 |
| Glucose (mg/dl) | 104.31 (22.63) | 107.36 (25.04) | 101.74 (20.08) | 0.014 |
| HbA1c (%) | 5.57 (0.72) | 5.63 (0.74) | 5.52 (0.71) | 0.121 |
| Diabetes | | | | 0.022 |
| No | 242 (61.1%) | 105 (58.0%) | 137 (63.7%) | |
| Prediabetes | 102 (25.8%) | 43 (23.8%) | 59 (27.4%) | |
| Diabetes | 52 (13.1%) | 33 (18.2%) | 19 (8.8%) | |
| Total cholesterol (mg/dl) | 218.05 (36.31) | 215.12 (34.54) | 220.51 (37.65) | 0.141 |
| HDL-C (mg/dl) | 62.00 (17.68) | 62.14 (17.62) | 61.89 (17.76) | 0.888 |
| LDL-C (mg/dl) | 139.68 (32.98) | 136.71 (31.51) | 142.17 (34.04) | 0.101 |
| Triglycerides (mg/dl) | 131.41 (85.12) | 133.42 (92.51) | 129.73 (78.54) | 0.668 |
| eGFR (ml/min/1.73 $m^2$) | 86.62 (12.96) | 86.18 (13.13) | 87.00 (12.83) | 0.531 |
| Serum potassium (mmol/L) | 4.29 (0.28) | 4.30 (0.29) | 4.27 (0.28) | 0.298 |
| Serum phosphate (mmol/L) | 1.04 (0.15) | 1.04 (0.15) | 1.05 (0.15) | 0.4 |
| Diabetic medication | 30 (7.6%) | 23 (12.7%) | 7 (3.3%) | 0.001 |
| Antihypertensive medication | 100 (25.3%) | 49 (27.1%) | 51 (23.7%) | 0.517 |
| Lipid lowering medication | 42 (10.6%) | 21 (11.6%) | 21 (9.8%) | 0.669 |
| Diuretics medication | 54 (13.6%) | 23 (12.7%) | 31(14.4%) | 0.728 |
| Anticoagulant therapy | 8 (2.0%) | 4 (2.2%) | 4 (1.9%) | 1 |
| (**b**) | | | | |
| | **All** | **Dietary Mg ≤ 155.2 mg/1000 kcal/day** | **Dietary Mg > 155.2 mg/1000 kcal/day** | ***p* Value** |
| | **N = 311 (77.9%)** | **n = 154 (49.5%)** | **n = 157 (50.5%)** | |
| Age (years) | 56.39 (9.10) | 55.86 (9.23) | 56.91 (8.97) | 0.311 |
| Male sex | 175 (56.3%) | 118 (76.6%) | 57 (36.3%) | <0.001 |
| Weight (kg) | 82.23 (16.60) | 85.79 (15.81) | 78.74 (16.67) | <0.001 |
| BMI ($kg/m^2$) | 27.95 (4.97) | 28.19 (4.64) | 27.71 (5.27) | 0.394 |
| Smoking | | | | 0.495 |
| Never smoker | 115 (37.0%) | 52 (33.8%) | 63 (40.1%) | |
| Ex-smoker | 136 (43.7%) | 70 (45.5%) | 66 (42.0%) | |
| Smoker | 60 (19.3%) | 32 (20.8%) | 28 (17.8%) | |
| Waist circumference (cm) | 97.99 (14.56) | 100.86 (13.90) | 95.18 (14.69) | 0.001 |
| Systolic BP (mmHg) | 120.05 (16.36) | 123.13 (16.31) | 117.02 (15.89) | 0.001 |
| Diastolic BP (mmHg) | 74.80 (9.90) | 76.30 (9.97) | 73.34 (9.64) | 0.008 |
| Physically active | 189 (60.8%) | 88 (57.1%) | 101 (64.3%) | 0.237 |
| Hypertension | 108 (34.7%) | 60 (39.0%) | 48 (30.6%) | 0.151 |
| Glucose (mg/dl) | 103.44 (18.28) | 105.61 (20.96) | 101.31 (14.96) | 0.038 |
| HbA1c (%) | 5.53 (0.59) | 5.56 (0.63) | 5.51 (0.54) | 0.438 |
| Diabetes | | | | 0.531 |
| No | 192 (61.7%) | 93 (60.4%) | 99 (63.1%) | |
| Prediabetes | 83 (26.7%) | 40 (26.0%) | 43 (27.4%) | |
| Diabetes | 36 (11.6%) | 21 (13.6%) | 15 (9.6%) | |
| Total cholesterol (mg/dl) | 217.67 (36.18) | 216.32 (37.37) | 218.89 (35.05) | 0.532 |
| HDL-C (mg/dl) | 62.63 (17.82) | 58.89 (16.89) | 66.29 (18.00) | <0.001 |
| LDL-C (mg/dl) | 139.31 (33.50) | 139.25 (34.12) | 139.36 (32.99) | 0.977 |
| Triglycerides (mg/dl) | 127.65 (79.43) | 144.12 (97.15) | 111.49 (52.43) | <0.001 |
| eGFR (ml/min/1.73 $m^2$) | 86.63 (13.10) | 86.98 (13.34) | 86.28 (12.88) | 1 |
| Energy intake (kcal/day) | 1841.53 (414.39) | 2004.73 (383.37) | 1681.45 (380.79) | <0.001 |

**Table 1.** *Cont.*

| (**b**) | | | | |
|---|---|---|---|---|
| | **All** | **Dietary Mg ≤ 155.2 mg/1000 kcal/day** | **Dietary Mg > 155.2 mg/1000 kcal/day** | ***p* Value** |
| | **N = 311 (77.9%)** | **n = 154 (49.5%)** | **n = 157 (50.5%)** | |
| Dietary calcium (mg/day) | 763.29 (205.97) | 739.29 (193.76) | 786.83 (215.30) | <0.001 |
| Dietary potassium (mg/day) | 2532.28 (503.22) | 2515.33 (470.74) | 2548.91 (534.16) | 0.557 |
| Dietary phosphate (mg/day) | 1111.75 (263.84) | 1130.93 (244.04) | 1092.94 (281.43) | 0.205 |
| Diabetic medication | 23 (7.4%) | 13 (8.4%) | 10 (6.4%) | 0.630 |
| Antihypertensive medication | 84 (27.0%) | 45 (29.2%) | 39 (24.8%) | 0.458 |
| Lipid lowering medication | 34 (10.9%) | 21 (13.6%) | 13 (8.3%) | 0.183 |
| Diuretics medication | 49 (15.8%) | 22 (14.3%) | 27 (17.2%) | 0.583 |
| Anticoagulant therapy | 8 (2.6%) | 4 (2.6%) | 4 (2.5%) | 1 |

Values are reported as mean (standard deviation) or n (%), unless otherwise indicated. BP, blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate.

Table 1b shows the clinical characteristics of the study participants stratified by dietary magnesium intake. Among 311 subjects with complete dietary data (mean age: 56.3 ± 9.1 years; 56.3% male), 154 (49.5%) had low dietary magnesium intake (mean age: 55.9 ± 9.2 years; 76.6% male). Men were more likely to have significantly lower dietary magnesium intake in comparison to women (*p*-value <0.001). Furthermore, individuals with low dietary magnesium intake were more likely to have higher body weight, waist circumference, glucose levels, systolic and diastolic blood pressure, triglycerides, energy intake, and lower HDL cholesterol and dietary calcium intake.

Concordance between dichotomized serum magnesium and dichotomized dietary magnesium is shown in Table S2. Among 311 participants, 75 (24.1%) had both low serum and dietary magnesium and 91 (29.3%) had both high serum and dietary magnesium.

Overall, mean MRI markers of subclinical cardiovascular disease within the study sample were within normal limits (Table 2a,b).

**Table 2.** (**a**): Imaging markers of subclinical cardiovascular disease by serum magnesium status. (**b**): Imaging markers of subclinical cardiovascular disease by dietary magnesium intake.

| (**a**) | | | | |
|---|---|---|---|---|
| | **All** | **Mg ≤ 2.07 mg/dL** | **Mg > 2.07 mg/dL** | ***p* Value** |
| **Left Ventricular Function** | **N = 366 (91.7%)** | **n = 168 (45.9%)** | **n = 198 (54.1%)** | |
| Early diastolic filling rate (mL/s) | 226.11 (115.89) | 223.95 (115.97) | 227.93 (116.09) | 0.744 |
| Late diastolic filling rate (mL/s) | 225.90 (109.28) | 223.95 (113.18) | 227.56 (106.11) | 0.754 |
| End diastolic volume (mL/m$^2$) | 66.14 (14.90) | 66.50 (16.21) | 65.84 (13.73) | 0.673 |
| End systolic volume (mL/m$^2$) | 20.73 (8.65) | 20.87 (9.67) | 20.60 (7.70) | 0.764 |
| Stroke volume (mL/m$^2$) | 45.43 (9.42) | 45.65 (10.06) | 45.24 (8.85) | 0.682 |
| Cardiac output (mL/min/m$^2$) | 3009.05 (586.74) | 3050.06 (617.36) | 2974.25 (558.66) | 0.219 |
| Ejection fraction (%) | 69.37 (7.78) | 69.52 (8.45) | 69.25 (7.18) | 0.741 |
| Peak ejection rate (mL/s) | 354.70 (132.64) | 352.74 (136.76) | 356.36 (129.37) | 0.795 |
| Myocardial mass (g/m$^2$) | 71.46 (13.37) | 72.46 (13.80) | 70.61 (12.97) | 0.188 |
| LGE | 11 (3.0%) | 3 (1.8%) | 8 (4.0%) | 0.341 |
| Remodeling index (g/mL/m$^2$) | 0.58 (0.15) | 0.59 (0.17) | 0.57 (0.14) | 0.418 |
| Mean diastolic thickness (mm/m$^2$) | 4.85 (0.67) | 4.88 (0.72) | 4.82 (0.61) | 0.33 |
| **Right ventricular function** | **N = 336 (84.2%)** | **n = 85 (25.3%)** | **n = 251 (74.7%)** | |
| End diastolic volume (mL/m$^2$) | 84.72 (17.46) | 87.63 (18.54) | 83.73 (17.00) | 0.075 |
| End systolic volume (mL/m$^2$) | 40.33 (11.78) | 42.70 (12.16) | 39.52 (11.56) | 0.031 |
| Stroke volume (mL/m$^2$) | 44.43 (9.11) | 44.95 (9.45) | 44.25 (9.00) | 0.539 |
| Cardiac output (mL/min/m$^2$) | 2938.74 (574.20) | 3004.43 (575.29) | 2916.49 (573.27) | 0.223 |
| Ejection fraction (%) | 52.85 (7.01) | 51.80 (6.74) | 53.21 (7.08) | 0.109 |

**Table 2.** *Cont.*

| (a) | | | | |
|---|---|---|---|---|
| | **All** | **Mg ≤ 2.07 mg/dL** | **Mg > 2.07 mg/dL** | ***p* Value** |
| **Carotid plaque** | **N = 248 (62.2%)** | **n = 76 (30.6%)** | **n = 172 (69.4%)** | |
| Presence of plaque | 50 (20.2%) | 12 (15.8%) | 38 (22.1) | 0.333 |
| Presence of plaque type | | | | 0.416 |
| AHA type I | 198 (79.8%) | 64 (84.2%) | 134 (77.9) | |
| AHA type III | 34 (13.7%) | 7 (9.2%) | 27 (15.7) | |
| AHA type V | 10 (4.0%) | 4 (5.3%) | 6 (3.5) | |
| AHA type VI or VII | 6 (2.4%) | 1 (1.3%) | 5 (2.9) | |
| Wall thickness left (mm) | 0.75 (0.11) | 0.76 (0.13) | 0.74 (0.10) | 0.112 |
| Wall thickness right (mm) | 0.76 (0.10) | 0.78 (0.10) | 0.75 (0.10) | 0.018 |
| (**b**) | | | | |
| | **All** | **Dietary Mg ≤ 155.2 mg/1000 kcal/day** | **Dietary Mg > 155.2 mg/1000 kcal/day** | ***p* Value** |
| **Left Ventricular Function** | **N = 287 (91.8%)** | **n = 141 (49.1%)** | **n = 146 (50.9%)** | |
| Early diastolic filling rate (mL/s) | 229.48 (115.39) | 226.99 (110.31) | 231.87 (120.42) | 0.721 |
| Late diastolic filling rate (mL/s) | 227.74 (110.88) | 226.97 (118.89) | 228.48 (102.96) | 0.908 |
| End diastolic volume (mL/m$^2$) | 66.65 (14.81) | 65.83 (14.37) | 67.45 (15.23) | 0.355 |
| End systolic volume (mL/m$^2$) | 20.73 (8.08) | 20.48 (7.81) | 20.97 (8.35) | 0.607 |
| Stroke volume (mL/m$^2$) | 45.94 (9.43) | 45.38 (9.17) | 46.47 (9.67) | 0.327 |
| Cardiac output (mL/min/m$^2$) | 3041.45 (574.70) | 3047.67 (561.30) | 3035.44 (589.22) | 0.857 |
| Ejection fraction (%) | 69.53 (7.27) | 69.46 (6.93) | 69.60 (7.61) | 0.875 |
| Peak ejection rate (mL/s) | 356.40 (133.62) | 361.26 (136.96) | 351.71 (130.61) | 0.546 |
| Myocardial mass (g/m$^2$) | 70.71 (12.72) | 73.57 (12.56) | 67.94 (12.29) | <0.001 |
| LGE | 9 (3.1%) | 3 (2.1%) | 6 (4.1%) | 0.532 |
| Remodeling index (g/mL/m$^2$) | 0.57 (0.14) | 0.58 (0.15) | 0.56 (0.13) | 0.091 |
| Mean diastolic thickness (mm/m$^2$) | 4.81 (0.63) | 4.88 (0.65) | 4.75 (0.61) | 0.090 |
| **Right ventricular function** | **N = 263 (84.0%)** | **n = 128 (48.7%)** | **n = 135 (51.3%)** | |
| End diastolic volume (mL/m$^2$) | 85.66 (17.65) | 85.92 (17.27) | 85.41 (18.07) | 0.817 |
| End systolic volume (mL/m$^2$) | 40.52 (12.03) | 41.36 (11.94) | 39.72 (12.10) | 0.271 |
| Stroke volume (mL/m$^2$) | 45.18 (9.00) | 44.58 (8.65) | 45.74 (9.32) | 0.296 |
| Cardiac output (mL/min/m$^2$) | 2974.97 (574.37) | 3000.75 (561.10) | 2950.51 (587.71) | 0.118 |
| Ejection fraction (%) | 53.21 (6.89) | 52.37 (6.83) | 54.01 (6.88) | <0.001 |
| **Carotid plaque** | **N = 188 (60.1%)** | **n = 100 (53.2%)** | **n = 88 (46.8%)** | |
| Presence of plaque | 41 (21.8%) | 23 (23.0%) | 18 (20.5%) | 0.807 |
| Presence of plaque type | | | | 0.265 |
| AHA type I | 147 (78.2%) | 77 (77.0%) | 70 (79.5%) | |
| AHA type III | 28 (14.9%) | 15 (15.0%) | 13 (14.8%) | |
| AHA type V | 7 (3.7%) | 6 (6.0%) | 1 (1.1%) | |
| AHA type VI or VII | 6 (3.2%) | 2 (2.0%) | 4 (4.5%) | |
| Wall thickness left (mm) | 0.75 (0.11) | 0.75 (0.12) | 0.74 (0.10) | 0.463 |
| Wall thickness right (mm) | 0.76 (0.10) | 0.76 (0.10) | 0.75 (0.11) | 0.248 |

Values are reported as the mean (SD), n (%), unless otherwise indicated. LGE: Late Gadolinium Enhancement, AHA: American Heart Association

### *3.2. Correlation between Serum and Dietary Magnesium with Clinical Characteristics and MRI-Derived Markers*

In our sample, there was no correlation between serum and dietary magnesium (Spearman's rho = 0.04, $p = 0.5$, Figure 2). Serum magnesium was negatively correlated with systolic blood pressure and fasting blood glucose ($p = 0.003$, Table S3), and positively correlated with total cholesterol and LDL-cholesterol ($p < 0.05$, Table S3). Regarding MRI outcomes, serum magnesium was negatively correlated with right carotid artery wall thickness ($p = 0.037$), but not with any other MRI parameter of cardiac structure and function. On the other hand, dietary magnesium intake was positively correlated with

systolic blood pressure and body weight ($p < 0.05$, Table S3). Expectedly, dietary magnesium was highly correlated with dietary potassium and dietary phosphate (Table S3). Regarding MRI outcomes, dietary magnesium was positively correlated with LV and RV end-systolic and end-diastolic volume and myocardial mass, and negatively correlated with ejection fraction, peak ejection rate, remodeling index, and mean diastolic wall thickness.

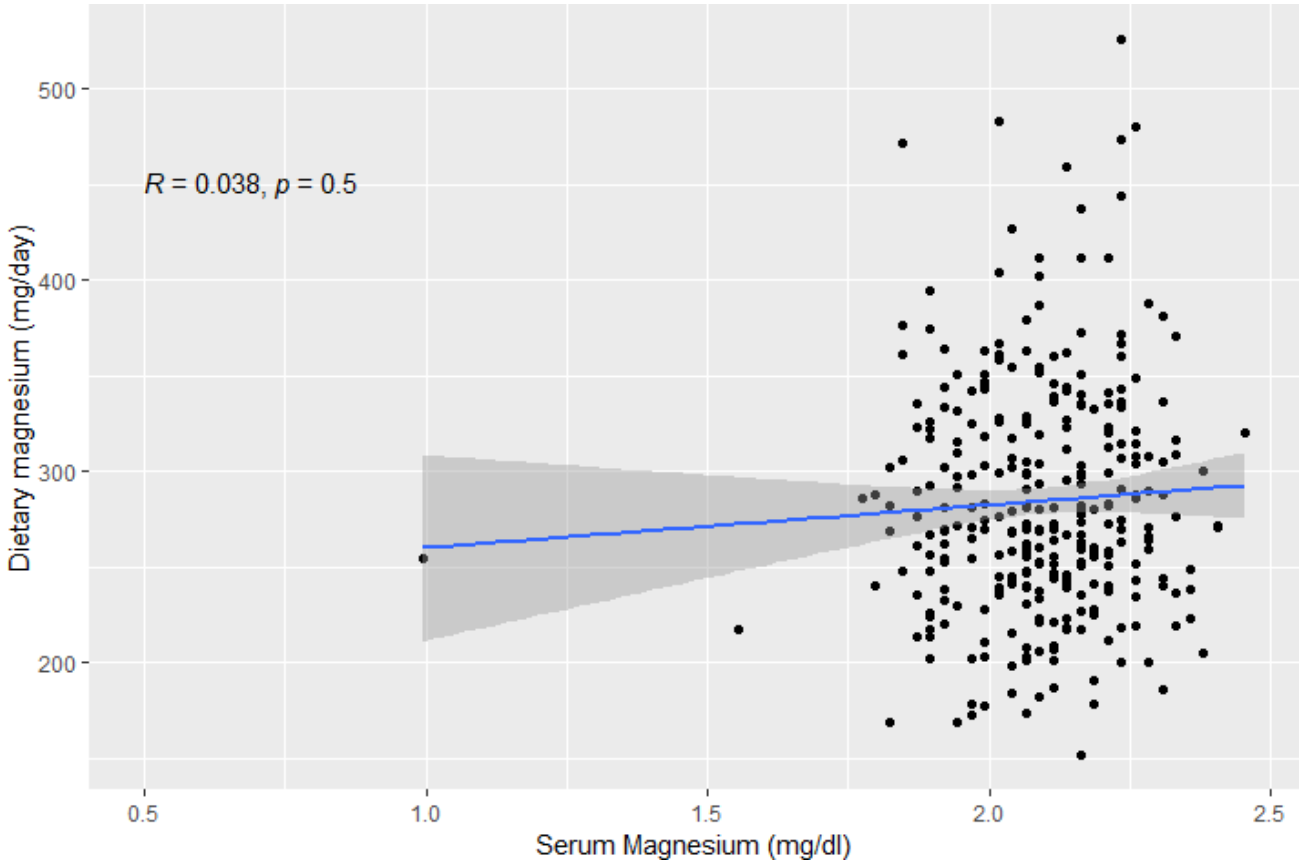


**Figure 2.** Correlation between serum and dietary magnesium, as assessed by Spearman correlation.

### *3.3. Association between Magnesium and MRI-Derived Subclinical Cardiovascular Disease Markers*

#### 3.3.1. Serum Magnesium

In univariate analysis, markers of early cardiac impairment and preclinical atherosclerosis were similar between groups with and without hypomagnesemia (Table 2a). However, right ventricular end-systolic volume and right carotid artery wall thickness were significantly higher in the group with low serum magnesium levels ($p$-value: 0.031, 0.018, respectively). Multivariable analysis adjusted for age, sex, BMI, systolic blood pressure, and diabetes status revealed a significant negative association of continuous serum magnesium with right ventricular end-systolic volume (coefficient: $-1.21 mL/m^2$; 95% CI $-2.39 mL/m^2$ to $-0.04 mL/m^2$). Higher ORs for LGE, presence, and type of carotid plaque were also observed, which remained significant even after additionally adjusting for serum cholesterol and smoking status (OR = 3.06, 95% CI 1.27 to 8.32; OR = 1.62, 95% CI 1.07 to 2.56; OR = 1.58, 95% CI 1.19 to 2.11, respectively) (Table 3). We note, however, that prevalence of LGE was low and this result has to be interpreted with caution.

**Table 3.** Results of multivariable-adjusted models for the relation between serum and dietary magnesium and imaging markers of subclinical cardiovascular disease.

| | Serum Magnesium | | | Dietary Magnesium | | |
|---|---|---|---|---|---|---|
| | **Estimate** | **95% CI** | ***p* Value** | **Estimate** | **95% CI** | ***p* Value** |
| **Left Ventricular Function** | | **N = 366** | | | **N = 287** | |
| Early diastolic filling rate (mL/s) | −3.29 | (−14.24, 7.67) | 0.556 | 0.17 | (−0.217, 0.556) | 0.388 |
| Late diastolic filling rate (mL/s) | −0.18 | (−11.17, 10.81) | 0.975 | 0.05 | (−0.347, 0.453) | 0.794 |
| End diastolic volume (mL/m$^2$) | −0.86 | (−2.32, 0.58) | 0.239 | 0.06 | (0.014, 0.114) | 0.013 |
| End systolic volume (mL/m$^2$) | −0.07 | (−0.94, 0.79) | 0.871 | 0.04 | (0.008, 0.065) | 0.011 |
| Stroke volume (mL/m$^2$) | −0.81 | (−1.72, 0.10) | 0.082 | 0.03 | (−0.006, 0.059) | 0.104 |
| Cardiac output (mL/min/m$^2$) | −52.73 | (−111.58, 6.11) | 0.078 | 1.42 | (−0.631, 3.471) | 0.174 |
| Ejection fraction (%) | −0.38 | (−1.16, 0.41) | 0.341 | −0.02 | (−0.048, 0.004) | 0.101 |
| Peak ejection rate (mL/s) | 5.38 | (−7.66, 18.42) | 0.418 | −0.22 | (−0.685, 0.253) | 0.365 |
| Myocardial mass (g/m$^2$) | 0.003 | (−1.17, 1.18) | 0.995 | −0.01 | (−0.052, 0.028) | 0.546 |
| LGE | OR 3.06 | (1.27, 8.32) | 0.018 | OR 1.01 | (0.988, 1.036) | 0.434 |
| Remodeling index (g/mL/m$^2$) | 0.005 | (−0.009, 0.019) | 0.479 | −0.001 | (−0.001. −0.0002) | 0.004 |
| Mean diastolic thickness (mm/m$^2$) | 0.02 | (−0.04, 0.08) | 0.565 | −0.002 | (−0.004, −0.0002) | 0.029 |
| **Right ventricular function** | | **(N = 336)** | | | **(N = 236)** | |
| End diastolic volume (mL/m$^2$) | −1.74 | (−3.50, 0.02) | 0.053 | 0.05 | (−0.011, 0.114) | 0.105 |
| End systolic volume (mL/m$^2$) | −1.21 | (−2.39, −0.04) | 0.043 | 0.03 | (−0.011, 0.071) | 0.171 |
| Stroke volume (mL/m$^2$) | −0.53 | (−1.47, 0.41) | 0.268 | 0.02 | (−0.011, 0.056) | 0.187 |
| Cardiac output (mL/min/m$^2$) | −32.62 | (−93.93, 28.70) | 0.296 | 1.51 | (−0.852, 3.474) | 0.169 |
| Ejection fraction (%) | 0.33 | (−0.38, 1.04) | 0.362 | −0.01 | (−0.034, 0.014) | 0.441 |
| **Carotid plaque** | | **(N = 248)** | | | **(N = 188)** | |
| Presence of plaque | OR 1.62 | (1.07, 2.56) | 0.033 | OR 0.99 | (0.975,1.000) | 0.056 |
| Presence of plaque type | OR 1.58 | (1.19, 2.11) | 0.002 | OR 0.99 | (0.980, 0.996) | 0.004 |
| Wall thickness left (mm) | −0.003 | (−0.016, 0.009) | 0.66 | −0.0001 | (−0.0006, 0.0003) | 0.562 |
| Wall thickness right (mm) | −0.008 | (−0.019, 0.004) | 0.211 | −0.0001 | (−0.0006, 0.0002) | 0.381 |

All models are adjusted for age, sex, BMI, systolic blood pressure, and diabetes. Daily caloric intake was additionally adjusted for models with dietary magnesium. Serum cholesterol and smoking status were additionally adjusted for the association between serum magnesium and LGE, presence and type of plaque. Estimates represent β-estimates from linear regression or OR from logistic regression.

In mediation analysis, the association of serum magnesium on carotid plaque was not mediated by serum cholesterol (coefficient: 0.003 mg/dl; 95% CI −0.06 mg/dl to 0.12 mg/dl) (Figure 3).

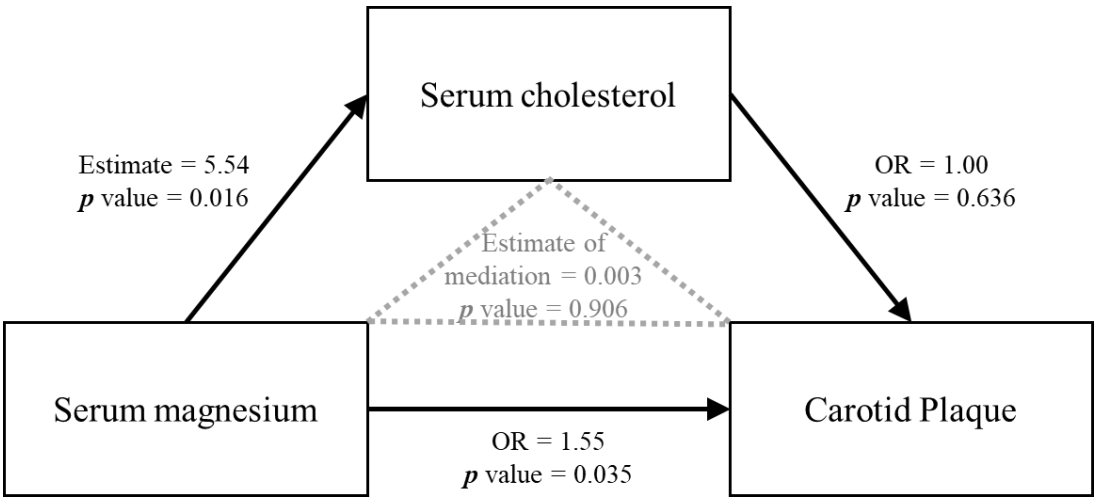


**Figure 3.** Direct acyclic graph (DAG) showing non-adjusted mediation analysis between serum magnesium, serum cholesterol, and carotid plaque.

#### 3.3.2. Dietary Magnesium

Table 2b presents the univariate analysis between dietary magnesium intake and MRI markers of subclinical cardiovascular disease. Left ventricular myocardial mass was significantly higher in individuals with low dietary magnesium intake (73.57 g/m$^2$ ± 12.56 g/m$^2$ vs. 67.94 g/m$^2$ ± 12.29 g/m$^2$, *p*-value < 0.001). In addition, with a decrease in dietary magnesium intake, there was a decrease in right ventricular ejection fraction (52.37% ± 6.83% vs. 54.01% ± 6.88%, *p*-value < 0.001). In multivariable analysis, we observed a significant positive association between dietary magnesium intake and left ventricular end-systolic and end-diastolic volume after adjusting for age, sex, BMI, systolic blood pressure, and diabetes status (coefficients: 0.04 mL/m$^2$, 0.06 mL/m$^2$; CI 0.008 mL/m$^2$ to 0.065 mL/m$^2$, 0.014 mL/m$^2$ to 0.114 mL/m$^2$, respectively) (Table 3). Furthermore, there was an inverse association between dietary magnesium intake and left ventricular mean diastolic thickness and remodeling index (coefficients: −0.002 mm/m$^2$, −0.001 g/mL/m$^2$; CI −0.004 mm/m$^2$ to −0.0002 mm/m$^2$, −0.001 g/mL/m$^2$ to −0.0002 g/mL/m$^2$, respectively). Dietary magnesium was also associated with lower risk of more severe plaque (type of carotid plaque OR 0.99; 95% CI 0.98 to 0.99).

When stratifying the sample by both serum and dietary magnesium, no clear pattern in the distribution of MRI markers could be observed (Table S4).

## 4. Discussion

### *4.1. Main Findings*

This comprehensive investigation of the association of serum and dietary magnesium with several imaging-derived markers of subclinical CVD burden in a population-based cohort revealed partly contradicting directions of results. While serum magnesium was associated with decreased right ventricular end-systolic volume, dietary magnesium intake was associated with increased left ventricular end-systolic and end-diastolic volume. Unlike dietary magnesium intake, serum magnesium was associated with a higher risk for carotid plaque. We also found an inverse association between dietary magnesium intake and left ventricular remodeling and mean left ventricular thickness. These associations were independent of sex, age, and common cardiovascular risk factors including hypertension and diabetes status.

### *4.2. Correlation between Serum and Dietary Magnesium*

These inconsistent results will partly be due to the low correlation between dietary and serum magnesium (r = 0.038, *p*-value = 0.501). Only 75 subjects had consistently low serum and dietary magnesium and only 91 subjects had consistently high serum and dietary magnesium. The poor correlation between dietary magnesium and plasma levels of this mineral has been reported in several previous studies [37–40]. This could be explained by the fact that the magnesium concentration is regulated by a balance between intestinal absorption and kidney excretion. Any condition impairing intestinal absorption such as aging, inflammation, bowel disorders, or reduced kidney function could affect magnesium bioavailability and thus contribute to the lack of correlation between serum and dietary magnesium [41]. Furthermore, serum magnesium constitutes only 0.3% of the total body magnesium and thus may not necessarily reflect true body magnesium content. Indeed, magnesium concentration is under tight hemostatic regulation and can be maintained as normal, even if intakes are low, by reducing urinary excretion and mineral release from bone, muscles, and internal organs [42]. This is supported by the metabolic unit magnesium balance experiments on menopausal women, showing that serum magnesium levels did not significantly change with the magnesium depletion–repletion protocol. Furthermore, they found that consuming a magnesium-deficient diet for 72 to 92 days did not markedly decrease serum magnesium concentration [43]. This indicates that normal serum magnesium concentrations do not rule out magnesium deficiency.

### *4.3. Association of Magnesium with Overt and Early Cardiac Impairment*

Magnesium has been implicated as a potential marker of CVD risk. In fact, evidence indicates that high serum and dietary magnesium are inversely associated with CVD [7,44]. There is a paucity of studies regarding the relationship of serum and dietary magnesium with early cardiac impairment markers. A population-based longitudinal study showed that low serum magnesium concentrations were associated with increased left ventricular mass [17]. The Jackson Heart Study found no association between quartiles of magnesium intake/Kg body weight and systolic function. However, dietary magnesium was inversely associated with Doppler peak mitral E wave velocity (a surrogate for diastolic function) and tricuspid regurgitation peak velocity (an estimate of pulmonary systolic pressures) [15]. Our results failed to demonstrate any association between serum magnesium and left ventricular parameters. On the other hand, we found an inverse association between serum magnesium and right ventricular end-systolic volume which could potentially imply a protective effect of serum magnesium to subclinical pulmonary vascular resistance. This is supported by an animal study which demonstrated that magnesium supplementation reduced pulmonary arterial pressure, right heart hypertrophy, and media wall thickness of pulmonary arteries [45].

Diastolic dysfunction is characterized by impaired myocardial relaxation and left ventricular filling and distensibility, which is associated with significant morbidity and mortality [46]. In our study, individuals with higher dietary intake of magnesium had larger end-diastolic volumes and less remodeling, indicating better diastolic function. These associations remained significant after multivariable adjustment. This is in good agreement with the Jackson Heart Study [15] as well as with an animal study showing an impaired relaxation with a decreased ratio between early and late diastolic velocity of the mitral valve in low-magnesium fed mice, which was reversed after magnesium repletion [11].

Notably, we observed increased rates of myocardial LGE—potential markers of minor myocardial infarction—in participants with high serum magnesium levels. It has been shown that in patients with acute coronary syndrome, there is a higher magnesium leakage from the infarcted myocardium leading to increased magnesium levels, with subsequent development of malignant ventricular arrhythmia and increased in-hospital deaths [47,48]. However, this cannot explain our findings since participants were not in an acute condition. We interpret our results with caution, since the absolute number of LGE was low.

It is crucial to note that despite the majority of observational studies favoring serum magnesium in the prevention of CVD, causal associations using Mendelian randomization remain inconsistent. One study found that a genetic predisposition to higher magnesium levels was inversely associated with coronary artery disease [49], whereas the other found no associations between serum magnesium and type 2 diabetes, coronary artery disease, heart failure, and atrial fibrillation [50]. Therefore, the effect of magnesium on the development of CVD remains equivocal.

### *4.4. Association of Magnesium with Lipids and Preclinical Atherosclerosis*

The relationship between magnesium with preclinical carotid atherosclerosis is not novel, as this association has been described in different studies, using ultrasound for the assessment of carotid-intima media thickness as a proxy for atherosclerosis. While some studies found an inverse association between serum magnesium and common carotid intima-media thickness [8,18,19], others demonstrated an effect of magnesium supplementation on improving endothelial function but not carotid intima-media thickness [21]. A population-based study in Japan showed a significant association between low serum magnesium and mean intima-media thickness and risk of ≥2 carotid plaques [20]. Our results, however, suggested a positive association between serum magnesium and carotid plaque. On the other hand, higher dietary magnesium in the current study was modestly associated with decreased carotid plaque type. This is in good agreement with the study reporting that greater magnesium intake was associated with slightly lower odds of high common carotid artery intima-media thickness [51]. The accuracy of intima-media thickness as a

marker of preclinical atherosclerosis is questioned since it can only measure the common carotid artery (laminal turbulent flow), rather than the internal carotid or carotid bulb. Furthermore, the main predictors of common carotid artery media hypertrophy are age and hypertension rather than atherosclerosis [22]. In contrast, carotid plaque has a better predictive ability of coronary artery disease. In fact, a meta-analysis of 11 population-based studies found that carotid plaque has a significantly higher diagnostic accuracy for the prediction of future myocardial infarction events in comparison to carotid intima-media thickness [52].

The relationship between magnesium status and dyslipidemia is ambiguous and studies present conflicting results. A study has suggested a possible magnesium–lipid interaction in atherosclerosis. They found that high LDL-cholesterol and triglyceride levels affect carotid intima-media thickness only when magnesium levels are low [53]. Nevertheless, we found that serum magnesium was positively associated with total cholesterol levels. This is consistent with previous research [8,18–20,54]. In causal mediation analysis, we found that the effect of magnesium on carotid plaque was not mediated by cholesterol. These results point to the theory suggesting a simple binding interaction between serum magnesium and lipoprotein particles rather than a complex physiological and pathological process [55].

### *4.5. Strengths and Limitations*

The strengths of our study include the availability of both serum and dietary magnesium as markers of magnesium concentration in the body. The accurate measurement of various functional and structural parameters of subclinical CVD burden using an advanced MRI technique contrary to previous studies that used echocardiography and ultrasound was a strength of this study. Additionally, the well-designed population-based study that included extensive measurements and assessment of the dietary intake and different confounding variables was also a strength. Nevertheless, we are aware that our study has limitations that may have influenced the reported results. We had a relatively small sample size of participants which became more prominent in complete-case analyses. This will have reduced the statistical power to show a potential association between magnesium with markers of subclinical CVD. Because our study design was cross-sectional, the temporality and causality of the associations could not be concluded. Moreover, since our analysis was performed on a selected cross-sectional sample, generalizability to other populations might be limited.

## 5. Conclusions

Our results showed that serum and dietary magnesium were associated with some markers of subclinical CVD burden among participants without manifest cardiovascular disease, and thus may be implicated in cardiovascular disease development already at the subclinical stage. However, the findings were inconsistent and highlighted the importance to consider both dietary and serum magnesium since these entities do not correlate well. Larger, well-characterized, population-based studies are required to increase statistical power and confirm and extend our findings.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14234954/s1, Table S1: Demographic and cardiovascular characteristics of excluded and included participants for the respective analyses. Table S2: Demographic and cardiovascular risk factors by serum and dietary magnesium. Table S3: Correlation between serum and dietary magnesium with cardiovascular risk factors and imaging markers of subclinical cardiovascular disease, assessed by Spearman correlation. Table S4: Imaging markers of subclinical cardiovascular disease by serum and dietary magnesium.

**Author Contributions:** Conceptualization, N.S.A. and S.R.; Methodology, N.S.A., C.L.S., F.B., B.T., J.L., J.S., A.P. and S.R.; Formal Analysis, N.S.A.; Resources, C.L.S., F.B., B.T., J.L. and A.P.; Data Curation, C.L.S., F.B., B.T., J.L., A.P. and S.R.; Writing—Original Draft Preparation, N.S.A. and S.R.;

Writing—Review and Editing, N.S.A., C.L.S., F.B., B.T., J.L., J.S., A.P. and S.R.; Supervision, J.S., A.P. and S.R.; Funding Acquisition, F.B., J.L. and A.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** The KORA study was initiated and financed by the Helmholtz Zentrum München—German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. The KORA-MRI study received funding from the German Research Foundation (Bonn, Germany) grant BA 4233/4-1, and German Centre for Cardiovascular Disease Research (Berlin, Germany) grants 81x2600209 and 81x2600214.

**Institutional Review Board Statement:** The study was performed according to the Declaration of Helsinki. It was approved by the ethics committee of Ludwig-Maximillian-University Munich (498-12) and the Bavarian Chamber of Physicians (FF4: EC No. 06068).

**Informed Consent Statement:** All participants gave written informed consent.

**Data Availability Statement:** The informed consent given by KORA study participants does not cover data posting in public databases. However, data are available upon request from the KORA database by means of a project agreement. Requests should be sent to kora.passt@helmholtz-muenchen.de and are subject to approval by the KORA Board.

**Conflicts of Interest:** The authors declare no conflict of interest.

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## Project III

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| Title: | Magnesium Depletion, Metabolic Impairment, and Cardiac Alterations: The NAKO-MRI Study with Mendelian Randomization |
| Authors: | Nuha Shugaa Addin, Christopher Schuppert, Peter M. Full, Hermann Brenner, Marcus Dörr, Thomas Keil, Ricarda von Krüchten, Felix G. Meinel, Thoralf Niendorf, Tobias Pischon, Börge Schmidt, Jeanette Schulz-Menger, Julia Schwichtenberg, Henry Völzke, Stefan N. Willich, Fabian Bamberg, Annette Peters, Christopher L. Schlett, Susanne Rospleszcz |
| Journal: | Journal of Clinical Endocrinology and Metabolism |
| Year: | 2025 |
| DOI: | https://doi.org/10.1210/clinem/dgaf476 |
| Impact factor: | 5 |
| Rank: | 30/186 in Endocrinology and Metabolism<br>(Journal Citation Reports™, year 2023) |

*The Journal of Clinical Endocrinology & Metabolism*, 2025, **00**, 1–13
https://doi.org/10.1210/clinem/dgaf476
Advance access publication 21 August 2025
**Clinical Research Article**

# Magnesium Depletion, Metabolic Impairment, and Cardiac Alterations: The NAKO-MRI Study With Mendelian Randomization

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## Abstract

**Context:** Magnesium deficiency may contribute to subclinical cardiac changes, particularly metabolic diastolic cardiomyopathy.

**Objective:** To investigate the association between magnesium depletion, metabolic syndrome (MetS), and magnetic resonance imaging (MRI)-derived cardiac alterations in a population-based sample.

**Methods:** We cross-sectionally analyzed participants (N = 9568) from the baseline examination of the German National Cohort who underwent whole-body MRI. Associations of serum magnesium and magnesium depletion score (MDS) with MetS and cardiac alterations were assessed using multivariable logistic and linear regression, respectively. Two-sample Mendelian Randomization was performed to evaluate the potential causal relationship between serum magnesium and MRI-derived cardiac parameters.

**Results:** Our analysis revealed no correlation between serum magnesium and MDS (Spearman's rho = 0.065; $P < .001$). A 1-SD increase in serum magnesium was associated with lower MetS prevalence (odds ratio, 0.93 [95% CI, 0.88-0.99]) and reduced left and right ventricular systolic and diastolic volumes. Higher MDS, indicating magnesium deficiency, was linked to increased MetS prevalence (OR per 1 unit, 1.32 [95% CI, 1.23-1.41]) and its individual components. Furthermore, higher MDS was associated with increased left ventricular remodeling index (estimate, 0.012 g/mL [95% CI, 0.008-0.017]) and decreased left ventricular end-diastolic volume (estimate, −1.132 mL/m$^2$ [95% CI, −1.538 to −0.727]), indicating concentric hypertrophy. Two-sample Mendelian Randomization suggested no causal relationship between serum magnesium and MRI-derived cardiac markers.

**Received:** 8 May 2025. **Editorial Decision:** 18 August 2025. **Corrected and Typeset:** 16 September 2025

**Conclusion:** Magnesium depletion may serve as an early indicator of cardiac impairment. However, Mendelian Randomization results do not support a causal role of serum magnesium on cardiac structure and morphology.

**Key Words:** magnesium, metabolic syndrome, cardiac function, cardiac morphology, magnetic resonance imaging

**Abbreviations:** BMI, body mass index; CKM, Cardiovascular-Kidney-Metabolic syndrome; CMR, cardiovascular magnetic resonance; CVD, cardiovascular disease; DAG, directed acyclic graph; eGFR, estimated glomerular filtration rate; GWAS, genome-wide association study; HbA1c, hemoglobin A1c; LVRI, left ventricular remodeling index; MDS, magnesium depletion score; MetS, metabolic syndrome; MRI, magnetic resonance imaging; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier test; NAKO, The German National Cohort; OR, odds ratio; SNP, single nucleotide polymorphism.

Magnesium is the second most abundant intracellular cation, primarily stored in bones and skeletal muscles, with less than 1% found in serum (1). Magnesium acts as a cofactor for numerous enzymatic reactions critical to hundreds of body processes, including the regulation of cardiovascular function. Evidence suggests that magnesium has anti-inflammatory, anti-ischemic, and antiarrhythmic properties (2). Chronic magnesium deficiency contributes to several pathophysiological processes, including inflammation, oxidative stress, endothelial dysfunction, and platelet aggregation. Consequently, it can lead to the development of various subclinical diseases, such as insulin resistance and altered lipid metabolism, all of which increase the risk of cardiovascular disease (CVD) (3).

The complex pathophysiological interplay between several cardiometabolic risk factors has led to the conceptualization of Cardiovascular-Kidney-Metabolic (CKM) syndrome (4). Poor CKM health significantly contributes to multimorbidity and premature mortality, imposing major burdens on public health systems and socioeconomics (5). CKM syndrome progresses through 4 stages, beginning with early excess or dysfunctional adiposity and insulin resistance, which can develop into metabolic syndrome (MetS) and chronic kidney disease in stage 2. Stage 3 marks the subclinical phase, which includes preclinical atherosclerosis and pre-heart failure, and can progress to clinical CVD if not treated (4). The American Heart Association emphasizes research on the first 3 stages to help prevent CVD events (4).

Advances in noninvasive cardiac imaging have enabled the precise quantification of ventricular morphology and function, as well as the detection of subtle cardiac abnormalities in asymptomatic individuals at high risk for heart failure, who would benefit from early intervention. Compared to echocardiography, cardiovascular magnetic resonance (CMR) imaging offers higher reproducibility, greater accuracy, and reduced susceptibility to poor image quality (6). Few studies have explored the relationship between magnesium and imaging-based cardiac morphology and function. Reffelmann et al found an inverse association between serum magnesium and left ventricular mass measured by echocardiography (7). Building on this, our team identified cross-sectional associations between serum and dietary magnesium and various magnetic resonance imaging (MRI)-derived subclinical CVD markers (8).

Because serum magnesium levels are affected by homeostatic mechanisms in chronic deficiency and modulated by factors such as gastrointestinal absorption and renal handling (9), there is a need for more comprehensive assessment methods. To address this, Fan and his team developed the Magnesium Depletion Score (MDS), a scoring system designed to predict magnesium deficiency. MDS incorporates 4 established risk factors, namely diuretic use, proton pump inhibitor use, estimated glomerular filtration rate, and alcohol abuse. These risk factors are known to compromise the kidneys' capacity to reabsorb magnesium. Higher MDS scores indicate greater severity of magnesium deficiency (10). However, the association between magnesium deficiency conceptualized by the MDS and CMR-derived cardiac alterations has not been investigated.

MetS represents the second stage of CKM syndrome, characterized by a constellation of interconnected metabolic risk factors, namely abdominal obesity, hyperglycemia, dyslipidemia, and elevated blood pressure (11). Previous studies have shown an inverse association between serum magnesium and MetS, and this association could be potentially causal (12, 13). Additionally, MetS has been linked to MRI markers of subclinical CVD, including left ventricular diastolic dysfunction (14, 15). To entangle these complex metabolic relationships and shed further insight on the role of magnesium depletion in this, the current study aimed to use a large population-based sample to investigate the association of serum magnesium and MDS with MetS, and with CMR-derived markers of left and right ventricular morphology and function. Additionally, we examined the potential mediating role of MetS in the relationship between magnesium depletion and cardiac alterations. Finally, we conducted a 2-sample Mendelian Randomization analysis to assess the causal link between serum magnesium and MRI-derived ventricular markers.

## Methods

### Study Population

We used cross-sectional data from the baseline examination of the German National Cohort (NAKO Gesundheitsstudie), an ongoing, multidisciplinary, population-based prospective study conducted across 18 study centers in Germany (16). Briefly, >205 000 women and men aged 19 to 74 years were randomly selected from residents' registration offices, covering both urban and rural areas. Recruitment took place between 2014 and 2019 and included face-to-face interviews, self-administered questionnaires, physical examinations, and the collection of various biological samples. A subgroup of 30 868 participants at 5 study sites underwent a comprehensive imaging protocol, including cardiac MRI (17). The NAKO study received approval from local ethics committees, and all participants gave written informed consent.

### Exposure Assessment

Serum magnesium concentrations (mmol/L) were measured using photometry (modified methylthymol blue method) on the Dimension VISTA 1500 analyzer (Siemens Healthineers, Erlangen, Germany). Calcium interference was minimized using Ba-EGTA, which selectively complexes with calcium. Residual interference from calcium at serum concentrations up to 5 mmol/L resulted in less than 10% bias in magnesium measurements and was therefore considered analytically

and clinically negligible. The magnesium-methylthymol blue complex was quantified at 600 and 510 nm. The coefficients of variation were 2.73% for low and 2.18% for high control concentrations.

MDS was used as an indicator of magnesium status in the body and was calculated based on the sum of the following scores: (1) 1 point for current diuretic use; (2) 1 point for current proton pump inhibitor use; (3) 1 point for an estimated glomerular filtration rate (eGFR) between 60 mL/min/1.73 $m^2$ and 90 mL/min/1.73 $m^2$, whereas 2 points were assigned for individuals with eGFR less than 60 mL/min/1.73 $m^2$; and (4) 1 point for heavy alcohol drinking, defined as >1 drink/day for women (14 g/day) and >2 drinks/day for men (28 g/day) (10). We calculated eGFR based on creatinine using the Chronic Kidney Disease Epidemiology Collaboration formula (18). We analyzed MDS both as a continuous variable and as a categorical variable (0, 1, 2, ≥3).

## Outcome Assessment

CMR was performed as part of a whole-body MRI protocol on 3T MR scanners (MAGNETOM Skyra, Siemens Healthineers, Erlangen, Germany) (17, 19) and included the acquisition of steady-state free precession, 2-dimensional, multislice, short-axis view CINE data covering the heart from the apex to the base with whole cardiac cycle coverage. Clinical CMR parameters were derived from segmentation of the epi- and endocardial borders using an extended nnU-net framework (20), including extensive quality control for image and segmentation quality and imaging artefacts (21). Final CMR parameters include left and right ventricular end-diastolic and end-systolic volume, stroke volume and ejection fraction, as well as left ventricular end-diastolic mass. Volumes and mass were indexed by body surface area using the Du Bois formula (body surface area $[m^2] = 0.007184 \times \text{weight (kg)}^{0.425} \times \text{height (cm)}^{0.725}$). The left ventricular remodeling index (LVRI) was calculated as mass divided by volume, and left ventricular concentricity was defined as LVRI > 1.3 g/mL (22).

## MetS Assessment

We defined MetS according to the harmonized definition (23) as the presence of at least 3 of the following criteria: (1) waist circumference ≥94 cm in men or ≥80 cm in women; (2) serum fasting triglycerides ≥150 mg/dL (1.69 mmol/L) or the use of fibrates; (3) serum high-density lipoprotein cholesterol <40 mg/dL (1.03 mmol/L) in men or <50 mg/dL (1.29 mmol/L) in women or the use of fibrates; (4) systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or treatment with antihypertensive medication, being aware of having hypertension; and (5) fasting serum glucose level ≥100 mg/dL (5.6 mmol/L) or intake of antidiabetic medication.

## Covariate Assessment

All participants provided information on sociodemographic and lifestyle factors as well as medication and previous disease history. Physical activity was categorized according to the World Health Organization recommendations, with individuals achieving >600 MET-min/week considered physically active. Smoking status was classified as never smoker, former smoker, and current smoker. We defined alcohol consumption as none (0 g/day), moderate (0.1-39.9 g/day for men and 0.1-19.9 g/day for women), and excessive (≥40 g/day for men and ≥20 g/day for women) (24).

Additionally, participants underwent comprehensive physical and laboratory examinations. Anthropometric and blood pressure measurements were performed by trained personnel using standardized protocols and instruments. Body mass index (BMI) was calculated as body weight divided by the square of body height (kg/$m^2$). Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. Serum biomarkers such as lipid profile, serum glucose, hemoglobin A1c (HbA1c), and serum electrolytes were measured by standard laboratory protocols. Chronic kidney disease was defined as an eGFR < 60 mL/min per 1.73 $m^2$.

## Statistical Analysis

We conducted all analyses using R version 4.1.2 (R CoreTeam, Vienna, Austria).

### Descriptive

Demographics, cardiovascular risk factors, and CMR outcomes are presented as means ± SD or medians (interquartile range) for continuous variables, and counts (percentages) for categorical variables. Descriptions were stratified by MetS status and MDS categories (0, 1, 2, ≥3), using 2-sample *t*-test, 1-way ANOVA, or chi-square test, where appropriate. We applied Spearman's rank correlation to assess the correlation between serum magnesium and MDS (continuous).

### Analysis of associations

Serum magnesium was *Z*-standardized (minus mean and divided by SD) on the overall sample prior to subsequent analyses. We estimated the associations of serum magnesium and MDS with MetS and its individual components using multivariable logistic regression. Since MDS already incorporates potential confounders of the association between serum magnesium and MetS, such as eGFR and diuretic use, the adjustment strategies differed between the 2 exposures. Models for serum magnesium were adjusted for age, sex, smoking status, physical activity, alcohol consumption, serum potassium, serum calcium, eGFR, and diuretic use. In contrast, models for MDS were adjusted for age, sex, smoking status, physical activity, serum potassium, and serum calcium. To account for multiple testing, *P* values were corrected using the false discovery rate method using Benjamini-Hochberg procedure (25).

We used multivariable linear regression to evaluate associations between serum magnesium (always continuous), MDS (as both a continuous and categorical variable), and CMR parameters, first in the total population and then stratified by MetS status. Given the complexity of the association, confounders were selected using a combined approach that incorporated clinical knowledge, evidence from previous literature, and the construction of directed acyclic graphs (DAGs). DAGs enable the visualization of the relationships between the outcome, exposure, and covariates, and assist in identifying potential sources of bias from confounders or colliders. They help determine the minimally sufficient set of variables required to block all backdoor paths from the exposure to the outcome, thereby reducing the risk of overadjustment (26). To achieve this, we used the web version of the program "DAGitty" (https://www.dagitty.net/dags.html). Models for

serum magnesium were adjusted for age, sex, smoking status, physical activity, alcohol consumption, serum potassium, serum calcium, HbA1c, eGFR, and diuretic use, whereas models for MDS were adjusted for age, sex, smoking status, physical activity, BMI, and serum total cholesterol [Fig. S1 (27)]. In models restricted to individuals with MetS, we excluded MetS-related risk factors, namely serum total cholesterol, HbA1c, and BMI, from the covariate set to reduce the risk of multicollinearity. We also adjusted for multiple testing using false discovery rate.

Associations of serum magnesium and MDS with left ventricular concentricity were assessed using multivariable logistic regression. Subgroup analyses were performed for this association, stratified by age categories, sex, MetS, BMI categories (≥25, <25), elevated waist circumference (waist circumference ≥94 cm in men or ≥80 cm in women), hypertension (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or taking antihypertensive medication being aware of having hypertension), and type 2 diabetes (fasting glucose ≥126, HbA1c ≥6.5%, or use of antidiabetic medication). Models were adjusted for age, sex, smoking status, physical activity, BMI, and serum total cholesterol. In MetS-stratified analysis, BMI and serum total cholesterol were excluded from the covariate list. Formal tests for multiplicative interaction were conducted.

Results were expressed as odds ratios (ORs) and β-coefficients per 1-SD increase in serum magnesium and per 1-unit increase in MDS when modeled as a continuous variable, or by comparison to MDS = 0 when modeled as a categorical variable with corresponding 95% CIs for logistic and linear regression models.

### Sensitivity analysis

We performed several sensitivity analyses to check the robustness of our results. First, because of a substantial number of participants excluded because of missing data on key variables, we compared the analysis sample (complete cases) to the original full sample. We evaluated key demographic and clinical characteristics to determine whether the excluded participants differed systematically from individuals included in the final analysis. Second, we conducted multivariable linear regression to explore the association between MetS and CMR parameters adjusted for age, sex, smoking status, physical activity, alcohol consumption, eGFR, and diuretic medication. Finally, we conducted a causal mediation analysis to examine the mediating role of MetS in the relationship between MDS and selected subclinical CVD markers, namely the left ventricular remodeling index and right ventricular end-diastolic volume. The models were adjusted for age, sex, smoking status, and physical activity. Briefly, using a counterfactual framework, the analysis decomposed the total effect of MDS into direct and indirect components. The direct effect represents the influence of MDS on the CVD markers independent of MetS, whereas the indirect effect captures the portion of this relationship that could be explained by MetS. To quantify the contribution of this mediation, the proportion of the total association attributable to MetS was estimated. We used the R package "mediation" for the mediation analyses (Fig. S2 (27)).

### Mendelian Randomization analysis

We performed a 2-sample Mendelian Randomization analysis to investigate the potential causal link between serum magnesium and MRI-derived cardiac morphology and function.

Mendelian Randomization relies on 3 assumptions: a strong association between the genetic variants and the exposure, no confounding factors affecting the genetic variants and outcome, and a direct effect of the genetic variants on the outcome exclusively through the exposure (28). We used publicly available genome-wide association study (GWAS) data of individuals with European ancestry, as outlined next. We included 6 single-nucleotide polymorphisms (SNPs) that showed strong, independent associations with serum magnesium at genome-wide significance ($P < 5 \times 10^{-8}$, Table S1 (27)), with a mean F-statistic of 64.3. These SNPs were previously identified through a combined analysis of a discovery cohort (n = 15 366) and a replication cohort (n = 8463), collectively explaining 1.6% of the variance in serum magnesium (29). We extracted SNP-outcome associations from the largest available GWAS for MRI-derived left and right ventricular morphology and function parameters. For the left ventricle, we used GWAS summary statistics for 6 measures: end-diastolic volume, end-systolic volume, ejection fraction, mass, remodeling index, and stroke volume (30). As left ventricular stroke volume data were unavailable in the primary GWAS (n = 16 923 European participants), we obtained it from a separate GWAS of 32 528 European participants (31). Right ventricular measures included end-diastolic volume, end-systolic volume, ejection fraction, and stroke volume, based on publicly available GWAS data. Analyses included 29 506 UK Biobank participants of European ancestry, free of preexisting myocardial infarction or heart failure (32). Detailed information on the GWAS studies included can be found in Table S2 (27).

We applied multiple Mendelian Randomization methods, including inverse variance-weighted, weighted median, MR-Egger, simple mode, and weighted mode approaches. Directional horizontal pleiotropy was indicated if the MR-Egger intercept significantly differed from zero ($P < .05$). However, the reliability of MR-Egger is limited in the context of a small number of SNPs, as reduced statistical power can affect its accuracy. Therefore, we further assessed horizontal pleiotropy using the Mendelian Randomization Pleiotropy RESidual Sum and Outlier test (MR-PRESSO). In summary, MR-PRESSO includes 3 key components: (1) the global test, which detects the presence of horizontal pleiotropy; (2) the outlier test, which corrects for pleiotropy by identifying and removing outlier variants; and (3) the distortion test, which evaluates whether causal estimates change significantly after outlier correction. Furthermore, heterogeneity was assessed using Cochran's Q test.

To further evaluate the robustness of the genetic instruments, we visually assessed individual SNP effects using single SNP and leave-one-out analyses. These plots helped identify whether the overall result was driven by any single variant or by SNPs estimating effects in divergent directions. Additionally, we used scatter plots to visualize the consistency of effect estimates across all SNPs. All ORs were scaled per 0.1 mmol/L increase in serum magnesium. All Mendelian Randomization analyses were performed using "TwoSampleMR" R-package (33).

## Results

### Study Sample

Of the total NAKO-MRI cohort comprising 30 868 participants, n = 29 602 had CMR measurements (Fig. S3 (27)). Of those, a large part of individuals had to be excluded from

the current analysis because of lack of magnesium measurements because magnesium was only assessed in selected study centers of the NAKO. Moreover, we excluded individuals with a history of CVD, including angina pectoris, myocardial infarction, heart failure, arrhythmia, ischemia, stroke, and heart surgeries. Additionally, individuals with missing data on covariates were excluded (Fig. S3 (27)). A final sample of 9568 individuals with complete data was included in the present analysis. As could be expected from the exclusion of individuals with prevalent CVD, excluded individuals had on average slightly worse cardiometabolic risk factor profiles (Table S3 (27)).

Of the final sample, 30.4% had MetS (mean age: 50.8 ± 11.0 years; 67.9% men). Individuals with MetS showed significantly higher sedentary lifestyle factors, anthropometric measures, and cardiometabolic risk factors. Moreover, a higher MDS and worse cardiac parameters were observed in individuals with MetS. For example, participants with MetS were more likely to have lower left and right end-diastolic volumes (68.97 vs 76.56 mL/m$^2$; 78.36 vs 86.08 mL/m$^2$; $P < .001$, respectively). Additionally, the proportion of left ventricular concentricity was significantly higher in individuals with MetS (3.2%) compared to those without MetS (0.5%; $P < .001$). A detailed description of the study population's clinical characteristics, stratified by MetS status, is provided in Table 1.

## Serum Magnesium and Magnesium Depletion

We found no correlation between serum magnesium and MDS ($R = 0.065$; $P < .001$). The distribution of serum magnesium levels across MDS categories showed a slight increase from category 0 to 1, remained stable between 1 and 2, and decreased from 2 to ≥3 (Fig. 1).

Table S4 (27) presents the clinical characteristics according to MDS categories. Overall, participants with higher MDS exhibited a less favorable metabolic and cardiovascular risk profile, including higher age, lipid levels, BMI, HbA1c, and serum glucose. Furthermore, individuals with higher MDS were more likely to exhibit worse cardiac alterations, such as lower left and right ventricular end-diastolic volume, end-systolic volume, and stroke volume, as well as a higher left ventricular remodeling index.

## Association of Magnesium With MetS and Its Components

Multivariable logistic regression models demonstrated associations between lower serum magnesium levels and a higher prevalence of MetS and its components (Fig. S4 (27)). A 1-SD higher serum magnesium concentration was associated with lower odds of MetS (OR, 0.93 [95% CI, 0.88-0.99]). Among MetS components, an inverse association was observed: A 1-SD higher serum magnesium concentration was associated with both, higher glucose (OR, 0.80 [95% CI, 0.76-0.84]) and lower high-density lipoprotein cholesterol (OR, 0.90 [95% CI, 0.85-0.96]). A 1-unit increase in MDS, indicative of magnesium deficiency, was associated with a higher prevalence of MetS (OR, 1.32 [95% CI, 1.23-1.41]) and each of its individual components (Fig. S4 (27)).

## Association of MetS With Cardiac Parameters

MetS was associated with CMR parameters, including reduced left and right ventricular end-systolic volume, end-diastolic volume, stroke volume, and an increased left ventricular remodeling index (Fig. S5 (27)).

## Association of Magnesium Deficiency With Cardiac Parameters

Higher MDS levels (from 1 to ≥3), compared to MDS = 0, were significantly associated with lower left and right ventricular end-systolic volume, end-diastolic volume, and stroke volume, as well as a higher remodeling index (Table 2). For example, left ventricular end-diastolic volume showed a graded decline, from −1.29 mL/m$^2$ (95% CI, −1.87 to −0.70) in level 1 to −5.14 mL/m$^2$ (95% CI, −7.63 to −2.65) in level ≥3 ($P$ for trend < .001). Similarly, left ventricular remodeling index exhibited a graded increase, from 0.01 g/mL (95% CI, 0.001-0.013) in level 1 to 0.06 g/mL (95% CI, 0.039-0.091) in level ≥3 ($P$ for trend < .001).

The associations between serum magnesium and MDS with various CMR parameters in the overall sample, as well as stratified by MetS status, are shown in Fig. 2 and Table S5 (27). A 1-SD higher serum magnesium concentration was associated with lower left and right ventricular end-systolic volume, end-diastolic volume, and left stroke volume and cardiac mass. However, these associations were no longer significant in individuals with MetS. There was no significant association between a 1-SD increase in serum magnesium and left or right ventricular ejection fraction, or the left ventricular remodeling index. In contrast, a 1-unit higher in MDS (magnesium depletion) was associated with lower left and right ventricular end-systolic volume, end-diastolic volume, and stroke volume, as well as a higher left ventricular remodeling index. These associations were attenuated but remained significant in individuals with MetS, except for right ventricular end-diastolic volume and stroke volume, where the associations were no longer observed. MDS was not significantly associated with left or right ventricular ejection fraction.

Causal mediation analysis indicated that MetS mediated 26.7% of the association between MDS and left ventricular remodeling index, with an effect of 0.005 g/mL (95% CI, 0.0003-0.006). Additionally, MetS mediated 25.4% of the association between MDS and right ventricular end-diastolic volume, with an effect of −0.431 mL/m$^2$ (95% CI, −0.485 to −0.228) (Table S6 (27)).

A 1-unit higher MDS was associated with a higher prevalence of left ventricular concentricity in the overall population (OR 1.33 [95% CI, 1.06-1.65]) (Table S5 (27)). Subgroup analyses revealed a significant positive association between MDS and left ventricular concentricity in men, individuals with overweight and obesity (BMI ≥ 25 kg/m$^2$), and individuals with MetS. However, tests for multiplicative interaction were not statistically significant (Fig. 3).

## Mendelian Randomization

In the primary analysis using inverse variance-weighted, genetically predicted serum magnesium did not support a causally association with CMR-derived measures of cardiac morphology and function. The findings were consistent across sensitivity analyses using various Mendelian Randomization methods, including the weighted median, MR-Egger, simple mode, and weighted mode (Fig. 4).

The MR-Egger intercept showed no indication of directional pleiotropy (Table S7 (27)). MR-PRESSO results

**Table 1. Characteristics of the study sample, stratified by presence of metabolic syndrome**

| | All (N = 9568) | No MetS (N = 6658) | MetS (N = 2910) | *P* value |
|---|---|---|---|---|
| Demographics | | | | |
| Age (y) | 45.8 (12.1) | 43.7 (12.0) | 50.7 (10.9) | <.001 |
| Male sex | 5539 (58.0%) | 3562 (53.5%) | 1977 (67.9%) | <.001 |
| Blood pressure measurement | | | | |
| Heart rate (bpm) | 63.5 (11.0) | 63.3 (11.04) | 63.8 (10.9) | .026 |
| Systolic BP (mm Hg) | 127.3 (15.4) | 123.9 (14.4) | 135.4 (14.4) | <.001 |
| Diastolic BP (mm Hg) | 78.9 (9.8) | 76.6 (9.3) | 83.8 (9.2) | <.001 |
| Hypertension | 2235 (23.4%) | 1038 (15.6%) | 1197 (41.1%) | <.001 |
| Anthropometrics | | | | |
| Waist circumference (cm) | 90.5 (13.2) | 85.9 (11.2) | 100.9 (11.2) | <.001 |
| BMI (kg/m$^2$) | 26.4 (4.6) | 24.9 (3.9) | 29.6 (4.5) | <.001 |
| Behavioral risk factors | | | | |
| Smoking | | | | <.001 |
| Never | 4945 (51.7) | 3639 (54.7%) | 1306 (44.9%) | |
| Former | 2842 (29.7) | 1810 (27.2%) | 1032 (35.5%) | |
| Current | 1781 (18.6) | 1209 (18.2%) | 572 (19.7%) | |
| Alcohol consumption | | | | .001 |
| None | 660 (6.9%) | 454 (6.8%) | 206 (7.1%) | |
| Moderate | 8351 (87.3%) | 5846 (87.8%) | 2505 (86.1%) | |
| Excessive | 557 (5.8%) | 358 (5.4%) | 199 (6.8%) | |
| Physical activity | 8328 (87.0%) | 5883 (88.4%) | 2445 (84.0%) | <.001 |
| Lipid profile | | | | |
| Total cholesterol (mg/dL) | 204.2 (40.9) | 198.4 (39.2) | 217.4 (41.7) | <.001 |
| LDL-C (mg/dL) | 125.4 (34.1) | 120.3 (32.9) | 136.9 (33.8) | <.001 |
| HDL-C (mg/dL) | 58.9 (16.1) | 62.9 (15.4) | 49.9 (13.9) | <.001 |
| Triglycerides (mg/dL), median (IQR) | 121.3 (85.9, 178.8) | 101.8 (76.9, 134.5) | 197.4 (155.8 261.9) | <.001 |
| Kidney function | | | | |
| eGFR (mL/min/1.73 m$^2$) | 98.1 (14.8) | 99.7 (14.5) | 94.3 (14.8) | <.001 |
| Serum creatinine (mg/dL) | 0.84 (0.16) | 0.83 (0.15) | 0.86 (0.18) | <.001 |
| Serum albumin (g/L) | 42.08 (2.85) | 42.20 (2.89) | 41.82 (2.73) | <.001 |
| CKD | 78 (0.8%) | 31 (0.5%) | 47 (1.6%) | <.001 |
| Blood markers | | | | |
| Serum magnesium (mmol/L) | 0.892 (0.064) | 0.892 (0.063) | 0.891 (0.067) | .587 |
| MDS categories | | | | <.001 |
| [0] | 5683 (59.4%) | 4272 (64.2%) | 1411 (48.5%) | |
| [1] | 3071 (32.1%) | 1998 (30.0%) | 1073 (36.9%) | |
| [2] | 710 (7.4%) | 355 (5.3%) | 355 (12.2%) | |
| [≥3] | 104 (1.1%) | 33 (0.5%) | 71 (2.4%) | |
| Serum sodium (mmol/L) | 140.6 (1.8) | 140.6 (1.8) | 140.6 (1.9) | .489 |
| Serum potassium (mmol/L) | 4.19 (0.27) | 4.19 (0.26) | 4.20 (0.27) | .008 |
| Serum calcium (mmol/L) | 2.29 (0.09) | 2.28 (0.08) | 2.29 (0.09) | .010 |
| Serum glucose (mg/dL) | 96.4 (22.4) | 90.8 (14.3) | 109.2 (30.7) | <.001 |
| HbA1c (%) | 5.41 (0.52) | 5.31 (0.35) | 5.62 (0.72) | <.001 |
| Medication | | | | |
| Magnesium supplementation | 346 (3.6%) | 241 (3.6%) | 105 (3.6%) | .873 |
| Use of PPI | 568 (5.9%) | 296 (4.4%) | 272 (9.3%) | <.001 |
| Use of diuretics | 409 (4.3%) | 105 (1.6%) | 304 (10.4%) | <.001 |
| Diabetic medications | 210 (2.2%) | 35 (0.5%) | 175 (6.0%) | <.001 |
| Antihypertensive medications | 1377 (14.4%) | 449 (6.7%) | 928 (31.9%) | <.001 |
| Lipid-lowering medications | 429 (4.5%) | 188 (2.8%) | 241 (8.3%) | <.001 |

(*continued*)

**Table 1. Continued**

| | All (N = 9568) | No MetS (N = 6658) | MetS (N = 2910) | *P* value |
|---|---|---|---|---|
| MetS components | | | | |
| Elevated waist circumference | 5075 (53.0%) | 2433 (36.5%) | 2642 (90.8%) | <.001 |
| Elevated blood pressure | 4784 (50.0%) | 2330 (35.0%) | 2454 (84.3%) | <.001 |
| Elevated Fasting glucose | 2720 (28.4%) | 925 (13.9%) | 1795 (61.7%) | <.001 |
| Elevated triglycerides | 3389 (35.4%) | 1079 (16.2%) | 2310 (79.4%) | <.001 |
| Reduced HDL-C | 1330 (13.9%) | 298 (4.5%) | 1032 (35.5%) | <.001 |
| Left ventricular function | | | | |
| End-diastolic volume (mL/m$^2$) | 74.2 (14.1) | 76.6 (13.9) | 68.9 (13.3) | <.001 |
| End-systolic volume (mL/m$^2$) | 27.4 (6.8) | 28.1 (6.6) | 25.6 (6.6) | <.001 |
| Stroke volume (mL/m$^2$) | 46.9 (9.5) | 48.5 (9.3) | 43.3 (8.9) | <.001 |
| Ejection fraction (%) | 63.2 (5.2) | 63.3 (5.1) | 62.9 (5.5) | <.001 |
| Myocardial mass (g/m$^2$) | 58.9 (10.1) | 58.2 (10.0) | 60.4 (9.98) | <.001 |
| Remodeling index (g/mL) | 0.81 (0.16) | 0.77 (0.14) | 0.90 (0.2) | <.001 |
| LV concentricity | 128 (1.3%) | 35 (0.5%) | 93 (3.2%) | <.001 |
| Right ventricular function | | | | |
| End-diastolic volume (mL/m$^2$) | 83.7 (16.3) | 86.1 (16.1) | 78.36 (15.3) | <.001 |
| End-systolic volume (mL/m$^2$) | 37.2 (9.8) | 38.2 (9.9) | 34.8 (9.1) | <.001 |
| Stroke volume (mL/m$^2$) | 46.6 (10.1) | 47.9 (10.1) | 43.6 (9.7) | <.001 |
| Ejection fraction (%) | 55.8 (6.9) | 55.8 (6.8) | 55.8 (6.9) | .421 |

Values are reported as mean (SD) or n (%), unless otherwise indicated.
MDS defined as the sum of the following 4 factors: (1) current use of diuretics scored 1 point; (2) current use of PPI scored 1 point; (3) an eGFR between 60 mL/min/1.73 m$^2$ and 90 mL/min/1.73 m$^2$ scored 1 point, whereas an eGFR less than 60 mL/min/1.73 m$^2$ scored 2 points; and (4) heavy drinking (>1 drink/d for women and >2 drinks/d for men) scored 1 point (1 drink defined as the amount of alcoholic beverage containing 0.6 fluid ounces or 14 g of ethanol). MDS categorized into 6 groups: MDS = 0, MDS = 1, MDS = 2, MDS = 3, MDS = 4, and MDS = 5. Hypertension defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg without considering antihypertensive drugs intake. Metabolic syndrome components: elevated waist circumference: waist circumference ≥94 cm in men or ≥80 cm in women. Elevated blood pressure: systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or treatment with antihypertensive medication being aware of having hypertension. Elevated fasting glucose: fasting serum glucose level ≥100 mg/dL or intake of antidiabetic medication. Elevated triglycerides: serum fasting triglycerides ≥150 mg/dL or the use of fibrates. Reduced HDL-C: serum HDL-C <40 mg/dL in men or <50 mg/dL in women or the use of fibrates. Excessive alcohol consumption: men with an alcohol intake ≥30 g/day and women ≥20 g/day. Left ventricular remodeling index was calculated as mass divided by volume. Left ventricular concentricity was defined as left ventricular remodeling index >1.3 g/mL.

(Table S8 (27)) indicated significant horizontal pleiotropy in left ventricular systolic and diastolic volumes, remodeling index, and right ventricular systolic and diastolic volumes. The identified outlier SNPs—rs13146355, rs7965584, and rs448378—are known to be associated with eGFR, hypertension, and systolic and diastolic blood pressure, respectively. After exclusion of these SNPs, the results remained consistent, suggesting no evidence of a causal association between serum magnesium and MRI-derived cardiac parameters. Significant heterogeneity was observed in left ventricular end-diastolic volume, stroke volume, and the remodeling index, as well as in right ventricular systolic and diastolic volumes (Table S9 (27)).

The single-SNP plots were consistent with the MR-PRESSO results, highlighting rs13146355, rs7965584, and rs448378 as outliers with divergent directions and effect estimates that influenced the overall Mendelian Randomization findings (Fig. S6 (27)). The leave-one-out analysis further highlighted the disproportionate influence of rs13146355, which showed a different effect direction compared to the other SNPs (Fig. S7 (27)). The MR scatter plot showed a generally flat slope across the different Mendelian Randomization methods, supporting the absence of a causal association between serum magnesium and MRI-derived cardiac parameters. Two distinct SNPs appeared as outliers, deviating noticeably from the cluster of other variants [Fig. S8 (27)].

## Discussion

This comprehensive analysis represents one of the largest studies to date investigating the complex association between magnesium, metabolic health, and cardiac function using CMR-derived parameters. We found that serum magnesium did not correlate well with MDS. The MDS showed stronger associations than serum magnesium with MetS and unfavorable alterations in cardiac morphology and function, especially those related to diastolic dysfunction. The relationship between magnesium depletion and adverse cardiac changes appears to vary depending on MetS prevalence, suggesting that metabolic health may play a partial mediating role. Finally, our Mendelian Randomization analysis did not support a causal relationship of serum magnesium with CMR-derived parameters.

Our findings suggest no correlation between serum magnesium and the MDS. Although serum magnesium is commonly used to assess magnesium status in clinical practice, it is not a reliable indicator of total-body magnesium. Notably, serum magnesium constitutes only 0.3% of the body's total magnesium, with the majority stored in bones, muscles, and soft tissues (1). Magnesium homeostasis is tightly regulated through a dynamic balance of intestinal absorption, renal excretion, and bone storage. As a result, serum magnesium typically remains within the normal reference range, especially in chronic magnesium deficiency (9).

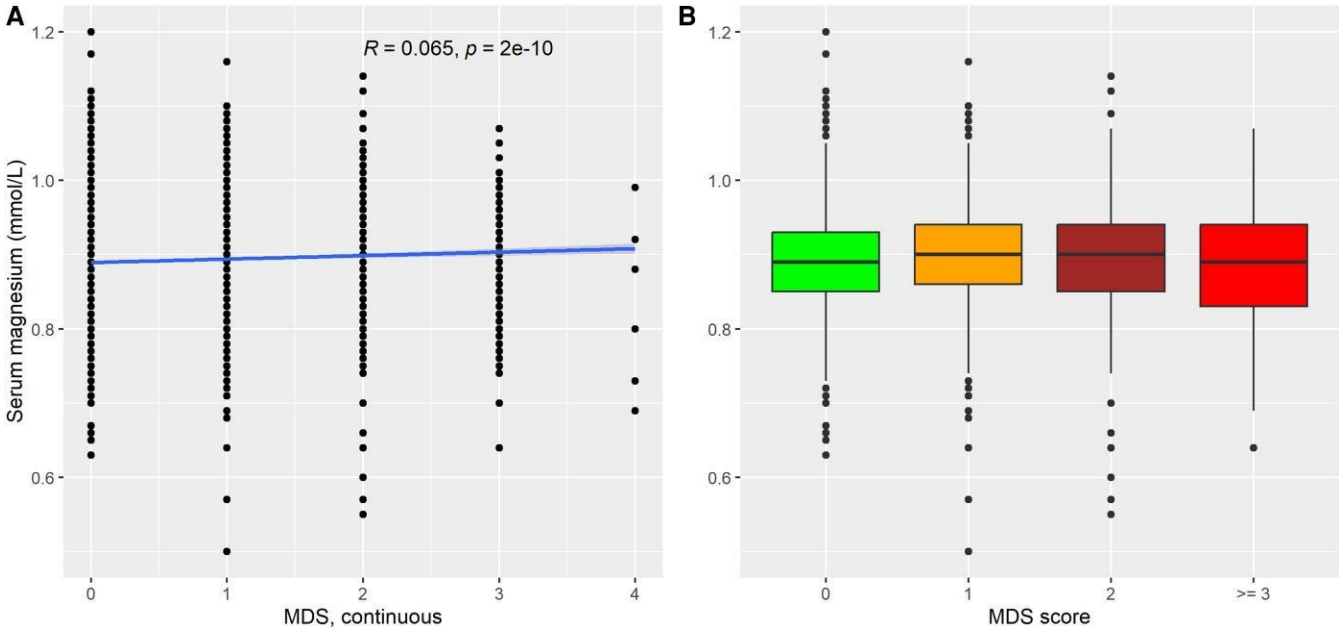


**Figure 1.** (A) Spearman correlation between serum magnesium (mmol/L) with magnesium depletion score (continuous scale). (B) Distribution of serum magnesium (mmol/L) by magnesium depletion score (MDS).

Magnesium homeostasis is essential for cellular enzymes and metabolic cycles, and its impairment is associated with various cardiometabolic diseases, including diabetes and MetS (3). We demonstrated that higher MDS, reflecting greater magnesium loss, was independently associated with MetS, consistent with previous reports (34). MDS was also linked to individual MetS components, including elevated glucose, waist circumference, blood pressure, and triglycerides. This suggests that MDS may contribute to MetS development by influencing its individual components. These findings are in line with results from a randomized clinical trial, which showed that oral magnesium supplementation improved MetS by reducing blood pressure, hyperglycemia, and hypertriglyceridemia (35). Compared to MDS, we found that serum magnesium was inversely associated with MetS, with diabetes being the primary driver of this relationship, confirming our earlier findings (13).

The MDS provides a more comprehensive assessment of magnesium status than serum magnesium by reflecting the kidney's capacity for magnesium reabsorption. Under normal physiological conditions, approximately 2400 mg of plasma magnesium is filtered daily by the glomeruli, with about 90% reabsorbed and 3% to 5% excreted in urine (36). The MDS accounts for factors that impair renal magnesium reabsorption, including alcohol consumption, diuretic use, proton pump inhibitor use, and kidney disease (10). Consequently, it may serve as a more reliable marker of magnesium deficiency linked to metabolic impairment. Indeed, our findings demonstrated stronger associations of MDS with metabolic impairment and cardiac alterations compared to serum magnesium.

We found that higher MDS was associated with reduced ventricular volumes, such as end-diastolic volume and stroke volume. Additionally, MDS was positively associated with the left ventricular concentricity, suggesting diastolic dysfunction—a condition characterized by increased resistance to ventricular diastolic inflow and impaired diastolic relaxation. Notably, this association was stronger in individuals with higher metabolic risk, including men and individuals with obesity and MetS. Evidence shows that magnesium deficiency has been shown to cause diastolic cardiomyopathy, whereas supplementation improved cardiac mitochondrial and diastolic function in diabetic mice by enhancing ATP production, reducing mitochondrial reactive oxygen species, and increasing calcium overload (37, 38). Moreover, previous population-based studies have found associations of dietary magnesium intake with better CMR markers of diastolic function (8, 39).

We observed a contrasting pattern of associations for serum magnesium and MDS in relation to cardiac volumetric measures. Higher serum magnesium was associated with lower left and right ventricular end-diastolic volumes, end-systolic volumes, and stroke volumes. on the other hand, magnesium depletion, as indicated by a higher MDS, was also associated with reduction in cardiac volumes. These findings could be comparable with the contradictory direction of results previously observed between serum and dietary magnesium in relation to cardiac alterations (8). A possible hypothesis could be a state of chronic magnesium deficiency during subclinical CVD, triggering a compensatory mechanism to increase serum magnesium through renal reabsorption and bone and muscle resorption.

Evidence on the relationship between serum magnesium and overt heart failure remains inconsistent. Interestingly, a meta-analysis of 7 studies on patients with chronic heart failure found that hypermagnesemia (≥1.05 mmol/L), but not hypomagnesemia, was associated with an increased risk of cardiovascular mortality (40). Moreover, genetically predicted serum magnesium showed no causal relationship with heart failure risk (41). This is in good agreement with our Mendelian Randomization findings, suggesting no causal association of serum magnesium with MRI-derived cardiac alterations.

Among 19 227 US adults, a higher MDS was linked to an increased risk of congestive heart failure, especially in those

**Table 2. Association of magnesium depletion score (class 1-≥3) with outcomes of MRI-derived cardiac function and morphology**

| | Estimate (95% CI) | *P* value | Adjusted *P* |
|---|---|---|---|
| Left ventricular function | | | |
| End-diastolic volume (mL/m$^2$) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −1.29 (−1.87, −0.70) | <.001 | <.001 |
| 2 | −1.60 (−2.64, −0.56) | .002 | .007 |
| ≥3 | −5.14 (−7.63, −2.65) | <.001 | .001 |
| End-systolic volume (mL/m$^2$) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −0.41 (−0.70, −0.13) | .004 | .008 |
| 2 | −0.71 (−1.21, −0.20) | .006 | .011 |
| ≥3 | −1.90 (−3.10, −0.69) | .002 | .006 |
| Stroke volume (mL/m$^2$) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −0.87 (−1.28, −0.46) | <.001 | <0.001 |
| 2 | −0.90 (−1.61, −0.18) | .014 | .011 |
| ≥3 | −3.24 (−4.96, −1.52) | .001 | .001 |
| Ejection fraction (%) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −0.12 (−0.36, 0.11) | .314 | .370 |
| 2 | 0.19 (−0.26, 0.57) | .369 | .419 |
| ≥3 | 0.08 (−0.99, 1.01) | .873 | .873 |
| Myocardial mass (g/m$^2$) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −0.54 (−0.89, −0.19) | .002 | .007 |
| 2 | 0.24 (−0.37, 0.87) | .437 | .480 |
| ≥3 | 0.93 (−2.42, 0.55) | .218 | .278 |
| Remodeling index (g/mL) | | | |
| 0 | Ref | Ref | Ref |
| 1 | 0.01 (0.001, 0.013) | .036 | .055 |
| 2 | 0.02 (0.015, 0.037) | <.001 | <.001 |
| ≥3 | 0.06 (0.039, 0.091) | <.001 | <.001 |
| LV concentricity | | | |
| 0 | Ref | Ref | Ref |
| 1 | 1.16 (0.76, 1.78) | .496 | .512 |
| 2 | 1.91 (1.12, 3.20) | .015 | .023 |
| ≥3 | 1.90 (0.68, 4.54) | .177 | .244 |
| Right ventricular function | | | |
| End-diastolic volume (mL/m$^2$) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −1.48 (−2.14, −0.81) | <.001 | <.001 |
| 2 | −1.78 (−2.95, −0.61) | .002 | .007 |
| ≥3 | −5.21 (−8.01, −2.40) | <.001 | <.001 |
| End-systolic volume (mL/m$^2$) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −0.51 (−0.90, −0.11) | .012 | .021 |
| 2 | −1.05 (−1.76, −0.35) | .003 | .007 |
| ≥3 | −2.46 (−4.14, −0.78) | .004 | .008 |
| Stroke volume (mL/m$^2$) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −0.97 (−1.41, −0.52) | <.001 | <.001 |
| 2 | −0.72 (−1.51, 0.05) | .069 | .100 |
| ≥3 | −2.74 (−4.62, −0.86) | .004 | .008 |
| Ejection fraction (%) | | | |
| 0 | Ref | Ref | Ref |
| 1 | −0.17 (−0.50, 0.11) | .204 | .314 |
| 2 | 0.34 (−0.28, 0.80) | .346 | .278 |
| ≥3 | 0.49 (−0.94, 1.65) | .589 | .491 |

Models adjusted for age, sex, smoking status, physical activity, body mass index, and serum total cholesterol. Adjusted *P* value using false discovery rate (FDR). Left ventricular remodeling index was calculated as mass divided by volume. Left ventricular concentricity was defined as left ventricular remodeling index (LVRI) >1.3 g/mL. Estimates represent β estimates from linear regression or odds ratio from logistic regression.

with inadequate magnesium consumption (42). Moreover, prior evidence using X-ray absorptiometry reported a positive association between MDS and abdominal aortic calcification scores, particularly in adults with lower magnesium intake (43). Our study extended these findings by examining a broader range of cardiac morphology and function markers using MRI, a technique less susceptible to imaging limitations. Whether magnesium depletion is causally linked to cardiac alterations is uncertain, given the absence of GWAS on MDS. Nevertheless, Mendelian Randomization results did not find evidence of a causal relationship between genetically predicted renal impairment and CVD (44, 45).

Several prospective interventional studies have investigated the effects of magnesium supplementation on metabolic and cardiovascular outcomes. Although most studies have focused on metabolic risk factors—such as hypertension and hyperglycemia (35, 46)—and subclinical cardiovascular markers, including arterial stiffness and endothelial function (47, 48), some research has explored more direct cardiac endpoints. For instance, in a prospective study among thiazide-treated hypertensive women, magnesium supplementation was associated with improved blood pressure control, enhanced endothelial function, and reduced subclinical atherosclerosis (47). Furthermore, magnesium supplementation has been suggested to improve heart rate variability in patients with heart failure (49), indicating potential benefits for cardiac autonomic regulation.

MetS is a well-established driver of worsened cardiac function and morphology. In our study, MetS was associated with decreased ventricular volumes and an increased remodeling index, suggesting impaired diastolic function. These findings are consistent with previous studies linking MetS to diastolic dysfunction, including increased left ventricular mass and ventricular dimensions (15, 50). It is crucial to note that the attenuation of the associations of magnesium depletion and unfavorable cardiac alterations in individuals with MetS suggests that metabolic dysfunction, such as insulin resistance and dyslipidemia, may partially mediate the link between magnesium depletion and subclinical CVD. Our causal mediation analysis revealed a modest mediation by MetS, indicating that although it

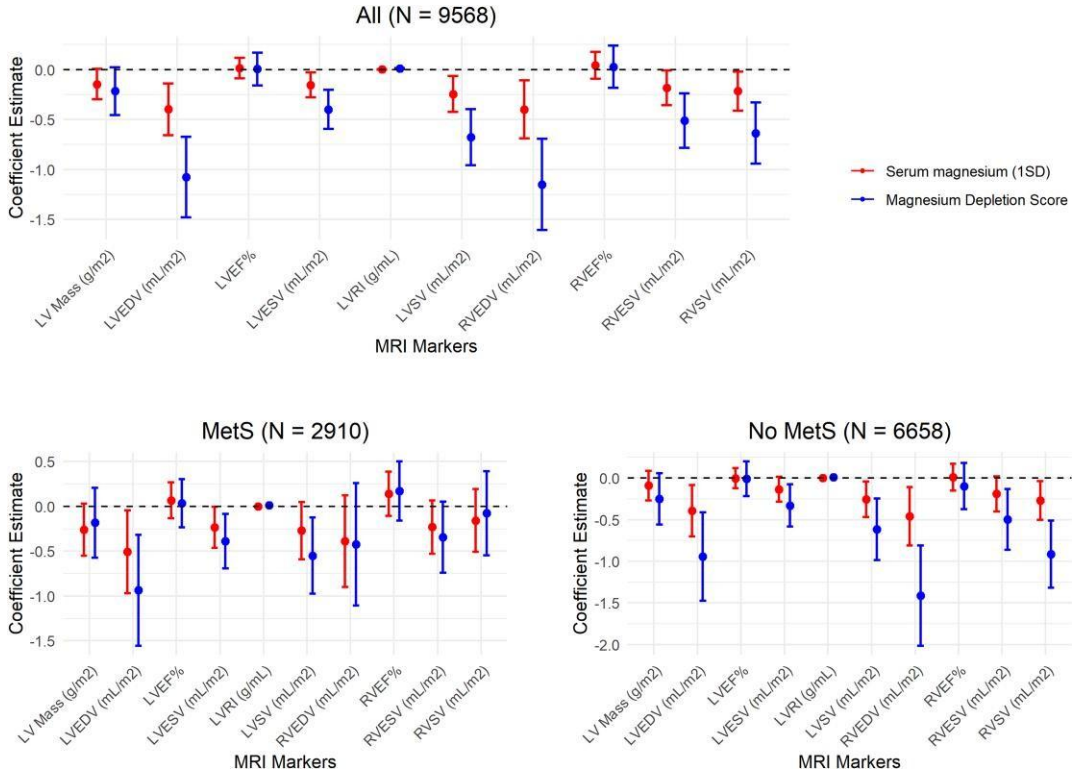


**Figure 2.** Multivariable adjusted linear results for the association of serum magnesium (1-SD) and magnesium depletion score (MDS) with MRI-derived cardiac parameters in all population and stratified by metabolic syndrome. Association between serum magnesium and MRI-derived cardiac disease parameters were adjusted for age, sex, smoking status, physical activity, alcohol consumption, serum potassium, serum calcium, HbA1c, eGFR, and diuretic use. Association between magnesium depletion score (continuous scale) and MRI-derived cardiac markers were adjusted for age, sex, smoking status, physical activity, BMI, and serum total cholesterol. In models restricted to MetS, serum total cholesterol, HbA1c, and BMI were excluded. Estimates represent β-estimates. Abbreviations: LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; LVRI, left ventricular remodeling index; LVSV, left ventricular stroke volume; MetS, metabolic syndrome; RVEDV, right ventricular end diastolic volume; RVEF, right ventricular ejection fraction; RVESV, right ventricular end systolic volume; RVSV, right ventricular stroke volume. Left ventricular remodeling index was calculated as mass divided by volume.

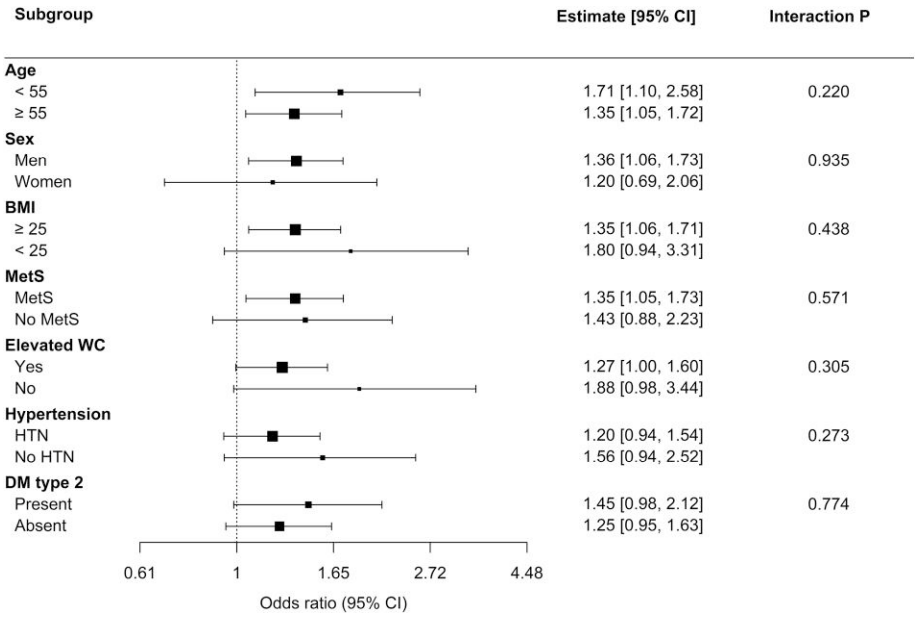


**Figure 3.** Association between magnesium depletion score (continuous scale) and left ventricular (LV) concentricity in different subgroups. Adjusted for age, sex, smoking status, physical activity, BMI, and serum total cholesterol. BMI and serum total cholesterol were excluded from MetS models. LV concentricity was defined as left ventricular remodeling index (LVRI) >1.3 g/mL. Elevated waist circumference: Waist circumference ≥94 cm in men or ≥80 cm in women. Hypertension: Systolic blood pressure ≥140, diastolic blood pressure ≥90 or taking antihypertensive medication being aware of having hypertension. DM type 2: Fasting glucose ≥126, HbA1c ≥ 6.5% or use of antidiabetic medication. Abbreviations: BMI, body mass index; DM, diabetes mellitus; MetS, metabolic syndrome; WC, waist circumference.

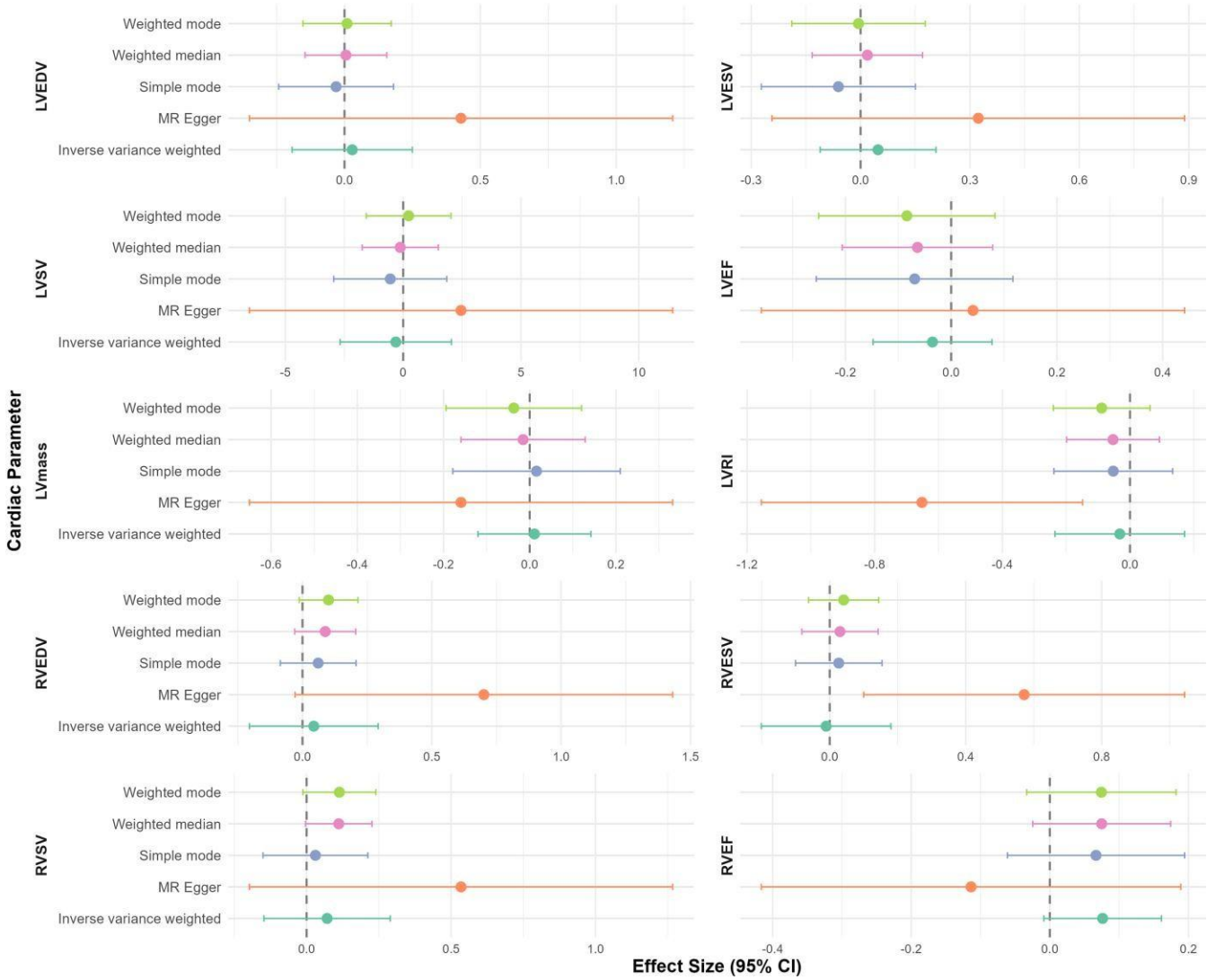


**Figure 4.** Two sample Mendelian Randomization results showing associations between genetically determined levels of serum magnesium and MRI-derived cardiac parameters. Number of SNPs = 6, estimates and 95% CIs were scaled per 0.1 mmol/L increase in serum magnesium. Abbreviations: LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; LVRI, left ventricular remodeling index; LVSV, left ventricular stroke volume; MetS, metabolic syndrome; RVEDV, right ventricular end diastolic volume; RVEF, right ventricular ejection fraction; RVESV, right ventricular end systolic volume; RVSV, right ventricular stroke volume.

partially explains the link between magnesium deficiency and cardiac alterations, other mechanisms, such as inflammation and oxidative stress, should be explored.

Our study has several strengths. This is the first large, population-based investigation to explore the association between magnesium status and comprehensive MRI-derived markers of cardiac alterations. Unlike previous studies that lacked serum magnesium measurements, we were able to evaluate whether the MDS serves as a better indicator of magnesium deficiency than serum magnesium. Additionally, the availability of GWAS data for serum magnesium and MRI-based cardiac parameters enabled the use of Mendelian Randomization analysis as a first step to explore potential causality. Nevertheless, some limitations should be acknowledged. The cross-sectional nature of the study limits the ability to assess temporal relationships between magnesium and cardiac parameters, and a single serum magnesium measurement may not reflect chronic magnesium concentration. Our results regarding the association between magnesium depletion and cardiac remodeling are not generalizable to individuals with prevalent CVD as these have been excluded from our analysis. The Mendelian Randomization should be regarded as a first step toward exploration of potential causality, and does not prove or disprove causal associations. Limitations related to the Mendelian Randomization analysis include the relatively small number of genetic instruments, which may have reduced statistical power and limited the ability to detect weak causal effects. Additionally, although horizontal pleiotropy was addressed using MR-PRESSO, residual pleiotropic effects and heterogeneity across SNPs could have still influenced the causal estimates. Moreover, although the exposure and outcome GWAS data and our main cohort all comprise individuals of European ancestry, differences in age and cohort characteristics may limit sample comparability. Finally, the absence of GWAS data for MDS restricts our ability to fully address its genetic determinants and causal role.

## Conclusion

In our CMR-based analysis of a large, CVD-free population, magnesium depletion was linked to metabolic impairment and adverse changes in cardiac morphology and function. Properly measured, magnesium depletion may serve as a potential diagnostic marker for early cardiometabolic disease. Further longitudinal studies are warranted to clarify the role of magnesium depletion as a prognostic marker.

## Acknowledgments

The authors thank all participants who took part in the NAKO study and the staff of this research initiative.

## Funding

This project was conducted with data (Application No. NAKO-696) from the German National Cohort (NAKO) (www.nako.de). The NAKO is funded by the Federal Ministry of Education and Research (BMBF) [project funding reference numbers: 01ER1301A/B/C, 01ER1511D, 01ER1801A/B/C/D and 01ER2301A/B/C], federal states of Germany and the Helmholtz Association, the participating universities and the institutes of the Leibniz Association. Nuha Shugaa Addin is supported by an individual doctoral scholarship from the Hanns-Seidel-Stiftung. Peter M. Full was funded by the Kaltenbach Scholarship of the German Heart Foundation.

## Author Contributions

Conceptualization: N.S.A., S.R.; Methodology: N.S.A., S.R.; Formal analysis: N.S.A.; Resources: N.S.A., C.S., P.M.F., H.B., M.D., T.K., R.v.K., F.G.M., T.N., T.P., B.S., J.S.M., J.S., H.V., S.N.W., F.B., A.P., C.L.S., S.R.; Data Curation: N.S.A., C.S., P.M.F., C.L.S., S.R.; Writing—original draft: N.S.A., S.R.; Writing-review and editing: N.S.A., C.S., P.M.F., H.B., M.D., T.K., R.v.K., F.G.M., T.N., T.P., B.S., J.S.M., J.S., H.V., S.N.W., F.B., A.P., C.L.S., S.R.; Supervision: A.P., S.R.; Funding acquisition: F.B., C.L.S.

## Disclosures

Fabian Bamberg reports an unrestricted research grant from Siemens Healthineers. Fabian Bamberg and Christopher L. Schlett report honoraria from the speaker's bureaus of Bayer Healthcare and Siemens Healthineers. Susanne Rospleszcz is a member of the Editorial Board of *JCEM*. All other Authors declare no competing interests.

## Data Availability

Access to and use of NAKO data and biosamples can be obtained via an electronic application portal (https://transfer.nako.de). Summary-level data from GWAS on serum magnesium and CMR parameters are publicly available through the GWAS Catalog (https://www.ebi.ac.uk/gwas/studies). Specific details of the studies used, including their accession numbers, are provided in Table S1.

## Ethics Approval and Consent to Participate

The study was conducted in accordance with national law and with the Declaration of Helsinki of 1975 (in the current, revised version). The research protocol was approved by all local Ethics Committees of the respective study centers. Written informed consent was obtained from all individual participants.

## Consent for Publication

Not applicable.

All authors have read and agreed on the final version of the manuscript.

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# Acknowledgement

The journey of this thesis has been filled with numerous challenges and invaluable growth. From the early days of drafting the research proposal, through the difficult process of securing funding, to the long and sometimes uncertain road toward achieving publications, each phase tested and shaped me in profound ways. Beyond the scientific and academic aspects of this work, I gained important personal and professional skills—resilience, patience, persistence, and many other soft skills—that I believe will serve me well as I take the next steps in my career.

I would first like to express my deepest gratitude to my supervisor, Dr. Susanne Rospleszcz, for her unwavering support, insightful guidance, and constant encouragement throughout the course of my PhD. Her mentorship has been instrumental not only in shaping the scientific quality of this work but also in fostering my growth as an independent researcher. I am especially grateful for the many opportunities she created for me—whether through new collaborations and projects, attending conferences, or learning experiences—which allowed me to thrive both personally and professionally during my PhD journey.

I am also sincerely thankful to my supervisor, Prof. Annette Peters for her huge support in helping me secure funding and for providing me with the opportunity to work at Helmholtz Munich. Her guidance, along with the valuable insights of Prof. Jochen Seissler during the Thesis Advisory Committee meetings, has been greatly appreciated. I would also like to thank the coauthors of all my projects for their constructive feedback, which significantly contributed to improving the quality of my work. Additionally, I am deeply grateful to the participants of the KORA and NAKO studies, whose involvement made my PhD projects possible.

I gratefully acknowledge the continuous support of the PhD Program Committee in Public Health and Epidemiology, including Dr. Annette Hartmann and Monika Darchinger, whose guidance and encouragement were always appreciated. A very special thanks goes to Prof. Ulrich Mansmann for believing in my project and awarding me the start-up scholarship. This opportunity had a profound impact on both my life and career. Being granted this scholarship opened the door to new possibilities and significantly shaped my professional path, providing me with opportunities that I would not have otherwise had.

Furthermore, I would like to express my sincere gratitude to the Hanns-Seidel-Stiftung for awarding me a scholarship, which enabled me to complete my PhD project. I am also deeply

thankful to all my colleagues for the moments we shared, as well as for their support and thoughtful advice throughout my PhD journey.

Finally, I would like to express my deepest gratitude to my late parents, whose memory continues to inspire me every day—I know they would have been proud of this achievement. I am also sincerely thankful to my husband and my brother for their unwavering support and encouragement in pursuing my dreams, especially in a context where such support for women is not always the norm. Above all, I dedicate this success to my daughter, Juman, whose presence fills my life with joy, love, and purpose.

# List of all scientific publications to date

1. Rospleszcz S, Burger T, **Shugaa Addin N**, et al. Association of habitual diet with skeletal muscle composition in a cross-sectional, population-based imaging study. *Nutrition J.* 2025;24:139. doi:10.1186/s12937-025-01222-5

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