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# **Ionized and total magnesium levels in patients with chronic kidney disease: associated factors and outcomes**

Maxime Pluquet<sup>1</sup>, Said Kamel<sup>1,2</sup>, Natalia Alencar de Pinho<sup>3</sup>, Nicolas Mansencal<sup>3,4</sup>, Christian Combe<sup>5,6</sup>, Marie Metzger<sup>3</sup>, Ziad A. Massy<sup>3,7</sup>, Sophie Liabeuf<sup>1,8</sup>, Solène M. Laville<sup>1,8</sup>

<sup>1</sup> MP3CV Laboratory, Jules Verne University of Picardie, F-80054 Amiens, France

<sup>2</sup> Department of Biochemistry, Amiens-Picardie University Medical Center, F-80054 Amiens, France

<sup>3</sup> Centre for Research in Epidemiology and Population Health (CESP), INSERM UMRS 1018, Université Paris-Saclay, Université Versailles Saint Quentin, F-94807 Villejuif, France

<sup>4</sup> Department of Cardiology, Ambroise Paré University Hospital, APHP, Boulogne-Billancourt, Paris, France

<sup>5</sup> Service de Néphrologie Transplantation Dialyse Aphérèse, Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France

<sup>6</sup> INSERM, U1026, Univ Bordeaux Segalen, Bordeaux, France

<sup>7</sup> Department of Nephrology, Ambroise Paré University Hospital, APHP, Boulogne-Billancourt, Paris, France

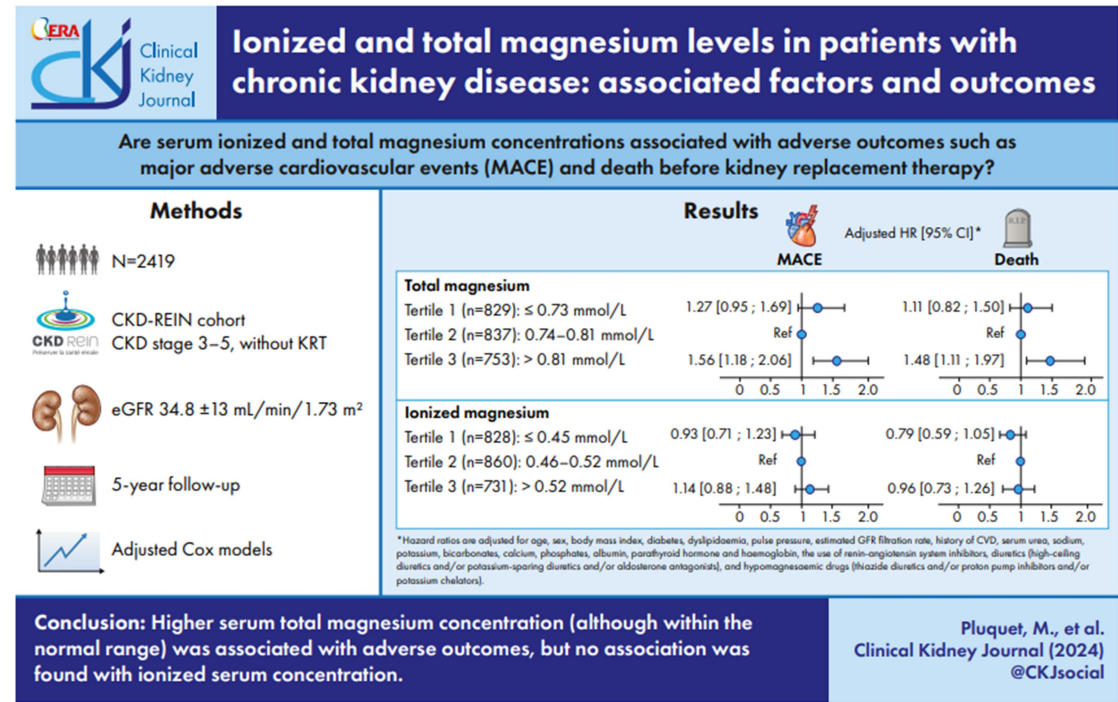
<sup>8</sup> Pharmacoepidemiology Unit, Department of Clinical Pharmacology, Amiens-Picardie University Medical Center, Amiens, France

Running Title: Ionized and total magnesium levels in patients with CKD

Correspondence to:

Sophie Liabeuf; E-mail: [liabeuf.sophie@chu-amiens.fr](mailto:liabeuf.sophie@chu-amiens.fr)

GRAPHICAL ABSTRACT



ABSTRACT

**Background and hypothesis.**

Association between hypo- and/or hypermagnesaemia and cardiovascular (CV) outcomes or mortality showed conflicting results in chronic kidney disease (CKD) and was conducted on total magnesium (tMg) levels. Thus, the objectives of the present study were to (i) describe the serum ionized Mg (iMg) concentration in patients at various CKD stages, (ii) measure the correlation between iMg and tMg concentrations, (iii) identify their associated factors and (iv) determine whether serum tMg and/or iMg concentrations are associated with major adverse cardiovascular events (MACE) and mortality before kidney replacement therapy (KRT) in CKD patients.

**Methods.**

CKD-REIN is a prospective cohort of CKD patients with an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m<sup>2</sup>. Baseline iMg and tMg serum concentrations were centrally measured. Adjusted cause-specific Cox proportional hazard models were used to estimate hazard ratios (HRs) for first MACE and for mortality.

**Results.**

Of the 2,419 included patients, median age was 68, and the mean eGFR was 34.8 mL/min/1.73 m<sup>2</sup>. Concentrations of serum iMg and tMg were strongly correlated (r=0.89, p<0.001) and are

independently associated with eGFR. The adjusted HR [95%CI] for MACE associated with the baseline serum tMg level was 1.27 [0.95; 1.69] for patients in tertile 1, and 1.56 [1.18; 2.06] for patients in tertile 3, relative to patients in tertile 2. The HR [95%CI] of death according to serum tMg concentration was increased in tertile 3 (1.48 [1.11; 1.97]). The adjusted risk for MACE and mortality (all-cause or CV) associated with the baseline serum iMg level was not significantly different between tertiles.

### **Conclusions.**

Our analysis of a large cohort of patients with moderate-to-advanced CKD demonstrated that individuals with higher serum tMg concentrations, although still within the normal range, had a greater likelihood of MACE and mortality. However, serum iMg levels were not associated with these outcomes.

**Keywords:** cardiovascular disease, chronic kidney disease, magnesium, mortality

ORIGINAL UNEDITED MANUSCRIPT

## KEY LEARNING POINTS

### What was known:

In patients with chronic kidney disease (CKD), previous studies have shown an association between high or low serum total magnesium (Mg) levels and all-cause mortality, with conflicting findings regarding cardiovascular events. However, no study has investigated the association between ionized magnesium and cardiovascular events or mortality in patients with CKD.

### This study adds:

In 3033 patients with moderate-to-advanced CKD, individuals with elevated serum total Mg concentrations, although still within the normal range, had a greater likelihood of major adverse cardiovascular events and mortality, even after adjustment for confounding factors. We failed to show an association between serum ionized magnesium on one hand and the occurrence of cardiovascular events or mortality on the other.

### Potential impact:

Total magnesium is routinely assayed in hospital laboratories and might be a useful variable to monitor in CKD patients. The safety of magnesium supplementation requires further evaluation.

## INTRODUCTION

Chronic kidney disease (CKD) is considered a major public health problem worldwide.<sup>1</sup> All patients with CKD should be considered to have a high risk of cardiovascular disease (CVD), although the underlying pathophysiological mechanisms are not fully understood.<sup>2,3</sup> Indeed, conventional cardiovascular risk factors do not appear to fully explain the elevated risk of CVD.

Magnesium is the fourth most abundant cation in the body. Although only about 1% of the total magnesium is located outside the cells, this is nevertheless a major fraction because it is involved in a multitude of enzyme reactions and essential physiological processes,<sup>4-6</sup> such as the inhibition of vascular smooth muscle cell calcification,<sup>7</sup> the regulation of inflammation and immune responses.<sup>8</sup> Magnesium homeostasis depends mainly on absorption through the intestine and excretion by the kidney.<sup>9</sup> In late-stage CKD, the growing impairment in renal excretion typically leads to the accumulation of magnesium in the blood.<sup>10</sup> However, hypomagnesaemia is also common in patients with CKD, due to variations in dietary magnesium intake and the use of drugs that can induce hypomagnesaemia (such as thiazide diuretics and proton pump inhibitors).<sup>9,11</sup>

In serum, around 20% to 30% of the total magnesium in circulation is bound to proteins and is believed to be biologically inert. Ionized magnesium comprises roughly 60% to 70% of total magnesium in circulation and is regarded as the active form of magnesium. It is plausible that ionized magnesium could serve as a more physiologically significant marker compared to total magnesium. The total serum magnesium concentration is most frequently used in routine clinical practice.<sup>12</sup> In fact, the fraction of ionized magnesium is not usually measured and is difficult to calculate.<sup>13,14</sup> Recent studies of various non-CKD cohorts have evidenced a moderate correlation between total magnesium and ionized magnesium.<sup>15,16</sup> Given the variations and/or impairments in serum binding proteins observed in patients with CKD, one could expect to find the same moderate correlation in this population. However, data on levels of ionized magnesium in patients with CKD are lacking.

A large body of data from studies of the general population shows that both hypermagnesaemia and hypomagnesaemia are associated with greater mortality and worse CVD outcomes.<sup>17-20</sup> On the same

lines, the results of a registry-based study of a cohort of more than 140,000 haemodialysis patients highlighted a U-shaped relationship between total magnesium levels and both CVD and non-CVD mortality rates.<sup>21</sup> All-cause mortality is the most frequently studied outcome in CKD populations.<sup>22–29</sup> The association between magnesium levels and cardiovascular outcomes has not been frequently evaluated, and the few published data are conflicting.<sup>22,23,25,28</sup> Furthermore, most studies of the link between magnesium and patient outcomes have focused on total magnesium concentrations and not ionized magnesium, and some studies have reported that total magnesium and ionized magnesium are not related in the same way to the predicted clinical outcomes.<sup>15,16</sup>

Thus, the objectives of the present study were to (i) describe the serum ionized magnesium concentration in patients at various CKD stages, (ii) measure the correlation between ionized magnesium and total magnesium concentrations, (iii) identify associated factors of the ionized magnesium concentration, and (iv) determine whether serum total and/or ionized magnesium concentrations are associated with adverse outcomes (such as fatal or non-fatal major cardiovascular events and mortality) before kidney replacement therapy (KRT, defined as the initiation of chronic dialysis, or kidney transplantation) in patients with CKD, after adjustment for the estimated glomerular filtration rate (eGFR) and other risk factors.

## MATERIALS AND METHODS

The results of this cohort study are reported in accordance with the *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE) guideline.<sup>30</sup>

### Study design and participants

CKD-REIN is a French prospective cohort of adult patients (over 18 years of age) with a confirmed diagnosis of CKD, with eGFR <60 mL/min/1.73 m<sup>2</sup> (stage 3 to 5), who were not on maintenance dialysis nor had undergone kidney transplantation by study entry. Details of the study protocol have already been published.<sup>31</sup> Patients were recruited from 40 nationally representative nephrology outpatient facilities in France with respect to geography and facility legal status, public or private. A total of 3,033 patients were included during a routine nephrology outpatient appointment between

2013 and 2016, and were followed up by clinical research associates (CRAs) for 5 years or until December 31<sup>st</sup>, 2020. The study protocol was approved by the institutional review board at the French National Institute of Health and Medical Research (INSERM; reference: IRB00003888) and was registered at ClinicalTrials.gov (NCT03381950).

For the purposes of the present analysis, we excluded patients who did not have a serum collection at baseline (n=442), patients for whom the total magnesium assay could not be performed (n=3) and patients for whom serum samples were collected more than 90 days after inclusion in the study (n=169). Hence, a total of 2,419 patients were included in the present analysis (**Figure S1**).

### **Study data**

CRAs collected data from patient interviews and medical records at baseline and then annually. The patients' characteristics were recorded, and the patients were screened for a history of hypertension, diabetes, CVD. Height and weight were measured to calculate body mass index (BMI). A specific electronic case report form (linked to the international Anatomical Therapeutic and Chemical (ATC) thesaurus) was used by the CRAs to record the drugs prescribed to patients in the three months prior to the enrolment visit. Standard blood and urine tests (those recommended by the French health authorities for the routine management of CKD) were carried out for all patients in their usual medical laboratory. The GFR was estimated using the Chronic Kidney Disease - Epidemiology Collaboration (CKD-EPI) creatinine equation.<sup>32</sup> The urinary albumin-to-creatinine ratio (ACR) was determined by either measurement or estimation using an equation based on proteinuria measurements.<sup>33</sup> We therefore classified patients according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 guideline stages as follows: A1 (normal or minimal increase): ACR<3 mg/mmol or albumin excretion rate (AER)<30 mg/24 h; A2 (moderate increase): ACR 3–30 mg/mmol or AER 30–300 mg/24 h; A3 (severe increase): ACR=30 mg/mmol or AER=300 mg/24 h.<sup>34</sup>

### **Total and ionized magnesium measurements**

Serum samples were collected at the time of patient's enrolment, stored at 4°C, and aliquoted within 6 hours without further processing. All samples were stored at -80°C at the Biobanque de Picardie



(Amiens-Picardie University Hospital, Amiens, France, BRIF number: BB-0033-00017). We used direct potentiometry with an ion-selective electrode (Stat Profile® PRIME™ ES Comp, Nova Biomedical, Waltham, MA, USA) to assay ionized magnesium levels. In healthy adults, the serum ionized magnesium concentration ranges from 0.43 to 0.54 mmol/L.<sup>35</sup> We used a photometric technique (a modified xylidyl blue reaction) to assay total magnesium assays in the same serum samples (Atellica® CH, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA; reference range: 0.66-1.07 mmol/L). For three patients whose serum ionized magnesium concentration was below the assay's limit of quantification (0.10 mmol/L), we set the value of 0.10 mmol/L. Hypomagnesaemia was defined as a total magnesium concentration below 0.66 mmol/L, and hypermagnesaemia as a magnesium concentration equal or above 1.07 mmol/L.

### **Study outcomes**

Hospitalizations occurring during study follow-up were identified from medical reports, hospital records, and/or patient interview; deaths were ascertained from death certificates, hospital records, reports by family members, and by linkage with the national vital status registry. A physician reviewed and coded all events using the International Classification of Diseases version 10 (ICD-10). Then, based on these codes, this physician identified and assessed cardiovascular events according to the Cardiovascular and Stroke Endpoint Definitions for Clinical Trials.<sup>36</sup> One senior cardiologist further adjudicated all CV deaths.

The primary endpoint was the occurrence of the first fatal or non-fatal major cardiovascular event (MACE), defined as any cardiovascular death, myocardial infarction, stroke, and hospitalisation for heart failure. The secondary endpoint was the occurrence of death i) whatever the cause and ii) from cardiovascular cause.

Only events that occurred before KRT were analysed. KRT events were defined as initiation of maintenance dialysis or pre-emptive kidney transplantation, identified from medical records, patient interviews, and/or by linkage with the national REIN (Renal Epidemiology and Information Network) registry. KRT events and non-cardiovascular deaths before KRT were considered competing events

for cardiovascular events and cardiovascular mortality. Patients were censored at the date of the competing event, the end of their 5-year follow-up, or at study loss to follow-up.

### Statistical analysis

Baseline characteristics were described first for the study population as a whole ( $n=2,419$ ). We then compared subgroups of patients by the serum total magnesium tertiles at baseline (Tertile 1 (T1):  $\leq 0.73$  mmol/L, T2:  $0.74\text{--}0.81$  mmol/L, T3:  $>0.81$  mmol/L). Continuous variables were reported as the mean (standard deviation (SD)) or the median [first-third quartiles], depending on the distribution. Categorical variables were reported as the frequency (percentage). Depending on the distribution, we used a chi-squared test or Fisher's exact test to compare values of categorical variables, and an analysis of variance (ANOVA) or the Kruskal-Wallis test to compare values of continuous variables.

Multivariable linear regressions (based on sociodemographic variables, clinical variables, and prescription drugs) were used to estimate independent associations with the serum ionized magnesium concentration and the serum total magnesium concentration (expressed as a non-standardized coefficient [95% confidence interval (CI)], and as a standardized coefficient). Our choice of the variables included in multivariable models was based on a literature review and a  $p$ -value  $<0.2$  in a univariable analysis. Hypomagnesaemic drugs were those negatively associated with magnesium concentration in multivariable models.

We used cause-specific Cox models to test the crude and adjusted associations between the risk of a first cardiovascular event on one hand and the serum ionized, total magnesium concentration at baseline on the other. Cox models were adjusted for a set of confounding factors selected from a directed acyclic graph (**Figure S2**).<sup>37</sup> This approach enables the selection of an optimal set of adjustment factors (i.e. closing non-causal pathways between exposure (magnesaemia) and outcome (cardiovascular event)). The selected covariates were: age, sex, history of CVD, diabetes, dyslipidaemia, BMI, pulse pressure, eGFR, levels of serum urea, sodium, potassium, bicarbonate, calcium, phosphate, serum albumin, parathyroid hormone (PTH) and haemoglobin, and prescriptions of renin-angiotensin system inhibitors (RASIs), hypomagnesaemic drugs (thiazide diuretics, proton

pump inhibitors (PPIs) and/or potassium chelators), and other diuretics (defined as all diuretics other than thiazide diuretics, such as high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists). We also tested the association between the baseline serum magnesium concentration (ionized and total) and all-cause mortality, as well as cardiovascular mortality. The adjustment factors were the same as those used to assess the risk of MACE (**Figure S3**). We tested the proportional hazards hypothesis by analysing the Schoenfeld residuals for each model. Using restricted cubic splines in the adjusted Cox models,<sup>38</sup> we investigated the functional relationship between the serum magnesium concentration (ionized and total) and the cardiovascular risk, all-cause and cardiovascular mortality.

After assuming that data were missing at random, we managed missing covariate data with multivariate imputation by chained equations (MICE) with predictive mean matching in the mice package in R.<sup>39,40</sup> A total of 25 datasets were created, and the number of iterations was set to 20. All covariates present in the final Cox models were included in the imputation model. Fitted Cox models were generated for each dataset, and pooled regression coefficients were obtained using Rubin's rules.

All tests were two-tailed, and the threshold for statistical significance was set to  $p < 0.05$ . All statistical analyses were performed with R software (version 4.1.3).<sup>40</sup>

## RESULTS

### Characteristics of the patients at baseline

Of the 3,033 patients enrolled in the CKD-REIN cohort, 2,419 were analysed (**Figure S1**). The median age was 68 [60-76], 66% of the patients were men, and the mean (SD) eGFR was 34.8 (13.4) mL/min/1.73 m<sup>2</sup>; 52% of the patients had a history of CVD, and 41% had diabetes (**Table 1**). For the study population as a whole, the mean (SD) serum total and ionized magnesium concentrations were respectively 0.77 (0.10) mmol/L and 0.48 (0.09) mmol/L. Compared with patients in T1 and T2, patients in T3 were older, were more likely to have a history of CVD history, had higher serum phosphate, PTH and urea concentrations, and had a lower baseline eGFR. However, patients in T1 had more prescribed daily medications, including higher use of PPI, thiazide diuretics or potassium

chelators, and had more frequently diabetes than those of T2 or T3. A small but significant negative correlation was found for serum magnesium (ionized and total) and the eGFR ( $r=-0.19$ ;  $p<0.001$  for both total and ionized). The more advanced CKD stages were associated with higher serum ionized and total magnesium concentrations (**Table 2**).

### **Associations between ionized magnesium and total magnesium**

The serum ionized and total magnesium concentrations were strongly correlated ( $r=0.89$ ,  $p<0.001$ ). The vast majority of patients had a normal total magnesium concentration (88%); 11.5% showed hypomagnesaemia ( $<0.66$  mmol/L), and only 0.5% showed hypermagnesaemia ( $\geq 1.07$  mmol/L). In T1 for ionized magnesium, a third of patients showed hypomagnesaemia and two thirds were in the normal range of total magnesium levels (**Table S1**). In T2, over 99% of the patients were in the normal range. In T3, only 2% of patients showed hypermagnesaemia, and 98% were in the normal range.

### **Factors associated with magnesium levels**

In linear regression models, both ionized and total magnesium levels were independently positively associated with age, serum levels of phosphate, albumin, and urea, and prescriptions for high-ceiling diuretics, and laxatives; and negatively associated with diabetes, BMI, eGFR, serum levels of triglycerides, and prescriptions for PPIs, and potassium chelators. Additionally, the serum ionized magnesium level was negatively associated with systolic blood pressure, serum levels of calcium, and prescriptions for thiazide diuretics (**Table S2**), while the serum total magnesium level was positively associated with the serum calcium, bicarbonate, and negatively associated with sodium, and 25-OH vitamin D levels (**Table S3**). Diabetes, prescription for high-ceiling diuretics, serum urea levels, prescriptions for PPIs and potassium chelators were the variables that most strongly predicted ionized magnesium levels (**Table S2**). For total magnesium levels, the best predictors were prescriptions for high-ceiling diuretics, diabetes, urea and serum albumin levels, and kidney function (**Table S3**).

### Major cardiovascular events

Over a median [IQR] follow-up period of 4.9 [3.3; 5.1] years, 351 patients experienced a first MACE. The crude incidence rate [95%CI] was 3.9 [3.5; 4.3] per 100 person-years (PYs). The incidence rate was higher in patients in T3 and (to a lesser extent) in T1 than in patients in T2 (**Figure 1, Table S4**). The adjusted HR [95%CI] for MACEs associated with the baseline serum total magnesium level was 1.27 [0.95; 1.69] for patients in T1, and 1.56 [1.18; 2.06] for patients in T3, relative to patients in T2 (**Figure 1, Table S5**). A restricted cubic spline analysis showed that the relationship between the serum total magnesium level and the MACE risk was non-linear, with a greater risk at high concentrations (**Figure 2A**).

The adjusted HR [95%CI] for MACEs associated with the baseline serum ionized magnesium level was 0.93 [0.71; 1.23] for patients in T1 and 1.14 [0.88; 1.48] for patients in T3, relative to patients in T2 (**Figure 1, Table S6**). Restricted cubic spline analysis confirmed this non-significant trend (**Figure 2B**).

### Mortality

In our study, 314 patients died before KRT resulting in a crude incidence rate [95%CI] of 3.3 [3.0; 3.7] per 100 PYs. The incidence rate was higher in patients in T3 and (to a lesser extent) T1 than in patients in T2 for both ionized and total magnesium (**Figure 3, Table S4**). After multiple adjustments, the risk of death before KRT according to serum total magnesium concentration was 1.11 [0.82; 1.50] for patients in T1 and 1.48 [1.11; 1.97] for patients in T3, compared with patients in T2 (**Figure 3, Table S7**). The adjusted risk of death before KRT according to serum ionized magnesium concentration did not differ significantly from one tertile to another (**Figure 3, Table S8**). The same trends have been found when the analysis was restricted to cardiovascular mortality, the risk being increased in patients in T3 of serum total magnesium (HR [95%CI], 1.98 [1.27; 3.10]) and non-significantly in patients in T1 (HR [95%CI], 1.38 [0.85; 2.25]), relative to patients in T2 (**Figure 4, Table S9**). The risk of cardiovascular death was not found to be significantly associated with serum

ionized magnesium levels in our study (**Figure 4, Table S10**). Restricted cubic spline analyses confirmed these trends (**Figure S4-S5**).

## DISCUSSION

Our analysis of 2,419 non-dialyzed CKD patients from a large prospective cohort showed that both ionized and total magnesium levels increased with the CKD stage, were strongly correlated, and were associated with similar factors. Higher serum total magnesium levels, although still within the normal range, were significantly associated with an elevated risk of MACEs, all-cause mortality, and cardiovascular mortality. However, the serum ionized magnesium level was not associated with these outcomes.

In our study, the serum magnesium concentrations (ionized and total) tended to rise with the CKD stage; indeed, magnesium excretion is known to decrease as renal function declines.<sup>10</sup> The correlation between ionized and total magnesium concentrations was very strong ( $r=0.89$ ;  $p<0.001$ ). This finding contrasts with published data on intensive care unit patients, where the correlation was weak and up to 85% of the patients with a low total magnesium level had a normal ionized magnesium level.<sup>15</sup> However, our finding is in line with the results of a small, single-centre study of chronic haemodialysis patients ( $n=42$ ), in which the ionized and total magnesium levels were strongly correlated ( $r=0.88$ ;  $p<0.001$ ).<sup>41</sup> It is noteworthy that the degree of inter-individual variability was lower for ionized Mg than for total Mg.

We found that serum magnesium concentrations were not particularly elevated, as it might have been the case in patients with a decreased kidney function.<sup>10</sup> It could be due, at least in part, to the use of hypomagnesaemic drugs.<sup>9</sup> We found that the use of PPIs, thiazide diuretics, and potassium chelators was associated with a decrease of serum magnesium levels, either ionized for thiazide diuretics, or both total and ionized for PPIs and potassium chelators. Indeed, hypomagnesaemia is a known adverse effect of these classes of medications, and is mentioned in the summaries of product characteristics. Furthermore, around 40% of patients suffered from diabetes, a factor known to be associated with a decrease in magnesemia.<sup>42</sup> These factors could partially explain why patients in our cohort have

magnesium concentrations close to normal. Overall, we found that ionized and total magnesium were associated with the same factors: age, eGFR, diabetes, BMI, serum calcium, phosphate, albumin, urea and triglycerides, and prescriptions of PPIs, high-ceiling diuretics, potassium chelators, and laxatives. These predictors of magnesium concentration have already been described in the literature for total magnesium levels.<sup>22,24,25,43,44</sup> So, we extended these findings for ionized magnesium levels.

With regard to the reference values for total magnesium (0.66-1.07 mmol/L), 11.5% of patients showed hypomagnesaemia and 0.5% showed hypermagnesaemia. Similar values were found in a study of more than 10,000 patients (at CKD stages 3-4) in the Cleveland Clinic registry: 12.4% of the patients had a magnesium concentration below 1.7 mg/dL (0.70 mmol/L), and 1.9% had a concentration over 2.6 mg/dL (1.07 mmol/L).<sup>24</sup>

Our present study highlighted an elevated risk of MACE and mortality in T3 of the serum total magnesium concentration. To note, the elevation of total magnesium is moderate (>0.81 mmol/L). A similar, non-significant trend was found for the serum ionized magnesium level and the risk of MACE. In a study of more than 65,000 hospitalized patients, a U-shaped association between the total magnesium level and mortality was found.<sup>19</sup> A registry-based study of a large cohort of haemodialysis patients also found that high levels of total magnesium were associated with both CVD and non-CVD mortality.<sup>21</sup> To note, the levels of total magnesium in this dialysis cohort were higher than in our non-dialyzed CKD population. In studies of non-dialyzed CKD patients, the total magnesium level (but not the ionized magnesium level) has sometimes (but not always) been linked to mortality or cardiovascular disease. Some studies (such as the present one) have found an association between a high total magnesium level on one hand and cardiovascular events<sup>23</sup> and all-cause mortality<sup>22-24</sup> on the other, whereas other studies failed to show an association with cardiovascular outcomes.<sup>22,25</sup> Furthermore, low total magnesium levels have been associated with mortality in CKD populations.<sup>22,24,25,27</sup> In the present study, we failed to find an association between low total magnesium levels and studied outcomes.

Moreover, we failed to show an association between serum ionized magnesium on one hand and the occurrence of cardiovascular events or mortality on the other. This lack of association with ionized

magnesium (the biologically active fraction) might suggest that serum total magnesium is not causally related to MACE and mortality, and that confounding factors affecting both total magnesium and the occurrence of these events caused the significant association in our study. The ionized fraction of magnesium might not be affected by these unknown confounding factors, which would explain why the association with MACE and mortality is not significant in our study. In contrast to total magnesium, ionized magnesium is not routinely assayed in hospital laboratories and is used in a research setting only. Furthermore, the measurement of ionized magnesium is not straightforward because the result can be influenced by other ions (especially calcium), temperature, pH, dilution of the sample, and/or the lack of normative reference values.<sup>45</sup>

It has been suggested that magnesium supplementation can reduce the cardiovascular burden in general and the vascular calcification burden in CKD patients in particular. A randomized, controlled trial in patients with stage 3-4 CKD showed that the median in the coronary artery calcification (CAC) score at two years was significantly smaller in participants taking magnesium oxide than in controls.<sup>46</sup> In another recent, randomized, controlled trial, supplementation with magnesium hydroxide for 12 months did not slow the progression of CAC (relative to a placebo) in 148 patients with stage 3b-4 CKD.<sup>47</sup> It is noteworthy that although no MACEs were reported in the placebo group, six (three sudden cardiac deaths, two strokes and one incident heart failure) were reported in the magnesium group. Hence, the safety of magnesium supplementation requires further evaluation, in addition to determination of whether lowering total magnesium concentrations will impact outcomes in the setting of kidney disease.

The present study had several strengths. Firstly, it is (to the best of our knowledge) the first to have investigated the association between both serum total and ionized magnesium concentrations and the occurrence of cardiovascular events and mortality in CKD patients not undergoing KRT. Secondly, our analysis of a large number of patients from a nationally representative, multicentre, prospective cohort enabled comprehensive adjustments for confounding factors. Thirdly, the serum ionized and total magnesium concentrations were measured centrally, which probably reduced the variability



between measurements. Lastly, all cardiovascular events were assessed carefully, according to standardized definitions.

Our study also had some limitations. First, 99% of the body's magnesium is located in the intracellular space (mainly in the bones), and so only 1% is present in extracellular fluids. Hence, the serum magnesium concentration might not adequately reflect the body's magnesium status; it might be more relevant to measure the intracellular magnesium concentration in specific tissues or cell types. However, this evaluation is particularly complex and, in contrast to a total magnesium assay, is not carried out on a routine basis. Secondly, the magnesium concentrations were measured once (at baseline), and so we were not able to explore putative associations between time-varying concentrations and outcomes. Lastly, the lack of data on the participants' diet prevented us from adjusting our analyses for the magnesium intake through diet.

In conclusion, we highlighted a strong correlation between total and ionized magnesium levels. Our analysis of a large cohort of patients with moderate-to-advanced CKD demonstrated that (i) elevated serum total magnesium concentrations (albeit still within the normal range) were associated with a greater likelihood of MACE and mortality but (ii) elevated serum ionized magnesium levels were not associated with these outcomes. Moreover, the safety of magnesium supplementation requires further evaluation.

## DATA AVAILABILITY STATEMENT

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

## AUTHORS' CONTRIBUTIONS

Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work - even one in which the author was not directly involved - are appropriately investigated and resolved, including with documentation in the literature if appropriate.

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## CKD-REIN STUDY GROUP

### *Steering committee and coordinators*

The CKD-REIN Study Group steering committee and coordinators include: Natalia Alencar de Pinho, Dorothée Cannet, Christian Combe, Denis Fouque, Luc Frimat, Aghilès Hamroun, Yves-Edouard Herpe, Christian Jacquelinet, Oriane Lambert, Céline Lange, Maurice Laville, Sophie Liabeuf, Ziad A. Massy, Marie Metzger, Pascal Morel, Christophe Pascal, Roberto Pecoits-Filho, Joost Schantsra, Bénédicte Stengel.

### *Investigators*

Alsace: Prs T. Hannedouche et B. Moulin (CHU, Strasbourg), Dr A. Klein (CH Colmar) Aquitaine: Pr C. Combe (CHU, Bordeaux), Dr J.P. Bourdenx (Clinique St Augustin, Bordeaux), Dr A. Keller, Dr C. Delclaux (CH, Libourne), Dr B. Vendrely (Clinique St Martin, Pessac), Dr B. Deroure (Clinique Delay, Bayonne), Dr A. Lacraz (CH, Bayonne) Basse Normandie: Dr T. Lobbedez (CHU, Caen), Dr I. Landru (CH, Lisieux) Ile de France: Pr Z. Massy (CHU, Boulogne – Billancourt), Pr P. Lang (CHU, Créteil), Dr X. Belenfant (CH, Montreuil), Pr E. Thervet (CHU, Paris), Dr P. Urena (Clinique du Landy, St Ouen), Dr M. Delahousse (Hôpital Foch, Suresnes) Languedoc – Roussillon: Dr C. Vela (CH, Perpignan) Limousin: Pr M. Essig, Dr D. Clément (CHU, Limoges) Lorraine: Dr H. Sekhri, Dr M. Smati (CH, Epinal) Dr M. Jamali, Dr B. Hacq (Clinique Louis Pasteur, Essey-les-Nancy), Dr V. Panescu, Dr M. Bellou (Polyclinique de Gentilly, Nancy), Pr Luc Frimat (CHU, Vandœuvre-les-Nancy) Midi-Pyrénées: Pr N Kamar (CHU, Toulouse) Nord-Pas-de-Calais: Prs C. Noël et F. Glowacki (CHU, Lille), Dr N. Maisonneuve (CH, Valenciennes), Dr R. Azar (CH, Dunkerque), Dr M. Hoffmann (Hôpital privé La Louvière, Lille) Pays-de-la Loire: Pr M. Hourmant (CHU, Nantes), Dr A. Testa (Centre de dialyse, Rezé), Dr D. Besnier (CH, St Nazaire) Picardie: Pr G. Choukroun (CHU, Amiens), Dr G. Lambrey (CH, Beauvais) Provence-Alpes - Côte d'Azur: Pr S. Burtay (CHU, Marseille), Dr G. Lebrun (CH, Aix-en-Provence), Dr E. Magnant (Polyclinique du Parc Rambot, Aix-en-Provence) Rhône-Alpes: Pr M. Laville, Pr D. Fouque (CHU, Lyon-Sud) et L. Juillard (CHU

Edouard Herriot, Lyon), Dr C. Chazot (Centre de rein artificiel Tassin Charcot, Ste Foy-les-Lyon), Pr P. Zaoui (CHU, Grenoble), Dr F. Kuentz (Centre de santé rénale, Grenoble).

## CONFLICT OF INTEREST STATEMENT

M.P., S.M.L., S.L., S.K., M.M. have nothing to declare.

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**Table 1: Baseline characteristics of the study population**

	Total (N=2419)	Total magnesium (mmol/L)			p-value	Imputed data (N=2419)
		Tertile 1 ≤0.73 (N=829)	Tertile 2 0.74-0.81 (N=837)	Tertile 3 >0.81 (N=753)		
Total magnesium (mmol/L)	0.77 (0.10)	0.66 (0.07)	0.78 (0.02)	0.88 (0.06)	<0.001	0%
Ionized magnesium (mmol/L)	0.48 (0.09)	0.40 (0.08)	0.49 (0.04)	0.57 (0.07)	<0.001	0%
Age (years)	68 [60-76]	67 [58-75]	68 [60-75]	70 [63-77]	<0.001	0%
Men	66%	68%	68%	63%	0.049	0%
Level of studies					0.23	
<9 years	14%	16%	14%	13%		1%
9-11 years	49%	48%	48%	52%		
≥12 years	36%	36%	38%	35%		
Alcohol abuse	1%	2%	1%	1%	0.16	0.7%
Smoking status					0.12	
Non-smoker	40%	40%	38%	44%		0.6%
Current	12%	13%	14%	10%		
Past	47%	48%	48%	46%		
Hypertension	96%	97%	96%	96%	0.46	0.2%
Diabetes	41%	51%	39%	33%	<0.001	0.2%
Dyslipidaemia	66%	68%	68%	63%	0.35	0.4%
History of cardiovascular disease	52%	50%	51%	56%	0.04	0.5%
Gastrointestinal bleeding	4%	4%	3%	4%	0.65	4.3%
Body mass index (kg/m <sup>2</sup> )	28.8 (5.8)	29.6 (6.0)	28.5 (5.6)	28.1 (5.7)	<0.001	1.9%
Systolic blood pressure (mmHg)	142 (21)	143 (21)	143 (20)	141 (21)	0.13	2.1%
Pulse pressure (mmHg)	64 (19)	64 (19)	64 (18)	64 (19)	0.88	2.3%
eGFR (mL/min/1.73m <sup>2</sup> )	34.8 (13)	36.9 (14)	36.1 (14)	31.0 (12)	<0.001	1.4%
Albumin- or protein-to-creatinine ratio classes					0.02	
Normal or minimal increase (A1)	28%	25%	30%	30%		8.5%
Moderate increase (A2)	32%	31%	31%	34%		
Severe increase (A3)	40%	44%	39%	37%		
CRP (mg/L)	2.5 [1.1-5.9]	2.7 [1.2-6.1]	2.4 [1.0-5.4]	2.6 [1.3-6.5]	0.12	0.8%
Sodium (mmol/L)	140 (2.8)	140 (2.8)	140 (2.7)	140 (2.8)	0.90	0.5%
Potassium (mmol/L)	4.5 (0.5)	4.5 (0.5)	4.6 (0.5)	4.5 (0.5)	0.10	0.4%
Bicarbonates (mmol/L)	24.9 (3.4)	24.5 (3.4)	25.0 (3.4)	25.2 (3.4)	<0.001	8.9%
Calcium (mmol/L)	2.35 (0.13)	2.34 (0.15)	2.36 (0.12)	2.35 (0.12)	<0.001	2.5%
Phosphates (mmol/L)	1.15 (0.23)	1.14 (0.24)	1.14 (0.21)	1.18 (0.24)	<0.001	3.8%
Serum albumin (g/L)	40.6 (4.1)	39.8 (4.3)	40.8 (4.0)	41.1 (3.8)	<0.001	0.1%
Urea (mmol/L)	13.3 [10.2-18.4]	12.8 [10.1-16.9]	12.9 [9.80-17.2]	14.8 [11.1-20.5]	<0.001	6.7%
Total cholesterol (mmol/L)	4.83 (1.29)	4.74 (1.29)	4.91 (1.30)	4.84 (1.27)	0.04	9.3%
HDL-cholesterol (mmol/L)	1.32 (0.47)	1.25 (0.44)	1.32 (0.48)	1.38 (0.47)	<0.001	10.0%
LDL-cholesterol (mmol/L)	2.69 (1.09)	2.58 (1.08)	2.76 (1.10)	2.73 (1.08)	0.005	11.8%
Triglycerides (mmol/L)	1.53 [1.10-2.25]	1.72 [1.19-2.52]	1.51 [1.08-2.22]	1.45 [1.04-1.98]	<0.001	9.6%
Haemoglobin (g/dL)	13.0 (1.7)	12.9 (1.7)	13.3 (1.7)	12.9 (1.6)	<0.001	0.8%
PTH (pg/mL)	79 [49-129]	73 [46-122]	73 [46-118]	95 [58-150]	<0.001	12.9%
25-OH Vitamin D (ng/mL)	28 [19-37]	27 [18-36]	28 [21-37]	28 [20-37]	0.24	10.6%
Number of prescriptions per patient	8 [5-10]	8 [6-11]	7 [5-10]	8 [5-10]	<0.001	0.3%
Proton pump inhibitors	32%	39%	30%	28%	<0.001	0.3%
Diuretics	54%	53%	51%	59%	0.01	0.3%
Thiazide diuretics	21%	27%	21%	16%	<0.001	0.3%
High-ceiling diuretics	35%	27%	33%	46%	<0.001	0.3%
Potassium-sparing diuretics	5%	5%	4%	5%	0.49	0.3%
Beta-blockers	42%	43%	40%	43%	0.38	0.3%
Calcium channel blockers	47%	45%	48%	48%	0.43	0.3%
Renin-angiotensin system inhibitors	76%	80%	78%	70%	<0.001	0.3%
Corticosteroids	7%	7%	6%	8%	0.42	0.3%
Potassium chelator	13%	15%	12%	12%	0.049	0.3%
Phosphate binders	4%	3%	3%	5%	0.06	0.3%
Magnesium supplementation	0.4%	0.4%	0.5%	0.4%	0.93	0.3%

Medications for constipation	4%	3%	4%	6%	0.02	0.3%
Lithium	0,2%	0,02%	0,2%	0,3%	0.34	0.3%

CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone

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**Table 2: Serum ionized and total magnesium concentrations, by CKD stage**

	Total (N=2394)	CKD stage			P-value
		Stage 2-3A (N=531)	Stage 3B (N=884)	Stage 4-5 (N=979)	
<b>Ionized magnesium (mmol/L)</b>	0.48 (0.09)	0.46 (0.08)	0.47 (0.09)	0.50 (0.10)	<0.001
<b>Total magnesium (mmol/L)</b>	0.77 (0.10)	0.74 (0.09)	0.76 (0.10)	0.79 (0.11)	<0.001

CKD, chronic kidney disease

	N events	Event rate per 100 person-years	Adjusted hazard ratio*
<b>Serum total magnesium</b>			
Tertile 1	118	3.8 [3.1; 4.5]	1.27 [0.95; 1.69]
Tertile 2	88	2.7 [2.2; 3.3]	Ref
Tertile 3	145	5.5 [4.6; 6.4]	1.56 [1.18; 2.06]
<b>Serum ionized magnesium</b>			
Tertile 1	105	3.3 [2.7; 3.9]	0.93 [0.71; 1.23]
Tertile 2	114	3.6 [2.9; 4.2]	Ref
Tertile 3	132	5.2 [4.3; 6.1]	1.14 [0.88; 1.48]

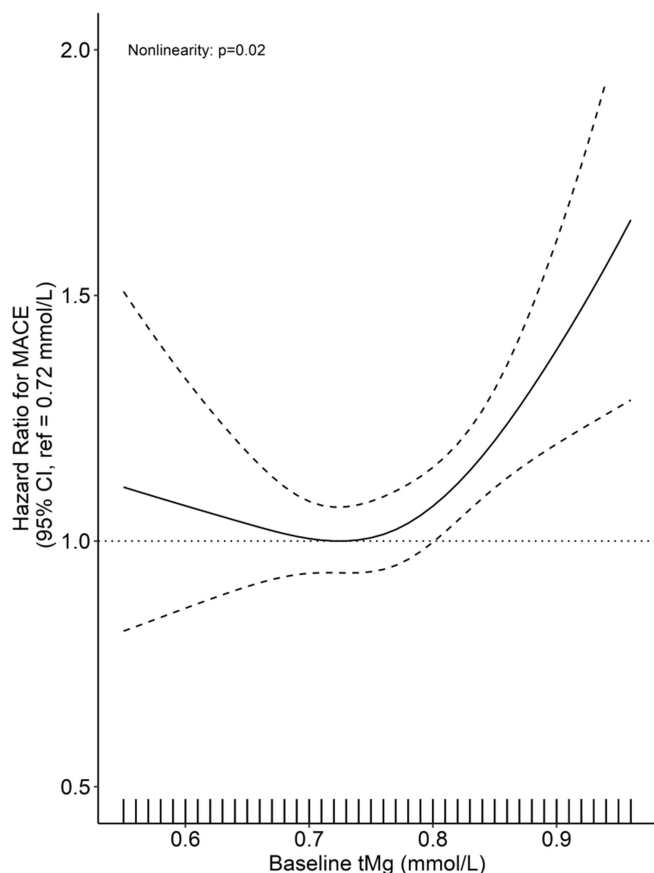
Serum total magnesium: Tertile 1 ( $\leq 0.73$  mmol/L), Tertile 2 (0.74-0.81 mmol/L), Tertile 3 ( $> 0.81$  mmol/L)

Serum ionized magnesium: Tertile 1 ( $\leq 0.45$  mmol/L), Tertile 2 (0.46-0.52 mmol/L), Tertile 3 ( $> 0.52$  mmol/L)

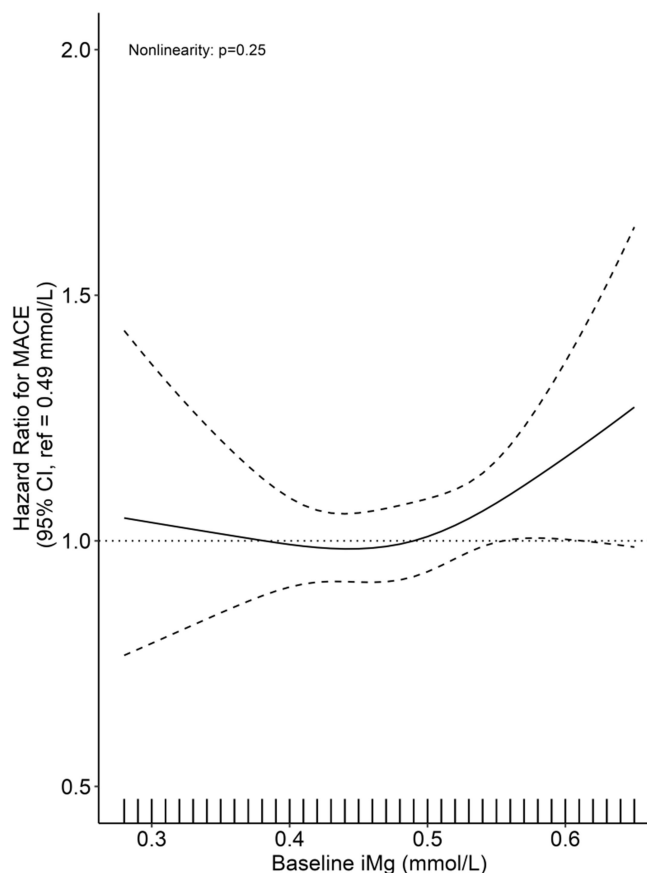
Note: Hazard ratios are adjusted for age, sex, body mass index, diabetes, dyslipidaemia, pulse pressure, estimated glomerular filtration rate, history of CVD, serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, parathyroid hormone and haemoglobin, the use of renin-angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists), and hypomagnesaemic drugs (thiazide diuretics, proton pump inhibitors (PPIs) and/or potassium chelators).

**Figure 1: Number of events, incidence rates, and adjusted hazard ratios for MACEs, as a function of the baseline serum total and ionized magnesium concentrations**

**A. Adjusted hazard ratio for the first MACE, as a function of the baseline serum total magnesium level**



**B. Adjusted hazard ratio for the first MACE, as a function to baseline serum ionized magnesium level**



CI, confidence interval; iMg, ionized magnesium; MACE, major cardiovascular event; tMg, total magnesium

Note: Hazard ratios are adjusted for age, sex, body mass index, diabetes, dyslipidaemia, pulse pressure, estimated glomerular filtration rate, history of CVD, serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, parathyroid hormone and haemoglobin, the use of renin-angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists), and hypomagnesaemic drugs (thiazide diuretics, proton pump inhibitors and/or potassium chelators). The continuous line represents predictions with restricted cubic splines in Cox models (dashed lines represent the 95% confidence intervals). Ticks on the x-axis represent the distribution of the baseline serum magnesium level. The 5% most extreme values of serum magnesium concentrations have been removed in this graph.

**Figure 2: Adjusted hazard ratio for the occurrence of the first MACE, as a function of the baseline serum total and ionized magnesium concentrations, using restricted cubic spline term. A) Adjusted hazard ratio for the first MACE, as a function of the baseline serum total magnesium level. B) Adjusted hazard ratio for first MACE, as a function to baseline serum ionized magnesium level.**

	N events	Event rate per 100 person-years	Adjusted hazard ratio*
<b>Serum total magnesium</b>			
Tertile 1	102	3.1 [2.5; 3.7]	1.11 [0.82; 1.50]
Tertile 2	82	2.5 [1.9; 3.0]	Ref
Tertile 3	130	4.7 [3.9; 5.5]	1.48 [1.11; 1.97]
<b>Serum ionized magnesium</b>			
Tertile 1	89	2.6 [2.1; 3.2]	0.79 [0.59; 1.05]
Tertile 2	112	3.3 [2.7; 4.0]	Ref
Tertile 3	113	4.2 [3.4; 5.0]	0.96 [0.73; 1.26]

Serum total magnesium: Tertile 1 ( $\leq 0.73$  mmol/L), Tertile 2 (0.74-0.81 mmol/L), Tertile 3 ( $> 0.81$  mmol/L)

Serum ionized magnesium: Tertile 1 ( $\leq 0.45$  mmol/L), Tertile 2 (0.46-0.52 mmol/L), Tertile 3 ( $> 0.52$  mmol/L)

Note: Hazard ratios are adjusted for age, sex, body mass index, diabetes, dyslipidaemia, pulse pressure, estimated glomerular filtration rate, history of CVD, serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, parathyroid hormone and haemoglobin, the use of renin-angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists), and hypomagnesaemic drugs (thiazide diuretics, proton pump inhibitors and/or potassium chelators).

**Figure 3: Number of events, incidence rates, and adjusted hazard ratios for the occurrence of overall mortality, as a function of the baseline serum total and ionized magnesium concentrations**

	N events	Event rate per 100 person-years	Adjusted hazard ratio*
<b>Serum total magnesium</b>			
Tertile 1	46	1.4 [1.0; 1.8]	1.38 [0.85; 2.25]
Tertile 2	30	0.9 [0.6; 1.2]	Ref
Tertile 3	69	2.5 [1.9; 3.1]	1.98 [1.27; 3.10]
<b>Serum ionized magnesium</b>			
Tertile 1	40	1.2 [0.8; 1.6]	0.86 [0.56; 1.34]
Tertile 2	47	1.4 [1.0; 1.8]	Ref
Tertile 3	58	2.2 [1.6; 2.7]	1.06 [0.71; 1.59]

Serum total magnesium: Tertile 1 ( $\leq 0.73$  mmol/L), Tertile 2 (0.74-0.81 mmol/L), Tertile 3 ( $> 0.81$  mmol/L)

Serum ionized magnesium: Tertile 1 ( $\leq 0.45$  mmol/L), Tertile 2 (0.46-0.52 mmol/L), Tertile 3 ( $> 0.52$  mmol/L)

Note: Hazard ratios are adjusted for age, sex, body mass index, diabetes, dyslipidaemia, pulse pressure, estimated glomerular filtration rate, history of CVD, serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, parathyroid hormone and haemoglobin, the use of renin-angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists), and hypomagnesaemic drugs (thiazide diuretics, proton pump inhibitors and/or potassium chelators).

**Figure 4: Number of events, incidence rates, and adjusted hazard ratios for the occurrence of cardiovascular mortality, as a function of the baseline serum total and ionized magnesium concentrations**