Circulating Magnesium and Cardiovascular Disease

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DEDICATION

The practice of epidemiology is often considered both a science and an art. This dissertation is dedicated to my parents, Paul and Sue Rooney, who always encouraged my scientific and artistic curiosity.

ABSTRACT

Low circulating magnesium (Mg) or hypomagnesemia is thought to be common, and is traditionally measured by circulating total Mg. Proton pump inhibitor (PPI) medication use is also common and has been linked with low circulating Mg. Both low circulating Mg and PPI use have been associated with elevated cardiovascular disease (CVD) risk. This dissertation further characterizes the complex relationship between circulating Mg and CVD among older adults.

Using data from a double-blind pilot Mg supplementation randomized controlled trial, the first manuscript characterizes the interrelationship of different circulating Mg status biomarkers (ionized and total Mg) at baseline and in response to Mg supplementation. Baseline ionized and total Mg were modestly and positively associated. Mg supplementation versus placebo over 10 weeks resulted in increased concentrations of ionized and total Mg.

In the second manuscript, we test cross-sectional associations of circulating total Mg with burden of atrial and ventricular arrhythmias as measured over 2 weeks on an ambulatory electrocardiographic monitoring patch in the Atherosclerosis Risk in Communities (ARIC) study. In this now elderly population, serum Mg was inversely associated with premature ventricular contraction burden. While effect estimates were in the hypothesized direction, we found little evidence of an association between circulating Mg and atrial arrhythmias. These findings were similar even among those without a history of CVD.

The third manuscript explores cross-sectional associations of PPI use with circulating total Mg and prospective associations of PPI use, hypomagnesemia and CVD risk in the ARIC study. One in four participants had used a PPI within the last 2 weeks, and PPI users had a greater prevalence of hypomagnesemia than non-users. Additionally, PPI users had modestly elevated risk of CVD; however, presence of hypomagnesemia did not explain this elevated risk of CVD.

Collectively, this dissertation helps refine our understanding of Mg homeostasis in relation to CVD.

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LIST OF ABBREVIATIONS

ACEi – angiotensin-converting enzyme inhibitor

AF – atrial fibrillation

ARB – angiotensin II receptor blocker

ARDL – Advanced Research and Diagnostics Laboratory

ARIC – Atherosclerosis Risk in Communities

BMI – body mass index

CABG – coronary artery bypass graft

CHD – coronary heart disease

CVD – cardiovascular disease

EAR – estimated average requirement

ECG – electrocardiogram

eGFR – estimated glomerular filtration rate

FDA – Food and Drug Administration

GERD – gastroesophageal reflux disease

GI -- gastrointestinal

GWAS – genome wide association study

HDL – high-density lipoprotein

HF – heart failure

ICD – International Classification of Disease

iMg – ionized magnesium

LDL – low-density lipoprotein

Mg – magnesium

MI – myocardial infarction

NHANES – National Health and Nutrition Examination Survey

NSVT – non-sustained ventricular tachycardia

PTH – parathyroid hormone

PAC – premature atrial contraction

PVC – premature ventricular contraction

PPI – proton pump inhibitor

RDA – recommended dietary allowance

RERI – relative excess risk due to interaction

SNP – single nucleotide polymorphism

SVT – supraventricular tachycardia

tMg – total magnesium

TRPM6 – transient receptor potential melatstatin ion channel 6

TRPM7– transient receptor potential melatstatin ion channel 7

CHAPTER 1 – OVERVIEW OF MAGNESIUM AND HEALTH

I. INTRODUCTION TO MAGNESIUM

Magnesium (Mg) is the fourth most common mineral in the human body. Approximately 75-90% of Mg is found within the bone and muscle, with the remaining found extra- or intra-cellularly.[1] Mg concentrations are reflective of a complex balance of intestinal absorption and renal handling.[2] This mineral plays a role in over 300 enzymatic reactions in the body and, notably, is involved in insulin metabolism, DNA synthesis, blood pressure regulation and serves as a calcium antagonist.[3,4] The first known use of Mg in medicine dates back to 1697 when Mg-sulfate—the major ingredient of Epsom salts—was identified. Epsom salts were used to treat a variety of conditions at the time, such as abdominal pain and constipation.[1]

The Institute of Medicine's (now National Academies of Medicine) nutritional guidelines for Mg intake are defined by age and sex.[5] The Estimated Average Requirement (EAR) for Mg intake—an intake value used for assessing population-level nutritional adequacy—is 350 mg/day for adult men and 265 mg/day for adult women. The Recommended Dietary Allowance (RDA) for Mg—a daily intake level thought to meet Mg requirements for 97.5% of the population—is 420 mg/day for adult men and 320 mg/day for adult women. The 2015 Dietary Guidelines Advisory Committee labeled Mg as "shortfall nutrient", as nearly 50% of Americans consume less than the EAR.[6] As shown in **Table 1.1**, in 2011-12, the prevalence of daily total Mg intake below the EAR is pervasive in the nationally representative U.S.-based National Health and Nutrition Examination Survey (NHANES) for most adults and both sexes.

Table 1.1. Percent of American adults with daily total magnesium intake (dietary and supplement) below the EAR: NHANES 2011-2012

	Age	EAR†
Men		**350 mg**
	30-50	47%
	51-70	47%
	≥70	55%
Women		**265 mg**
	30-50	50%
	51-70	36%
	≥70	48%

† EAR: average daily level of intake estimated to meet the requirements of 50% of healthy individuals; estimated average requirement

In research and clinical settings, serum total Mg (tMg) concentrations are traditionally used to assess magnesium status. tMg is reflective of a complex and dynamic interplay of dietary intake, bone exchange, excretion and intestinal reabsorption. Reasons for clinically low Mg concentrations may arise from a variety of factors, such as inadequate dietary Mg intake and mutations in the *TRPM6* gene, which is related to Mg absorption. It can also be induced by certain medications (e.g. diuretics, proton pump inhibitors).

The estimated prevalence of clinical hypomagnesaemia [traditionally defined by serum tMg <0.75 mmol/L (1.82 mg/dL; multiply mmol/L by 2.43 to convert to mg/dL)] in the U.S. is unclear, as serum tMg has not been measured in NHANES since 1974, though it appears common. The estimated prevalence in the U.S. may range from 3% to 15%, while in intensive care settings the prevalence may near 65%.[10,11] In 2016, serum tMg was assessed among older participants (aged 66-90 years) of the Atherosclerosis Risk in Communities (ARIC) study and 19.9% had clinical hypomagnesemia. Serum tMg was also measured in a nationally

representative Canadian sample in 2012-2013, in which 10-17% of adults across sex-age groups had clinical hypomagnesemia.[7]

A. Magnesium homeostasis

Mg undergoes regulation in the human body to ensure adequate stores. Mg homeostasis is based on complex feedback loops and is reflective of a balance of dietary intake, absorption, bone exchange and excretion. An overview of each aspect involved in Mg homeostasis is depicted in **Figure 1.1**.[1]

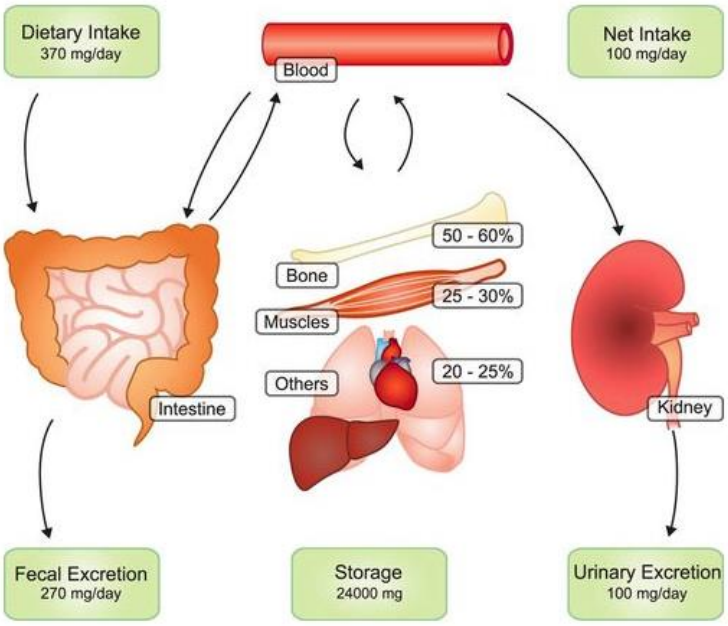


Figure 1.1. Magnesium homeostasis, De Baaij, 2015

1. *Diet*

As described earlier, the majority of American adults consumed less than their daily recommendation for dietary Mg intake.[6] The RDA for adult men is 420 mg per day for men and for adult women is 320 mg per day. A variety of dietary sources include Mg, such as plant

sources, animal products, hard tap water and supplements. Plant sources (e.g. green leafy vegetables, beans, nuts, whole grains, dark chocolate) are among the richest sources of Mg. The majority of Mg is consumed in the form of vegetables, fruits, grains and nuts, with lesser amounts arising from milk, meat and eggs.[8] Water intake also contributes a small amount to daily Mg consumption. Concentrations of Mg within water (in the form of Mg salts) is positively correlated with the degree of hardness of tap water.[9]

Dietary supplements are commonly used among American adults. In 2011-2012, 28% of adult NHANES participants consumed at least one Mg-containing supplement.[10] Notably, like many other nutrients, individuals who consume supplements also tend to have higher dietary intake of the nutrient than non-supplement users.[11,12] The Upper Limit for Mg intake is 350 mg/day of supplemental Mg. Mg toxicity is relatively rare, and generally only occurs from non-food sources, such as antacids or laxatives.[8]

2. *Absorption*

The majority of Mg is absorbed in the intestines through both passive and active transport. In the small intestine, Mg is primarily absorbed passively. Later, when passing through the large intestine, Mg is primarily absorbed transcellularly through the transient receptor potential melastatin type 6 (TRPM6) and the transient receptor potential melastatin type 7 (TRPM7).[1] Mg absorption depends on a variety of conditions such as usual Mg intake and concurrent intake of other nutrients such as fiber or protein. When Mg is consumed in the form of dietary supplements, the chemical composition also influences the fractional absorption in the intestines. Additionally, not all Mg that is consumed is absorbed within the body. Under adequate conditions, approximately 40-60% of dietary Mg is absorbed. When dietary Mg intake decreases, the fractional absorption of Mg increases. Conversely when dietary Mg increases, the fractional

absorption of Mg decreases.[8]

3. *Bone exchange*

As described previously, the majority of Mg stores (~50-60%) in the human body reside in the bone. Bone metabolism is closely linked to circulating Mg concentrations. Exchangeable pools of Mg are found bound to the surface of hydroxyapatite crystals or within fluid surrounding the crystals.[13] When circulating Mg and dietary Mg are low, Mg is excreted from bone into circulation to maintain physiological concentrations.[1] Mg in the bone affects the solubility of phosphorus and calcium (found within hydroxyapatite crystals) and affects crystal size and formation.[14]

4. *Excretion*

The kidneys serve as the primary organ involved in Mg balance and the rate of excretion is inversely related to circulating Mg concentrations. Mg undergoes a filtration-reabsorption process in the kidneys. The majority (65%) of renal Mg is reabsorbed in the loop of Henle, while 20-30% is reabsorbed in the proximal convoluted tubule.[9] When circulating Mg is high, the kidneys filter more Mg out of the blood to be excreted in urine. In the presence of kidney failure, Mg toxicity may occur due to impaired filtering of circulating Mg.[8] The majority of unabsorbed Mg is excreted primarily through feces and also urination.[1] Small amounts of Mg are excreted through perspiration.[15]

B. Biomarkers of magnesium status

While there are a variety of laboratory methods available to assess Mg status, currently, the most common method to assess Mg status is to measure total circulating Mg (tMg) in blood

specimens. Herein, we focus on circulating tMg and ionized Mg (iMg) concentrations followed by a brief discussion of other measurements for assessing Mg status.

1. *Circulating total magnesium*

Mg status has traditionally been assessed using tMg (in serum or plasma), likely owing to its relatively inexpensive and straightforward assay. There are a few types of assays to quantify tMg: 1) atomic absorption spectrophotometry, 2) colorimetric methods, or 3) enzymatic methods.[16] However, there are important limitations of tMg as a biomarker. As previously described, only <0.3% of total body Mg stores are in circulation. Of circulating tMg, ~30% is protein-bound and thought to be physiological inactive.[1,17] Additionally, serum or plasma tMg can be impacted by time of day of the blood draw. Low albumin can also lead to spuriously low tMg concentrations.[16]

2. *Circulating ionized magnesium*

iMg is thought to be the physiologically active form of circulating Mg, as protein-bound Mg is not active.[18] To date, very few studies have incorporated measurement of iMg concentrations; similarly iMg is not widely used in clinical settings.[16,18] This is likely because specialized equipment is required, it is recommended that laboratory analysis take place immediately after the blood draw and iMg measurement can be prone to interference (e.g. pH level, calcium). Ion selective electrodes are available to measure serum iMg concentrations (usually in whole blood), but results may be instrument dependent. However, recent advances in laboratory assays of ionic electrolytes and limitations of tMg as a biomarker of Mg status have contributed to growing research interest in iMg.[16] Most epidemiologic studies that have examined iMg have done so cross-sectionally in populations with medical conditions (e.g.

chronic kidney disease, hypertension, pre-term labor).[18] Whether iMg is better indicator of Mg adequacy and how iMg relates to health outcomes is an ongoing area of research. [16]

3. *Other*

As the contents of this dissertation revolve around circulating Mg measurements (specifically in serum), only brief mention of other Mg measurements are described. For a review of all measures available to assess Mg status, see a review by Costello et al (2017).[16] Briefly, urinary Mg concentration is another relatively common method to assess Mg status. To minimize effects of circadian rhythm, 24-hour urinary samples are preferred. Urinary Mg concentrations are also influenced by co-morbidities (e.g. poor diabetic control or renal function), or use of certain medications (e.g. diuretics).[16] In instances of short-term changes in Mg intake, urinary Mg responds more quickly than circulating concentrations. As such, urinary Mg might not be indicative of cumulative Mg intake.[17] Several urinary Mg measurements over multiple time-points may be more informative of Mg adequacy. [19] In instances of Mg loss, fractional excretion of Mg can be used to assess the route (gastrointestinal or renal).[16]

C. Clinical cut-points of magnesium adequacy

The reference interval for serum tMg cut-points was based on the distribution of concentrations in a healthy young population of NHANES participants between 1971-1974.[20] In a recent review, it has been suggested that subclinical Mg deficiency may begin to occur with tMg concentrations between 0.75-0.85 mmol/L, which is within the current reference range of 0.75-0.955 mmol/L or 1.82-2.32 mg/dL for normal Mg as found in healthy participants.[17]

Clinical hypomagnesemia is traditionally defined by a serum tMg concentration of <0.75 mmol/L (1.82 mg/dL; multiply mmol/L by 2.43 to convert to mg/dL). This is inclusive of both

asymptomatic hypomagnesemia 0.50-0.75 mmol/L (1.22-1.82 mg/dL) and symptomatic hypomagnesemia <0.50 mmol/L (<1.22 mg/dL). Symptomatic hypomagnesemia, while relatively rare, may warrant immediate medical attention and can result in adverse biochemical, neuromuscular or cardiac electrophysiology changes. Hypomagnesemia may manifest through largely non-specific symptoms such as convulsions, muscle cramps or coma. Other electrolyte disturbances, such as hypokalemia and hypocalcemia, may coexist or arise secondary to hypomagnesemia.[21,22]

Hypermagnesemia is less common, particularly in clinical settings, relative to hypomagnesemia.[23] Severe hypermagnesemia typically arises as a result of therapeutic use of Mg in cases of chronic renal failure and eclampsia, or from misuse of Mg-containing laxatives, antacids, or Epsom salts. Symptomatic hypermagnesemia (>2.00 mmol/L or >4.86 mg/dL) can also result in diarrhea, adverse ECG changes (e.g. prolonged PR interval) or coma.[22]

The interval for iMg is not clear and can vary based on numerous components related to laboratory analysis, pH and the concentration of calcium in the sample.[18] A recent review suggested a reference interval for iMg as 0.50-0.75 mmol/L.[16]

D. Intervening upon low circulating magnesium

Circulating Mg concentration is not necessarily a direct reflection of dietary intake and may not reflect intracellular stores.[24] Despite the complexity of Mg homeostasis, serum tMg has generally been responsive to Mg supplementation.[24-26] A meta-analysis of randomized controlled trials (RCT) indicates that supplementation with Mg increases serum tMg concentrations relative to control groups.[26]

There are small randomized controlled trials (RCTs) that have looked at oral Mg

supplementation in relation to other biologic parameters (e.g. iMg), though these have generally been conducted in populations with existing health conditions and results have been inconsistent.[26] Four small oral Mg supplement RCTs (Range $N_{randomized}$ = 26-60) have included measurements of both circulating iMg and tMg (summarized in **Table 1.2**).[27-30] In a RCT of elderly participants with type 2 diabetes,[27] those randomized to Mg supplementation had, after 1 month of treatment, a statistically significant increase in iMg (but not tMg) from baseline. No changes in iMg or tMg from baseline were found in the placebo group.[27] In contrast, other RCTs have found no effect of Mg supplementation on bio-distribution of circulating tMg or iMg.[28-30] Notably, these trials vary in regards to supplement formulation and dose as well as the duration of prescribed study treatment.

Table 1.2. Summary characteristics of oral magnesium supplement randomized controlled trials with both ionized and total magnesium measurements

				Baseline[a,b]				**Post-intervention**[a,b]			
		Treatment	**Duration**	**Mg (active)**		**Placebo**		**Mg (active)**		**Placebo**	
Study population	**N**[c]	**(Mg form)**	**(months)**	**iMg**	**tMg**	**iMg**	**tMg**	**iMg**	**tMg**	**iMg**	**tMg**
Elderly with type 2 diabetes & hypertension[27]	60	Mg pidolate; 368 mg/day	1	0.42 ± 0.05	0.91 ± 0.05	0.43 ± 0.05	0.92 ± 0.05	0.49 ± 0.06 [d]	0.93 ± 0.05	0.42 ± 0.06	0.91 ± 0.05
Adults with mild to moderate asthma[28]	55	Mg citrate; 340 mg/day	6.5	0.58 (0.01)	0.78 (0.07)	0.57 (0.01)	0.76 (0.06)	0.58 (0.0)	0.79 (0.0)	0.58 (0.0)	0.78 (0.0)
Idiopathic infertile men[29]	26	Mg orotate; 197 mg/day	3	0.51 ± 0.05	0.81 ± 0.06	0.54 ± 0.02	0.81 ± 0.1	0.56 ± 0.04	0.88 ± 0.07	0.45 ± 0.03	0.84 ± 0.05
Healthy male volunteers[30]	30	Mg lactate; 48 mg/day	1	0.47 ± 0.06	0.84 ± 0.06	0.49 ± 0.05	0.87 ± 0.04	0.45 ± 0.06	0.86 ± 0.05	0.48 ± 0.07	0.87 ± 0.08

[a] units=mmol/L; [b] mean ± SD or mean (SE); [c] N randomized, all studies used 1:1 randomization; [d] Statistically significant ($p<0.05$) change from baseline

Relatively few observational studies have examined both iMg and tMg,[16,18] in part likely owing the iMg assay recommending immediate analysis of whole blood and iMg measurement, and also since it can be prone to interference by factors such as pH level and serum calcium. As such, little is known about a) the correlation of iMg and tMg in a relatively healthy population, b) whether iMg changes in response to Mg supplementation, or c) whether individuals with low iMg experience a greater change in iMg due to supplementation. Importantly, while Mg homeostasis is complex, it is possible that Mg levels can be intervened upon among those with hypomagnesemia.

E. Correlates of low circulating magnesium

Below we describe correlates of low serum Mg. To date, the majority of epidemiologic studies examined tMg. As such, except where indicated (e.g. iMg), herein we describe correlates of low serum tMg. For ease and convention, we refer to tMg as Mg in the remainder of this section.

1. *Age*

As described previously, serum Mg has not been measured in a nationally representative sample of the United States since 1971-1974.[20] In both men and women, serum Mg was highest in childhood with declines until adolescence or early adulthood. After early adulthood, average serum Mg tended to slightly increase thereafter.[20] In 2012-2013, serum Mg was measured in a nationally representative sample of 5,561 Canadians aged 3-79.[7] Within 11 sex-age categories, serum Mg followed a fairly normal distribution, though there were slight fluctuations in absolute levels across the lifespan. Notably, the prevalence of hypomagnesemia generally tended to be higher among older age categories for both sexes.[7] Several characteristics of the elderly and/or

aging process may explain the higher prevalence of hypomagnesemia in this population, including: chronic low dietary Mg intake, reduced intestinal Mg absorption, increased urinary Mg excretion, or low Mg may arise secondary to other comorbidities or medications (which are more common among the elderly).[31]

2. *Sex*

Differences in circulating Mg concentrations by sex across the lifespan are thought to be relatively small, based on a nationally representative Canadian sample in 2012-2013.[7] However, women tend to have slightly lower serum Mg concentrations and higher prevalence of hypomagnesemia.[20] In women, reproductive health is correlated with Mg status. In the NHANES 1971-74 sample, serum Mg was measured in pregnant women, women taking oral contraceptives, post-menopausal women and reproductive aged women not on contraceptives (referent group). Pregnant women tended to have lower serum tMg than those who were not pregnant or taking oral contraceptives (the control group) regardless of age and race. To a lesser extent, women taking oral contraceptives had lower Mg concentrations than women not taking oral contraceptives. Post-menopausal women had higher serum Mg levels by 0.08 mmol/L compared with pre-menopausal women.[32] Notably, Mg (in the form of intravenous $MgSO_4$) is advocated by the World Health Organization is advocated for preventing and treating convulsions in severe pre-eclampsia and eclampsia.[33]

3. *Race*

When serum Mg was measured in the 1971-74 NHANES survey cycle, concentrations were only measured in whites and blacks. Slight race differences were noted, whereby blacks tended to have lower serum Mg across all age groups and in both sexes.[20] In the 2012-2013 Canadian sample, racial diversity was limited to comparisons of white versus non-white. Similar

to white vs black comparisons in 1971-1974, non-white Canadians tended to have lower serum Mg concentrations as compared to whites.[7] As race-specific Mg concentrations have not been previously measured in a large sample in race groups other than whites and blacks, it is unclear how serum Mg (including iMg) concentrations compare across other race/ethnic groups.

4. *Diet and Supplements*

Low serum Mg can be reflective of inadequate Mg intake. Metabolic ward studies indicate Mg-deficient diets in the short term (e.g. 72-92 days) do not dramatically affect serum Mg. Mg supplementation in these Mg-depleted individuals resulted in increased serum Mg concentrations. Notably, low dietary Mg intake over months or years may contribute to slow declines in serum Mg.[34] However, Mg homeostasis is dynamic and depends on numerous conditions as described throughout this Chapter.

In epidemiologic studies, Mg intake and circulating Mg tend to be poorly correlated. In the ARIC study, for example, the correlation between tMg and reported Mg intake as estimated from the Willet 61 item food-frequency questionnaire (FFQ) was poor[35,36] (e.g. Pearson partial correlation coefficient r = 0.04 in white men, r = 0.06 black men, r = 0.01 white women, r = -0.02 black women).[35] A complicating factor to interpretation of epidemiologic studies is that individuals who consume Mg in the form of dietary supplements, which increase serum Mg concentrations, also tend to have higher intakes of minerals, such as Mg, from food sources.[11,12] Importantly, as described earlier in section I.D of this Chapter, circulating Mg concentrations appear responsive to supplements as reported in meta-analyses of RCTs of oral Mg supplementation effects of circulating Mg concentrations.[26]

5. *Diabetes*

Individuals with type 2 diabetes tend to have a higher prevalence of hypomagnesaemia, with an estimated prevalence of 14%-50% as compared to 3-15% among those without diabetes. Low serum tMg has been associated with higher fasting insulin, glucose[40,41] and HbA1c.[37] Among diabetic individuals, hypomagnesemia has been associated with diabetic complications.[38] Based on a nationally representative sample of Canadian adults, individuals with diabetes had lower serum Mg concentrations than those without diabetes by an estimated 0.04-0.07 mmol/L.[7] Some studies suggest that serum tMg is inversely associated with risk of diabetic complications, such as retinopathy and albuminuria.[37-39] A study with both tMg and iMg measurements reported low iMg concentrations even in the presence of normal tMg concentrations among elderly individuals with type 2 diabetes.[40]

Low circulating Mg has also been associated with a higher type 2 diabetes risk in 4 prospective observational studies. A meta-analysis of these observational studies [31,284 total participants; 2,680 type 2 diabetes events; mean 9 years follow-up, reported a pooled relative risk for type 2 diabetes of 0.64 (95% CI: 0.50, 0.81) for the highest versus the lowest category of circulating Mg.[41]

Furthermore, in meta-analyses of randomized controlled trials, oral Mg supplementation had beneficial effects as compared to placebo on insulin resistance[42,43] and fasting glucose[44] among those with type 2 diabetes as well as on insulin sensitivity parameters[45] and glucose concentrations[46] among those with high diabetes risk. In an experimental Mg-depletion study, during glucose tolerance tests, serum glucose peaked at a higher concentration (and remained higher throughout the tolerance test) when the test was conducted during Mg-depletion as compared to after Mg repletion. The rate of decrease in glucose concentration or insulin response during the glucose tolerance test was not affected.[47]

There are several proposed mechanisms which may account for these findings of a relationship of Mg with glucose and insulin. Individuals with diabetes tend to have increased urinary Mg excretion. This higher renal excretion may arise from glucose-induced osmotic diuresis.[38,48] In rats, Mg deficiency adversely influenced glucose homeostasis *in vivo* and in isolated islet cells *in vitro*.[49] Interestingly, dietary Mg restriction in female rats lead to greater adiposity and insulin resistance in their rat pups as compared to pups born to rats fed a control diet.[50] Other mechanisms include impaired signaling or secretion of insulin or altered glucose transport.[38]

6. *Alcohol*

Numerous studies have examined Mg homeostasis following ethanol intake and among those with chronic alcoholism.[51] Alcoholics are prone to developing hypomagnesemia, particularly when their Mg dietary intake is already deficient.[52] Chronic alcohol intake tends to increase Mg excretion via the kidneys.[9] Acute intake appears to influence Mg homeostasis in the short term within liver cells but to a lesser extent than in the context of chronic alcoholism. Mg content and transport in liver cells of male rats were restored following withdrawal from chronic ethanol exposure.[53] In experimental studies of male rats, acute intravenous ethanol infusion resulted in minimal effects on Mg transport in hepatocytes;[54] chronic alcohol impaired Mg transport mechanisms[54] and Mg concentrations within hepatocytes.[55] Two studies have measured both iMg and tMg in relation to alcohol. One study among chronic alcoholics found that the correlation between iMg and tMg was instrument dependent. When the NOVA CRT was used, iMg was significantly lower in alcoholics than controls. When the AVL 988-4 ion-selective electrode was used, iMg and tMg were not correlated.[56] Another study among emergency care patients with confirmed ethanol ingestion found significantly lower iMg than hospitalized controls, while concentrations of tMg did not significantly differ.[52]

7. *Genetics*

Hereditary Mg disorders are relatively rare, but when they exist they are generally more clinically important and are generally related to Mg transport disorders.[57] Some examples of familial hypomagnesemia conditions include familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) and Bartter's syndrome. Gene-linkage studies in families with hereditary hypomagnesemia played important roles in identifying Mg transport proteins (i.e. claudin 16, claudin 19) and advancing understanding of Mg homeostasis.[57]

In the general population, Mg homeostasis is estimated to have a heritability estimate of approximately 30%.[2,58] Single nucleotide polymorphisms (SNPs) associated with Mg concentrations have been identified in magnesium transporter genes, such as CNNM2.[59] A genome wide association study (GWAS) in those of European ancestry within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium identified and replicated six loci (*MUC1, TRPM6, SHROOM3, DCDC5, ATP2B1, PRMT7)* statistically significantly associated with serum tMg, with *MUC1* rs4072037 most strongly associated. Together these SNPs explained only 2% of variability in tMg.[59] Another GWAS among ARIC participants of African ancestry identified and replicated three loci (*MUC1, TRPM6, SHROOM3*) that had been previously associated with tMg in those of European ancestry. Three SNPs met genome-wide significance in ARIC and the replication cohort (MUC1 rs2974937, SHROOM3 rs9993810, TRPM6 rs113607577), with MUC1 rs2974937 most strongly associated with tMg. In this population, these SNPs explained 3% of variability in tMg concentrations.[60]

8. *Medications*

There are numerous medications which are thought to affect Mg status. Relevant to this dissertation, proton pump inhibitors (PPIs) have been linked with hypomagnesemia.[61] As

described further in Chapter 2, PPIs are a commonly used class of drugs used to treat gastroesophageal reflux disease (GERD) and other acid-related conditions.[62] PPIs can be purchased over-the-counter or as a prescribed medication and are commonly used in the U.S..[63] A series of case reports regarding long-term PPI use and hypomagnesemia prompted the U.S. Food and Drug Administration to publish a safety communication.[61] PPIs are thought to reduce intestinal Mg absorption. Diuretics are also known to affect Mg status through increased urinary Mg excretion. Moreover, it has been suggested that concomitant diuretic and long-term PPI use may increase the risk for hypomagnesemia.[64,65]

9. *Other*

Gastrointestinal issues such as vomiting and diarrhea are also common contributors to Mg deficiency. Low Mg may arise as a result of poor intestinal uptake of water, which is important for Mg reabsorption. Briefly, Mg homeostasis is intertwined in the metabolism of other minerals (e.g. calcium and potassium) and vitamin D. Briefly, Mg deficiency is related to low 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] and reduced parathyroid hormone (PTH) response. $1,25(OH)_2D$ increases intestinal Mg absorption, though to a lesser extent than for calcium.[8] That said, while relatively rare nowadays, when left unresolved, magnesium-related vitamin D resistant rickets can also occur.[66]

II. MAGNESIUM AND ARRHYTHMIAS

Mg plays an important role in cardiac electrophysiology,[3,4] and extreme concentrations of circulating Mg can lead to ECG changes.[67] Less is understood about the role, if any, that subclinical deficiencies in circulating Mg may have with atrial and ventricular arrhythmias. It warrants mention that ECG changes may not be specific to Mg, abnormal Mg homeostasis may coexist with (and/or exacerbate) other electrolyte abnormalities, namely abnormalities in

concentrations of calcium and potassium, which also themselves can result in ECG abnormalities as well.[68] This first half of this section includes a summary of epidemiologic literature on circulating Mg and supraventricular arrhythmias followed by potential pathophysiologic mechanisms; the second half covers the epidemiologic and pathophysiologic literature on Mg and ventricular arrhythmias. Except where otherwise indicated, Mg refers to circulating total Mg concentrations. Associations between dietary intake of Mg and arrhythmias will not be discussed, given the low correlation between dietary and serum Mg (e.g. r ~ 0.03 in the ARIC study).

A. Magnesium and atrial arrhythmias

1. *Epidemiology of magnesium and atrial arrhythmias*

The majority of the epidemiologic literature on circulating Mg and supraventricular arrhythmias relates to atrial fibrillation (AF), likely in part because 1) AF is the most common sustained clinical arrhythmia[69] and 2) AF is a common complication following cardiac surgery. Estimates of how common AF is after cardiac surgery vary widely, ranging from 10-65% of post-operative patients.[70] Mg supplementation is often administered as a prophylaxis to prevent AF events after cardiac surgeries. A Cochrane systematic review and meta-analysis of RCTs assessing high-dose intravenous Mg supplementation for post-operative AF prevention reported a pooled $OR_{\text{Mg vs placebo}} = 0.55$ (95% CI 0.41, 0.73);[71] however, its efficacy remains controversial.[72,73]

Inverse associations between serum Mg and incident AF has also been suggested in three prospective observational studies.[74-76] In the ARIC study, after multivariable adjustment, participants in the lowest serum Mg quintile had an HR for incident AF of 1.34 (95% CI: 1.16-1.54) as compared to those in the middle quintile.[74] Comparable effect estimates were reported in the Framingham Offspring study, whereby those in the lowest (versus highest) serum Mg quartile

had a HR_{AF}=1.52 (95% CI: 1.00-2.31)].[75] In an Israeli health maintenance organization, mild and moderate hypomagnesemia were both associated with higher AF risk over a follow-up of up to 25 months. Neither mild nor moderate hypomagnesemia were associated with AF risk in the short term (e.g. 90 days).[76]

Less has been published on circulating Mg and other supraventricular arrhythmias (e.g. premature atrial contractions (PACs) and supraventricular tachycardia (SVT)). Higher burden of these arrhythmias may be intermediate phenotypes of AF.[77] While a PAC or SVT beat are common, there is appreciation that a higher burden of these arrhythmias can be associated with increased risk of clinically important outcomes (such as AF and stroke). A small RCT (Manuscript 1) found that oral Mg supplementation increased serum Mg relative to placebo, but no effect was found on PAC burden (though statistical power to detect an association was low).[78] Another small RCT with oral Mg supplements reported decreased intensity of PACs in individuals without known ischemic or structural cardiac diseases.[79] Additionally, as described in Chapter 1 section II.B, decreases in premature ventricular contraction (PVC) intensity were also reported in that study. In an experimental study among patients with paroxysmal SVT, intravenous $MgSO_4$ administration lead to termination or slowing of SVT (specifically when the atrioventricular node was in the reentrant circuit).[80] Specifically, intravenous Mg atrioventricular node conduction was prolonged starting with 5 mmol (vs 0 mmol) intravenous Mg; effects were not further prolonged at higher doses (10 or 20 mmol).[81]

A non-randomized experimental feeding study lends support to these epidemiologic findings. Of 14 healthy women who were fed an extremely low diet in Mg, 3 of the women developed AF. Their AF resolved quickly after Mg repletion.[47] Whether intervening upon low Mg status (particularly subclinical Mg deficiency) is an effective primary prevention strategy for AF in the general population remains an ongoing area of research.

2. *Pathophysiology of magnesium and atrial arrhythmias*

The pathophysiology linking circulating Mg and supraventricular arrhythmias in the general population or following cardiac surgery is not well understood and may arise through many potential pathways. Plausibly, the association may act through traditional risk factors related to both low circulating Mg and supraventricular arrhythmias (namely hypertension or inflammation[17]), which predispose individuals to arrhythmogenesis.

Beyond traditional risk factors, Mg is involved in hundreds of enzymatic reactions throughout the body and is important in cardiac electrophysiology.[82] Ionic flow of electrolytes, including Mg as well as calcium and potassium, are important for generating action potentials and maintaining the membrane potential of cardiac cells.[83] During phase 4 of the action potential, potassium flows into the cell at rate that can depend on Mg availability.[67]

Mg may affect the cardiac substrate and promote increased atrial myocardial sensitivity, such as cardiac automaticity, sinus node recovery time and atrioventricular nodal conduction.[84] Additionally, Mg competes with calcium for membrane–binding sites to the L-type Ca^{2+} current.[84,85] As a natural calcium antagonist, Mg blocks flow of calcium into the cell, which plausibly prolongs atrioventricular conductance and reduces the rate of sinus node firings.

Experimental and animal studies provide insights into potential pathways connecting low circulating Mg to supraventricular arrhythmia occurrence and burden. In an experimental study conducted among individuals without known cardiac disease, intravenous Mg administration prolonged sinoatrial node conduction time and atrioventricular conduction time.[86] Intravenous Mg may also increase atrial (and ventricular) refractoriness and sinus node function.[87] In animal studies, severe Mg deficiency lead to changes in cardiac automaticity and conduction velocity.[21]

Additionally, specific to post-operative AF, intracellular Mg, which is moderately correlated (r = 0.46) with serum tMg,[88] is commonly depleted after cardiac surgery.[89] Moreover, hypomagnesemia inhibits nitric oxide release from coronary endothelium after cardiac operations.[90]

B. Magnesium and ventricular arrhythmias

1. *Epidemiology of magnesium and ventricular arrhythmias*

Intravenous Mg has widely used in managing torsade de pointes (also known as polymorphic ventricular tachycardia), a type of ventricular arrhythmia, in the setting of long QT-interval syndrome. A few small studies in the 1980s suggested a benefit to Mg administration; however, no large-scale RCT has been conducted to test for its efficacy.[1]

In the context of the general population, low serum Mg has been cross-sectionally associated with a higher prevalence of PVCs in two studies conducted in community-based settings.[91,92] Among 750 obese Canadians with type 2 diabetes, participants with low serum Mg (defined as ≤0.70 mmol/L) had a 2.5-fold higher prevalence of premature ventricular contraction (PVC) on a Holter monitor compared to those with serum Mg above 0.70 mmol/L (50% vs 21%).[91] In the Framingham Offspring Study, lower serum Mg concentrations (per 1 SD 0.08 mmol/L decrement) were associated with lower odds of having ≥1 PVC during 1 hour ECG monitoring [adjusted OR=1.18 (1.02, 1.37)].[92] As described in Chapter 1 section II.A, a small RCT with oral Mg supplements reported decreased intensity of PVCs in individuals without known ischemic or structural cardiac diseases.[79] Little is known about the association of serum Mg with the prevalence or burden of non-sustained ventricular tachycardia in the general population.

Among cardiac patients, more studies have been conducted evaluating Mg and ventricular arrhythmias. Typically, these studies have evaluated ventricular rate control in AF patients or in populations with other heart conditions (e.g. congestive heart failure (HF), myocardial infarction (MI)). Intravenous Mg has had conflicting results in reducing the frequency of ventricular arrhythmias after an acute MI. In a small RCT among 140 patients undergoing coronary artery bypass graft (CABG) surgery, randomization to intravenous Mg (70 mmol $MgSO_4$) was associated with fewer PVCs (as measured over 48 hours of Holter monitoring).[93] In individuals with HF, intravenous $MgSO_4$ had beneficial effects on the number of isolated and couplet PVCs and on the number of NSVT episodes compared to placebo infusions.[94]

Serum Mg has also been considered in relation to digitalis toxicity that arises due to AF treatment with digoxin—an anti-arrhythmic medication.[67] Among digoxin-treated AF patients with low serum Mg concentrations, oral Mg supplementation was associated with a reduction in PVC prevalence.[95] A small randomized uncontrolled trial among patients undergoing a CABG procedure found that intraoperative correction of plasma iMg (using $MgSO_4$ infusion) was associated with lower occurrence of ventricular tachyarrhythmia and longer continuous sinus rhythm in the day following the procedure.[96]

2. *Pathophysiology of magnesium and ventricular arrhythmias*

Similar to the potential pathophysiology of Mg and supraventricular arrhythmias, the mechanisms connecting Mg and ventricular arrhythmias are not fully understood. There is some overlap in the potential pathophysiologic mechanisms of circulating Mg to both supraventricular and ventricular arrhythmias. Herein, we briefly reiterate overlapping mechanisms (addressed in Chapter 1 section II.B) and then describe the potential mechanisms specific to ventricular arrhythmias.

As described earlier, Mg is important in energy utilization and its involvement in balancing potassium influx to cardiac cells. As electrolytes play important roles in sinus rhythm, disruptions in electrolyte balance can plausibly stimulate pro-arrhythmic or anti-arrhythmic effects.[85] Mg is also a calcium antagonist and these electrolytes compete for binding sites on contractile proteins. Inadequate stores of Mg may in turn plausibly influence electrical activity and contractility of the myocardium.[1]

Specific to the ventricular conduction path, intravenous Mg may prolong His-ventricular conduction and suppress ventricular ectopic activity.[84] Mg helps regulate potassium (K) transport through channels into the cell and serves as a cofactor of Na/K ATPase, which transports potassium (K) into the cell during the action potential. When cellular Mg is deficient, this makes the Na/K ATPase system less efficient. This results in a membrane potential that is less negative;[67] [82] this can result in QT interval prolongation, which may promote the development of ventricular arrhythmias.[82] However, experimental and *in vivo* studies involving digitalis toxicity, Mg administration has yielded conflicting results on shortening the QT interval.[97] In sum, much remains to be understood regarding the role of Mg in the development of ECG abnormalities.

III. MAGNESIUM AND CARDIOVASCULAR DISEASES

For the purposes of this section, we will focus on epidemiologic findings specific to circulating (plasma or serum) Mg and CVD. Associations between dietary intake of Mg and CVD will not be discussed, given the low correlation between dietary and serum Mg (e.g. r ~ 0.03) in the ARIC study. This section is followed by a summary of potential pathophysiologic pathways through which circulating Mg may influence cardiovascular function.

A. Epidemiology of magnesium and cardiovascular diseases

The strongest evidence that serum Mg may be causally related to CVD risk comes from a Mendelian Randomization study published in 2018, which evaluated Mg-related SNPs in relation to CHD risk. Each 0.1 mmol/L (approximately 1 SD) higher increment of genetically predicted serum Mg concentrations was associated with a lower odds of coronary artery disease [OR=0.88 (0.78, 0.99)].[98]

Circulating Mg has been described in relation to CVD risk in 3 meta-analyses of prospective cohort studies.[41,99,100] A 2013 meta-analysis by Del Gobbo et al found that each 0.2 mmol/L higher increment in circulating Mg was associated with a 30% lower risk of total CVD (including stroke) (95% CI: 0.56, 0.88), and a non-statistically significant lower risk of ischemic heart disease [RR=0.83 (0.65, 1.05)] and fatal ischemic heart disease [0.61 (0.37, 1.00)].[99] Another 2013 meta-analysis by Qu et al found that each 0.05 mmol/L higher increment in circulating Mg was associated with a 9% lower risk of total CVD (0.85, 0.97).[100] A 2017 meta-analysis examined circulating Mg in relation to CHD risk, specifically. Wu et al found that highest category in circulating Mg was associated with a marginally statistically significant lower CHD risk as compared to the lowest category [RR=0.86 (0.74, 1.00)]. When circulating Mg was modeled linearly per 0.1 mmol/L, the Mg-CHD association was not statistically significant [RR=0.89 (0.77, 1.03)].[41]

To our knowledge, four additional prospective or nested case-control studies have since been published and/or were not included in the previous meta-analyses. Low serum Mg has been associated with higher risk of fatal CHD [$HR_{\text{per 1SD increment}} = 0.82$ (0.70-0.96)][101] and CVD mortality [$HR_{Mg \leq 0.73 mmol/L \text{ vs } >0.73 mmol/L} = 2.30$ (1.43-3.71)].[102] Nested case-control studies within the Nurses' Health Study reported that low plasma Mg (<0.82 mmol/L) was associated with a higher risk of ischemic stroke [RR=1.57 (1.03-2.65)][103] but was not associated with CHD after adjustment for traditional cardiovascular risk factors.[104]

Additionally, HF and AF were not included as an outcome in the prior meta-analyses on circulating Mg and CVD. In the ARIC study, those in the lowest serum Mg quintile (≤0.7 mmol/L) were associated with a HR of 1.71 (95% CI: 1.46, 1.99) for incident HF as compared to those in the highest quintile (≥0.9 mmol/L).[105] Among the older male participants of the British Regional Heart Study, low serum Mg was associated with higher risk of HF [HR_{Q5vsQ1} = 0.56 (0.36, 0.86)].[106] Low serum Mg has also been predictive of increases in left ventricular mass over 5 years independently of clinical CVD risk factors in the Study of Health in Pomerania, which included German adults aged 45 years and older.[107] Moreover, as described in Chapter 1 section II.A, low serum Mg has been associated with a higher risk of AF in 3 prospective observational studies.[74-76]

Cross-sectionally, low serum Mg has been associated with markers of subclinical CVD. In the ARIC study, low serum Mg was associated with greater carotid wall thickness among women (but not men).[35] Low serum Mg has also been associated with higher coronary artery calcification scores among Koreans with low CVD risk[108] and among Mexican individuals with diabetes (Genetics of Atherosclerosis Disease Study).[109] Additionally, low serum Mg was associated with carotid intima media thickness and mitral valve calcification in diabetic patients with mild to moderate chronic kidney disease.[110]

Mg therapy has been examined as a secondary prevention strategy among patients with acute MI in two large trials—the Fourth International Study of Infarct Survival (ISIS-4)[111] and Magnesium in Coronaries (MAGIC)[112]—but no survival benefit was found in either trial. The ISIS-4 trial, which included patients with suspected acute MI, had a 2x2x2 factorial design with one arm including randomization of 58,050 patients to an intravenous bolus of 8 mmol $MgSO_4$ administration followed by 72g $MgSO_4$ (or matching placebo) over 24 hours (oral captopril and mononitrate were the other 2 arms). In the MAGIC trial of patients with acute ST-elevation MI,

6,213 patients were randomized to a 2g intravenous $MgSO_4$ bolus followed by 17g over 24 hours or to a matching placebo treatment. Two meta-analyses of RCTs reported a dose dependent relationship of oral Mg supplementation on blood pressure reduction.[113,114] To our knowledge, no RCT has examined Mg supplementation in relation to overall CVD risk.

B. Pathophysiology of magnesium and cardiovascular diseases

There are numerous potential mechanisms connecting low circulating Mg and increased CVD risk[1,17,115,116] as depicted in **Figure 1.2**.[115]

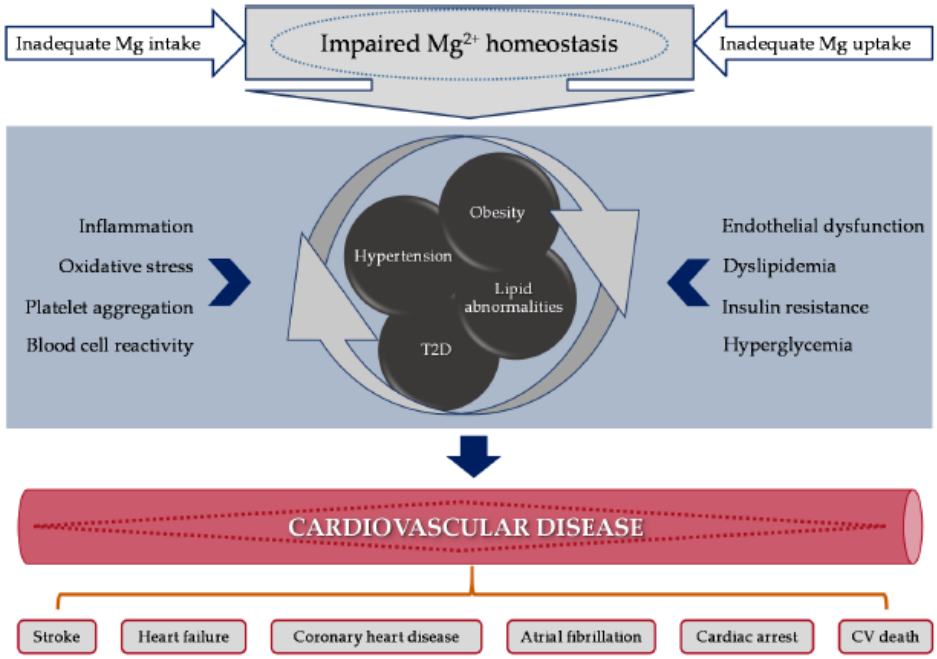


Figure 1.2. Potential mechanisms linking Mg to CVD, Rosique-Esteban, 2018.

This may occur in part through established CVD risk factors such as hypertension, diabetes or inflammation.[17] A recent meta-analysis of RCTs found that Mg supplementation at a dose of 300 mg/d was significantly associated with increased serum Mg and decreased blood

pressure.[117] Oral Mg supplementation was found to raise high-density lipoprotein cholesterol among type 2 diabetics.[44] In meta-analyses of RCTs, oral Mg supplementation had beneficial effects on fasting glucose among those with type 2 diabetes[44] and those with or at high risk of type 2 diabetes[46] (as described in Chapter 1 section I.E.5).

Relatedly, Mg also plays roles in platelet formation,[1,118] vascular smooth muscle tone, endothelial function,[1,116] and in maintaining normal sinus rhythm.[1] As described in Chapter 1 section II, Mg acts as a natural calcium antagonist and helps regulate ion channel transport (namely, calcium and potassium influx) in cardiac cells.[119] This in turn could influence electrophysiologic activity and predispose to arrhythmogenesis.[1]

Findings from experimental studies lend support to the hypothesis that Mg inadequacy may induce atherosclerosis and other adverse cardiovascular effects. As described previously in Chapter 1 section II, experimental nutrition studies in metabolic wards indicate that extreme dietary Mg depletion led to glucose intolerance, heart rhythm changes (e.g. AF) and decreases in serum cholesterol, which resolved upon Mg repletion.[47] An experimental study reported that low dietary Mg induced PACs,[25] which have been associated AF.[120-127] In mice, a long-term diet that is moderately deficient in Mg worsened cardiovascular risk factors and was related to increased mortality,[128] as well as oxidative stress.[129,130]

Hypomagnesemia inhibits oxidative DNA damage in cardiac tissue.[131] Moreover, Mg concentration in cardiac muscle of those who died from heart disease were lower than those who died from an acute trauma.[132] Mg concentrations within the affected myocardial tissue was 50% lower, while in the non-infarcted myocardial concentrations were 20% lower than controls.[132] Significant drops in serum Mg concentrations have been reported immediately after an infarction, with a return to normal levels within 12 days after the infarction.[133]

CHAPTER 2 – OVERVIEW OF PROTON PUMP INHIBITOR USE AND HEALTH

I. INTRODUCTION TO PROTON PUMP INHIBITORS

Proton pump inhibitors (PPIs) are medications used to treat gastroesophageal reflux disease (GERD) and other acid-related disorders, which are highly common and can substantially affect an individuals' quality of life. The American College of Gastroenterologists defines GERD, one of the most common gastroesophageal conditions, as "symptoms or complications resulting from the reflux of gastric contents into the esophagus or beyond, into the oral cavity (including larynx) or lung".[134] PPIs are generally considered superior in efficacy of treating GERD as compared to histamine 2 receptor antagonists (H_2-blockers).[134]

The first PPI commercially available in the U.S. was omeprazole (name brand = Prilosec), which was introduced in 1989. Since then, as of 2015, several other drugs of this class have been introduced: esomeprazole (Nexium), lansoprazole (Prevacid), dexlansoprazole (Dexilent), pantoprazole (Protonix) and rabeprazole (Aciphex). PPIs may be prescribed and some varieties are also available over-the-counter.[62]

Chemical structures of the medications within this class vary slightly but have relatively similar pharmacologic profiles.[62] The half-life of these products are generally short (ranging from 0.5 to 2 hours), but can have longer lasting effects with a time to peak plasma level of up to 5 hours (depending on dose). The primary route of metabolism is via the liver.[62] PPIs act (as the name implies) by inhibiting acid secretion of the proton pump (H+/K+ ATPase pump) of the parietal cells in the stomach. PPIs are packaged (e.g. coated tablet or granule, gelatin capsule) to safeguard the inactivated medication as it passes through the stomach until activation in the small intestine. PPIs are generally consumed orally but intravenous formulations are available for

hospitalized patients in particular.[62]

Since their introduction to the U.S. marketplace starting in the late 1980s, PPIs have become one of the most widely used medications among American adults.[62] In 2009, an estimated 9% of outpatient visits involved patients who use PPIs.[63] PPI use has become increasingly common across the entire lifespan, ranging from as early as infancy to the elderly. The prevalence of PPI use among infants even rose 4-fold over the time period of 1999-2014.[135] A claim-based analysis of Belgian pediatricians prescribing habits indicated that PPI prescriptions for children increased between 1997 and 2009 (as did prescriptions of H_2-blockers, another drug class used for acid-related disorders) but the prevalence of GERD did not increase in this age group during this timeframe.[136] In the 2004 U.S.-based National Nursing Home Survey, 27% of residents were using one or more PPI, of which an estimated 49% were not evidence-based in their usage of PPIs.[137] Additionally, in unpublished data from ARIC visit 5, 25% of participants reported use of PPIs in the prior 2 weeks.

Acid suppressant medications can have important benefits, specifically when used as prescribed and when considered necessary. PPIs are generally regarded as having superior efficacy to H_2-blockers in the treatment and symptom management of GERD and other acid-related disorders.[138] In the majority of cases, the standard treatment with PPIs can help abate symptoms or treat the condition. Common side effects of PPIs include headache, diarrhea, abdominal pain and nausea.[139] However, over the last decade, PPIs have also been associated with serious clinical outcomes in case reports and other observational settings (e.g. bone fracture, community-acquired pneumonia, clostridium difficile infection, chronic kidney disease). Additionally, chronic high-dose use of PPIs can affect intestinal absorption of certain nutrients[140] (e.g. Mg, calcium, vitamin B_{12}) as further described in section II of this Chapter. The elderly[141,142] and men[142] are thought to have a higher risk of hypomagnesemia due to PPI use than their

counterparts. Additionally, drug interactions have been noted, whereby individuals who take diuretics may have a higher risk of hypomagnesemia compared to those only taking PPIs.[143] This is particularly concerning as the elderly have a particularly high prevalence of PPI use as well as concomitant polypharmacy. That said, risks and benefits need to be weighed, particularly when considering high-dose and/or chronic use of PPIs.[144]

Among those already on PPIs, the risks versus benefits of reducing dose or stopping treatment (also known as deprescribing) PPI use is an ongoing area of research. Oftentimes, acid-related symptoms later recur and use may be on-demand as symptoms recur or continuous to prevent symptoms (or minimize severity). A 2017 Cochrane review suggested that in those with mild GERD, deprescribing 'on-demand' PPI users led to more GI symptoms as compared to continuous PPI users.[145] In light of the important potential benefits and suggestions of adverse effects, the choice to treat and/or deprescribe can be a personal one (e.g. willingness to tolerate minor symptoms, fear of symptom recurrence, severity of symptoms, concern about adverse effects of PPI use) and is an important topic for patients to communicate with a health care provider.[146]

A. Correlates of PPI use

PPIs are used to treat acid-related disorders, namely for GERD, peptic ulcer disease, and dyspepsia, which affect the upper gastroesophageal region.[147] PPIs may also be used as a component of *Helicobacter pylori* eradication treatment or for the prevention or treatment of gastric injury related to nonsteroidal anti-inflammatory drug use.[142] PPIs are generally approved for short-term use (e.g. 4-8 weeks), though some may have an ongoing indication for longer term treatment (e.g. prevention of upper GI bleeding in high-risk patients, Barrett's Esophagus). Yet, PPIs are often used without a specific ongoing indication, particularly as some PPIs are available

over-the-counter directly to patients.[148] Plausible correlates of PPI use may relate to insurance and access to care (in relation to prescription PPI use in particular) or socioeconomic status (such as ability to pay for medications).

Risk factors and correlates of conditions also serve as indication for medication. As GERD is one of the more common acid-related disorders, affecting an estimated 10-20% of individuals in the U.S. and Western Europe[149], we focus on risk factors for GERD. Obesity is considered a strong modifiable risk factor for GERD.[150,151] Possible pathways for the obesity-GERD association are multiple and may relate to higher intra-abdominal pressure, more frequent sphincter relaxation or impaired gastric emptying.[152] Other risk factors for GERD or exacerbating GERD symptoms relate to dietary habits (e.g. caffeine, spicy foods, alcohol), tobacco smoking, post-prandial body position or pregnancy.[150,151] It appears GERD and related complications may be more common among men than in women; however, this has not been found consistently.[151,153]

II. PROTON PUMP INHIBITORS AND LOW CIRCULATING MAGNESIUM

A summary of the epidemiologic literature on PPI use and serum magnesium is described in Chapter 1 section II.A, which is followed by a discussion of the pathophysiology of PPI-induced hypomagnesemia (in Chapter 1 section II.B). PPIs potentially also induce other nutritional deficiencies, including vitamin B_{12}, iron and calcium.[140] However, as the focus of this dissertation is on Mg, we focus our discussion on Mg.

A. Epidemiology of PPI use and low circulating magnesium

PPIs are commonly used and can have important benefits for those with acid-related diseases; however, this class of medication has been linked with several adverse health outcomes since

their introduction. Relevant to this dissertation, a series of case reports and studies regarding hypomagnesemia among PPI users were published[143,154-165] prompting the U.S. Food and Drug Administration (FDA) to release a safety communication regarding potential for PPIs to induce hypomagnesemia when taken for a year or longer.[61] It is thought that this may be a class effect (rather than for individual medications) as all 6 commercially available medications have had a case report published linking them with hypomagnesemia.[142]

After the series of case reports, other observational studies (9 in total; 3 cohort, 1 case-control, 5 cross-sectional) have been conducted on the PPI-hypomagnesemia association in population-based settings, which individually yielded inconclusive findings (some null, some positive). In a meta-analysis of these 9 observational studies, PPI use was associated with a pooled RR for hypomagnesemia of 1.43 (95% CI: 1.08, 1.88) compared to those who do not use PPIs.[165]

Additionally, a cross-sectional study was conducted among 48 hospitalized Italian patients with torsades de pointes. Torsades de pointes patients taking PPIs had lower serum Mg concentrations versus those not taking PPIs. In contrast, other electrolytes measured in serum (calcium, potassium, sodium) did not differ statistically by PPI user status among this patient group.[166]

Since that meta-analysis, another cross-sectional study on PPI use and hypomagnesemia was published in the population-based Rotterdam Study, whereby serum Mg concentrations were slightly lower in PPI users versus non-users by 0.01 mmol/L (95% CI: 0.16, 0.01 mmol/L). PPI use was associated with hypomagnesemia (≤0.35 mmol/L) after prolonged use [OR=2.99 (1.73, 5.15)] (range 182-2,618 days). The association of PPI use with hypomagnesemia was stronger among diuretic users than in non-diuretic users. H_2-blockers — a medication with similar

indications as for PPIs but with no known link to hypomagnesemia— were associated with lower serum Mg concentrations than non-users and higher odds of hypomagnesemia as well.[159] Considering H_2-blockers have not been linked with hypomagnesemia, this may be reflective of confounding and other biases inherent to observational examinations of medication effects.

A population-based case-control was done using Ontario-based health care databases to examine the association of PPI use with hospitalization with hypomagnesemia. Among those hospitalized for hypomagnesemia, the odds of being a PPI user was OR=1.43 (1.06, 1.93) compared to those not hospitalized for hypomagnesemia. Effect measure modification was present by diuretic use, whereby the association was present in diuretic users [OR=1.73 (1.11, 2.70)] but not among those who do not use diuretics [OR=1.25 (0.81-1.91)]. Moreover, H_2-blockers were not associated with hospitalization with hypomagnesemia.[167]

Interestingly, a case-control study among 133 chronic PPI users (those with hypomagnesemia were cases and those without were controls) was conducted to see how common SNPs in the candidate gene *TRPM6* relate to hypomagnesemia (SNPs included were rs2274925, rs2274924, rs3750425, rs45616231). Among PPI users, those with the TGAC haplotype had a nearly 6-fold (2.00-17.02) higher odds of hypomagnesemia compared to those with the wild-type haplotype (TAGC).[168]

Last, an important concern is whether Mg status is restored upon de-prescribing PPI use. In many cases, it is thought that after stopping PPI use following prolonged use, hypomagnesemia tends to resolve.[169] Moreover, hypocalcemia and/or hypokalemia may also arise secondary to hypomagnesemia. These electrolyte abnormalities were refractory to supplementation until after the circulating Mg was intervened upon.[142] It is also unclear how or if PPIs are related to subclinical Mg deficiency and public health implications, if this is the case.

B. Pathophysiology of PPI use and low circulating magnesium

Over the last decade, there has been a growing body of research related to mechanisms of PPI-induced hypomagnesemia, which is thought to be attributed to reduced intestinal absorption of dietary Mg.[170,171] Much of the mechanistic research to date has focused on the active Mg transport channel, TRPM6. There is some evidence to suggest that PPIs may also interfere slightly with passive absorption.[172,173] PPIs are not thought to majorly influence urinary Mg excretion or reabsorption in the kidneys.

PPIs affect the H+/K+ ATPase enzyme. This enzyme helps pump a hydrogen ion into the stomach in exchange for a potassium ion, which lowers the acidity of the gastrointestinal region. As TRPM6 is a pH-dependent channel, PPI use plausibly affects affinity of TRPM6 for Mg. In less acidic environments, TRPM6 activity decreases. That said, since PPIs act by decreasing intestinal pH, a lower pH would plausibly downregulate TRPM6. As *TRPM6* should over-express during times of Mg insufficiency, certain genetic profiles may help promote continued Mg absorption in the presence of PPI use. Yet not all PPI users develop hypomagnesemia and risk factors for developing PPI-induced hypomagnesemia are an ongoing area of research. Whether genetic or epigenetic factors play a role in why only some PPI users develop hypomagnesemia is largely unexplored, but there are suggestions of associations between common *TRPM6* gene SNPs with hypomagnesemia among PPI users.[168]

As PPI-induced hypomagnesemia is a relatively new phenomenon, there is much to be understood regarding mechanisms for this relatively rare but potentially serious side effect. PPI-induced hypomagnesemia is generally thought to arise as a result of long-term or chronic PPI use. Yet, a modeling study of PPI use suggests that even short-term PPI use can slightly diminish intestinal Mg absorption rates.[174]

III. PROTON PUMP INHIBITORS AND CARDIOVASCULAR DISEASES

Considering the pervasiveness of PPI use and their implication with adverse outcomes, it is important to understand how or if their use may affect cardiovascular health. Herein, we first summarize the epidemiologic literature related to PPI use and CVD in section A, which is followed by section B with a description of possible pathophysiologic mechanisms accounting for the association.

A. Epidemiology of PPI use and cardiovascular diseases

PPIs have been controversially associated with increased risk of CVD outcomes. Initial discussion over whether PPI affect cardiovascular health arose primarily due to concerns over potential interactions with the antiplatelet drug, clopidogrel, in RCTs. PPIs were proposed to interfere with clopidogrel bioactivation by CYP2C19, as the medications compete for this same enzyme. However, the association is not thought to be causal as PPI users have been found to have a higher risk of CVD irrespective of clopidogrel use.[175]

After initial concern over PPI-clopidogrel drug-drug interactions, several prospective studies examined PPI in relation to risk of CVD outcomes. In a meta-analysis of RCTs where incident CVD endpoints were reported, PPI users had a pooled RR for incident CVD of 1.70 (1.13, 2.56) as compared to non-users. In subgroup analyses, the risk of CVD was higher among long-term PPI users [RR=2.33 (1.33, 4.08)].[176]

In observational settings, PPI users tend to have a modestly higher stroke risk as compared non-users.[177-179] PPI use was associated with a modestly higher risk of MI, by 16% (95% CI: 9-24%) versus non-users, but H_2-blocker use was not associated with MI risk.[180] In the

Taiwan National Health Insurance Research Database, PPI users were matched using propensity scores to non-users, and PPI users had a 1.58-fold higher risk of MI (1.11, 2.25) than non-users.[181] Another study among privately insured adults found no association between prescribed PPI use with risk of MI,[182] while an analysis within a population-based database reported that PPI use was associated with higher odds of both MI (OR=1.8 (1.7-1.9)) and HF (1.8 (1.7-1.9)). However, medications with no known cardiac toxicity (H_2-blockers and benzodiazepines) were both associated with higher odds of these adverse cardiac events.[179]

PPI use has also been examined in relation to incident CVD outcomes in populations with other existing cardiac diseases, such as coronary artery disease and HF. Among 706 patients with coronary artery disease, PPI users (versus non-users) had a 5.71-fold (95% CI: 1.63-20.04) higher risk of a composite HF or mortality outcome but was not associated with acute ischemic events (acute coronary syndrome, stroke, or transient ischemic attack). In sensitivity analyses, similar results were found when users and non-users were matched using propensity scores.[183] However, power was low and variance estimates were imprecise. In HF patients, PPI use was actually associated with a lower risk of CVD mortality compared to those in the H_2-blockers and non-acid suppressive therapy group.[184]

Among critically ill patients (n>8000), PPI use has been cross-sectionally associated with a marginally higher prevalence of any arrhythmias (as measured on a 12-lead ECG upon admission to the hospital) in crude models but after multivariable adjustment was no longer associated with arrhythmias. In subgroups, PPI use was not associated with arrhythmias that were atrial or ventricular in origin. Moreover, the null association did not differ by diuretic user status.[185]

The relationship between PPI use and CVD risk has also been explored in populations

with GERD, the primary indication for PPI use. Among a population with diagnosed GERD, PPI use was associated with a higher risk of AF[186] and CHD[176] as compared to non PPI users. Little is known about the association of PPI use with AF. The condition GERD has also been examined in relation to incident CVD, and it has been associated with a higher risk of AF[187] and CHD.[188]

As described in the next section, hypomagnesemia is one of the proposed mechanisms linking PPI use and CVD. One cross-sectional analysis examined PPIs in relation to serum Mg and prevalent arrhythmias among 421 intensive care or critical care unit patients with a MI or unstable angina diagnosis. Patients administered PPIs soon after hospital admission tended to have lower serum Mg concentrations and a greater prevalence of cardiac arrhythmias compared to those not exposed to PPIs.[189] Notably, this study is in a selected sample of critically ill patients, was a cross-sectional design and data on diuretic use (which may increase risk of hypomagnesemia among PPI users) was not collected. Whether serum Mg mediates the PPI and CVD association has not yet been tested in an unselected population.

B. Pathophysiology of PPI use and cardiovascular diseases

There are several proposed mechanisms through which PPI use may increase risk of CVD, as shown in **Figure 2.1**.[175]

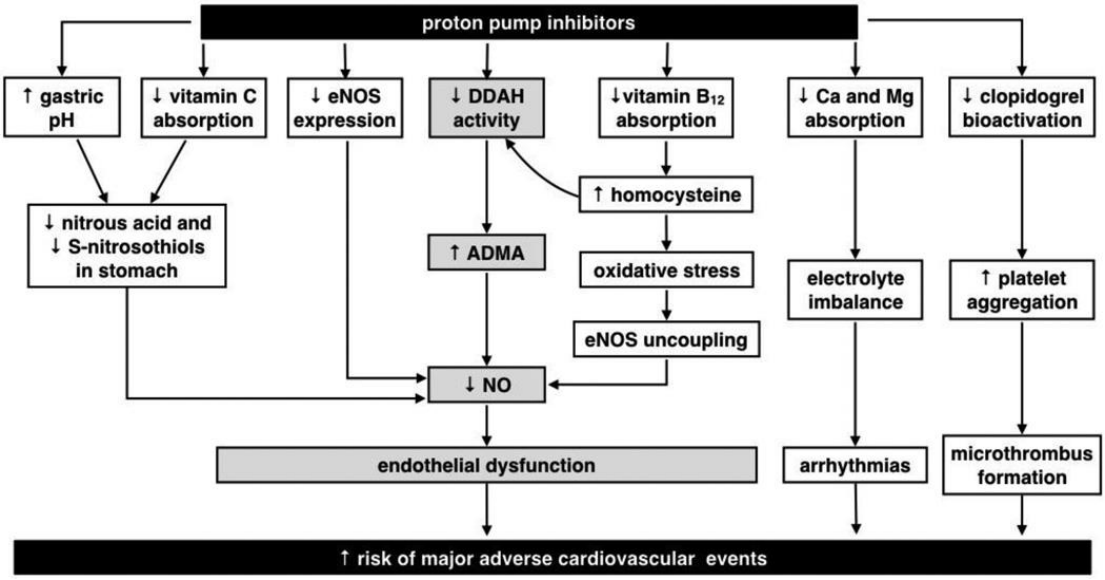


Figure 2.1. Potential mechanisms linking PPIs to CVD, Sukhovershin, 2016.

As described throughout, low circulating Mg has been studied in relation to higher risk of arrhythmias as well as with other CVD outcomes. Moreover, as PPIs can induce lower concentrations of circulating Mg, it is plausible that Mg may mediate (at least in part) the epidemiologic association between PPIs and CVD. Specific to AF and other arrhythmias, Mg is a cofactor of the sodium-potassium ATP pump, which is important for myocardial excitability.[190] Abnormal Mg homeostasis (in this case via PPI-induced hypomagnesemia) could adversely influence electrophysiology.[191]

Basic science studies with *ex vivo* human tissue lend support to epidemiologic findings. Other plausible mechanisms relate to greater endothelial dysfunction, such as accelerated endothelial senescence, telomere erosion[192] and inhibition of a cardiac enzyme, dimethylaminohydrolase (DDAH), which is involved in reducing oxidative stress.[193] Nitric oxide (NO) synthase has vasodilative effects. Reduced NO synthase activity can lead to oxidative stress and other adverse vascular effects. [175] Plasma asymmetrical dimethylarginine (ADMA) is

considered an inhibitor of NO. Elevated ADMA concentrations have been associated with higher CVD risk. In mice, PPI administration led to an increase in ADMA.[193]

Aside from PPIs affecting Mg absorption, PPIs are also thought to decrease absorption of other nutrients, such as calcium and vitamin B_{12}, which may themselves relate to an increased risk of CVD. PPIs have also been associated with acute kidney injury and chronic kidney disease, which, if PPIs truly affect renal health, may also explain epidemiologic associations between PPI and cardiovascular health. Additionally, presence of GERD or greater severity of GERD has been associated with an increased risk of AF, plausibly through increased inflammation or vagal nerve stimulation.[186,194] Last, as H_2-blockers which also suppress acid secretion through different mechanisms and have no known cardiac toxicity, it seems unlikely that the act of acid suppression itself exerts a direct role on cardiac health.

CHAPTER 3 – STUDY DESIGNS AND DATA COLLECTION

I. OVERVIEW

The objectives of this dissertation were evaluated using data from 2 sources. Manuscript #1, which examines interrelations between iMg and tMg, overall and in response to supplementation, used data from a pilot randomized controlled trial entitled 'Magnesium Supplementation for the Prevention of Supraventricular Arrhythmias'. Manuscripts #2 and 3, which evaluated the association of tMg to arrhythmias and whether low tMg mediates the association between PPI use and CVD risk, used data from the ARIC study, an ongoing community-based prospective cohort that began in 1987. Within Chapter 3, section II describes the pilot randomized trial used for Manuscript #1; section III describes the ARIC study used for Manuscripts #2-3.

II. MAGNESIUM SUPPLEMENTATION PILOT RANDOMIZED CONTROLLED TRIAL

A. Study design

Manuscript #1 was conducted using data from a double-blind pilot Mg supplementation trial, which sought to examine oral Mg supplementation in the primary prevention of supraventricular arrhythmias (assessed using 2-week portable heart monitor, Zio® XT Patch) [Clinical Trials Registration #: NCT02837328].[78] Briefly, between March to August 2017, 59 individuals from the general population aged >55 years and with no prior CVD history, were recruited using 4 methods: 1) fliers, 2) the University of Minnesota StudyFinder website, 3) ResearchMatch research volunteer database, and 4) invitations to University of Minnesota School of Public Health employees. Individuals with a history of CVD, kidney disease, inflammatory

bowel disease or any severe gastrointestinal disorder, or allergy/intolerance to Mg were excluded, as were those who reported use of type I or III antiarrhythmic drugs, digoxin, or Mg supplements. Use of multivitamins containing Mg was permitted, since these generally contain relatively low Mg doses (e.g. 50 mg Mg).

Eligible participants were randomized to 400 mg/day of oral Mg oxide or lactose placebo for 12 weeks. Block randomization within two strata of age (<65y and ≥65y) was used. At the baseline visit, blood was drawn, weight, height and blood pressure were measured, and several questionnaires were administered. At the end of the visit, the Zio® XT Patch heart rhythm monitoring device was applied, which was worn for 2 weeks. The study treatment was mailed to participants 2 weeks after the baseline visit. After 10 weeks on study treatment, participants returned for a second blood draw, and a second Zio® XT Patch was applied. They continued on study treatment until the Zio® XT Patch was removed.

Study participants and staff were blinded to the treatment status. The University of Minnesota Investigational Drug Service managed bottling of the active supplement and matched placebo in accordance with the randomization scheme. Participants provided written informed consent and the University of Minnesota Institutional Review Board approved the study protocol. Details of the trial (e.g. treatment compliance, assessment of blinding, adverse events) have been previously published.[78]

B. Biomarker measurements

In the pilot trial, participants were asked to fast for 8 hours prior to the blood draw. Blood samples were obtained at baseline and at the follow-up visit by a trained phlebotomist. Date and time of phlebotomy were recorded. iMg was measured in whole blood immediately after the blood draw using the pHOx® Ultra blood gas analyzer from Nova Biomedical. Centrifuged blood

samples yielded up to 3 aliquots of plasma and up to 6 aliquots of serum. Unused specimens were stored in the refrigerator and in the freezer at -80°C until analysis. Serum tMg was measured 'in batch' at the end of the study at the University of Minnesota Advanced Research and Diagnostics Laboratory (ARDL), using the Roche Cobas 6000 colorimetric analyzer (Roche Diagnostics; Indianapolis, Indiana). Glucose was also measured in serum at ARDL using the Roche Cobas 6000. In a subset of participants, iMg from previously frozen and refrigerated samples was measured in serum. Date and time of specimen storage and analysis was recorded.

C. Other data collection

At the baseline and follow-up visit, height (using a research stadiometer) and weight (using a scale with the participant in light clothing) were measured. Resting blood pressure (after 5 minutes sitting) was measured using a random zero sphygmomanometer three times; the average of the three measurements was calculated.

III. ATHEROSCLEROSIS RISK IN COMMUNITIES STUDY

A. Study design

The ARIC study[195] began in 1987-89, when participants were aged 45-64 years old, and now has over 30 years of follow-up. Of the 15,792 ARIC study participants at baseline, 27% were black and 73% white, while 55% were women and 45% men. Participants were recruited from 4 communities (suburbs of Minneapolis, MN; Forsyth County, NC; Jackson, MS; Washington County, MD). Since the baseline visit, there has been continuous surveillance for hospitalization (linkage with local hospitals, state and national death indices and through annual or semi-annual follow-up telephone calls). Several clinic visits have since been conducted (Visit 2: 1990-92,

Visit 3: 1993-95, Visit 4: 1996-98, Visit 5: 2011-13, Visit 6: 2016-2017, Visit 7: 2018-2019). At each visit, informed consent was obtained.

Relevant to this dissertation, visit 5 was attended by 6,538 participants and visit 6 by 4,003. At these visits, participants were interviewed, underwent anthropometric measurements and sitting blood pressure measurements, and a fasting blood draw. Participants were asked to bring bottles of current medications to each visit; medication information was transcribed and coded as described in the next section.

B. Exposures

1. *Magnesium*

Fasting blood samples were obtained at each ARIC visit, and were frozen until analysis. Specific to this dissertation, tMg was analyzed from previously frozen sera samples obtained at visit 5 (2011-13) and visit 6 (2016-17). As described in greater detail within the methods sections of Manuscripts #2-3, tMg was measured at ARDL using similar colorimetric methods from samples obtained at both visits 5 and 6 (Roche Cobas 6000 Chemistry Analyzer; Roche Diagnostics, Indianapolis, Indiana).

2. *Medication use*

Participants were asked to bring bottles of medications (including over-the-counter and prescription) used during the prior two weeks to the visit; medications were transcribed and coded. From 2006 to 2011, medication use was also assessed over the telephone annually where participants reported medication names from prescription bottles. Use of PPIs was identified at this time as well.

C. Outcomes

1. *Arrhythmias*

Data from the Zio® XT Patch was processed using the ZEUS algorithm.[196] Physician ECG readers at EPICARE downloaded the iRhythm reports and verified the accuracy of reports, which were then sent to the ARIC Coordinating Center. Details of definitions and modeling approaches to arrhythmias are described further within Manuscript #2's methods section.

2. *Cardiovascular diseases*

Prevalent CVD was defined by a history of CHD, heart failure (HF), stroke or AF based on ARIC ascertainment on or prior to the participants' clinic visit date. Specific to Manuscript 3, we define incident CVD events by: incident CHD, HF, stroke, AF and CVD mortality (both as a composite outcome and individual outcomes) through December 31, 2017. Detailed descriptions of prevalent and incident CVD events are described within the methods sections of Manuscripts #2 and #3. Briefly, potential CHD events were identified by (1) recent hospitalizations identified during follow-up phone calls to participants (twice annually since 2012); (2) ongoing surveillance of community hospital discharge lists and death certificates; and (3) linkage to State and National Death Indices. International Classification of Disease (ICD) codes were recorded from all hospitalizations.

D. Covariate measurements

Similar methods for covariate measurement were used across study visits. Participants self-reported their sociodemographic characteristics and lifestyle habits (including smoking status, alcohol consumption, physical activity), underwent measurements of anthropometry and blood pressure, as well as a fasting blood draw. Alcohol consumption habits were used to

estimate ethanol intake in grams per week. Physical activity (sports index) was quantified using the validated Baecke questionnaire.[197] Height and weight were measured and used to calculate BMI (kg/m^2). After 5 minutes rest, blood pressure was measured three times using a random zero sphygmomanometer. Systolic and diastolic blood pressure was quantified based on the mean of the second and third blood pressure measurements. Fasting (>8hrs) serum glucose was measured using a hexokinase method at visit 5 and 6. Diabetes was defined as a having a fasting glucose level ≥126 mg/dL, non-fasting glucose level ≥200 mg/dL, self-reported use of diabetes medication or self-reported physician diagnosis. Serum potassium was measured using an indirect ion selective electrode (refer to Chapter 5 section III for more detail).

CHAPTER 4 (MANUSCRIPT 1) – CIRCULATING IONIZED MAGNESIUM: COMPARISONS WITH CIRCULATING TOTAL MAGNESIUM AND RESPONSE TO MAGNESIUM SUPPLEMENTATION IN A RANDOMIZED CONTROLLED TRIAL

I. OVERVIEW

Introduction: Ionized Mg (iMg) is considered the biologically active fraction of circulating total Mg (tMg). It is possible that iMg may be a more physiologically relevant marker than tMg.

Objectives: Using data from a double blind pilot randomized controlled trial, we tested 1) whether oral Mg supplementation will increase iMg concentrations compared to placebo, and 2) the relationship between iMg and tMg cross-sectionally at baseline and in response to supplementation. Additionally, we evaluated the agreement between iMg measured in fresh whole blood versus stored samples.

Methods: Participants were randomized to oral Mg supplementation (400 mg/day, Mg Oxide) or placebo for 10 weeks. Fasting blood samples were obtained at baseline and the follow-up visit. The analysis used linear regression and an intent-to-treat approach.

Results: The 59 participants were generally healthy, mean aged 62 years old and 73% female. Baseline iMg and tMg were modestly and positively associated; the ratio of baseline iMg to tMg was 53%. The mean supplement effect on iMg was 0.03 mmol/L (95% CI: 0.01, 0.05) for those randomized to Mg supplementation as compared to placebo. The supplement effect on iMg did not differ significantly by baseline iMg. For lab stability, iMg was consistently higher in previously refrigerated and frozen samples by 0.14 and 0.20 mmol/L, respectively, versus fresh

samples.

Discussion: In this relatively healthy adult population, Mg supplementation over 10 weeks resulted in increased iMg concentrations. Whether iMg is a more appropriate measure of Mg status than tMg and the public health or clinical utility of measuring iMg remains to be determined.

II. INTRODUCTION

Magnesium (Mg) homeostasis reflects a complex and dynamic interplay between dietary intake, absorption and excretion.[8,9] The majority of total body Mg resides within the bone tissue while less than 1% of total body Mg lies extra-cellularly. Serum total Mg (tMg) has traditionally been used to assess Mg status in both clinical and research settings, with a reference range of 0.75-0.95 mmol/L (multiply mmol/L by 2.43 for mg/dL; 1.82-2.31 mg/dL).[16] There are important considerations to be cognizant of when using tMg to reflect Mg status. Of the circulating tMg in serum, approximately 20-30% is bound to proteins, and is thought to be physiologically inactive. Ionized Mg (iMg) constitutes approximately60-70% of circulating tMg[18,66] and is considered the biologically active form of circulating Mg.[198] It is possible that iMg may be a more physiologically relevant marker than tMg.[18,66]

iMg is infrequently measured in research or clinical settings,[16,18] likely because the iMg assay protocol recommends immediate analysis of whole blood, specialized equipment is required for measurement, and iMg measurement can be prone to interference by individual-level factors such as pH level and serum calcium. While tMg and iMg are generally thought to be correlated, the literature has been mixed in both observational studies and randomized controlled trials (RCTs) of Mg supplementation. [27-30] Furthermore, these studies have primarily been conducted in populations with comorbidities thought to influence Mg homeostasis.

Since relatively little is known about iMg in healthy populations, using data from a Mg supplementation RCT we tested the following hypotheses: 1) oral Mg supplementation will increase iMg and tMg concentrations compared to placebo, particularly in those with low baseline iMg and tMg concentrations, respectively; and 2) iMg and tMg will be modestly associated at baseline and in response to supplementation. Additionally, to better understand considerations

related to iMg laboratory measurement, we evaluated the agreement between iMg concentrations measured in fresh whole blood as compared to refrigerated or frozen samples.

III. METHODS

A. Study design

We examined interrelations between iMg and tMg, overall and in response to supplementation. To do this we used data from a pilot RCT entitled 'Magnesium Supplementation for the Prevention of Supraventricular Arrhythmias' [Clinical Trials Registration #: NCT02837328].[78] This double-blind trial examined oral Mg supplementation for the primary prevention of supraventricular arrhythmias.

Between March and June of 2017, 59 individuals from the general population aged >55 years and with no prior history of CVD were randomized to 400 mg/day of oral Mg (in the form of Mg oxide) or lactose placebo for 10 weeks. Block randomization within two strata of age (<65y and ≥65y) was used. Within each stratum randomly permuted block sizes of 2, 4 or 6 were used to generate the randomization schedule.

At the baseline visit, blood was drawn, weight, height and blood pressure were measured, and several questionnaires administered. The study treatment was mailed to participants 2 weeks after the baseline visit, and the intervention then ensued. After 10 weeks on study treatment, participants returned for a second blood draw.

At the follow-up visit, participants brought the bottle containing the supplement or matching placebo, and treatment compliance was estimated by a pill count. Further details of the trial have been previously published[78] including measures of adverse effects and assessment of

blinding.

B. Biomarker measures

Fasting (>8 hours) blood samples were obtained at baseline and at the follow-up visit. Time of blood draw was recorded. iMg was measured in whole blood approximately 10 minutes after the blood draw using the pHOx® Ultra blood gas analyzer (machine and reagents were donated by Nova Biomedical; Waltham, MA). The pHOx® Ultra blood gas analyzer provides both crude iMg concentration and iMg concentration adjusted for pH (i.e. normalized iMg concentrations). As the concentration and activity of iMg can differ by sample pH, herein we present normalized iMg concentrations, except where indicated otherwise. Blood specimens were centrifuged and separated into plasma and serum. Serum tMg was measured 'in batch' at the end of the study using the Roche colorimetric analyzer at the University of Minnesota Advanced Research and Diagnostics Laboratory. Ionized calcium also measured in whole blood using the pHOx® Ultra blood gas analyzer.

In order to evaluate the impact of specimen storage on iMg concentrations, for a sub-sample (n=39) and using split specimens, iMg was also measured in serum that had been refrigerated for approximately 1 hour and serum that had been stored in the freezer. Freezer specimens were measured 'in batch' at the end of the study. The time that whole blood samples were placed in the refrigerator and freezer was recorded, as was time of iMg measurements.

C. Statistical analysis

Mean and median iMg concentrations at baseline are reported overall and by treatment group. Baseline characteristics across study treatment arms and across baseline iMg concentrations above/below the median are also reported.

We used a linear regression model to test whether change in iMg differs by treatment group. Change in iMg was the dependent variable with treatment group as an indicator variable adjusted for age stratum (randomization stratification factor, <65y vs ≥65y) and baseline iMg concentration. Baseline iMg was included as a covariate for added precision.[199,200] Confidence intervals were based on robust variance estimation. Pre-specified subgroup analyses to assess whether the intervention effect differs by baseline iMg status (above/below median) were also conducted by including a cross-product term in the model (treatment group*baseline iMg status). We also report baseline, follow-up and change in the iMg to tMg ratio by treatment group, as well as testing whether the intervention effect differs by baseline ratios. Additionally, results for change in tMg are also provided as previously reported,[78] using this approach. Our primary analysis for change in iMg was based on the intent-to-treat principle. In secondary analyses, we excluded those who did not take at least 80% of the supplements as instructed. In post-hoc analyses we additionally adjusted for sex and ionized calcium (separately).

To examine baseline associations of iMg with tMg, we used a linear model with iMg as the dependent variable, with tMg as the predictor variable as well as treatment group, age stratum (<65y vs ≥65y) and baseline iMg concentration. We used the slope to examine the association between iMg and tMg. Additionally, we used Pearson's partial correlation coefficients for baseline iMg and tMg, overall (adjusted for treatment arm, age and sex) and by treatment group (adjusted for age and sex). A scatter plot was used to visualize the association of baseline iMg and tMg. Additionally, Bland-Altman plots were used to visualize the comparative agreement of iMg in response to supplementation stratified by treatment arm. A similar set of Bland-Altman plots were used for tMg.

To evaluate whether iMg concentrations differ according to sample processing method we report mean/median concentrations for iMg measured from fresh whole blood, after

refrigeration and after one freeze-thaw cycle. We report, by processing method, the mean difference (95% confidence intervals) in iMg concentrations and distribution of the difference in percentiles. We also report mean time from blood draw to processing. We used Bland-Altman plots to visualize the agreement between iMg quantified in whole blood soon after blood draw with serum iMg as measured from samples stored in the refrigerator and samples stored in the freezer.

Two-tailed p-values<0.05 were considered statistically significant. STATA version 14.1 was used for analyses (College Station, TX).

IV. RESULTS

A. Study participants

Table 4.1 describes study participant characteristics at baseline by treatment group and by baseline iMg status (above and below median). Baseline characteristics by treatment group were largely similar but for sex; the group randomized to Mg supplements was comprised of 86.2% women, while the group randomized to placebo was 60.0% women.

The average baseline iMg to tMg ratio was 64%. The median baseline iMg and tMg concentrations in the treatment group were 0.56 mmol/L (Percentile: 25^{th} = 0.50 mmol/L, 75^{th} = 0.60 mmol/L), and 0.86 mmol/L (25^{th} = 0.82 mmol/L, 75^{th} = 0.90 mmol/L), respectively. In the placebo group, baseline iMg was 0.54 mmol/L (25^{th} = 0.52 mmol/L, 75^{th} = 0.57 mmol/L) and tMg was 0.86 mmol/L (25^{th} = 0.82 mmol/L, 75^{th} = 0.90 mmol/L). Baseline characteristics stratified by baseline iMg status above or below the median were comparable.

B. Effect of magnesium supplementation on magnesium biomarkers

In **Table 4.2** are mean and standard deviations for iMg and tMg at baseline, follow-up, and change in iMg and tMg by treatment arm. Also presented in Table 4.2 are age- and baseline-adjusted differences by assigned treatment arm. At the end of intervention period, the change in iMg for those randomized to 400 mg/day of supplemental Mg was significantly higher than the change for those randomized to placebo [mean supplement effect=0.03 mmol/L (95% CI: 0.01, 0.05); p-value=0.009]. The supplement effect on iMg did not statistically significantly differ by baseline iMg concentrations (above vs below the median, p-interaction=0.86). There was not a significant effect on the ratio of iMg to tMg [mean supplement effect of 0.6% (95% CI: -1.7%, 3.0%); p-value=0.58]; the supplement effect did not differ by the ratio of baseline iMg to tMg (p-interaction=0.47). As previously reported,[78] there was a significant supplement effect on tMg of 0.04 mmol/L (0.01, 0.06); p-value=0.004; which also did not differ significantly by baseline tMg status (p-interaction=0.23).

In secondary analyses, among those with compliance >80% (based on pill count), results were largely similar (**Supplemental Table 4.1**). When we adjusted the intervention effect for sex and baseline ionized calcium, results were also largely similar (data not shown).

C. Relationship between magnesium biomarkers

Baseline concentrations of iMg and tMg were correlated at r = 0.50 (p-value<0.001) overall, while in the treatment group it was r = 0.47 (p-value=0.02) and in the placebo group it was r = 0.58 (p-value=0.002). Using linear regression, the slope between iMg (outcome) and tMg (predictor) was 0.417 (intercept=0.187); the slope was 0.422 (intercept=0.186) when adjusted for treatment group and age stratum. **Figure 4.1** provides a scatterplot of iMg and tMg measurements at baseline, which shows a positive and even scatter across the association between baseline iMg and tMg. Bland-Altman plots show the comparative agreement between change in iMg and tMg

in response to supplementation stratified by treatment group (**Figure 4.2**). In the treatment group, there was slight variation between change in iMg across the mean of iMg measurements. Specifically, those with lower averaged iMg measurements tended to have positive change in iMg. There were not clear patterns for iMg in the placebo group or change in tMg.

D. Comparisons of iMg in fresh, refrigerated and frozen blood samples

There were 39 participants with baseline iMg measured in fresh as well as in stored samples. The average time from blood draw to analysis of baseline samples was 71 ± 29 minutes for refrigerated serum while for frozen serum it was 84 ± 15 days. Overall, the average iMg concentration was 0.54 ± 0.05 mmol/L in fresh whole blood samples; 0.68 ± 0.04 when measured in the refrigerated samples and 0.73 ± 0.05 in the frozen serum samples, respectively. The mean pH was also higher in previously refrigerated samples (7.45) and frozen samples at (7.53) than in fresh baseline samples (7.38).

After refrigeration, iMg concentrations were higher by, on average, 0.14 mmol/L (95% CI: 0.12, 0.16) than iMg in fresh whole blood. After one freeze-thaw cycle, serum iMg was higher than in fresh whole blood by an average of 0.20 mmol/L (95% CI: 0.18, 0.21). Bland-Altman plots depict the comparative agreement between iMg in whole blood measured soon after blood draw against iMg in serum refrigerated for approximately 1 hour (**Figure 4.3A**) and iMg after one freeze-thaw cycle (**Figure 4.3B**). The difference in iMg measured in refrigerated vs fresh was higher by about 0.14 mmol/L and did not appreciably differ by the average of the two measurements (Figure 4.3A), while previously frozen vs fresh was consistently higher by about 0.20 mmol/L. The difference between iMg using refrigerated vs fresh samples ranged from 0.07 mmol/L to 0.30 mmol/L, while for frozen vs fresh differences ranged from 0.11 to 0.29 mmol/L. Pearson's partial correlations were r = 0.34 (p-value=0.04) for refrigerated versus fresh samples

and r = 0.46 (p-value=0.005) for frozen versus fresh samples.

V. DISCUSSION

In this randomized controlled trial, oral Mg supplementation over 10 weeks increased iMg in whole blood compared to placebo. The change did not differ by baseline iMg concentration, though we were not powered to detect subgroup differences. Mg supplementation did not have an effect on the ratio of iMg/tMg, and as previously reported,[78] Mg supplementation resulted in increased tMg. With regard to lab stability, concentrations of iMg measured in refrigerated serum were consistently overestimated based on previously refrigerated and frozen sera samples.

The distribution of iMg in this relatively healthy population is largely consistent with those from other studies. One study suggested a reference interval for whole blood iMg of 0.44-0.59 among 125 healthy participants, while among 200 consecutively recruited ICU patients, the range of iMg concentrations was wider (0.35-0.78 mmol/L).[201] Another study measured iMg in plasma using the same assay in the previous study[201] and reported a higher range of 0.53-0.67 mmol/L.[202] Currently, however, there is not an established threshold for defining optimal iMg, particularly in relation to predicting longer term health outcomes.

Four small oral Mg supplement RCTs (range N randomized = 26-60), conducted primarily in populations with comorbidities, have included both blood measurements of iMg and tMg.[27-30] Also complicating the ability to draw comparisons between RCTs incorporating iMg is that some of these studies utilized different iMg assays. In a RCT of 60 elderly participants with type 2 diabetes,[27] those randomized to 1 month of Mg supplementation had experienced a statistically significant increase in iMg (but not tMg) from baseline, relative to placebo.[27] Other RCTs have found no effect of Mg supplementation on biodistribution of circulating tMg or

iMg.[28-30] However, in addition to being small, the studies are heterogeneous (Mg dose/formulation, population, duration) and thus are difficult to directly compare.[27-30]

Most observational studies that have examined iMg in populations with medical conditions (e.g. chronic kidney disease, hypertension, pre-term labor) have done so cross-sectionally.[18] Many of these conditions are known to influence tMg concentrations.[18] A pilot study among 173 surgical intensive care unit patients reported poor agreement (weighted kappa=0.35) between iMg and tMg status.[203] It warrants mention, importantly, that classifications of low, normal or high tMg were based on established clinical cut-points, while classifications of iMg as low, normal or high were based on a reference interval in a healthy population.[201]

In the present manuscript we also evaluated the impact of processing on iMg concentrations. When iMg was measured using previously refrigerated and frozen samples, iMg concentrations were higher when compared to iMg measured soon after blood draw as recommended by the assay manufacturer. When compared to the gold standard processing (measurements made <15 minutes after blood draw), the pattern of higher iMg in refrigerated and frozen samples did not vary across iMg concentrations. If iMg were found to be a stronger biomarker of 'true' Mg status, then plausibly iMg could be measured using stored samples and then corrected for processing method. It would also be important to compare similarities or differences in circulating iMg by sample type (i.e. whole blood, serum, plasma). Previously, in a study published in 1996 using an earlier generation of this assay, the laboratory stability of iMg was tested in a cross-sectional analysis among relatively healthy participants under a variety of conditions (uncapped at room temperature, capped at room temperature and capped at 4°C) after 2, 4 and 6 hours storage. [201] Average fresh whole blood iMg (0.52 mmol/L) was similar when measured after storage in capped tubes for 2-6 hours at room temperature or at 4°C. Mean iMg concentrations were lower in uncapped room temperature samples. The pH of the blood increased

over time, particularly in uncapped samples. In that study, iMg was not corrected to pH of 7.4.[201] Further research is needed to determine if iMg is more strongly associated with health outcomes, and if so, enough so that it outweighs challenges of using stored samples.

It is important to be cognizant of limitations of this analysis. First, given how little research has been conducted on iMg, optimal concentrations of iMg, specifically in relation to health outcomes, are not well characterized. Second, the sample in the present analysis is of modest size. We were powered to detect overall supplement effects, but not subgroup comparisons such as differences by baseline iMg concentrations. Last, fresh iMg (i.e. iMg measured soon after blood draw) was measured in whole blood, while serum was used for measurements of iMg in previously refrigerated and frozen samples. It is possible differences in specimen may account for the apparent difference in fresh iMg versus iMg in stored serum. However, previously one study noted minimal differences in fresh iMg as measured in whole blood, plasma or serum.[204] Nevertheless, the major strengths of this analysis are that it is one of the first randomized controlled supplement trials to examine both tMg and iMg in a relatively healthy population. Additionally, we were able to examine the (lack of) laboratory stability of iMg when measured soon after blood draw and when using previously refrigerated or frozen samples in a RCT.

In conclusion, we found that Mg supplementation over 10 weeks resulted in increased iMg concentrations. Baseline concentrations of iMg and tMg were modestly and positively associated. Using refrigerated and frozen samples, iMg concentrations consistently overestimated iMg as measured in fresh whole blood. Whether iMg is a more appropriate measure of Mg status than tMg and the public health or clinical utility of measuring iMg remains to be determined. Further research is needed to learn how (or if) iMg relates to longer-term health outcomes, and whether iMg is a better predictor of health outcomes than tMg.

Table 4.1. Baseline participant characteristics stratified by study arm, and by baseline ionized magnesium concentration (above vs. below median), n=59

	Intervention status		**Baseline iMg concentration**	
	Magnesium (400 mg daily)	**Placebo**	**≥ median**[a]	**< median**
N	29	30	28	26
Age, years [b]	61.3 ± 5.3	61.6 ± 5.2	61.0 ± 4.3	62.2 ± 6.0
Age category				
≥65 years	6 (20.7)	8 (26.7)	5 (17.9)	8 (30.8)
<65 years	23 (79.3)	22 (73.3)	23 (82.1)	18 (69.2)
Sex				
Female	25 (86.2)	18 (60.0)	23 (82.1)	16 (61.5)
Male	4 (13.8)	12 (40.0)	5 (17.9)	10 (38.5)
Race				
White	27 (93.1)	29 (96.7)	26 (92.9)	25 (96.2)
Non-white	2 (6.9)	1(3.3)	2 (7.1)	1(3.8)
Education				
High school graduate or GED	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)
Some college	6 (20.7)	4 (13.3)	4 (14.3)	5 (19.2)
College graduate	10 (34.5)	16 (53.3)	10 (35.7)	14 (53.9)
Graduate or professional school	13 (44.8)	9 (30.0)	13 (46.4)	7 (26.9)
BMI, kg/m^2	27.7 ± 4.9	28.0 ± 4.5	26.9 ± 3.2	28.4 ± 5.4
Systolic blood pressure, mmHg	118.4 ± 14.9	119.3 ± 18.4	116.8 ± 12.1	122.0 ± 20.4
Diastolic blood pressure, mmHg	71.9 ± 8.7	71.2 ± 10.2	71.0 ± 7.0	72.46 ± 11.2
Glucose, mg/dL	94.2 ± 10.6	103.2 ± 40.2	94.1 ± 9.4	104.9 ± 43.1
Sensitivity analysis [c]	94.2 ± 10.6	96.2 ± 11.64	94.1 ± 9.4	96.8 ± 12.7
pH	7.38 ± 0.02	7.38 ± 0.03	7.38 ± 0.02	7.38 ± 0.03
Total magnesium, mmol/L	0.86 ± 0.06	0.85 ± 0.05	0.87 ± 0.05	0.84 ± 0.06
Ionized magnesium, mmol/L [d]	0.56 ± 0.06	0.55 ± 0.04	0.59 ± 0.03	0.51 ± 0.04
Total calcium, mmol/L	2.35 ± 0.09	2.34 ± 0.09	2.34 ± 0.09	2.35 ± 0.08
Ionized calcium, mmol/L [d]	1.19 ± 0.03	1.18 ± 0.03	1.19 ± 0.03	1.18 ± 0.03

Abbreviations: GED, general education diploma; BMI, body mass index; iMg, ionized magnesium; [a] iMg median = 0.55 mmol/L; [b] N (%) or mean ± standard deviation; [c] Omission of one participant with a baseline glucose value of 307 mg/dL; [d] Ionized calcium and magnesium are both 'normalized' to pH 7.4

Table 4.2. Ten-week change in ionized and total magnesium concentrations by treatment group, overall and stratified by baseline magnesium concentrations, n=59

	Intervention status				**Baseline Mg concentration**[a]				
					≥ median		**< median**		
	Magnesium (400 mg daily) Mean (SD)	Placebo Mean (SD)	Mean Intervention Effect (95% CI)[b]	p-value	Mean Intervention Effect (95% CI)	p-value	Mean Intervention Effect (95% CI)	p-value	p-interaction
N	29	30							
iMg,[c] mmol/L	22	27	0.03 (0.01, 0.05)	0.009	0.03 (0.00, 0.07)	0.07	0.03 (0.00, 0.07)	0.07	0.86
Baseline	0.56 (0.06)	0.54 (0.04)							
Follow-up[d]	0.57 (0.03)	0.53 (0.04)							
Change	0.01 (0.05)	-0.01 (0.05)							
tMg, mmol/L	24	30	0.04 (0.01, 0.06)	0.004	0.05 (0.01, 0.08)	0.01	0.02 (-0.00, 0.05)	0.08	0.27
Baseline	0.86 (0.06)	0.85 (0.05)							
Follow-up[d]	0.89 (0.06)	0.85 (0.05)							
Change	0.03 (0.05)	0.00 (0.05)							

Abbreviations: SD, standard deviation; CI, confidence interval; iMg, ionized magnesium; tMg, total magnesium; [a] iMg median = 0.55 mmol/L; tMg median = 0.86 mmol/L; [b] Adjusted for age (≥65 or <65), and baseline concentration (e.g. when change in iMg is the outcome, models were adjusted for baseline iMg). The numbers of observations included in linear models are 49 and 54 for the outcomes ionized magnesium (whole blood) and total magnesium (serum); [c] Normalized iMg concentration which is adjusted for blood pH; [d] Follow-up information obtained at intervention week 10

Table 4.3. Mean ionized magnesium concentrations in fresh, refrigerated and frozen blood samples and mean difference from baseline (fresh) after refrigeration and freezing, n=39

	Time from draw to analysis [a]	**pH** [a]	**Concentrations,** [a] mmol/L	**Mean Difference (95% CI)**, mmol/L	**Percentiles of Difference from Fresh iMg,** [c] mmol/L				
					1st	**25th**	**50th**	**75th**	**99th**
iMg (normalized)									
Fresh[b]	4.8 min (3.4)	7.38 (0.03)	0.54 (0.05)	Reference	-	-	-	-	-
Refrigerated	69.6 min (25.3)	7.45 (0.04)	0.68 (0.04)	0.14 (0.12,0.16)	0.07	0.10	0.13	0.17	0.31
Frozen	82.2 days (15.4)	7.51 (0.04)	0.73 (0.05)	0.19 (0.18,0.21)	0.11	0.15	0.19	0.25	0.29
iMg (not normalized)									
Fresh	4.8 min (3.4)	7.38 (0.03)	0.54 (0.05)	Reference	-	-	-	-	-
Refrigerated	69.6 min (25.3)	7.45 (0.04)	0.65 (0.04)	0.11 (0.10,0.13)	0.04	0.07	0.09	0.13	0.25
Frozen	82.2 days (15.4)	7.51 (0.04)	0.68 (0.05)	0.14 (0.12,0.15)	0.05	0.09	0.13	0.18	0.20

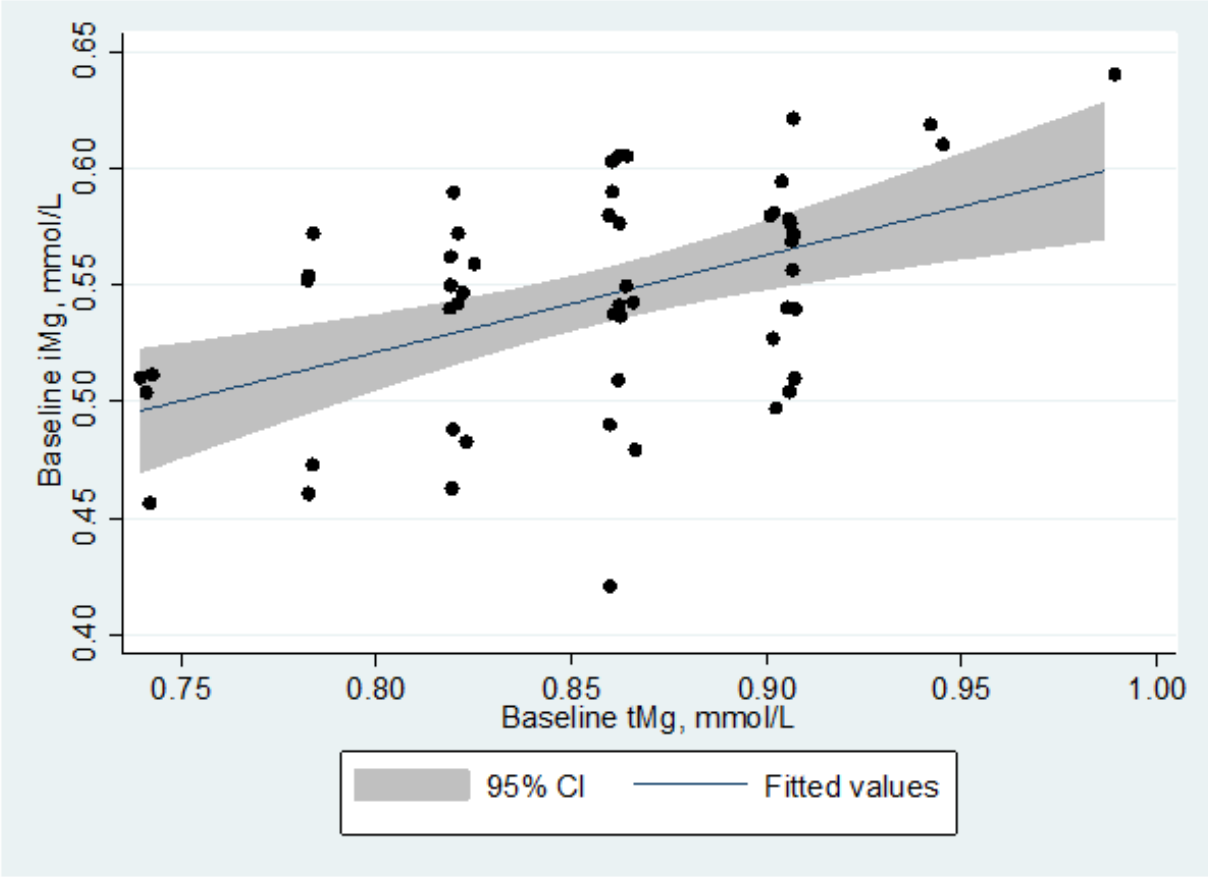
Abbreviations: CI, confidence interval; iMg, ionized magnesium; tMg, total magnesium; [a] Mean (standard deviation); [b] Refrigerated and frozen blood samples were measured in serum, while fresh was measured in whole blood; [c] The distribution (in percentiles) of the difference between refrigerated vs fresh and frozen vs fresh. A value of 0 indicates that iMg measured in refrigerated (or frozen) and fresh were identical.

Supplemental Table 4.1. Ten-week change in ionized and total magnesium concentrations by treatment group, overall and stratified by baseline magnesium concentrations, excluding those who did not take >80% capsules as assigned, n=38

	Treatment arm				Baseline Mg concentrations[a]				
					≥ median		< median		
	Magnesium (400 mg daily) Mean (SD)	Placebo Mean (SD)	Mean Intervention Effect (95% CI)[b]	p-value	Mean Intervention Effect (95% CI)	p-value	Mean Intervention Effect (95% CI)	p-value	p-interaction
N	15	23							
iMg,[c] mmol/L	11	22	0.04 (0.01, 0.06)	0.007	0.04 (-0.01, 0.09)	0.14	0.06 (0.01, 0.10)	0.02	0.37
Baseline	0.57 (0.06)	0.54 (0.04)							
Follow-up[d]	0.58 (0.02)	0.54 (0.04)							
Change	0.01 (0.05)	-0.01 (0.05)							
tMg, mmol/L	13	23	0.05 (0.02, 0.08)	0.003	0.05 (0.01, 0.10)	0.02	0.03 (-0.00, 0.07)	0.07	0.47
Baseline	0.85 (0.05)	0.85 (0.05)							
Follow-up[d]	0.89 (0.06)	0.84 (0.06)							
Change	0.04 (0.04)	-0.01 (0.05)							

Abbreviations: SD, standard deviation; CI, confidence interval; iMg, ionized magnesium; tMg, total magnesium; [a] iMg median = 0.55 mmol/L; tMg median = 0.86 mmol/L; [b] Adjusted for age (≥65 or <65), and baseline concentration (e.g. when change in iMg is the outcome, models were adjusted for baseline iMg). The numbers of observations included in linear models are 49 and 54 for the outcomes ionized magnesium (whole blood) and total magnesium (serum); [c] Normalized iMg concentration which is adjusted for blood pH; [d] Follow-up information obtained at intervention week 10

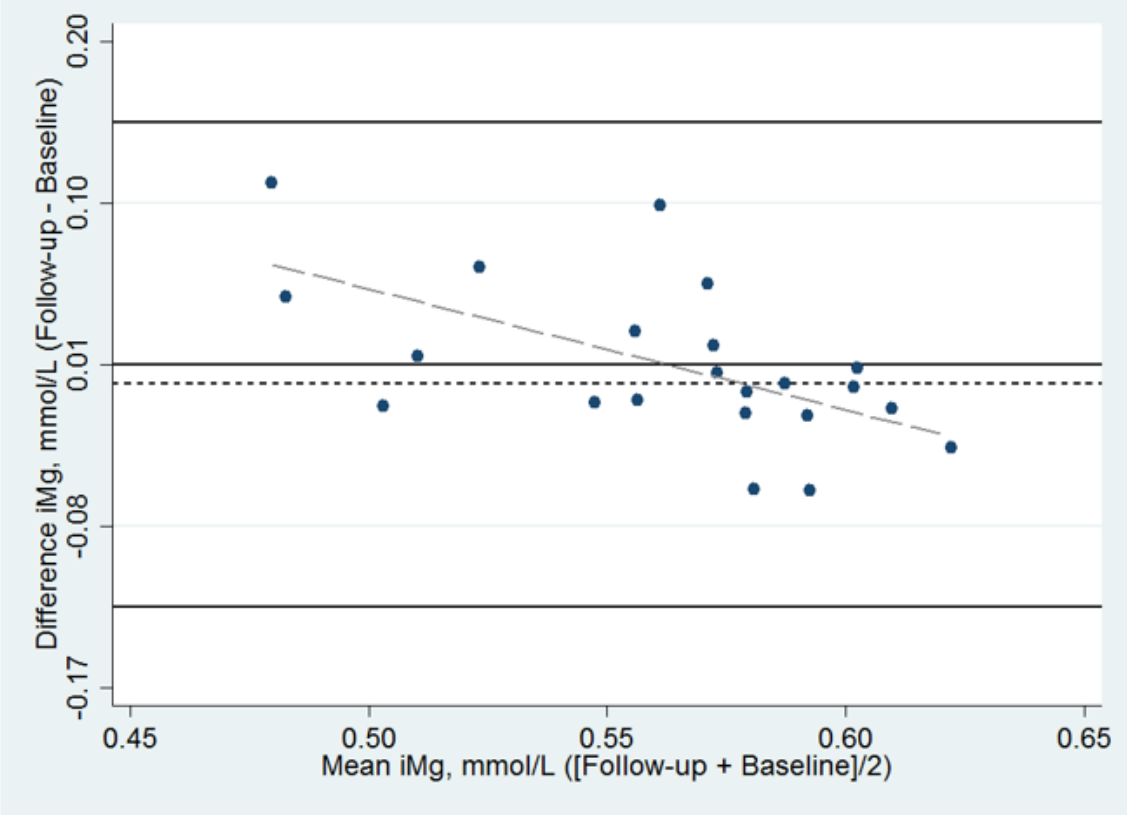
Figure 4.1. Scatterplot and linear fitted line between ionized[a] and total magnesium at baseline, unadjusted, n=49



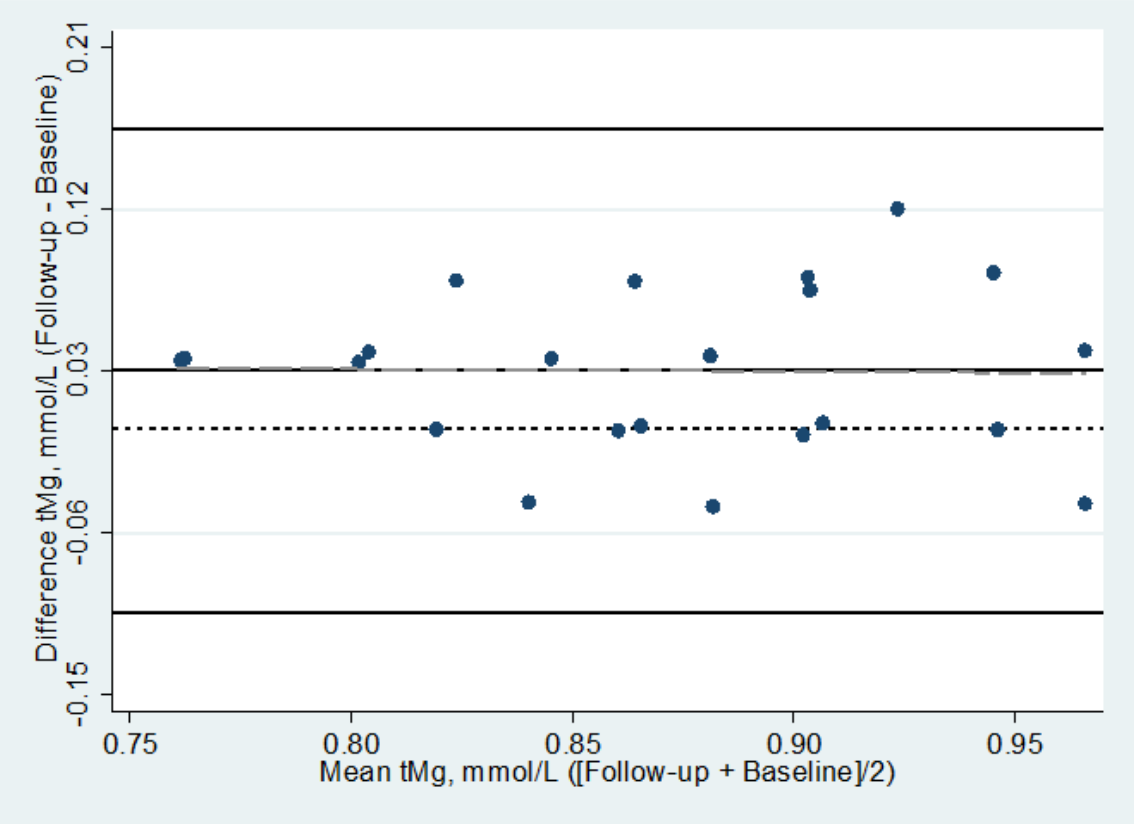
[a] Normalized iMg concentration which is adjusted for blood pH

Figure 4.2. Bland-Altman plots assessing the association between change in ionized and total magnesium in response to magnesium supplementation over 10 weeks, stratified by treatment arm[a,b]

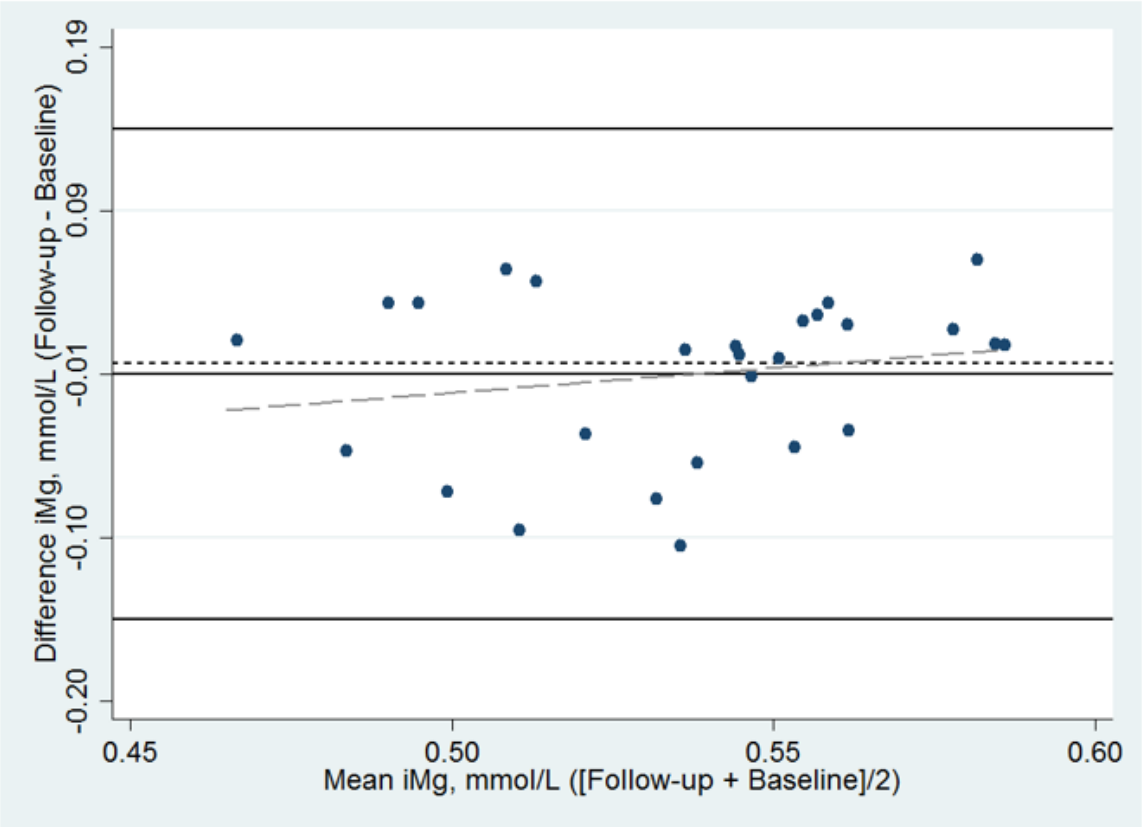
a) Change in ionized magnesium (iMg) in treatment group, n=22



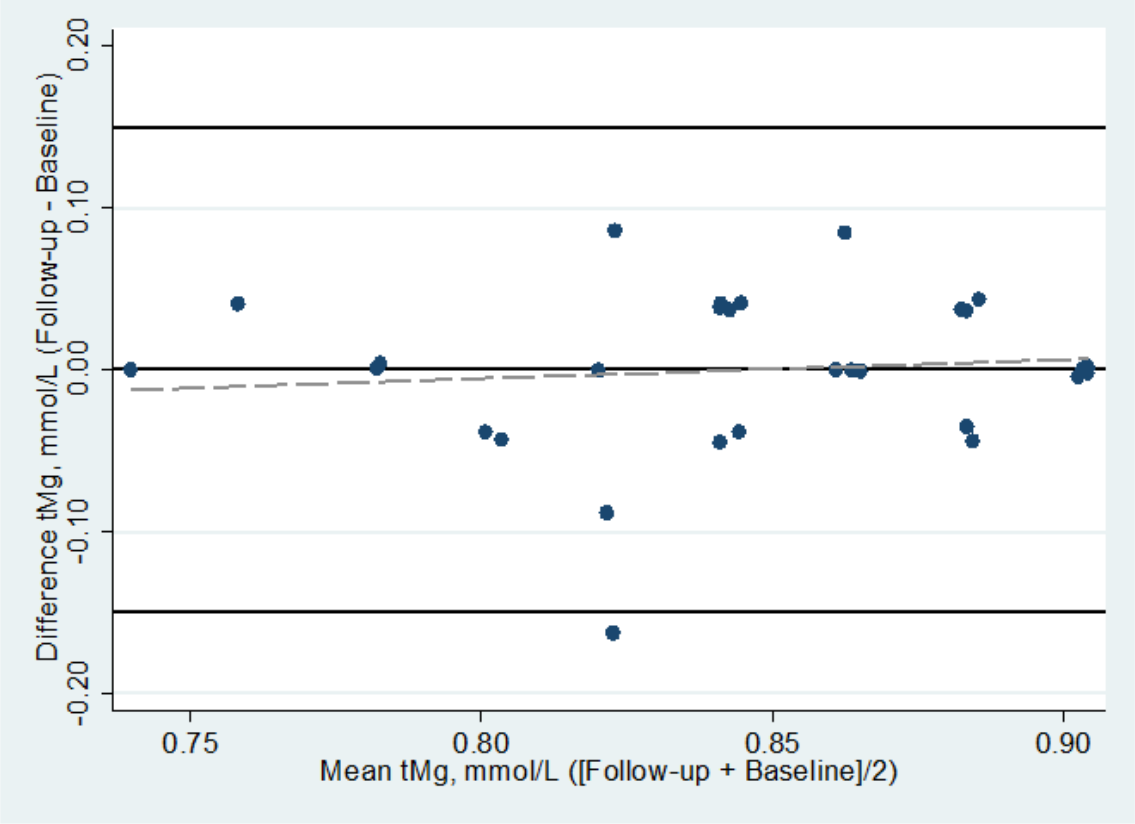
b) Change in total magnesium (tMg) in treatment group, n=24



c) Change in ionized magnesium (iMg) in placebo group, n=27



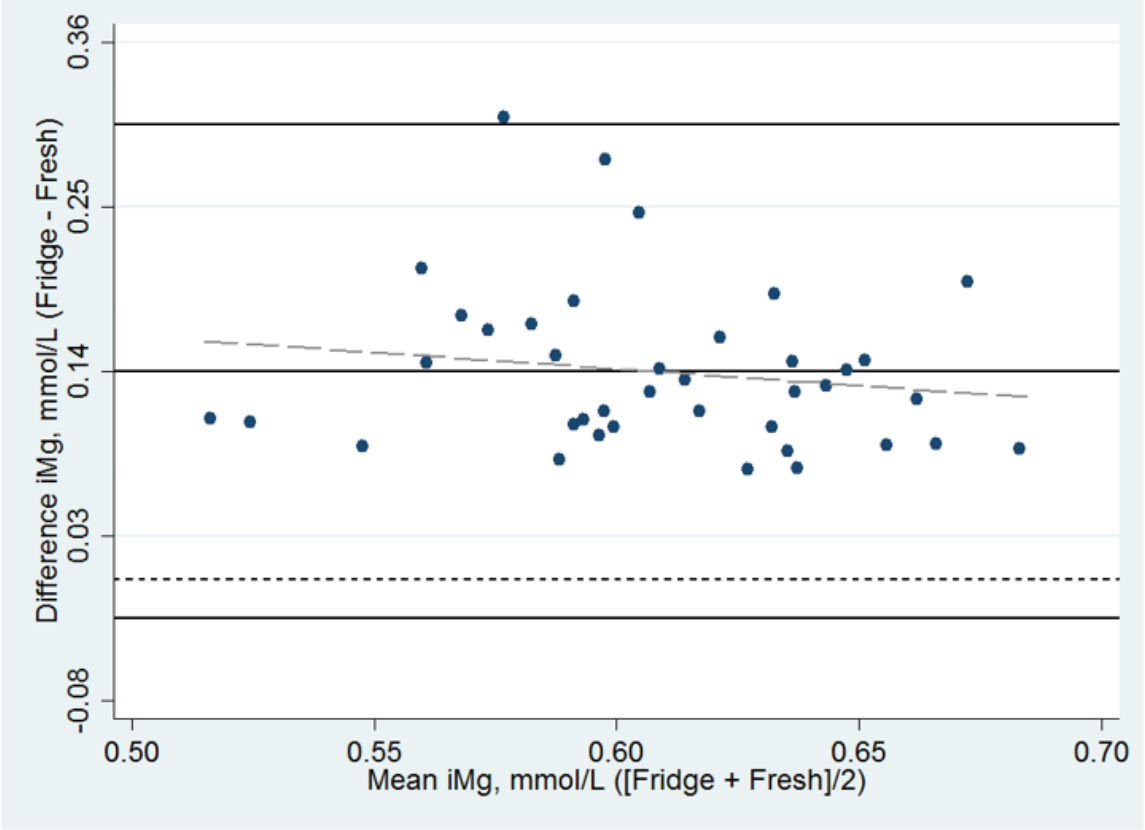
d) Change in total magnesium (tMg) in placebo group, n=30



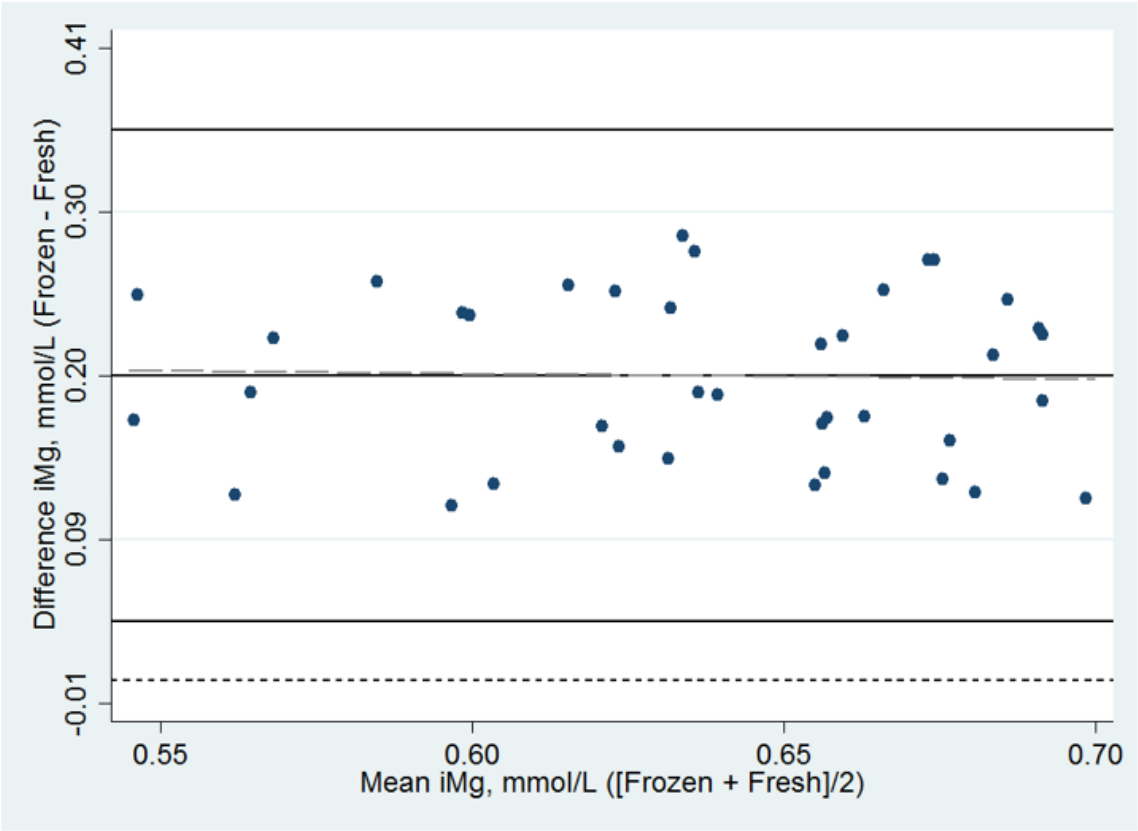
[a] Normalized iMg concentration which is adjusted for blood pH
[b] Solid lines (black) are mean difference ± 3 standard deviations; Long dash line (gray) are fitted values; short dash line (black) is reference line for mean difference of 0 mmol/L

Figure 4.3. Bland-Altman plots assessing the association between concentrations of ionized magnesium at baseline in fresh whole blood and in serum after refrigeration and freezing[a,b,c]

a) Ionized magnesium (iMg) concentrations in fresh whole blood vs refrigerated serum, n=39



b) Ionized magnesium (iMg) concentrations in fresh whole blood vs frozen serum, n=39



[a] Normalized iMg concentration which is adjusted for blood pH
[b] Median time in fridge = 71 minutes; median time frozen = 81 days
[c] Solid lines (black) are mean difference ± 3 standard deviations; Long dash line (gray) are fitted values; short dash line (black) is reference line for mean difference of 0 mmol/L

CHAPTER 5 (MANUSCRIPT 2) – SERUM MAGNESIUM AND BURDEN OF ATRIAL AND VENTRICULAR ARRHYTHMIAS: THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

I. OVERVIEW

Introduction: Magnesium (Mg) is thought to play a role in cardiac electrophysiology. Little is known about Mg in relation to subclinical atrial and ventricular arrhythmias among a community-based population of elderly individuals.

Objectives: To evaluate cross-sectional associations of serum Mg with atrial arrhythmias [atrial fibrillation (AF), premature atrial contractions (PAC), supraventricular tachycardia (SVT)], and ventricular arrhythmias [premature ventricular contractions (PVC), non-sustained ventricular tachycardia (NSVT)].

Methods: We included 2,513 ARIC study visit 6 participants who wore a leadless, ambulatory ECG-monitoring device. Serum Mg was modeled using cut-points and continuously. AF burden was categorized as intermittent or continuous based on the percent of analyzable time spent in AF. Other arrhythmia burdens were defined by the average number of abnormal beats per day. Linear regression was used to evaluate associations with continuous outcomes; while logistic and multinomial regression was used for binary and categorical outcomes, respectively.

Results: Participants were mean±SD age 79±5 years, 59% were women and 28% black. Mean serum Mg was 0.82±0.08 mmol/L and 19% had hypomagnesemia (<0.75 mmol/L). Across all models, serum Mg was inversely and monotonically associated with PVC burden. In the

demographic-adjusted model, serum Mg was inversely associated with odds of both continuous and intermittent AF. The association was no longer statistically significant with further adjustment for lifestyle characteristics and traditional AF risk factors. There was no statistically significant evidence of an association between serum Mg and other arrhythmias examined.

Conclusions: In this elderly population, low serum Mg was cross-sectionally associated with greater PVC burden. We found little evidence of an association between serum Mg and atrial arrhythmias. Whether Mg is associated with subclinical arrhythmia burden in mid-life is unclear.

II. INTRODUCTION

Magnesium (Mg) plays many essential physiologic functions, including having a role in cardiac electrophysiology.[3,4] The reference interval for normal serum Mg concentrations is traditionally defined by serum Mg concentrations between 0.75-0.95 mmol/L; however, some experts have suggested that individuals with serum Mg concentrations of 0.75-0.85 mmol/L may also exhibit subclinical or chronic latent magnesium deficiencies.[17] In nutrition sciences, severe Mg deficiency is widely thought to result in dysrhythmias, including atrial fibrillation (AF).[8,205] While extreme Mg concentrations are also thought to lead to adverse electrocardiographic (ECG) changes,[67] less is understood about subclinical deficiencies in circulating Mg in relation to burden of atrial and ventricular arrhythmias.

Most research on Mg and arrhythmias in the community have examined clinically recognized AF as the outcome. Three prospective observational studies, including ARIC,[8] have documented associations between low serum Mg and an increased risk of developing AF.[74-76] In ARIC, serum Mg was examined in relation to incident AF, as identified by study visit 12-lead ECGs and ICD codes on hospital discharges and death certificates. The other studies used similar AF ascertainment approaches.

Whether Mg is related to subclinical arrhythmias is unclear, particularly because these arrhythmias can often be asymptomatic and intermittent in nature. Current ECG technology has evolved to allow continuous monitoring for longer periods, such as 2 weeks,[206-208] which leads to the identification of additional arrhythmic events.[209] As such, relatively little research has explored the association between Mg and subclinical atrial arrhythmias [e.g. intermittent AF, premature atrial contractions (PAC) or supraventricular tachycardia (SVT)] or subclinical ventricular arrhythmias [e.g. premature ventricular contractions (PVC) or non-sustained

ventricular tachycardia (NSVT)] in the community.

Using data from over 2,000 ARIC participants we characterized cross-sectional associations of serum Mg concentrations across the spectrum of AF burden and other arrhythmias (PAC, SVT, PVC, NSVT) based on up to 2 weeks of continuous ECG recording. We hypothesized that low serum Mg concentrations were associated with a higher prevalence and burden of atrial and ventricular arrhythmias in this elderly community-dwelling population.

III. METHODS

A. Study design

The multi-center prospective ARIC study[195] began in 1987-89, when the eventual 15,792 participants were aged 45-64 years old. Participants were recruited from 4 communities (suburbs of Minneapolis, MN; Forsyth County, NC; Jackson, MS; Washington County, MD). Since the baseline visit, several clinic visits have been conducted. There has also been continuous surveillance for hospitalizations. At each ARIC visit, written informed consent was obtained.

Relevant to this manuscript, visit 6 occurred in 2016-17 and was attended by 4,003 participants (48% of those living). At visit 6, ARIC participants were invited to wear the Zio® XT Patch for 2 weeks provided they did not report a history of an allergic reaction to skin adhesive. Participants completed a brief questionnaire which asked about prior arrhythmias and treatments (e.g. previous arrhythmia diagnosis, anticoagulation status), and the device was applied to the upper left chest. Participants returned the devices by mail, in a pre-paid and labeled envelope to iRhythm (the manufacturer) for processing. Of the 2,650 participants who received a device, 17 devices were lost, and 17 devices were returned without data; thus resulting in 2,616

devices returned with analyzable data. Individuals who wore the Zio® XT Patch and had serum Mg measurement at visit 6 were eligible for this analysis. For analyses of atrial arrhythmias other than AF, we excluded those with prevalent AF as detected during Zio monitoring, or identified by prior ARIC AF diagnosis.[210]

1. *Biomarker Measures*

Fasting blood samples were obtained at ARIC visit 6, and were frozen until analysis. Serum total Mg was measured using colorimetric methods on the Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics; Indianapolis, Indiana). Serum potassium was measured using an ion selective electrode (Roche C501 Chemistry Analyzer). Serum glucose was measured using a hexokinase assay (Roche Cobas 6000 Chemistry Analyzer). Coefficients of variations (based on duplicate samples) were 1.6%, 2.2%, and 2.0%, for Mg, potassium, and glucose, respectively.

2. *Covariates*

At ARIC clinic visit 6, participants were interviewed, underwent anthropomorphic measurements and sitting blood pressure measurements, as well as a blood draw. Participants were asked to bring bottles of current medications to the visit, where medication information was transcribed and coded. Diabetes was defined as a having a fasting glucose level ≥126 mg/dL, non-fasting glucose level ≥200 mg/dL, self-reported use of diabetes medication or self-reported physician diagnosis. Systolic blood pressure was quantified based on the mean of the second and third blood pressure measurements. Body mass index (BMI) was calculated based on weight (kg) divided by height (m^2) squared. Physical activity (sports index) was quantified using the validated Baecke questionnaire.[197] Detailed definitions of coronary heart disease (CHD),[211] heart failure (HF)[212] and stroke[213] have been previously published. Briefly, trained staff abstracted possible

hospitalized CHD and stroke events onto standardized forms, which were classified by physicians using computer-assisted classification algorithms. HF was classified based on a prior hospital discharge code including '428' (428.0 – 428.9) or outpatient HF using previously published criteria.[212]

3. *Outcomes*

AF was defined by an irregularly irregular rhythm with absent P-waves lasting >30 seconds. AF burden was defined by percent of recording time spent in AF, which we categorized as no AF (0%), intermittent AF (0 to <100%) and continuous AF (100%).

SVT was defined by narrow complex tachycardia >4 beats with a rate >100/min, while NSVT was defined by wide complex tachycardia >4 beats with a rate >100/min. PAC count refers to the number of isolated PACs, while PVC count refers to the number of isolated PVCs. PAC, SVT, NSVT and PVC burden refer to the average number of arrhythmic beats per day (e.g. PAC count divided by duration of recording time).

B. Statistical analysis

We used multiple imputation with chained equations to avoid dropping observations due to missing covariates (<10% of observations for each covariate were missing).[214] The imputation model included the exposure (Mg, continuous) and all other covariates adjusted for in Model 3, as listed below. We created 10 imputed data sets using SAS PROC MI, separately analyzed each dataset, and used SAS PROC MIANALYZE to combine these results.

Restricted cubic splines were used initially to visualize the association between Mg and arrhythmias. We present unadjusted mean ± SD and proportions for the covariates stratified by

serum Mg categories. Specifically, we modeled Mg using clinical cut-points (i.e. >0.95 mmol/L hypermagnesemia, 0.85-0.95 mmol/L normal, 0.75-<0.85 mmol/L subclinical hypomagnesemia, <0.75 mmol/L hypomagnesemia).[17]

Linear regression was used for analyses involving continuous outcomes. We log-transformed PAC and PVC burden due to the right-skew distribution of these variables. Unconditional logistic regression was used to assess the association between serum Mg and binary measures of arrhythmias (NSVT). Multinomial logistic regression was used for categorical outcomes (AF burden). Confidence intervals were estimated based on model-based standard errors. In Model 1, we adjusted for demographic characteristics: age, sex and race-center (white-Minneapolis, MN; black-Jackson, MS; black-Forsyth County, NC; white-Forsyth County, NC; white-Washington County, MD). In Model 2, we additionally adjusted for educational attainment (less than high school; high school or GED; high school or more), smoking status (current, former, never), and ethanol intake (grams per week). In Model 3 (our fully adjusted model), we further adjusted for diabetes, systolic and diastolic blood pressure (continuous), and antihypertensive medication use. Additionally, circulating potassium plays an important role in cardiac electrophysiology and hypokalemia can frequently co-occur with hypomagnesemia. We added serum potassium (continuous) to Model 3 to test whether the serum Mg-arrhythmia associations are independent of serum potassium concentrations (Model 4).

To test the robustness of our findings, we conducted several sensitivity analyses: 1) excluding those taking antiarrhythmic medications, 2) excluding users of ACEI/ARBs and diuretics, 3) excluding users of proton pump inhibitors (PPIs) as well as 4) excluding those with a history of CVD (CHD, HF, stroke).

IV. RESULTS

The 2,513 participants were mean±SD aged 79±5 years, 58% were women and 25% were black. Mean serum Mg was 0.82±0.08 mmol/L and hypomagnesemia (<0.75 mmol/L) prevalence was 19%. Median analyzable time was 13.7 days (IQR=12.7-13.9). As shown in **Table 5.1**, participants with low serum Mg tended to take more medications and have a higher prevalence of cardiometabolic diseases as compared to those with normal magnesium (0.75-0.95 mmol/L).

As shown in **Figure 5.1**, low serum magnesium tended to be associated with higher model-predicted probability of any AF. Odds ratios (95% CIs) from multinomial logistic regression between serum Mg clinical categories with supraventricular arrhythmias are shown in **Table 5.2**. Due to small numbers individuals with AF among those with hypermagnesemia (>0.95 mmol/L), results are not reported for AF. In the demographic adjusted model, compared to those with hypomagnesemia (<0.75 mmol/L) the odds of having continuous AF were lower among those with serum Mg between 0.75-0.85 mmol/L [OR (95% CI) 0.59 (0.37, 0.95)] and 0.85-0.95 mmol/L [0.53 (0.33, 0.85)]. The association was attenuated with adjustment for lifestyle characteristics and particularly after further adjustment for traditional CVD risk factors. Similarly, each 0.1 mmol/L increment (approximately 1 SD) of serum Mg was associated with a 0.79 (0.74, 0.84) lower odds of continuous AF in the demographic-adjusted model. This association was also attenuated with further adjustment.

Effect estimates for intermittent versus no AF were in the hypothesized direction but smaller, and in most instances not statistically significant. No statistically significant associations were observed for serum Mg clinical cut-points in relation to intermittent versus no AF. Each 0.1 mmol/L increment was associated with lower odds of having intermittent AF [0.92 (0.84, 1.00)] with demographic adjustment. The effect estimate per 0.1 mmol/L was largely unchanged with further adjustment, but confidence intervals were wider. Among those with no AF, we also found

little evidence of an association of serum Mg with either PAC burden or SVT burden.

Associations between serum Mg and ventricular arrhythmias are presented in **Table 5.3**. Across all models, there was a monotonically inverse association between serum Mg clinical cut-points in relation to PVC burden. Similarly, when Mg was modeled continuously, each SD increment was associated with a lower PVC burden. There was no evidence of an association between serum Mg and the presence of NSVT.

In sensitivity analyses, the primarily null results for serum Mg and atrial arrhythmias were largely unchanged after we excluded participants taking anti-arrhythmic medications (**Supplemental Table 5.1a**), participants taking ACEi, ARB, and diuretics (**Supplemental Table 5.1b**), as well as participants taking PPIs (**Supplemental Table 5.1c**). The association was also similar when examined among those without a history of CVD (**Supplemental Table 5.2**). A similar set of sensitivity analyses were conducted for ventricular arrhythmias outcomes as shown in **Supplemental Tables 5.3-5.4**, and these results were largely similar to those in the main analyses.

V. DISCUSSION

In this community-based study of elderly individuals, we found that higher serum Mg was cross-sectionally associated with a lower burden of PVCs based on 2 week ambulatory ECG monitoring. We also found that participants with low magnesium concentrations had higher odds of continuous and intermittent AF in the demographic adjusted model; however, this association was attenuated and no longer statistically significant with further adjustment for lifestyle and CVD risk factors. These findings were similar even among those without a history of CVD.

A. Magnesium & atrial arrhythmias

Three prospective observational studies, including a prior ARIC publication,[74] have documented associations between low serum Mg and an increased risk of developing AF.[74-76] In ARIC, serum Mg was examined in relation to incident AF (hospital discharge, study visit ECGs and death certificates). Those in the lowest serum Mg quintile had a hazard ratio (HR) of 1.34 (95% CI: 1.16-1.54) compared to those in the middle quintile after multivariable adjustment.[74] Similarly, in the Framingham Offspring study, participants in the lowest serum Mg quartile had a higher AF risk compared to participants in the highest quartile [HR=1.52 (95% CI: 1.00-2.31)].[75] In an Israeli HMO, both mild and moderate hypomagnesemia were associated with higher AF risk over a follow-up period of about 2 years but not with AF risk over a short-term (3 month) follow-up.[76] However, outcomes in these studies were based on clinically recognized AF and/or shorter term ECG monitoring (e.g. 12-lead ECG), which might not capture those with intermittent AF episodes.[215,216] Additionally, an experimental feeding study lends support to these epidemiologic findings. Of 14 healthy women who were fed an extremely low diet in Mg, 3 of the women developed AF. Their AF resolved quickly after Mg repletion.[47] Furthermore, intravenous (high dose) Mg is used in the context of cardiac surgery to prevent post-operative AF.[217]

The pathophysiology linking circulating Mg and supraventricular arrhythmias is not well characterized. Mg is involved in hundreds of enzymatic reactions throughout the body.[82] Ionic flow of Mg, as well as calcium and potassium, are important for generating action potentials and maintaining the membrane potential of cardiac cells.[83] Mg is also considered a natural calcium antagonist, as Mg competes with calcium for membrane–binding sites to the L-type Ca^{2+} current.[84,85] Additionally, it is possible that low circulating Mg could act through known AF risk factors (namely hypertension, inflammation, or diabetes[17]) to promote arrhythmogenesis. The lack of a robust association in the present analysis is surprising and may be related to the

agedness of our study population when they wore the Zio® XT Patch. Prospective evaluations of Mg and AF began when participants were middle-aged; the present study population was on average 79 years old.

B. Magnesium & ventricular arrhythmias

Intravenous Mg is commonly used to manage torsade de pointes, a type of ventricular arrhythmia, in the setting of long QT-interval syndrome.[218] Generally, there has been little characterization of any relationship of serum Mg concentrations and ventricular arrhythmias in the community. A small oral Mg supplement RCT reported decreased PVC intensity among participants without prior cardiac diseases.[79] In the Framingham Offspring Study, each SD (0.08 mmol/L) decrement was associated with lower odds of having a PVC identified over 1 hour ECG monitoring.[92]

Aside from the aforementioned studies, much of the research on Mg and ventricular arrhythmias has been conducted in populations with existing medical conditions (e.g. congestive HF, MI, diabetes). For example, among 750 obese Canadian participants with type 2 diabetes, participants with serum Mg ≤0.70 mmol/L had a 2.5-fold higher prevalence of premature ventricular contraction (PVC)—as measured using a Holter monitor—than those with serum Mg >0.70 mmol/L (50% vs 21%).[91] This is similar to our findings among individuals either with or without prior cardiometabolic disease as detected over 2 weeks ECG monitoring.

Similar to supraventricular arrhythmias, potential mechanisms between low Mg and ventricular arrhythmias are not fully understood. There is some overlap in the potential pathophysiologic mechanisms of circulating Mg to both supraventricular and ventricular arrhythmias, as described in the previous section. However, specific to ventricular arrhythmias,

intravenous Mg administration may lead to suppression of ventricular ectopic activity.[84] Mg serves as a cofactor to Na/K ATPase, which, during the action potential, acts to aid transport of potassium into cardiac cells. When cellular Mg is deficient, this results in a less efficient Na/K ATPase system and influences the membrane potential.[67,82] As such, it is possible that QT interval prolongation could arise, and could promote abnormal ventricular rhythm.[82]

C. Strengths & limitations

There are strengths and limitations to the present study. First, given the small number of participants with intermittent AF, precision was limited to detect an association (if one truly exists). Second, as this analysis was cross-sectional the temporality of the association is difficult to disentangle, particularly considering the complexity of Mg homeostasis and cardiac electrophysiology. Third, residual confounding is another limitation of the cross-sectional design; it is plausible that those with arrhythmias are sicker and have other confounding characteristics shared by those with low circulating Mg. Relatedly, ECG abnormalities may not be specific to Mg. For example, abnormal Mg homeostasis may coexist with (and/or exacerbate) other electrolyte abnormalities, particularly calcium and potassium, which themselves are known to be involved in cardiac electrophysiology.[68] Fourth, this time frame in late-life may not be the optimal time to characterize associations between Mg and arrhythmias, as opposed to younger adults. Lastly, like virtually all studies of Mg and arrhythmias, we did not measure ionized Mg, which may be the more physiologically relevant form of circulating Mg.[16] Nevertheless, there are important strengths to these findings. Major strengths are the community-based population and the extensive characterization of arrhythmia burden using a novel ECG monitor worn for up to 2 weeks.

D. Conclusions

In conclusion, we found that low serum magnesium was associated with greater PVC burden as measured over 2 weeks of ECG monitoring. We found little evidence of a cross-sectional association between serum Mg and atrial arrhythmias in this elderly community-dwelling population. Future research should test whether serum Mg is associated with subclinical arrhythmia burden in younger adults and further explore the possible Mg-PVC association prospectively.

Table 5.1. Descriptive characteristics by serum magnesium cut-points, the ARIC study, 2016-2017

	Serum magnesium, mmol/L[a]			
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**
N	478	986	1004	45
Serum magnesium, mmol/L[b]	0.70 (0.37-0.74)	0.82 (0.78-0.82)	0.86 (0.86-0.95)	0.99 (0.99-1.11)
Age, y	79.0 ± 4.6	79.2 ± 4.6	79.3 ± 4.7	79.9 ± 4.4
Women	285 (59.6)	5549 (55.7)	581 (57.9)	30 (66.7)
Race				
White	330 (69.0)	734 (74.4)	796 (79.3)	33 (73.3)
Black	148 (31.0)	252 (25.6)	208 (20.7)	12 (26.7)
Educational attainment				
<High school	70 (14.6)	121 (12.3)	116 (11.6)	4 (8.9)
High school or GED	213 (44.5)	390 (39.5)	425 (42.3)	24 (52.7)
>High school	195 (40.9)	475 (48.2)	463 (46.1)	17 (38.4)
Current smoker	35 (7.2)	70 (7.1)	66 (6.6)	5 (11.6)
Current drinking status	213 (44.5)	501 (50.8)	538 (53.6)	22 (48.4)
Ethanol intake, g/week	25.4 ± 39.0	27.9 ± 36.6	28.3 ± 36.0	26.5 ± 32.7
Body mass index, kg/m^2	29.7 ± 5.7	28.3 ± 5.2	27.7 ± 5.0	27.0 ± 4.7
Diabetes	257 (53.8)	209 (21.2)	115 (11.4)	7 (15.6)
Systolic blood pressure, mmHg	136.7 ± 19.7	134.6 ± 18.3	134.7 ± 18.9	134.7 ± 20.1
Diastolic blood pressure, mmHg	67.7 ± 10.6	67.2 ± 10.6	67.2 ± 10.4	61.9 ± 10.5
Antihypertensive medication use	438 (91.7)	747 (75.7)	702 (69.9)	40 (88.9)
Diuretics	177 (37.1)	261 (26.4)	208 (20.7)	15 (33.3)
ACEi/ARB	314 (65.7)	467 (47.4)	392 (39.0)	22 (48.9)
Antiarrhythmic medication use	7 (1.5)	15 (1.5)	20 (2.0)	1 (2.2)
Serum potassium, mmol/L	4.1 ± 0.4	4.1 ± 0.4	4.2 ± 0.4	4.2 ± 0.4
Prevalent coronary heart disease	43 (9.0)	75 (7.6)	79 (7.9)	7 (15.6)
Prevalent heart failure	49 (10.3)	72 (7.3)	65 (6.5)	7 (15.6)
Prevalent stroke	15 (3.1)	40 (4.1)	44 (4.4)	1 (2.2)
PPI medication use	167 (34.9)	253 (25.7)	210 (20.9)	70 (15.6)

Abbreviations: Atherosclerosis Risk in Communities, ARIC; general education development, GED; angiotensin converting enzyme inhibitor / angiotensin receptor blocker, ACEi/ARB; proton pump inhibitor, PPI.

[a] N (%) or mean ± standard deviation except where indicated otherwise

[b] Median (Range)

Table 5.2. Associations of serum magnesium with atrial arrhythmias: the ARIC study, 2016-2017[a]

	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
AF Burden			**OR (95% CI)** [b]		
N	478	986	1004	45	
Continuous AF vs. no AF					
Continuous AF, N	33	46	43	1	
Model 1	1 (Ref)	0.59 (0.37,0.95)	0.53 (0.33,0.85)	-	0.79 (0.74,0.85)
Model 2	1 (Ref)	0.88 (0.68,1.14)	0.80 (0.62,1.04)	-	0.81 (0.65,1.01)
Model 3	1 (Ref)	0.90 (0.69,1.18)	0.89 (0.67,1.18)	-	0.89 (0.69,1.14)
Model 4	1 (Ref)	0.90 (0.68,1.18)	0.90 (0.67,1.19)	-	0.90 (0.70,1.15)
Intermittent AF vs. no AF					
Intermittent AF, N	17	32	32	0	
Model 1	1 (Ref)	0.83 (0.45,1.51)	0.77 (0.42,1.41)	-	0.92 (0.84,1.00)
Model 2	1 (Ref)	0.96 (0.70,1.30)	0.90 (0.66,1.20)	-	0.91 (0.69,1.20)
Model 3	1 (Ref)	0.95 (0.69,1.30)	0.89 (0.64,1.23)	-	0.90 (0.67,1.21)
Model 4	1 (Ref)	0.95 (0.69,1.30)	0.90 (0.65,1.25)	-	0.91 (0.67,1.22)
			Ratio of Geometric Means (95% CI)		
N [b]	400	851	870	42	
Isolated PAC burden					
Geometric Mean	226	234	192	159	
Model 1	1 (Ref)	1.01 (0.82,1.25)	0.84 (0.68,1.02)	0.68 (0.39,1.19)	0.92 (0.90,0.95)
Model 2	1 (Ref)	1.02 (0.84,1.26)	0.84 (0.69,1.04)	0.68 (0.39,1.19)	0.92 (0.84,1.01)
Model 3	1 (Ref)	1.01 (0.81,1.26)	0.84 (0.66,1.04)	0.71 (0.41,1.23)	0.91 (0.84,1.01)
Model 4	1 (Ref)	1.02 (0.82,1.27)	0.84 (0.68,1.06)	0.72 (0.41,1.26)	0.92 (0.84,1.02)
SVT burden					
Geometric Mean	1.9	2.2	2.2	1.9	
Model 1	1 (Ref)	1.11 (1.07,1.14)	1.12 (1.08,1.15)	0.94 (0.87,1.02)	1.04 (1.03,1.05)
Model 2	1 (Ref)	1.11 (1.00,1.21)	1.01 (1.11,1.22)	0.94 (0.73,1.21)	1.04 (1.00,1.08)
Model 3	1 (Ref)	1.05 (0.95,1.16)	1.03 (0.97,1.15)	0.91 (0.71,1.19)	1.01 (0.96,1.05)
Model 4	1 (Ref)	1.05 (0.95,1.16)	1.04 (0.96,1.15)	0.91 (0.71,1.19)	1.01 (0.96,1.05)

Abbreviations: Atherosclerosis Risk in Communities, ARIC; standard deviation, SD; confidence interval, CI; atrial fibrillation, AF; premature atrial contraction,

PAC; supraventricular tachycardia, SVT.
[a] Model 1 = age, sex, race-center
Model 2 = Model 1 + educational attainment, smoking status, ethanol intake, physical activity, body mass index
Model 3 = Model 2 + diabetes, systolic and diastolic blood pressure, antihypertensive medication use
Model 4 = Model 3 + serum potassium
[b] Excluding those with atrial fibrillation

Table 5.3. Associations of serum magnesium with ventricular arrhythmias: the ARIC study, 2016-2017[a]

	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
N	478	986	1004	45	
			OR (95% CI)		
NSVT (yes/no)					
NSVT N	150	311	281	13	
Model 1	1 (Ref)	0.97 (0.77,1.24)	0.83 (0.65,1.06)	0.90 (0.45,1.79)	0.94 (0.84,1.04)
Model 2	1 (Ref)	1.05 (0.86,1.29)	0.90 (0.73,1.11)	0.98 (0.59,1.61)	0.94 (0.85,1.05)
Model 3	1 (Ref)	1.06 (0.86,1.30)	0.92 (0.74,1.13)	1.01 (0.61,1.66)	0.97 (0.86,1.09)
Model 4	1 (Ref)	1.06 (0.86,1.30)	0.92 (0.74,1.13)	1.01 (0.61,1.66)	0.97 (0.86,1.09)
			Ratio of Geometric Means (95% CI)		
Isolated PVC burden					
Geometric Mean	94.6	68.6	64.6	44.3	
Model 1	1 (Ref)	0.68 (0.53,0.87)	0.65 (0.51,0.83)	0.46 (0.23,0.92)	0.82 (0.73,0.91)
Model 2	1 (Ref)	0.69 (0.54,0.89)	0.66 (0.52,0.85)	0.47 (0.24,0.94)	0.83 (0.74,0.92)
Model 3	1 (Ref)	0.67 (0.52,0.87)	0.64 (0.49,0.84)	0.45 (0.23,0.91)	0.81 (0.72,0.90)
Model 4	1 (Ref)	0.68 (0.53,0.87)	0.65 (0.50,0.85)	0.46 (0.23,0.92)	0.81 (0.72,0.91)

Abbreviations: Atherosclerosis Risk in Communities, ARIC; standard deviation, SD; confidence interval, CI; non-sustained ventricular tachycardia, NSVT; premature ventricular contraction, PVC.

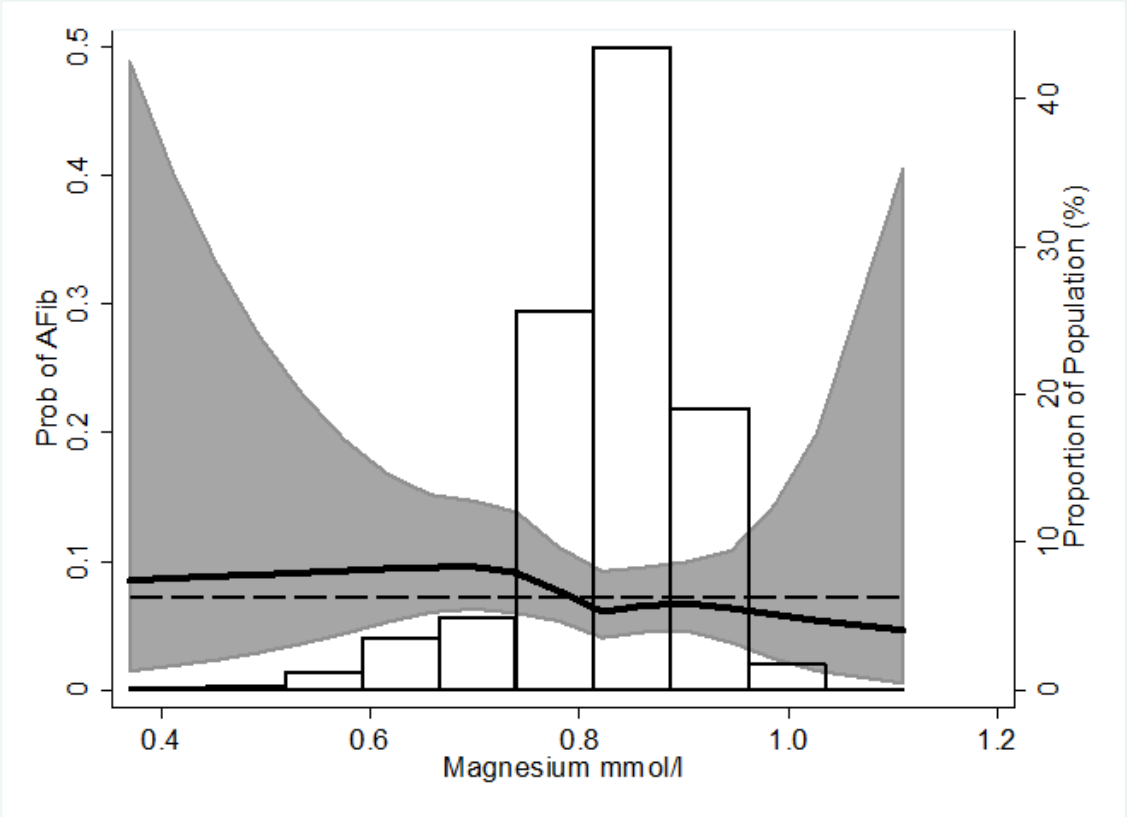
[a] Model 1 = age, sex, race-center

Model 2 = Model 1 + educational attainment, smoking status, ethanol intake, physical activity, body mass index

Model 3 = Model 2 + diabetes, systolic and diastolic blood pressure, antihypertensive medication use

Model 4 = Model 3 + serum potassium

Figure 5.1. Restricted cubic splines for serum magnesium with atrial fibrillation (intermittent or continuous) detected over 2 weeks ambulatory ECG monitoring[a]



[a] Serum magnesium modeled as restricted cubic splines with knots at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles with adjustment for age, race-center, sex. Dashed line reflects average probability of atrial fibrillation equal to 0.07.

Supplemental Table 5.1. Sensitivity analyses for the associations of serum magnesium with atrial arrhythmias, excluding a) anti-arrhythmic, b) ACEi/ARB/diuretic and c) PPI users: the ARIC study, 2016-2017[a]

A) Excluding anti-arrhythmic medication users					
	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
AF Burden	**OR (95% CI)**				
N	467	966	981	44	
Continuous AF vs. no AF					
Continuous AF, N	33	46	42	1	
Model 3	1 (Ref)	0.90 (0.69,1.19)	0.90 (0.67,1.20)	-	0.90 (0.70,1.15)
Intermittent AF vs. no AF					
Intermittent AF, N	16	28	29	0	
Model 3	1 (Ref)	0.96 (0.69,1.32)	0.87 (0.62,1.23)	-	0.89 (0.66,1.21)
	Ratio of Geometric Means (95% CI)				
N [b]	395	845	862	42	
Isolated PAC burden					
Geometric Mean	222	234	193	159	
Model 3	1 (Ref)	1.02 (0.82,1.26)	0.84 (0.68,1.05)	0.71 (0.41,1.25)	0.91 (0.84,1.01)
SVT burden					
Geometric Mean	1.9	2.2	2.2	1.9	
Model 3	1 (Ref)	1.05 (0.95,1.16)	1.03 (0.93,1.15)	0.91 (0.71,1.19)	1.01 (0.96,1.05)
B) Excluding ACEi/ARB and diuretic users					
	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
AF Burden	**OR (95% CI)**				
N	95	382	510	14	
Continuous AF vs. no AF					
Continuous AF, N	6	11	16	1	
Model 3	1 (Ref)	0.75 (0.40,1.39)	0.90 (0.50,1.60)	-	1.06 (0.60,1.85)
Intermittent AF vs. no AF					

Intermittent AF, N	4	12	18	0	
Model 3	1 (Ref)	0.93 (0.53,1.62)	0.96 (0.56,1.65)	-	0.99 (0.58,1.67)
			Ratio of Geometric Means (95% CI)		
N [b]	78	340	446	13	
Isolated PAC burden					
Geometric Mean	203	243	188	218	
Model 3	1 (Ref)	1.08 (0.70,1.68)	0.84 (0.54,1.31)	0.92 (0.34, 2.51)	0.85 (0.71,1.02)
SVT burden					
Geometric Mean	2.0	2.4	2.2	2.2	
Model 3	1 (Ref)	1.01 (0.80,1.27)	0.93 (0.73,1.17)	0.90 (0.55,1.49)	0.95 (0.87,1.04)
C) Excluding PPI users					
			Serum magnesium, mmol/L		
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
AF Burden			**OR (95% CI)**		
N	308	730	791	38	
Continuous AF vs. no AF					
Continuous AF, N	27	36	37	1	
Model 3	1 (Ref)	0.81 (0.59,1.10)	0.84 (0.61,1.15)	-	0.80 (0.59,1.07)
Intermittent AF vs. no AF					
Intermittent AF, N	9	20	26	0	
Model 3	1 (Ref)	0.88 (0.59,1.31)	0.89 (0.60,1.33)	-	0.87 (0.59,1.29)
			Ratio of Geometric Means (95% CI)		
N [b]	255	638	687	36	
Isolated PAC burden					
Geometric Mean	195	238	194	196	
Model 3	1 (Ref)	1.13 (0.87,1.48)	0.91 (0.70,1.20)	0.97 (0.53,1.79)	0.95 (0.85,1.07)
SVT burden					
Geometric Mean	1.9	2.1	2.2	2.0	
Model 3	1 (Ref)	1.09 (0.97,1.22)	1.08 (0.96,1.22)	1.00 (0.76,1.31)	1.02 (0.97,1.07)

[a] Model 3 = age, sex, race-center, educational attainment, smoking status, ethanol intake, body mass index, physical activity, diabetes, systolic and diastolic blood pressure, antihypertensive medication use

[b] Excluding those with atrial fibrillation

Supplemental Table 5.2. Sensitivity analyses for associations of serum magnesium with atrial arrhythmias, excluding those with a history of cardiovascular diseases: the ARIC study, 2016-2017[a]

	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
AF Burden			**OR (95% CI)**		
N	393	829	841	35	
Continuous AF vs. no AF					
Continuous AF, N	22	25	25	0	
Model 3	1 (Ref)	0.75 (0.52,1.08)	0.83 (0.57,1.21)	-	0.76 (0.54,1.07)
Intermittent AF vs. no AF					
Intermittent AF, N	13	26	30	0	
Model 3	1 (Ref)	0.91 (0.64, 1.29)	1.02 (0.72,1.45)	-	1.02 (0.73,1.42)
			Ratio of Geometric Means (95% CI)		
N [b]	344	739	752	34	
Isolated PAC burden					
Geometric Mean	209	235	189	124	
Model 3	1 (Ref)	1.12 (0.89,1.42)	0.90 (0.71,1.15)	0.64 (0.35,1.17)	0.93 (0.84,1.03)
SVT burden					
Geometric Mean	2.0	2.2	2.2	1.9	
Model 3	1 (Ref)	1.06 (0.95,1.19)	1.06 (0.95,1.19)	0.92 (0.70,1.23)	1.01 (0.95,1.06)

[a] Model 3 = age, sex, race-center, educational attainment, smoking status, ethanol intake, body mass index, physical activity, diabetes, systolic and diastolic blood pressure, antihypertensive medication use

[b] Excluding those with atrial fibrillation

Supplemental Table 5.3. Sensitivity analyses for the associations of serum magnesium with ventricular arrhythmias, excluding a) anti-arrhythmic, b) ACEi/ARB and diuretic and c) PPI medication users: the ARIC study, 2016-2017[a]

A) Excluding anti-arrhythmic medication users					
	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
N	467	966	981	44	
	OR (95% CI)				
NSVT (yes/no)					
NSVT, N	143	306	275	13	
Model 3	1 (Ref)	1.05 (0.86,1.29)	0.92 (0.74,1.13)	1.03 (0.62,1.71)	0.97 (0.86,1.09)
	Ratio of Geometric Means (95% CI)				
Isolated PVC burden					
Geometric Mean	94.1	70.0	65.5	46.8	
Model 3	1 (Ref)	0.68 (0.52,0.88)	0.64 (0.49,0.84)	0.48 (0.24,0.96)	0.81 (0.72,0.91)
B) Excluding ACEi/ARB and diuretic users					
	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
N	95	382	510	14	
	OR (95% CI)				
NSVT (yes/no)					
NSVT, N	24	110	131	2	
Model 3	1 (Ref)	1.39 (0.90,2.17)	1.24 (0.80,1.93)	0.50 (0.16,1.58)	0.96 (0.76,1.21)
	Ratio of Geometric Means (95% CI)				
Isolated PVC burden					
Geometric Mean	91.3	64.7	56.1	53.1	
Model 3	1 (Ref)	0.69 (0.40,1.19)	0.59 (0.34,1.00)	0.50 (0.14,1.82)	0.84 (0.67,1.04)

C) Excluding PPI users					
	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
N	308	730	791	38	
	OR (95% CI)				
NSVT (yes/no)					
NSVT, N	93	227	220	12	
Model 3	1 (Ref)	0.99 (0.79,1.25)	0.87 (0.69,1.09)	1.16 (0.68,1.98)	0.95 (0.83,1.10)
	Ratio of Geometric Means (95% CI)				
Isolated PVC burden					
Geometric Mean	91.2	69.4	66.5	44.7	
Model 3	1 (Ref)	0.64 (0.47,0.89)	0.61 (0.44,0.84)	0.44 (0.20,0.94)	0.81 (0.70,0.94)

[a] Model 3 = age, sex, race-center, educational attainment, smoking status, ethanol intake, body mass index, diabetes, physical activity, systolic and diastolic blood pressure, antihypertensive medication use

Supplemental Table 5.4. Sensitivity analyses for associations of serum magnesium with ventricular arrhythmias, excluding those with a history of cardiovascular diseases: the ARIC study, 2016-2017[a]

	Serum magnesium, mmol/L				
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**	**Per 0.1 mmol/L**
N	383	829	841	35	
	OR (95% CI)				
NSVT (yes/no)					
NSVT, N	107	239	214	9	
Model 3	1 (Ref)	1.06 (0.83,1.34)	0.93 (0.73,1.19)	1.06 (0.59,1.92)	0.99 (0.87,1.14)
	Ratio of Geometric Means (95% CI)				
Isolated PVC burden					
Geometric Mean	81.8	59.8	58.0	29.4	
Model 3	1 (Ref)	0.64 (0.49,0.85)	0.66 (0.49,0.88)	0.39 (0.18,0.85)	0.79 (0.70,0.90)

[a] Model 3 = age, sex, race-center, educational attainment, smoking status, ethanol intake, body mass index, physical activity, diabetes, systolic and diastolic blood pressure, antihypertensive medication use

CHAPTER 6 (MANUSCRIPT 3) – PROTON PUMP INHIBITOR USE, HYPOMAGNESEMIA AND RISK OF CARDIOVASCULAR DISEASES: THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

I. OVERVIEW

Introduction: Use of proton pump inhibitors (PPI) has been associated with hypomagnesemia, primarily in case reports or within insurance databases. Both PPI use and low serum magnesium (Mg) have been associated with modestly higher cardiovascular disease (CVD) risk. Yet, the interrelation between PPI use and Mg in relation to CVD risk is unclear.

Objective: To evaluate whether PPI use is cross-sectionally associated with hypomagnesemia and whether hypomagnesemia mediates the prospective association between PPIs and CVD risk in the Atherosclerosis Risk in Communities (ARIC) study.

Methods: The 4,431 ARIC participants without prevalent CVD at visit 5 (baseline, 2011-13) were included. Multivariable relative risk regression was used for cross-sectional analyses between PPI and hypomagnesemia prevalence (≤0.75 mmol/L). Incident CVD (defined by atrial fibrillation, coronary heart disease, CVD mortality, heart failure, stroke) was identified through 2017. Multivariable Cox regression was used to examine the PPI-CVD association.

Results: Participants were mean±SD aged 75±5 years; 63% were women, 23% black, and 24% were PPI users. Cross-sectionally, PPI users had 1.24-fold (95% CI: 1.08-1.44) higher prevalence of hypomagnesemia than non-users. Over a median 5 years of follow-up, 712 incident CVD

events occurred. PPI users had higher CVD risk [HR (95% CI) 1.30 (1.10-1.55))] than non-users. The effect estimate was largely unchanged when hypomagnesemia was added to the model as a potential mediator.

Discussion: In this elderly community-based study, PPI users had a higher prevalence of hypomagnesemia than in non-users. PPI users also had higher CVD risk than non-users; however, it appears unlikely that hypomagnesemia explains associations of PPIs with CVD risk.

II. INTRODUCTION

Proton pump inhibitors (PPIs) are medications used to treat gastroesophageal reflux disease (GERD) and other acid-related disorders, and may be prescribed or purchased over-the-counter.[62] PPIs are approved for short-term use, though are often misused.[148] This class of medication was first introduced into the U.S. marketplace in the late 1980s. Since then, PPI use has increased dramatically. They are among the most widely used medications among American adults.[62] In 2009, an estimated 9% of outpatient visits involved patients who use PPIs.[63]

Since their introduction, there has been concern over PPI-induced hypomagnesemia as evident by numerous case reports and case series,[155,157,160-164] particularly among long-term PPI users. In 2011, the U.S. Food and Drug Administration released a warning regarding potential for PPI-induced hypomagnesemia.[61] While risk factors for PPI-induced hypomagnesemia are not yet well characterized, the elderly and men are thought to have a higher risk of hypomagnesemia due to PPI use than their counterparts.[142] Additionally, drug interactions have been noted. Individuals who take diuretics may have a higher risk of hypomagnesemia compared to those only taking PPIs.[143] It is also unclear how or if PPIs are related to subclinical Mg deficiency, and the public health implications, if this is the case.

PPIs have also been controversially associated with increased risk of CVD outcomes in observational studies.[176-182,186] Additionally, low circulating Mg has been associated with elevated CVD risk.[99] As such, an intriguing possibility exists that hypomagnesemia may link PPI use to CVD outcomes. To our knowledge, whether hypomagnesemia mediates associations between PPI use and elevated CVD risk has not yet been tested.

The two primary aims of this paper were to evaluate 1) whether PPIs are cross-sectionally

associated with hypomagnesemia in a community-based population, and 2) whether low serum Mg mediates the prospective association between PPIs and CVD risk using up to 6 years of longitudinal data in the Atherosclerosis Risk in Communities (ARIC) study. In exploratory analyses, we also assessed whether this association was stronger among diuretic users. H_2-blockers are medications with similar indications as PPIs and no known link with hypomagnesemia or with cardiac toxicity. As a "negative control", we repeated the analyses using H_2-blockers instead of PPIs. We hypothesized that H_2-blockers are not associated with hypomagnesemia and that hypomagnesemia does not mediate the null relationship between H_2-blocker use and CVD risk.

III. METHODS

A. Study design

The ARIC study[195] is an ongoing longitudinal cohort study which includes white and black men and women who were aged 45-64 years at baseline in 1987-1989 (n=15,792) and were recruited from 4 US communities (Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; Washington County, MD). Several clinic visits have occurred since then. A total of, 6,538 participants attended visit 5, which occurred in 2011-2013 and serves as baseline for this analysis. To obtain information on CVD events, follow-up phone calls to participants (twice annually since 2012) has occurred as has continuous surveillance of hospitals in ARIC communities and linkage to vital statistics.

Of the 6,538 participants who attended visit 5, we excluded participants with prevalent CVD at visit 5 (n=1,837), missing information needed to adjudicate prevalent or incident CVD (n=171), or missing serum Mg measurements (n=56). Additionally, due to small numbers, we

excluded participants who were neither black nor white (n=18) and blacks at the MN and MD centers (n=25). This resulted in a final analytic sample of 4,431 participants.

1. *Exposure and covariate data collection*

At visit 5, participants were interviewed, answered questionnaires, underwent anthropomorphic measurements and provided fasting (>8 hour) blood samples. Participants self-reported their smoking habits and alcohol intake. Participants were asked to bring the containers of all prescription and over-the-counter medications (including dietary supplements) used during the previous 2 weeks to the visit. Information on these containers was transcribed and coded. Physical activity was quantified as a continuous sports index based on the validated Baecke questionnaire, with possible scores of 1 (low) to 5 (high).[197] Body mass index (BMI) was derived based on measured weight (kg) divided by height squared (m^2). Systolic blood pressure was measured 3 times after 5 minutes of rest; the average of the second and third measurements was used.

Serum Mg was measured using colorimetric methods on the Roche Cobas 6000 Chemistry Analyzer at visit 5 (Roche Diagnostics; Indianapolis, Indiana). The laboratory intra-assay coefficient of variation (CV) based on blind duplicate samples for serum Mg was 1.9%. Serum glucose was analyzed using a hexokinase assay (Roche Cobas 6000 Chemistry Analyzer). Diabetes was defined by a fasting glucose ≥126 mg/dL, non-fasting glucose ≥200 mg/dL, self-reported physician diagnosis or use of diabetes medication. High-density lipoprotein (HDL) cholesterol was measured at visit 5 using colorimetric methods (Beckman Coulter Olympus AU400®), and total cholesterol using enzymatic methods (Beckman Coulter Olympus AU400e®). Serum creatinine was quantified using the Jaffe method (soon after visit 5), and cystatin C was estimated using the Gentian cystatin C reagent (analyzed in 2012-13 from

previously frozen visit 5 samples). Both creatinine and cystatin C were used to determine estimated glomerular filtration rate (eGFR) based on the Chronic Kidney Epidemiology Collaboration formula.[219]

2. *Outcome ascertainment*

We defined incident CVD events by: incident CHD, heart failure (HF), stroke, AF and CVD mortality through December 31, 2017. Both the composite and individual outcomes were analyzed. Definitions of CVD events have been described previously. Briefly, potential CVD events were identified by (1) recent hospitalizations identified during follow-up phone calls to participants; (2) ongoing surveillance of community hospital discharge lists and death certificates; and (3) linkage to State and National Death Indices. International Classification of Disease (ICD) codes were recorded from all hospitalizations. Any possible coronary deaths occurring out-of-hospital were investigated through physician questionnaires and next-of-kin interviews. Then, possible CHD events were abstracted onto standardized forms, and adjudicated by physician review. CHD events were defined as definite or probable myocardial infarction (MI) or definite fatal CHD based on ARIC criteria or coronary revascularization. Fatal CHD was determined by history of CHD or chest pain with consistent underlying cause of death or no non-cardiac cause.[211] Heart failure was identified by hospitalization with an ICD-9 code of 428 or death with ICD-9 428 or ICD-10 I50 as the underlying cause; outpatient HF has also been captured using previously described criteria.[212] Stroke was classified based on a computer algorithm and physician review using National Survey of Stroke criteria.[213] Incident AF was based on a hospital discharge diagnosis for atrial fibrillation or flutter.[210] CVD mortality was defined by deaths with an underlying cause in ICD-9 codes 390 to 459 or ICD-10 codes I00 to I99. Prevalent CVD was defined by a history of CHD, heart failure, stroke or AF based on ARIC ascertainment on or

before the participants' visit 5 clinic date.

B. Statistical analysis

Visit 5 serves as baseline for the present analysis. All covariates were assessed at visit 5 except educational attainment (visit 1). We present baseline characteristics, including mean serum Mg concentrations, stratified by PPI use at visit 5. PPI use was modeled as a binary variable based on use in the 2 weeks prior to the ARIC clinic visit. Two-tailed p-values of 0.05 were used for cut-offs of statistical significance. SAS 9.4 (SAS Institute Inc.; Cary, NC) was used to conduct these analyses.

1. *Cross-sectional Association of PPI use and Hypomagnesemia*

We used relative risk regression (generalized linear model with log link, model-based standard errors) to compare the adjusted prevalence of hypomagnesemia in PPI users versus non-users. Hypomagnesemia was modeled as <0.75 mmol/L vs 0.75-0.95 mmol/L; participants with clinical hypermagnesemia >0.95 mmol/L were not included due to small numbers (n=95). In model 1, we adjusted for age, race-center and sex. In model 2, we additionally adjusted for education (<high school, high school or GED, >high school); smoking status (current, former, never, unknown); drinking status (current, former, never, unknown); physical activity sports index (continuous), and obesity (≥30 kg/m^2 vs <30 kg/m^2). Also, as a "negative control", we compared the adjusted prevalence of hypomagnesemia among H_2-blocker users versus non-users, using similar models.

2. *Prospective Association of PPI use and Incident CVD*

Multivariable Cox proportional hazards regression (model-based standard errors) was

used to examine the association between PPI use and incident CVD composite and individual endpoints (incident CHD, heart failure, stroke, AF and CVD mortality) through December 31, 2017. Person-years were calculated from baseline (date of visit 5 participation) to the first outcome of interest, death, loss-to-follow-up or the end of 2017, whichever came first.

We used a series of models to adjust for potential confounders and the hypothesized mediator (serum Mg). In model 1, we adjusted for age, race-center and sex. In model 2, we additionally adjusted for education (<high school, high school or GED, >high school); smoking status (current, former, never, unknown); drinking status (current, former, never, unknown); physical activity sports index (continuous), obesity (≥30 kg/m^2 vs <30 kg/m^2). In model 3, we further adjusted for established CVD risk factors: diabetes, systolic blood pressure, antihypertension medication use, lipid-lowering medication use, HDL-c, total cholesterol, eGFR clinical categories (≥90, 60-<90, <60 mL·min^{-1}·1.73 m^{-2}). Model 4 additionally included serum Mg (modeled categorically as hypomagnesemia vs not using the clinical cut-point of <0.75 mmol/L[17]). After including serum Mg (the hypothesized mediator) in the model, we qualitatively inspected for attenuation of the PPI-CVD association, and quantitatively estimated the proportion of the association mediated by hypomagnesemia.[220] There was no statistical evidence of an exposure-mediator interaction (p-value=0.20).

To test the robustness of our findings, we conducted sensitivity analyses including: 1) using H_2-blockers as an active comparator to test the specificity of the association,[221] and 2) matching PPI users and non-users using propensity scores to help balance potential confounding characteristics.[222] Propensity scores were generated based on all covariates included in Model 3 (age, race-center, sex, education, smoking status, drinking status, physical activity, obesity, diabetes, systolic blood pressure, antihypertension medication use, lipid-lowering medication use,

HDL, total cholesterol, eGFR).

We tested for multiplicative interactions with diuretic use, race and sex by including a cross-product term [e.g. PPI*diuretic] in the model. Additive interactions were tested by calculating the relative excess risk due to interaction (RERI) using model-based differences in probability, and 95% confidence intervals.[223] As we may not have sufficient power to examine interactions, we interpret these findings as exploratory in nature.

IV. RESULTS

A. Cross-sectional association of PPI use and hypomagnesemia

The 4,431 ARIC participants in this analysis were mean aged 75.2±5.1 years, 63.2% were women and 23.2% were black. At visit 5, 24.1% of participants were PPI users and 5.6% were H_2-blocker users. A small proportion were on both PPI and H_2-blockers at visit 5 (1.0%; n=44). Mean serum Mg (expressed as mmol/L) was 0.81±0.09 and 0.83±0.08 among PPI users and non-users, respectively. Specifically, serum Mg was 0.02 (95% CI: 0.01, 0.02) lower among PPI users than non-users. Overall, PPI users tended to be female, obese, taking other medications (lipid and blood pressure lowering medications), have poorer kidney function and have a higher prevalence of clinical hypomagnesemia (**Table 6.1**). Among H_2-blocker users serum Mg was 0.82±0.08 and among non-users was 0.83±0.08 [mean difference = 0.01 (95% CI: -0.00, 0.02)].

Table 6.2 shows the prevalence of clinical hypomagnesemia by PPI and H_2-blocker use status. After adjusting for demographic characteristics, PPI users had a 1.37-fold (95% CI: 1.17-1.55) higher prevalence of clinical hypomagnesemia than non-users. The association remained after additional adjustment for educational attainment and health behaviors [1.24 (1.08-1.44)]. No

statistically significant associations were found between H_2-blocker use and clinical hypomagnesemia, although the direction and magnitude of association [PR=1.19 (0.93-1.53)] was similar to that observed for PPI use.

B. Prospective association of PPI use and incident CVD

Over a median 5.6 years of follow-up, 712 incident cardiovascular events occurred (CHD n=112; heart failure n=285; stroke n=121; AF n=376; CVD mortality n=121). **Table 6.3** presents adjusted HRs (95% CIs) for the association of PPI use with incident CVD as well as the individual endpoints (AF, CHD, HF, CVD mortality, stroke). With adjustment for demographic characteristics, PPI users were at higher risk of CVD [1.38 (1.18-1.63)] as compared to non-users. The association was slightly attenuated with further adjustment for educational attainment and health behaviors [1.32 (1.11-1.55)] and established CVD risk factors [1.29 (1.09-1.54)]. Clinical hypomagnesemia did not substantially mediate the association between PPI use and elevated CVD risk (proportion mediated = -0.8%, total effect = 1.30, controlled direct effect = 1.29).

We tested whether the PPI and CVD composite association differed by diuretic use, sex or race on both the additive and multiplicative scales. For diuretic use, there was statistical evidence to suggest effect measure modification on the additive scale [RERI (95% CI) 0.13 (0.05-0.22)] but not on the multiplicative scale (p-interaction=0.15).As shown in **Supplemental Table 6.1**, the association between PPI use and incident CVD was somewhat stronger among diuretic users [HR (95% CI) 1.59 (1.17-2.16)] than nonusers [1.21 (0.98-1.50)]. When clinical hypomagnesemia was added to Model 3, results were largely unchanged among both diuretic users and non-users. There was no statistical evidence of effect measure modification by sex or race on either the additive scale or the multiplicative scale.

Precision was poor when evaluating the CVD endpoints individually, and PPI use was not consistently statistically significantly associated with the individual CVD endpoints. PPI use was associated with higher risk of AF with adjustment for demographic and lifestyle behaviors, but not after adjustment for traditional CVD risk factors. For CVD mortality and heart failure, PPI use was associated with elevated risk in model 1, but not after multivariable adjustment.

Participants with clinical hypomagnesemia (<0.75 mmol/L) had a higher risk of CVD after demographic adjustment [1.44 (1.18-1.76)] compared to those with concentrations between 0.85-0.95 mmol/L. The association was attenuated with further adjustment [Model 2 1.25 (1.01-1.56); Model 3 1.20 (0.95-1.50)]. For individual CVD endpoints, clinical hypomagnesemia was associated with higher risk of AF, CHD, CVD mortality and heart failure with demographic adjustment. The statistical association between hypomagnesemia and heart failure persisted after multivariable adjustment (**Supplemental Table 6.2**). CVD risk was similar among those with concentrations between 0.75-0.85 mmol/L to those with 0.85-0.95 mmol/L. Additionally, participants with hypermagnesemia (>0.95 mmol/L) had higher risk of CVD than those with concentrations between 0.85-0.95 mmol/L.

We conducted several sensitivity analyses. Of note, we tested whether H_2-blockers (a similar drug with no known cardiac toxicity) were associated with incident CVD, as a negative control. As shown in **Supplemental Table 6.3**, we detected no association between H_2-blocker use and incident CVD. Results were also largely similar when we matched PPI users and non-users using propensity scores to help balance potential confounding characteristics (**Supplemental Table 6.4**).

V. DISCUSSION

In this community-based study of elderly individuals, serum Mg was lower among PPI users than among non-users, however H_2-blocker user status was not significantly associated with serum Mg concentrations. Additionally, PPI use was associated with higher CVD risk. Counter to our hypothesis, low serum Mg concentrations did not explain the association of PPI use with elevated risk of CVD in these data.

A. PPI use and hypomagnesemia

In 2011, the U.S. FDA released a safety communication regarding the potential for PPIs to lead to reductions in circulating magnesium.[61] Case reports have tended to be written about individuals who did not adhere to label instructions and either took higher doses than instructed or took PPIs for extended periods. Yet, a modeling study indicated that even short-term PPI use, which would coincide with recommended intake for most, can diminish rates of intestinal absorption of Mg.[174]

PPI-induced clinical hypomagnesemia is a potentially serious side effect albeit relatively rare. A case-control performed within Ontario-based health care databases reported that among those hospitalized for hypomagnesemia, the odds of being a PPI user was 1.43 (1.06-1.93) compared to those hospitalized for reasons other than hypomagnesemia.[167] When stratified by diuretic use, the PPI-hypomagnesemia association was present only among diuretic users. This study also used H_2-blockers as a negative control; H_2-blocker use was not associated with hospitalization involving hypomagnesemia.[167] In a meta-analysis of 9 generally small observational studies (3 cohort, 1 case-control, 5 cross-sectional), the pooled RR (95% CI) for hypomagnesemia (cut-points were generally $\leq$0.7 mmol/L) was 1.43 (1.08-1.88) for PPI users compared non-users.[165] Since that meta-analysis, the population-based Rotterdam Study examined PPI use and hypomagnesemia cross-sectionally, and reported that serum Mg

concentrations were 0.01 mmol/L (95% CI: 0.02, 0.01 mmol/L) lower in PPI users as compared to non-users (though this difference may not be of clinical significance). PPI use was more strongly associated with hypomagnesemia among diuretic users than in non-diuretic users.[159] In the present analysis, serum Mg concentrations were 0.02 mmol/L (95% CI: 0.02, 0.01 mmol/L) lower among PPI users than non-users. In contrast to our findings, they found that users of H_2-blockers also had lower serum Mg as well as with higher odds of hypomagnesemia in comparison with those who do not use H_2-blockers.[159] This may be indicative of confounding and other biases inherent studying medication effects in observational settings.

Mechanisms by which PPIs may induce hypomagnesemia are an area of active scientific inquiry. PPI-induced hypomagnesemia is thought to arise from reductions in the absorption of dietary Mg within the intestines.[170,171] PPIs influence the enzyme, H+/K+ ATPase, which pumps a hydrogen ion into the stomach in exchange for a potassium ion. This exchange results in reductions in gastrointestinal acidity. To date, much of the mechanistic research has focused on the active Mg transport channel, TRPM6, which is a pH-dependent channel (i.e. TRPM6 activity decreases in less acidic environments). When considered together with the concept that the gene for TRPM6 should be over-expressed during times of Mg insufficiency, this suggests that certain genetic profiles may help promote continued Mg absorption in the presence of PPI use. Yet, this hypothesis has yet to be tested in large-scale human studies and the identification of potential risk factors for developing PPI-induced hypomagnesemia are ongoing areas of research.

B. PPI use, hypomagnesemia and incident CVD

PPIs have been inconsistently associated with increased CVD risk in several prospective studies. In a meta-analysis of RCTs which included incident CVD endpoints, PPI users had a higher risk of CVD RR=1.70 (1.13-2.56) when compared to non-users. In observational settings,

PPI users versus non-users have a similar modestly higher risk of stroke[177-179] and MI.[180-182] Observational studies examining the PPI and CVD association have generally been conducted within insurance databases, which are based on data collected during routine clinical encounters. A limitation of these studies is that circulating Mg is not routinely measured. One study, within the Taiwan National Health Insurance Research Database used propensity scores to match PPI users to non-users, and found that PPI users had a 1.58-fold higher risk of MI (1.11-2.25) than non-users.[181] The relationship between PPI use and CVD risk has also been characterized among individuals with GERD—the primary indication for PPI use. In populations with previously diagnosed GERD, PPI use was associated with a higher risk of AF[186] and CHD[176] as compared to nonuse.

A cross-sectional analysis within the National Health and Nutrition Examination Survey (NHANES), which has extensive data measurements on confounding characteristics, reported that PPI users tended to have slightly higher LDL cholesterol and apolipoprotein B than those who do not use PPIs, but no differences were found in total cholesterol, HDL cholesterol or triglycerides.[224] One cross-sectional study has examined PPIs in relation to serum Mg and arrhythmias. In this study of 421 intensive care or critical care unit patients with a MI or unstable angina diagnosis, patients administered PPIs soon after hospital admission tended to have lower serum Mg concentrations and a greater prevalence of cardiac arrhythmias compared to those not exposed to PPIs. This study did not collect data on diuretic use.[189]

No prospective studies, to date, have tested the hypothesis that hypomagnesemia underlies the observation that PPI use is associated with CVD. This mechanism is plausible considering that PPIs can induce hypomagnesemia, and low circulating Mg has been associated with greater risk of CVD.[99] Low Mg may exert adverse cardiovascular effects through poor CVD

risk factors, such as elevated blood pressure, chronic inflammation and hyperglycemia.[17,225] Mg also plays important roles in cardiovascular processes, such as in platelet formation,[1,118] vascular smooth muscle tone, and endothelial function.[1,116] Reductions in Mg (in this case arising due to PPI use) could thereby contribute to adverse vascular effects such as atherosclerosis and as a result CVD. Relatedly, Mg plays important roles in maintaining myocardial excitability, as Mg serves as cofactor of the sodium-potassium ATP pump.[190] Alterations in Mg homeostasis (via PPI-induced hypomagnesemia) could adversely influence electrophysiology and lead to arrhythmogenesis.[191] However our findings do not provide evidence that low Mg mediates the associations between PPI use and CVD.

Basic science studies also suggest other plausible mechanisms relating PPIs to CVDs beyond magnesium. For example, in *ex vivo* human tissue, PPIs may accelerate endothelial senescence, telomere erosion[192] and inhibit the cardiac enzyme, dimethylaminohydrolase (DDAH), which helps mitigate oxidative stress.[193] In mice, administration of PPIs led to increases in asymmetrical dimethylarginine (ADMA), which is considered an inhibitor of the vasodilator, nitric oxide. Elevated ADMA concentrations have been associated with higher CVD risk. [193]

C. Strengths and limitations

There are limitations to this analysis. First, our study was conducted among elderly individuals, and it is possible that this was not the optimal age-group in which to test this hypothesis. Our design was driven by, in ARIC, the availability of serum Mg concentrations following the widespread use of PPIs. Second, precision of the analyses of PPI use and the composite CVD outcome was modest, and precision was poor for analyses of the individual CVD endpoints. Additionally, H_2-blockers had a lower prevalence of use resulting in lower precision

for this sensitivity analysis. Third, misclassification of exposure may occur as PPIs are intended to be used on a short-term basis (e.g. 2-4 weeks at a time). Our modeling of PPIs was based on reported use in the past 2 weeks. Participants may stop or start using PPI over the follow-up period, and many may use PPIs chronically[148]—potentially elevating their risk of hypomagnesemia.[61] Nevertheless, this analysis has important strengths. The ARIC study was uniquely suited to address this research question with a prospective design and wealth of high-quality measurements. Considering the pervasiveness of PPI use and their implication with adverse outcomes, it is important to understand how or if their use may affect cardiovascular health.

D. Conclusion

In this community-based population of elderly individuals, PPI use was cross-sectionally associated with lower serum Mg and was prospectively associated with higher risk of CVD. We found little evidence that hypomagnesemia mediates the association between PPI use and CVD risk. Future research might explore risk factors and mechanisms for hypomagnesemia among PPI users.

Table 6.1. Unadjusted baseline characteristics by proton pump inhibitor (PPI) use: the ARIC study, 2011-2013, N=4431 [a]

	PPI user status	
Characteristic	**Yes**	**No**
N	1066	3365
Age, y	75.3 ± 5.1	75.2 ± 5.1
Gender		
Women	716 (67.2)	2084 (61.9)
Men	350 (32.8)	1281 (38.1)
Race		
White	850 (79.7)	2554 (75.9)
Black	216 (20.3)	811 (24.1)
Education		
> High School	423 (39.7)	1607 (47.8)
High School or GED	498 (46.7)	1345 (40.0)
< High School	144 (13.5)	409 (12.2)
Diabetes	324 (30.4)	916 (27.2)
Body mass index, kg/m^2	29.5 ± 5.5	28.2 ± 5.5
Obese >30 kg/m^2	404 (37.9)	1043 (31.0)
Overweight 25.0-29.9 kg/m^2	424 (39.8)	1283 (38.1)
Normal 18.5-25.0 kg/m^2	184 (17.3)	892 (26.5)
Underweight <18.5 kg/m^2	12 (1.1)	83 (2.5)
Smoking status		
Current	43 (4.0)	201 (6.0)
Former	478 (44.8)	1482 (44.0)
Never	426 (40.0)	1343 (39.9)
Unknown	119 (11.2)	339 (10.1)
Drinking status		
Current	462 (43.3)	1668 (49.6)
Former	302 (28.3)	870 (25.9)
Never	238 (22.3)	680 (20.2)
Unknown	64 (6.0)	147 (4.4)
Physical activity score	2.5 ± 0.8	2.7 ± 0.8
Systolic blood pressure, mmHg	131.0 ± 17.1	130.3 ± 17.8
Antihypertension medication use	828 (77.7)	2241 (66.6)
HDL cholesterol, mg/dL	51.9 ± 13.4	53.9 ± 14.2
LDL cholesterol, mg/dL	105.8 ± 33.0	109.8 ± 33.9
Total cholesterol, mg/dL	183.5 ± 40.8	188.3 ± 40.8
Lipid-lowering medication use	626 (58.7)	1568 (46.6)
eGFR mL/min/1.73m^2	64.9 ± 18.1	68.4 ± 17.1
>90 mL/min/1.73m^2	82 (7.7)	318 (9.5)
60-90 mL/min/1.73m^2	565 (53.0)	2021 (60.1)
<60 mL/min/1.73m^2	419 (39.3)	1026 (30.5)
H_2-blocker medication use	44 (4.1)	205 (6.1)
Diuretic use	310 (29.1)	847 (25.2)
Serum magnesium, mmol/L	0.81 ± 0.09	0.83 ± 0.08
Clinical hypomagnesemia [b]	225 (21.1)	525 (15.6)
Subclinical hypomagnesemia	396 (37.2)	1258 (37.4)

[a] Data presented as n (%) or mean ± standard deviation

[b] Clinical hypomagnesemia, <0.75 mmol/L; Subclinical hypomagnesemia-0.75-<0.85 mmol/L

Table 6.2. Prevalence ratios (95% CI) for proton pump inhibitor and H_2-blocker use in relation to clinical hypomagnesemia using relative risk regression: the ARIC Study, 2011-2013, N=4336

	PPI user	
	No	**Yes**
N	3284	1052
Clinical hypomagnesemia, N (%)	525 (16.0)	225 (21.4)
Model 1[a,b]	1 (ref)	1.37 (1.17-1.55)
Model 2	1 (ref)	1.24 (1.08-1.44)
	H_2-blocker user	
	No	**Yes**
N	4092	244
Clinical hypomagnesemia, N (%)	701 (17.1)	49 (20.1)
Model 1[a,b]	1 (ref)	1.18 (0.91-1.52)
Model 2	1 (ref)	1.19 (0.93-1.53)

[a] Hypomagnesemia (<0.75 mmol/L vs 0.75-0.95 mmol/L); participants with clinical hypermagnesemia >0.95 mmol/L not included due to small numbers

[b] Model 1 = age, race-center, sex

Model 2 = Model 1 + education, smoking status, drinking status, physical activity index score, obesity

Table 6.3. Hazard ratios (95% CI) for proton pump inhibitor use and cardiovascular disease risk: the ARIC study, 2011-2017, N=4431

	PPI user status		Proportion mediated by hypomagnesemia[b]
	No	Yes	
N	3365	1066	
CVD composite			
# events	502	210	
Model 1	1 (Ref)	1.38 (1.18-1.63)	--
Model 2	1 (Ref)	1.32 (1.11-1.57)	--
Model 3	1 (Ref)	1.30 (1.10-1.55)	--
Model 4	1 (Ref)	1.29 (1.09-1.54)	-0.8%
Individual CVD endpoints			
Atrial fibrillation			
# events	269	107	
Model 1	1 (Ref)	1.25 (1.00-1.57)	--
Model 2	1 (Ref)	1.29 (1.02-1.63)	--
Model 3	1 (Ref)	1.25 (0.99-1.59)	--
Model 4	1 (Ref)	1.24 (0.98-1.58)	-0.9%
Coronary heart disease			
# events	79	33	
Model 1	1 (Ref)	1.36 (0.90-2.05)	--
Model 2	1 (Ref)	1.24 (0.80-1.91)	--
Model 3	1 (Ref)	1.19 (0.76-1.84)	--
Model 4	1 (Ref)	1.17 (0.75-1.82)	-1.0%
CVD mortality			
# events	82	39	
Model 1	1 (Ref)	1.49 (1.01-2.14)	--
Model 2	1 (Ref)	1.31 (0.84-2.02)	--
Model 3	1 (Ref)	1.36 (0.87-2.12)	--
Model 4	1 (Ref)	1.34 (0.86-2.09)	-2.4%
Heart failure			
# events	199	86	
Model 1	1 (Ref)	1.36 (1.06-1.76)	--
Model 2	1 (Ref)	1.30 (1.01-1.68)	--
Model 3	1 (Ref)	1.21 (0.91-1.61)	--
Model 4	1 (Ref)	1.19 (0.90-1.58)	2.0%
Stroke			
# events	94	27	
Model 1	1 (Ref)	0.93 (0.60-1.42)	--
Model 2	1 (Ref)	0.95 (0.61-1.48)	--
Model 3	1 (Ref)	0.93 (0.60-1.45)	--
Model 4	1 (Ref)	0.92 (0.59-1.44)	-0.8%

[a] Model 1 = age, race-center, sex
Model 2 = Model 1 + education, smoking status, drinking status, physical activity, obesity
Model 3 = Model 2 + diabetes, systolic blood pressure, antihypertension medication use, lipid-lowering medication use, HDL, total cholesterol, eGFR
Model 4 = Model 3 + clinical hypomagnesemia
[b] Proportion of the total effect of PPI use on CVD endpoint that can be explained through the indirect effect, hypomagnesemia; $[RR_{NDE}*(RR_{NIE}-1)/(RR_{NDE}-RR_{NIE}-1)]$

Supplemental Table 6.1. Hazard ratios (95% CI) for proton pump inhibitor use and cardiovascular disease risk, stratifying by diuretic use: the ARIC study, 2011-2017, N=4431

	PPI user status	
Diuretic users	**No**	**Yes**
N	847	310
CVD composite		
# events	157	79
Model 1 [a]	1 (Ref)	1.63 (1.24-2.14)
Model 2	1 (Ref)	1.49 (1.11-2.01)
Model 3	1 (Ref)	1.59 (1.17-2.16)
Model 4	1 (Ref)	1.59 (1.17-2.16)
	PPI user status	
Not Diuretic users	**No**	**Yes**
N	2518	756
CVD composite		
# events	345	131
Model 1 [a]	1 (Ref)	1.25 (1.03-1.54)
Model 2	1 (Ref)	1.23 (1.00-1.52)
Model 3	1 (Ref)	1.21 (0.98-1.50)
Model 4	1 (Ref)	1.19 (0.96-1.48)

[a] Model 1 = age, race-center, sex
Model 2 = Model 1 + education, smoking status, drinking status, physical activity, obesity
Model 3 = Model 2 + diabetes, systolic blood pressure, antihypertension medication use, lipid-lowering medication use, HDL, total cholesterol, eGFR
Model 4 = Model 3 + clinical hypomagnesemia

Supplemental Table 6.2. Hazard ratios (95% CI) for serum magnesium clinical cut-point categories and cardiovascular disease risk: the ARIC study, 2011-2017, N=4431

	Serum Magnesium, mmol/L			
	<0.75	**0.75-0.85**	**0.85-0.95**	**>0.95**
N	750	1654	1932	95
CVD composite				
# events	151	250	291	20
Model 1 [a]	1.44 (1.18-1.76)	1.02 (0.86-1.21)	1 (Ref)	2.12 (1.35-3.34)
Model 2	1.25 (1.01-1.56)	1.01 (0.84-1.21)	1 (Ref)	1.97 (1.21-3.20)
Model 3	1.20 (0.95-1.50)	0.97 (0.81-1.16)	1 (Ref)	1.89 (1.15-3.10)
Individual CVD endpoints				
Atrial fibrillation				
# events	74	131	163	8
Model 1	1.32 (1.00-1.74)	0.97 (0.77-1.22)	1 (Ref)	1.06 (0.52-2.16)
Model 2	1.21 (0.90-1.62)	0.97 (0.76-1.23)	1 (Ref)	1.16 (0.57-2.36)
Model 3	1.20 (0.88-1.64)	0.96 (0.75-1.22)	1 (Ref)	1.13 (0.55-2.31)
Coronary heart disease				
# events	25	37	46	4
Model 1	1.63 (1.00-2.67)	0.99 (0.64-1.53)	1 (Ref)	1.50 (0.47-4.83)
Model 2	1.49 (0.88-2.52)	1.01 (0.64-1.59)	1 (Ref)	1.71 (0.53-5.55)
Model 3	1.23 (0.69-2.18)	0.90 (0.56-1.45)	1 (Ref)	1.74 (0.54-5.66)
CVD mortality				
# events	30	40	48	3
Model 1	1.66 (1.04-2.63)	0.95 (0.62-1.45)	1 (Ref)	1.39 (0.43-4.36)
Model 2	1.49 (0.88-2.52)	1.01 (0.64-1.59)	1 (Ref)	1.71 (0.53-5.55)
Model 3	1.23 (0.69-2.18)	0.90 (0.56-1.45)	1 (Ref)	1.74 (0.54-5.66)
Heart failure				
# events	82	97	97	9
Model 1	2.24 (1.66-3.02)	1.16 (0.87-1.54)	1 (Ref)	2.07 (1.66-3.02)
Model 2	1.81 (1.30-2.51)	1.10 (0.81-1.50)	1 (Ref)	1.81 (0.84-3.93)
Model 3	1.57 (1.10-2.24)	1.02 (0.74-1.40)	1 (Ref)	1.70 (0.78-3.69)
Stroke				
# events	29	38	50	4
Model 1	1.44 (0.91-2.30)	0.87 (0.57-1.33)	1 (Ref)	1.81 (0.65-5.02)
Model 2	1.23 (0.75-2.01)	0.80 (0.52-1.25)	1 (Ref)	1.89 (0.68-5.26)
Model 3	1.16 (0.68-1.97)	0.77 (0.49-1.22)	1 (Ref)	1.96 (0.70-5.47)

[a] Model 1 = age, race-center, sex
Model 2 = Model 1 + education, smoking status, drinking status, physical activity, obesity
Model 3 = Model 2 + diabetes, systolic blood pressure, antihypertension medication use, lipid-lowering medication use, HDL, total cholesterol, eGFR

Supplemental Table 6.3. Hazard ratios (95% CI) for H2-blocker use and cardiovascular disease risk: the ARIC study, 2011-2017, N=4431 [a]

	H_2-blocker user status		Proportion mediated by hypomagnesemia[b]
	No	Yes	
N	4182	249	
CVD composite			
# events	666	46	
Model 1	1 (Ref)	1.16 (0.86-1.57)	--
Model 2	1 (Ref)	1.15 (0.84-1.55)	--
Model 3	1 (Ref)	1.10 (0.81-1.51)	--
Model 4	1 (Ref)	1.10 (0.80-1.50)	-0.4%
Individual CVD endpoints			
Atrial fibrillation			
# events	359	17	
Model 1	1 (Ref)	0.80 (0.49-1.29)	--
Model 2	1 (Ref)	0.78 (0.48-1.27)	--
Model 3	1 (Ref)	0.78 (0.48-1.28)	--
Model 4	1 (Ref)	0.78 (0.48-1.28)	-0.2%
Coronary heart disease			
# events	104	8	
Model 1	1 (Ref)	1.37 (0.67-2.82)	--
Model 2	1 (Ref)	1.41 (0.68-2.91)	--
Model 3	1 (Ref)	1.37 (0.66-2.84)	--
Model 4	1 (Ref)	1.37 (0.66-2.85)	-1.0%
CVD mortality			
# events	109	12	
Model 1	1 (Ref)	1.94 (1.07, 3.52)	--
Model 2	1 (Ref)	2.26 (1.23, 4.14)	--
Model 3	1 (Ref)	2.16 (1.14, 4.08)	--
Model 4	1 (Ref)	2.17 (1.14, 4.11)	9.9%
Heart failure			
# events	270	15	
Model 1	1 (Ref)	0.96 (0.57-1.61)	--
Model 2	1 (Ref)	0.90 (0.53-1.55)	--
Model 3	1 (Ref)	0.73 (0.41-1.31)	--
Model 4	1 (Ref)	0.72 (0.40-1.29)	-0.5%
Stroke			
# events	113	8	
Model 1	1 (Ref)	1.18 (0.57-2.42)	--
Model 2	1 (Ref)	1.14 (0.55-2.36)	--
Model 3	1 (Ref)	1.14 (0.55-2.36)	--
Model 4	1 (Ref)	1.14 (0.55-2.34)	-0.7%

[a] Model 1 = age, race-center, sex
Model 2 = Model 1 + education, smoking status, drinking status, physical activity, obesity
Model 3 = Model 2 + diabetes, systolic blood pressure, antihypertension medication use, lipid-lowering medication use, HDL, total cholesterol, eGFR
Model 4 = Model 3 + clinical hypomagnesemia
[b] Proportion of the total effect of H_2-blocker use on CVD endpoint that can be explained though the indirect effect, hypomagnesemia; $[RR_{NDE}*(RR_{NIE}-1)/(RR_{NDE}-RR_{NIE}-1)]$

Supplemental Table 6.4. Hazard ratios (95% CI) for proton pump inhibitor use and cardiovascular disease risk, sensitivity analyses of 1:3 propensity score matching: the ARIC study, 2011-2017, N=3912 [a]

	PPI user status	
	No	**Yes**
N	2934	978
CVD composite		
# events	418	186
Model 1 [b]	1 (ref)	1.39 (1.17-1.66)
Model 2	1 (ref)	1.35 (1.14-1.61)
Individual CVD endpoints		
Atrial fibrillation		
# events	226	101
Model 1	1 (ref)	1.34 (1.06-1.70)
Model 2	1 (ref)	1.32 (1.05-1.68)
Coronary heart disease		
# events	65	29
Model 1	1 (ref)	1.39 (0.90-2.16)
Model 2	1 (ref)	1.36 (0.87-2.11)
CVD mortality		
# events	63	29
Model 1	1 (ref)	1.38 (0.89-2.15)
Model 2	1 (ref)	1.34 (0.86-2.09)
Heart failure		
# events	158	71
Model 1	1 (ref)	1.34 (1.02-1.78)
Model 2	1 (ref)	1.27 (0.96-1.69)
Stroke		
# events	83	26
Model 1	1 (ref)	0.95 (0.61-1.47)
Model 2	1 (ref)	0.92 (0.59-1.44)

[a] Matched on age, race-center, sex, education, smoking status, drinking status, physical activity, obesity, diabetes, systolic blood pressure, antihypertension medication use, lipid-lowering medication use, HDL, total cholesterol, eGFR

[b] Model 1 = Adjusted for age, race-center, sex

Model 2 = Model 1 + clinical hypomagnesemia

CHAPTER 7 – SYNTHESIS

To briefly summarize, this dissertation includes 3 manuscripts, which served to 1) better characterize the interrelationship of Mg status biomarkers (circulating ionized and total magnesium) using data from a double-blind pilot Mg supplementation RCT; 2) test cross-sectional associations of circulating Mg with burden of atrial and ventricular arrhythmias among elderly ARIC study participants; and 3) explore cross-sectional and longitudinal associations of PPI use with circulating Mg and CVD risk in the ARIC study. The remainder of this dissertation summarizes and discusses the potential public health (Section I) and clinical (Section II) implications of these findings.

I. PUBLIC HEALTH IMPLICATIONS

As presented in this dissertation, approximately 1 in 5 elderly ARIC study participants had clinical hypomagnesemia and nearly 2 in 5 may have a subclinical Mg deficiency.[17] Considering clinical and subclinical hypomagnesemia have been associated with adverse cardiovascular risk profiles as well as cardiovascular disease; this has potentially important public health implications.

As shown in Manuscript 1, Mg supplementation can increase circulating iMg and tMg as compared to placebo. Whether this effect translates into an effective primary prevention strategy for CVD in the general population is unclear, particularly because there is not clinically established cut-points for defining iMg status. Nevertheless, if improving Mg concentrations (even small beneficial changes) within healthy limits translates to improvements in cardiovascular health, it may have notable effects when viewed at the population level.

Manuscript 2 reported that low Mg was associated with greater PVC burden, but

documented weak associations with continuous and intermittent AF burden based on 2 week ambulatory ECG monitoring. It is possible the lack of robust association in our elderly population may not generalize to mid-life. Nevertheless, in light of the expected rise in AF incidence[226] and the current lack of primary prevention strategies, further research is still needed.

Manuscript 3 suggests that individuals who take PPIs have a higher prevalence of clinical hypomagnesemia compared to those who do not take PPIs. Considering the widespread use of PPIs and the link between PPIs and low circulating Mg, it is possible that PPIs are shifting the population level curve of circulating Mg lower. If this is the case, implications of this shift on CVD (including cardiac arrhythmias) and other outcomes are unclear. However, as reported in Manuscript 3, PPI use was associated with only modestly elevated CVD risk in this elderly community-based population, and we did not find statistical evidence that low circulating Mg mediated the association.

Taken together, this dissertation supports the notion that hypomagnesemia and PPI use are both highly prevalent among elderly individuals, as well as that Mg homeostasis is complex. Importantly, low Mg can be intervened upon through dietary modification or supplementation. While the focus of this dissertation was on circulating Mg, relatedly, most American adults do not meet nutritional guidelines for adequate Mg intake (similar to many other vitamins and minerals).[6] Efforts to improve dietary quality and the promotion of Mg-rich foods should continue to be emphasized for cardiovascular health. To date, however, no RCT has tested whether intervening on low circulating Mg is an effective primary prevention strategy for CVD.

II. CLINICAL IMPLICATIONS

Mg status tends to be most commonly assessed based on circulating tMg, as discussed

throughout this dissertation; however, there are important limitations to tMg as a biomarker. In contrast, there has been relatively little characterization of circulating iMg, which is the physiologically active form of Mg in circulation.

Whether iMg is the more clinically relevant biomarker of 'true' Mg status needs to be clarified, as it is possible that there may be substantial misclassification with tMg, resulting in attenuated effect estimates of evaluations of the association between Mg and CVD, and misidentification and treatment of hypomagnesemia in clinical settings. That said, whether it is practical to measure iMg in clinical settings is also unclear. As such, in Manuscript 1 we evaluated the feasibility of measuring iMg under different specimen-processing scenarios. We found that measurement of iMg after brief refrigeration and freezing was substantially higher than iMg measured soon after blood draw. However, it is possible that if iMg is found to be a stronger predictor of outcomes, then this may outweigh the inconvenience of testing iMg soon after blood draw in certain clinical or research settings.

In Manuscript 2, we found that low tMg was associated with a higher burden of PVCs as compared to those with normal tMg concentrations. Additionally, we found little evidence of associations between tMg with AF burden categories and other arrhythmias examined (PAC, SVT, NSVT). However, relevant to both Manuscripts 2 and 3, assessments were conducted in the elderly, and old-age may not be the optimal time-frame in the aging process to assess these questions. It is possible that Mg status may be of greater relevance when measured in mid-life rather than late-life.

PPI use is common among the elderly, as reported in Manuscript 3; approximately 1 in 4 ARIC study participants were PPI users. PPI users had a higher prevalence of hypomagnesemia when compared to non-users. While we did find an association between PPI use and elevated

CVD risk, it appears unlikely that hypomagnesemia is the mechanism (or at least the primary mechanism) through which PPIs may affect cardiovascular health. Nevertheless, it is important for clinicians and patients to be aware of potential side effects of PPI use and, where possible, minimize the use (or duration) of this class of medication. Whether health care providers should screen PPI users—especially long-term users—for low circulating Mg is unclear. Such screening might be considered if the patient presents with the relatively vague symptoms that can accompany severely low circulating Mg concentrations.

Overall, this dissertation helps address knowledge gaps in research on circulating Mg and CVD. Collectively, the findings reported in this dissertation help refine our understanding of the relationship between altered Mg homeostasis and CVD.

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