Magnesium, Genetic Risk, and Risk Factors for Diabetes and Heart Disease

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ABSTRACT

Background: Magnesium (Mg) is a vital but chronically under-consumed nutrient and low intake has been linked to type 2 diabetes (T2D) and cardiovascular disease (CVD). Recent, novel genetic findings related to fasting glucose (FG) and insulin (FI) have pointed to a diverse set of single nucleotide polymorphisms (SNPs) that elevate risk of disordered glycemic homeostasis or Mg status. The extent to which genetic variation affects Mg's associations with these traits is largely unknown. Further, Mg's long-term associations with progression of metabolic impairment (i.e., prediabetes or insulin resistance (IR)) merits further investigation. Meanwhile, arterial calcification—the "hardening" of the arteries—has been quantified in the last decade via computed tomography (CT) in the coronary arteries (CAC) and abdominal aorta (AAC), and experimental evidence points to a protective benefit of Mg in arterial health. IR and T2D are significant risk factors for calcification, and these disorders as well as CAC and AAC elevate risk of CVD morbidity and mortality.

Aims: The aims of this dissertation included analyses of: *Aim 1*: interactions between Mg intake and SNPs related to elevated FG and FI, and lower serum Mg, on FG and FI concentrations; *Aim 2*: Mg intake and risk of incident prediabetes/IR in those with normal glycemic status, and risk of progressing to T2D in those with prediabetes/IR; and *Aim 3*: cross-sectional associations of Mg intake with CAC and AAC.

Methods: *Aim 1*: 15 studies from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium provided data from up to 52,684 participants of European descent without diabetes. In fixed-effect meta-analyses, quantified were (1) associations of dietary Mg with FG and FI, and (2) interactions between Mg and SNPs related to FG (16 SNPs), FI (2 SNPs), or serum Mg (8 SNPs) on FG and FI. *Aim 2*: relative risks of developing prediabetes/IR among those normal at baseline, and developing T2D in those impaired at baseline, were estimated over 7 years of follow-up in 2,576 participants of the Framingham Heart Study (FHS). *Aim 3*: cross-sectional associations of Mg intake on CAC and AAC were assessed in 2,695 individuals free of CVD who participated in the FHS CT sub-study using CT-based calcium measures.

Results: *Aim 1*: after adjustment for age, sex, energy intake, body mass index (BMI), and behavioral risk factors, Mg (per 50 mg/d) was inversely associated with FG (-0.0091 mmol/L, $P<0.0001$) and FI (-0.0204 ln-pmol/L, $P<0.0001$). No Mg-related SNP or interaction between any SNP and Mg reached statistical significance after correction for multiple testing. However, rs2274924 in *TRPM6* (encoding an Mg transporter) showed nominal association (uncorrected $P=0.03$) with FG, and 2 other SNPs showed nominal interaction (uncorrected both $P=0.02$) with Mg on FG. *Aim 2*: after adjustment for age, sex, and energy intake, normal individuals in the highest category of Mg intake had 37% lower risk of developing prediabetes/IR (P trend=0.02), and impaired individuals had 32% lower risk of developing T2D (P trend = 0.05), compared to those with in the lowest category. *Aim 3*: after adjustment for age, BMI, major CVD risk factors and treatment for CVD risk factors, as well as intakes of calcium, vitamins D and K, saturated fat, fiber, alcohol, and energy, higher Mg intake (per 50 mg/d) was associated with 22% lower CAC ($P<0.001$) and non-significantly with 12% lower AAC ($P=0.07$). Compared to those with the lowest Mg intake, those with the highest intake also had lower odds of having any CAC or AAC. Stronger inverse associations were observed in women than in men.

Summary and Conclusion: Consistent with other studies, higher Mg intake was associated with lower FG and FI, generally irrespective of genetic risk for elevated concentrations of these traits. Further, higher Mg intake was associated not only with lower risk of developing metabolic disorders, but also with lower risk of progressing to T2D from disordered states. Finally, higher Mg intake was inversely associated with CAC and AAC. This research provides epidemiologic evidence that increasing intake of Mg—a low-cost, widely available nutrient—may improve health by reducing the burden of arterial calcification and disordered glucose and insulin metabolism, and subsequently reduce risk of T2D and CVD morbidity and mortality.

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William Shakespeare
in *Henry VIII*, Act I, Scene IV (1613)

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CHAPTER 1. INTRODUCTION

Magnesium is a wonder mineral.
Emergency Department Physician
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1.1 Background

Cardiovascular disease (CVD) is a leading cause of death in the US (1). In recent decades, the assessment of subclinical CVD has become increasingly important as a means of identifying, and thus mitigating disease progression in those not otherwise classified as high risk by traditional measures. One such measure of subclinical CVD, and overall atherosclerotic burden, is the computed tomography (CT) assessment of calcification of arteries of the heart and other major vessels, such as the aorta.

Calcification of the coronary arteries (CAC) (2–7) and the abdominal aorta (AAC) (8–10) are established, independent risk factors for CVD morbidity and mortality. Individuals with moderate levels of CAC (i.e., Agatston Scores of 1–100) are approximately four times more likely, and those with high CAC scores (i.e., Agatston Scores >300) are as much as 10 times more likely to suffer a coronary event than those without CAC (11). Both AAC and CAC, in particular, improve discrimination and reclassify individuals for risk of future clinical coronary events, over and above other risk factors and established risk score schemes, such as the Framingham Risk Score (10,12,13). One of the significant risk factors for calcification is type 2 diabetes (T2D) and related disorders of glucose metabolism (14–23). These disorders also substantially elevate overall CVD burden as well as risk of coronary events (24–26).

It is well-known that healthy diets offset risk of T2D and CVD. One mineral that may be key to the etiology of both T2D and CVD—particularly calcification—is magnesium. To

date, magnesium has been identified as a critical element in over 300 metabolic and nucleic acid reactions, and thus, it has pleiotropic effects in many different organ systems (27). Dietary and supplemental magnesium has been shown to be protective against or beneficial for T2D, insulin resistance (IR), and glucose impairment (28–45). Further, individuals with T2D and related metabolic disorders tend to have hypomagnesaemia (46–50). Despite a large body of scientific evidence supporting magnesium's beneficial roles in disorders of glucose and insulin metabolism, two areas of further research in this field are warranted. First, the extent to which magnesium intake interacts with genetic variants associated with fasting glucose and insulin measures, or with magnesium transport and homeostasis, to modify risk of impaired glucose and insulin metabolism has not been assessed. Understanding potential interactions between genetic risk variants and magnesium intake could introduce personalized treatment options in those at high genetic risk for impaired glucose and insulin metabolism. In addition, evidence supporting genetic interaction could expand current understanding of magnesium's physiologic actions in affecting glucose and insulin metabolism. Second, the association of magnesium with intermediate measures of disease progression (e.g., from normal fasting glucose to impaired fasting glucose to frank T2D, or from insulin sensitive to insulin resistant) is not well-characterized. Higher magnesium intake may, for example, be particularly beneficial in offsetting risk of progression to T2D in those with underlying metabolic impairment, perhaps owing in part to their predisposition to hypomagnesaemia. Understanding magnesium's benefits in groups at different risk strata for T2D may provide evidence in support of concrete nutritional recommendations for maintaining health and offsetting risk of future metabolic disease.

In contrast to the fairly extensive magnesium-T2D literature, just one observational study (51) has evaluated magnesium intake in relation to CAC in a generally healthy

population, with no association reported. However, a large body of *in vitro* and animal studies show that low magnesium status or magnesium-poor diets generate predisposition to both an atherogenic process and calcification (52–62). Extensive calcification is present in individuals with conditions known to exacerbate urinary magnesium excretion, such as chronic kidney disease (CKD) and end-stage renal disease (ESRD) (63). In addition, supplemental magnesium has slowed CAC progression in one very small clinical study in the high-risk hemodialysis population (64). Importantly, no study in humans has examined magnesium intake in relation to calcification in major arteries other than CAC, such as the abdominal aorta. Therefore, identifying a protective role of dietary magnesium in calcification in humans may underlie previous observations of lower risk of stroke (65,66), sudden cardiac death (67), and non-fatal myocardial infarction (MI) and fatal coronary heart disease (CHD) (68–70) in those with higher magnesium intake. Finally, as higher magnesium intake is associated with lower risk of T2D and related disorders, and as these disorders elevate risk of CAC and AAC, as well as CVD, understanding whether potential associations of dietary magnesium and calcification are mediated by magnesium's effects on T2D or related metabolic disorders, may further clarify the role of magnesium in lowering risk of CVD.

1.2 Central Hypothesis and Specific Aims

The central hypothesis of the research described in this dissertation is that higher magnesium intake is protective against two major sets of risk factors—impaired glucose and insulin metabolism, and CAC/AAC—implicated in the etiology of T2D and CVD. Within these pathways, genetic variation may affect magnesium's associations with risk factors in ways that mitigate individual genetic risk. The working framework of this research begins with the application of nutritional epidemiology—specifically focusing on magnesium

intake in relation to glucose and insulin metabolism and calcification—and from there expands into an examination of nutrient-nutrient and nutrient-gene interactions and their potential modification of the hypothesized magnesium-disease relationships.

Specific Aim 1. To examine the interaction of genetic variants associated with serum magnesium, and fasting glucose and insulin, on the cross-sectional association of dietary magnesium and fasting glucose and insulin in a meta-analysis of 15 European and US cohorts of the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium.

Specific Aim 2. To examine magnesium intake in relation to progression of glucose impairment and insulin resistance over approximately 7 years of follow-up in the Framingham Heart Study Offspring cohort.

Specific Aim 3. To examine associations between magnesium intake and CAC and AAC in the Framingham Heart Study Offspring and Generation 3 cohorts, and to assess potential effect-modification by total calcium intake.

1.3 Significance

Given the astronomic atherosclerotic disease burden in the US, the proposed research is significant because it may provide epidemiologic evidence for the role of magnesium in vascular calcification, spurring much-needed treatment and prevention trials, and it will add to the relatively limited knowledge we currently have about the role of diet in calcification. To our knowledge, this is the first epidemiologic investigation of magnesium intake in relation to AAC, and only the second to investigate the relationship with CAC. It is also the first study to examine the role of the magnesium-calcium intake relationship with respect to these two sites of vascular calcification.

Further, while higher magnesium intake is associated with improved measures of glucose and insulin metabolism in both healthy and diabetic populations, currently

unknown is the mechanism through which magnesium is acting to improve related measures of metabolism, and to that end, investigating genetic interactions between magnesium and known genetic risk variants may provide considerable insight into magnesium's mechanisms of action on impaired fasting glucose and insulin. To our knowledge, this research represents the first known large-scale epidemiologic investigation of interactions between magnesium intake and genetic risk factors associated with fasting glucose and insulin, and serum magnesium. Finally, furthering our understanding of magnesium's associations with steps along the trajectory of glucose and insulin impairment will complement the existing literature on magnesium's effects in T2D.

Targeting these conditions with a simple, accessible dietary treatment such as magnesium may ultimately reduce CVD morbidity and mortality. Magnesium is widely available in the food supply, inexpensive, and easily obtainable in supplemental form; alas it is a neglected but potentially promising therapy for a range of cardiometabolic conditions.

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CHAPTER 2. LITERATURE REVIEW

People don't expect too much from literature.
They just want to know they're not alone in being confused.
Jonathan Ames
in *Wake Up, Sir!* (2005)

This chapter presents a review of the literature on the experimental, clinical, and epidemiological evidence regarding magnesium in relation to type 2 diabetes, vascular calcification, and related traits. Each section touches upon mechanisms underlying magnesium's putative effects on these conditions. **Tables 2.1–2.5**, included at the end of this chapter, summarize many of the relevant experimental and observational studies. The chapter begins, however, with a general overview of magnesium in the body and in the diet.

2.1 Magnesium

Magnesium is a widely distributed mineral: it is the eighth most abundant element on earth, and the fifth most abundant element in seawater (1,2). It is found throughout the world as either a free cation [Mg^{2+}] in aqueous environments, or in salt or mineral forms (1,3). In the human body, magnesium is the fourth most abundant cation, and the second most abundant intracellular ion (1,3). Owing to its unique chemical properties and its high solubility, magnesium is found in all cells (1). To date, magnesium has been identified as a critical element for over 300 essential metabolic and nucleic acid reactions, and thus, it has pleiotropic effects in many different organ systems. It is crucial for glucose and lipid metabolism, protein synthesis and degradation, second messenger systems, anti-oxidant capacity, regulation of cellular ion concentrations, and muscle contraction (4–7). For example, magnesium is a cofactor for adenosine triphosphatase (ATPase), which catalyzes the breakdown of adenosine triphosphate (ATP) into adenosine diphosphate (ADP), an

energy-releasing reaction that is essential to the body's energy "currency." Two magnesium ions are required for another ubiquitous enzyme, adenylyl cyclase, responsible for transducing extracellular signals into intracellular responses. Adenylyl cyclase catalyzes the conversion of ATP to cyclic adenosine monophosphate (cAMP), a second messenger in the G protein signaling cascade. In addition to directly binding to ligands (e.g., ATP in ATP-requiring enzymes), magnesium is known to influence enzyme activity by directly binding to active sites of enzymes (e.g., pyrophosphatase, in lipid metabolism; pyruvate kinase, in glycolysis); by inducing conformational changes in enzymes (e.g., in the sodium-potassium pump [Na^+,K^+-ATPase], for maintaining sodium-potassium balance); or by a combination of factors (8).

Magnesium has received considerable attention since the early part of the 20th century for its putative roles in glucose and lipid metabolism, endothelial dysfunction, inflammation, cardiovascular disease, and bone health (7,9). Approximately 60% of total body magnesium is found in bone, where it serves both a structural role and as the body's magnesium reservoir (4,10). Only 1% of the body's magnesium is found extracellularly, and the remainder is intracellular, with 27% of total body magnesium found in muscle cells (4) where it is needed for contraction and other metabolic processes.

Magnesium homeostasis is tightly regulated and serum levels are roughly constant across a wide range of intake. Dietary magnesium correlates with serum magnesium, but only weakly (11,12). Age-, sex-, and energy-adjusted correlations between serum and dietary magnesium of 0.27 including supplement users, and just 0.15 excluding supplement users, have been reported (11), while others have reported no linear association between dietary and serum magnesium (r = 0.05) (12). Serum magnesium is therefore a poor marker of magnesium intake since it is not particularly sensitive to intake, except in cases of prolonged deficiency. Depletion studies (13,14) and supplementation trials (15,16) show

that serum magnesium concentrations change slowly, over periods of two to four months from depletion before they stabilize or before the onset of adverse events such as heart arrhythmias or impaired reflexes (14). As such, serum concentrations may not accurately reflect total body magnesium stores, and by the time magnesium deficiency is clinically recognized based on serum magnesium (usually <0.75 mmol/L (17)), an individual's deficiency may already be moderate to severe (9). As such, some experts have emphasized the problem of chronic latent magnesium deficiency, which may contribute to the incidence of or exacerbate conditions such as T2D, hypertension, cardiovascular disease, and osteoporosis (9,18).

The kidney is the main site of magnesium regulation; magnesium excretion decreases rapidly in response to decreased intake, long before serum or plasma levels fall below the normal range (19). It follows that disruption of normal function of the kidneys, or in the presence of renal disease, magnesium homeostasis is also generally impaired. Individuals at specific risk for magnesium deficiency include those with inadequate diets or nutritional supplementation, gastrointestinal disorders with malabsorption, endocrine and metabolic disorders (i.e., T2D, hyper- and hypoparathyroidism), primary aldosteronism, hungry bone syndrome, as well as those with conditions accompanied by diarrhea or excessive urinary magnesium losses or other renal dysfunction (9,19).

2.2 Magnesium in the Diet

In the US, the Recommended Dietary Allowance (RDA) for magnesium from food and water for adult men is 400–420 mg/day, and for adult women (not pregnant or lactating), it is 310–320 mg/day (20).

Magnesium is found in a wide variety of foods and beverages, including nuts and seeds, bran and whole grains, green leafy vegetables (magnesium is at the heart of the

chlorophyll molecule), and animal products, including dairy, meat, and certain types of fish. Magnesium is also found in the drinking water (i.e., in communities with "hard" water) or where mineral-rich drinking water is available. Despite the widespread availability of magnesium-rich food sources, the average daily intake of magnesium by Americans from diet alone does not meet the recommended level (21,22). According to the USDA, only 50% of Americans one year or older achieve the RDA for magnesium, with more pronounced inadequate intake in adolescents and in adults over 70 years old (22). Not surprisingly, then, most Americans appear to rely on only moderately-rich food sources of magnesium to attempt to meet magnesium needs, such as milk (which accounts for 5.6% of average daily intake), coffee (3.7%), beer (3.2%), and bananas (1.8%) (23). However, magnesium supplements can help individuals achieve the recommended intake (21): a study in the Multi-Ethnic Study of Atherosclerosis (MESA) observed that 36% of the study population reported supplemental magnesium intake. With supplement use, 49.9% of the study population met the RDA, while only 15.7% of those not using supplements met the RDA. In supplement users, median magnesium contributions from diet were 216 and 265 mg/day in women and men, respectively; with supplements, median intake totals reached 347 and 377 mg/day, in women and men, respectively (21).

2.3 Magnesium and Genetics

Despite magnesium's tight homeostatic regulation, a recent genome-wide association study (GWAS) identified six common variants associated with serum magnesium (24). These polymorphisms are in or near genes related to magnesium transport and homeostasis, and thus may modify individual risk for hypothesized magnesium-related conditions in the face of differential magnesium intake. Among the most investigated of these include variants in transient receptor potential cation channel, subfamily M (*TRPM*), members 6 (*TRPM6*) or 7

(*TRPM7*), which encode magnesium transporters—*TRPM7* being expressed ubiquitously, while *TRPM6* expression occurs mainly in the kidney and intestine (i.e., for reabsorption) (25). Note that purported functions of serum-magnesium-related SNPs as well as others relevant to this dissertation are listed in **Table A-1** of the Appendices. More on interactions between magnesium intake and these SNPs follow below (see **Section 2.4.5. Magnesium, Diabetes-Related Traits, and Genetic Interactions**).

2.4 Magnesium in Type 2 Diabetes and Related Traits

Impaired glucose and insulin metabolism lie along the etiologic trajectory that results in T2D (26). While the exact mechanism of magnesium's role in these processes remains to be elucidated, experimental evidence points to a role for magnesium in both beta-cell dysfunction and in insulin resistance in peripheral tissues. **Table 2-1** summarizes proposed mechanisms supported by experimental evidence.

2.4.1 Mechanisms of Magnesium in Insulin Resistance

Insulin resistance is a state in which insulin-sensitive tissues, such as skeletal muscle and adipose, become less responsive to insulin, thus impairing the uptake of glucose. As a consequence, glucose remains elevated, forcing the pancreas to produce more insulin to overcome the impaired responsiveness and to drive glucose into peripheral tissues thereby causing hyperinsulinemia. One mechanism through which magnesium may be acting within peripheral tissue is via its effect on tyrosine kinase, a component of the beta subunit of the insulin receptor for which magnesium is a co-factor. Activation of tyrosine kinase produces a signaling cascade that ultimately translocates GLUT4 (the major insulin-regulated glucose transporter expressed in muscle and other insulin-responsive tissues) to the membrane, and allows the cell to take up glucose. Suarez, *et al.*, reported that alongside a 50% reduction in the insulin sensitivity in rats fed a magnesium-deficient diet, there was also

50% reduced autophosphorylation of the beta subunit of the insulin receptor in isolated gastrocnemius muscle tissue of these rats as compared to controls, and that the tyrosine kinase activity of insulin receptors in these hypomagnesaemic animals was also significantly reduced (27). In another study, rat epidydmal adipocytes exposed to regular or reduced ambient and intracellular free magnesium ion concentrations showed significantly reduced insulin-stimulated (but not basal) glucose oxidation when cultured in low versus physiologic magnesium. The authors concluded that their study provides evidence for magnesium's role distal to glucose entry into the cell, and further, that impaired glucose oxidation may be reversible (28).

Insulin itself may be a regulatory hormone of magnesium metabolism. The mechanism whereby insulin modifies intracellular magnesium is via the activity of ion transport channels, such as Na/H antiporters, calcium-adenosine triphosphatases (Ca-ATPases), and ATPase-dependent pumps. Interestingly, insulin-mediated cellular uptake of magnesium may additionally depend on the activation of the tyrosine kinase of the insulin receptor, as there is evidence that inhibiting the receptor via monoclonal antibody nullifies insulin's intracellular magnesium-raising effects (29). (Notably, low intracellular magnesium does not affect insulin-insulin receptor binding, additionally pointing to a downstream (i.e., tyrosine kinase activation) regulatory focal point (30).) In addition, prolonged high concentrations of circulating insulin, such as those known to occur in insulin resistance, induce increases in renal magnesium excretion, thus perpetuating a deleterious cycle (30). Barbagallo and colleagues (29) have examined the relationship between basal intracellular magnesium levels and responsiveness of cells to both insulin and glucose: with lower basal intracellular magnesium concentrations, cells become less responsive to insulin and glucose. Further, they noted that while higher glucose induces magnesium efflux, the lower the basal concentration of magnesium, the less that concentration is responsive to the

modifying effects of insulin and glucose—that is, there is a non-linear response and it appears the cells must retain some basal level of magnesium at which they are not as responsive to fluctuations in insulin or glucose. The authors postulate that hyperglycemia induces cellular hypomagnesaemia, which subsequently contributes to the inability of the cell to respond to insulin (29). It should be noted that the effects of glucose and insulin on intracellular magnesium are tissue-specific; that is, effects in peripheral tissues, such as heart muscle or erythrocytes, are not necessarily observed in other cells or tissues, such as pancreatic islets.

2.4.2 Mechanisms of Magnesium in Beta-Cell Function

Beta-cell dysfunction is a condition in which the beta cells of the pancreas no longer produce sufficient insulin to respond to blood glucose. Glucose-induced insulin secretion is initiated by the uptake of glucose. ATP is generated via glycolysis (rate limiting step at phosphorylation of glucose by magnesium-ATP-dependent glucokinase) and the Krebs cycle in the beta cell. Subsequently, a higher ATP/ADP ratio stimulates magnesium-ATP-dependent closure of the potassium channels, which in turn stimulates membrane depolarization and voltage-gated calcium channel opening, triggering increased cytosolic calcium, and insulin granule exocytosis.

While the exact mechanisms of magnesium's direct and indirect effects on insulin production and secretion are unknown, several pathways are hypothesized. First, magnesium's direct roles as a cofactor for ATPases affects many steps of the glycolytic pathway. Second, magnesium may be acting as an inhibitor of the inositol 1,4,5-triphosphate (IP3)-gated calcium channel—thus magnesium may be acting as a calcium antagonist (31). Relatedly, it has been suggested that the calcium-magnesium ratio within cells may be a more powerful regulator of insulin secretion, and that inhibitors and/or

potentiators of ion balance or channel activity ultimately regulate insulin secretion (32,33). A third proposed pathway of magnesium's actions is via its role in the activation of acetyl-coA carboxylase, which ultimately catalyzes the formation of long-chain fatty acids, which have a role in insulin secretion. It has been shown in rat islet cells that acetyl-coA carboxylase activity is associated with magnesium in a dose-dependent manner (34). A fourth mechanism may be related to genomic regulation of transcription. In obese Zucker rats (spontaneous T2D animals) fed a magnesium-supplemented diet for 6 weeks beginning at 6-weeks old showed lower fasting and fed-state blood glucose concentrations, better glucose disposal, *higher* insulin and C-peptide concentrations, *and* increased pancreatic GLUT2 and insulin mRNA expression than animals on the control diet (35). While all of the eight control animals developed diabetes by 12 weeks of age, only one of eight of the magnesium-supplemented animals did (35).

Glucose itself likely plays a regulatory role in the magnesium concentration of beta cells. In rat islets, D-glucose (and certain other sugars metabolized by islets) induces a dose-dependent increase in magnesium, independent of insulin release (36). After experimentation with various inhibitors, researchers concluded that the magnesium-increasing effect of glucose in islets is not merely a consequence of the depolarization of the beta-cell membrane (which accounted for approximately one third of magnesium uptake), but also of the islets themselves metabolizing glucose (36).

These mechanisms related to insulin secretion and insulin resistance, coupled with plausible roles of pro-inflammatory cytokines with which low magnesium has also been implicated, may be processes that depend on magnesium both directly and indirectly (30).

2.4.3 Epidemiological Evidence

Many observational and clinical studies have shown strong associations between serum and dietary magnesium and fasting insulin or insulin resistance (37–49), and impaired glucose metabolism or T2D (47–55) (**Table 2-2** and **Table 2-3**). Prospective studies (47,48,51,54,55) have observed that individuals with high magnesium intake are 10–47% less likely to develop T2D. A recent meta-analysis of 13 prospective cohort studies concluded that the relative risk of T2D was 0.78 (95%CI 0.73–0.84) (dose response analysis: for every 100 mg/day increment of magnesium intake, the relative risk was 0.86 (95%CI 0.82–0.89)), and authors suggested that the inverse association was stronger in overweight/obese than normal-weight individuals (56).

Few studies have prospectively evaluated insulin sensitivity or resistance over the long term (i.e., >5 years). One prospective investigation of magnesium intake in 1,036 US adults (56.4% women) participating in the Insulin Resistance Atherosclerosis Study who were initially free of T2D, estimated that the optimal magnesium intake in relation to insulin sensitivity was at least 325 mg/day (46). The authors observed progressively poorer insulin sensitivity below that threshold, but no evidence for improvement of sensitivity above that threshold. In this study, insulin sensitivity was assessed by intravenous glucose tolerance tests, considered to be a criterion test for this measure of insulin metabolism.

2.4.4 Clinical Evidence

In addition, a body of clinical evidence supports a role for magnesium supplementation in glucose and insulin metabolism (**Table 2-4**). A meta-analysis of nine magnesium supplement trials in those with T2D found that a median magnesium dose of 360 mg/day was associated with significantly lower post-intervention fasting glucose in the treatment groups, suggesting improved glucose control (52). A recent randomized, placebo-controlled

trial in 25 obese, non-diabetic, normo-magnesaemic individuals who supplemented with 365 mg/day of magnesium for six months improved plasma fasting glucose from 5.1 to 4.8 mmol/L, fasting serum insulin from 109.4 to 100.0 pmol/L the Matsuda insulin sensitivity index from 3.4 to 4.0, and homeostatic model assessment of insulin resistance (HOMA-IR) from 3.5 to 3.0, differences that were not evident in the 22 placebo controls, in which no improvements were observed (39). Interestingly, Gutt's insulin sensitivity index, 2-hour post-OGTT glucose, and 2-hour post-OGTT insulin did not change significantly between groups (39). These results suggest that supplemental magnesium may play a more prominent role in glucose and insulin homeostasis, and insulin sensitivity, rather than response. Another recent cross-over supplementation trial in 14 overweight but otherwise healthy adults also showed that 4 weeks of 500 mg/day of magnesium citrate led to lower C-peptide and fasting insulin concentrations (16), suggesting reduced pancreatic insulin secretion which could have resulted from improved insulin sensitivity and subsequent lowered demand on the pancreas. Supplementation with magnesium in individuals with other risk factors, such as mild hypertension or hypomagnesaemia, has also been found to be effective in improving insulin sensitivity and beta-cell function (37,43,38). In these studies, each of which lasted three months, supplemental magnesium doses ranged from 300 to 600 mg/day. In a small sample of six healthy, normo-magnesaemic participants, insulin sensitivity was mildly, but significantly reduced in all subjects (as measured by modified intravenous glucose tolerance test) after three weeks on a low-magnesium diet (3.69±0.6 vs. 2.75±0.5 min^{-1} per uU/ml×10^{-4}, P <0.03, paired analysis). *Interestingly*, neither fasting glucose nor fasting insulin concentrations were significantly changed by this low magnesium diet (57), which may not be surprising in this short-term study, as decreasing insulin sensitivity may precede changes in tightly controlled fasting measures.

2.4.5 Magnesium, Diabetes-Related Traits, and Genetic Interactions

As mentioned above, a GWAS meta-analysis of serum magnesium, a biomarker of magnesium status, identified six SNPs in genes linked to magnesium transport and homeostasis (24). Among these six SNPs, the C allele of rs4072037 in *MUC1*, which was associated with lower serum magnesium, was also associated with lower fasting glucose concentrations (24). Three studies have thus far investigated associations between loci in these genes and diabetes or glycemic traits (25,58,59). One of these studies observed an association between carriers of the *TRPM6* rs2274924 variant and elevated total glycosylated hemoglobin and greater prevalence of gestational diabetes in 997 women following delivery (58). *TRPM6* encodes a magnesium-permeable epithelial channel with a critical role in magnesium reabsorption in the kidney. The missense mutation (A>G) at rs2274924 in exon 27 in *TRPM6* causes a Lys1584Glu amino acid change of the resulting channel protein. Nair, *et al*. (58) reported that in the presence this polymorphism, the insulin signaling cascade is unable to activate the phosphorylation of the amino acid adjacent to the substituted amino acid resulting from the polymorphism, thereby rendering the variant TRPM6 channel insensitive to the activating effects of insulin. Furthermore, increased glycosylated hemoglobin and greater risk of gestational diabetes was observed in *GG* homozygotes compared to *AA* homozygotes in that cohort of 997 pregnant women (58).

The other two studies to have investigated many loci in these two genes found that variants did not modify either risk of T2D (25,59) or glycemic traits (25); however, these were small studies, and one included women only (59). The latter study—a small case-control study of T2D in predominantly Caucasian, older women followed for 10 years—also examined interactions between magnesium intake and loci in *TRPM6* and *TRPM7*. The authors reported that women who were carriers of two rare alleles from non-synonymous

SNPs in *TRPM6* (rs3750425 and rs2274924) had nearly five times the odds of T2D, but only when their magnesium intake was <250 mg/day (59).

2.5 Magnesium in Vascular Calcification and Atherogenesis

To begin with, we wonder why researchers are interested in measuring calcification. Calcification has long been recognized as an integral part of the progression of atherosclerosis. Beginning in the 1950s, and continuing through 2011, autopsy studies of young men and women (soldiers dying during deployment in the Korean (60), Vietnam (61), and Iraqi (62) wars, or due to other trauma (63,64)), began to illuminate atherosclerosis and its progression from fatty streaks, to fibrous plaques, to complicated and eventually calcified lesions, as a product of age, sex, race/ethnicity, lifestyle characteristics, and health parameters, such as blood lipids and diabetes. These studies suggested that calcium appearing in and around atherosclerotic plaques likely indicates advanced disease.

Over this period, calcification became recognized as a biomarker that can aid in further classifying heart disease risk (e.g., risk of a future cardiovascular event) beyond what may already be known about an individual's risk of an adverse event based on "traditional" risk factors. Like any relatively novel biomarker, its value is in part determined by the proportion of individuals who end up in a different, "clinically relevant risk threshold" as a result of using a novel risk marker (65). The greater the degree of accuracy with which we can predict an individual's risk, the more that tells us about how or what we might to do in terms of appropriate therapy. For example, if the Framingham Risk Score predicts 60–65% of cardiovascular risk, then other factors, such as CRP, carotid intima-media thickness (IMT), or a coronary artery calcification (CAC) score may add to our predictive power (65–67). Indeed, this is what we observe. CAC (67–74) and abdominal

aortic calcification (75–77) are known, *independent* risk factors for cardiovascular disease (CVD) morbidity and mortality. CAC, in particular, discriminates between and reclassifies risk of heart disease, among those already classified by, for example, metabolic syndrome (78–80) or Framingham Risk Score (67–73) criteria. This is evident in generally healthy populations and particularly in individuals with T2D or impaired renal function or related metabolic disorders, who are at risk for higher levels of calcification (79–83), with concomitantly increased risk of CVD.

2.5.1 The Calcification Process

Vascular calcification is, at its most basic, the deposition of calcium phosphate, as hydroxyapatite, in vascular tissue. It can occur in blood vessels, the myocardium, and cardiac valves. Research over the last decade indicates that calcification is not just a passive process of crystal deposition, but rather, is a highly regulated and active form of biomineralization involving relevant markers of bone formation, but occurring in soft tissue (84,85). In the vernacular, arterial calcification is often referred to as the "hardening of the arteries" and is generally thought to increase with age, but age itself may not play a direct role in its pathogenesis. Interestingly, unlike many chronic conditions associated with modern lifestyles, vascular calcification is not strictly a condition arising from 20th-century ways; 5,300-year-old mummies discovered in the ice floes of the Alps (85) and other ancient peoples (86) have evidence of aortic and coronary calcification.

As an active cellular process, calcification is the biomineralization of extra-skeletal tissue by osteoblast-like cells, similar to the mineralization process in bone (84). These cells may arise through a process of osteogenic differentiation of vascular smooth muscle cells (VSMCs), from stem cells in arterial walls, or may migrate from bone (84,87). The process is likely (a) triggered by vascular tissue injury and associated inflammation, leading to *intimal*

calcification, typically associated with the atherosclerotic process of tissue injury, plaque formation, and subsequent atheroma calcification; or (b) it may be induced by mineral imbalances associated with long-standing kidney dysfunction, T2D, and CKD/ESRD, most often leading to *medial* calcification (84,88,87). Intimal and medial calcification can and frequently do exist simultaneously and may unfold in similar pathways; however, this field of research is rapidly evolving, and some argue that the two types of calcification are very different entities (89). Current CT imaging technology does not differentiate between the two types of calcification (90), however, calcification found in individuals free of (long-standing) kidney dysfunction, T2D, and CKD/ESRD, is *intimal*, not medial. This means CT-detected calcification in generally healthy populations is part of atherosclerotic plaque.

2.5.2 Atherosclerotic Calcification

With respect to atherosclerotic calcification, a brief review of atherosclerosis—a "chronic disease of the arterial wall" (91)—is provided. As depicted in **Figure 2-1** (from (92)) atherosclerosis is generally characterized by some kind of injury to the endothelium (e.g., oxidative, inflammatory, hyperglycemic, hyperinsulinemic, or physical, such as shear stress), which initiates a process of macrophages devolving into foam cells, generating a fatty build-up (a "plaque") in the intimal endothelium, which then gets progressively pushed up into the interior of the lumen while thickening the vessel wall. Plaques are thought of as "stable" or "unstable." Unstable plaques, with thinner barriers between the plaque and the vessel, are more vulnerable and higher risk — more likely to lead to rupture and thrombosis. Stable plaques are less likely to rupture, but by pushing up into the lumen, they narrow the lumen thereby creating a stenosis, reducing blood flow, and thus increasing pressure (91).

As shown in the figure, at some point in the atherosclerotic process, VSMCs recruited from the media also end up proliferating in the intima of the vessel. Once there, VSMCs secrete extracellular matrix proteins, including interstitial collagen. This process causes the atherosclerotic lesion to evolve from a lipid-rich plaque to a fibrotic (and ultimately, calcified) plaque. While stabilizing the plaque, the fibrosis and calcification may also create a stenosis (92,93), which may rupture or stabilize. At the present time, not all experts agree that the sequence of events is: necrosis, atheroma, calcification. Some suggest that the sequence is: calcification, necrosis, atheroma (93).

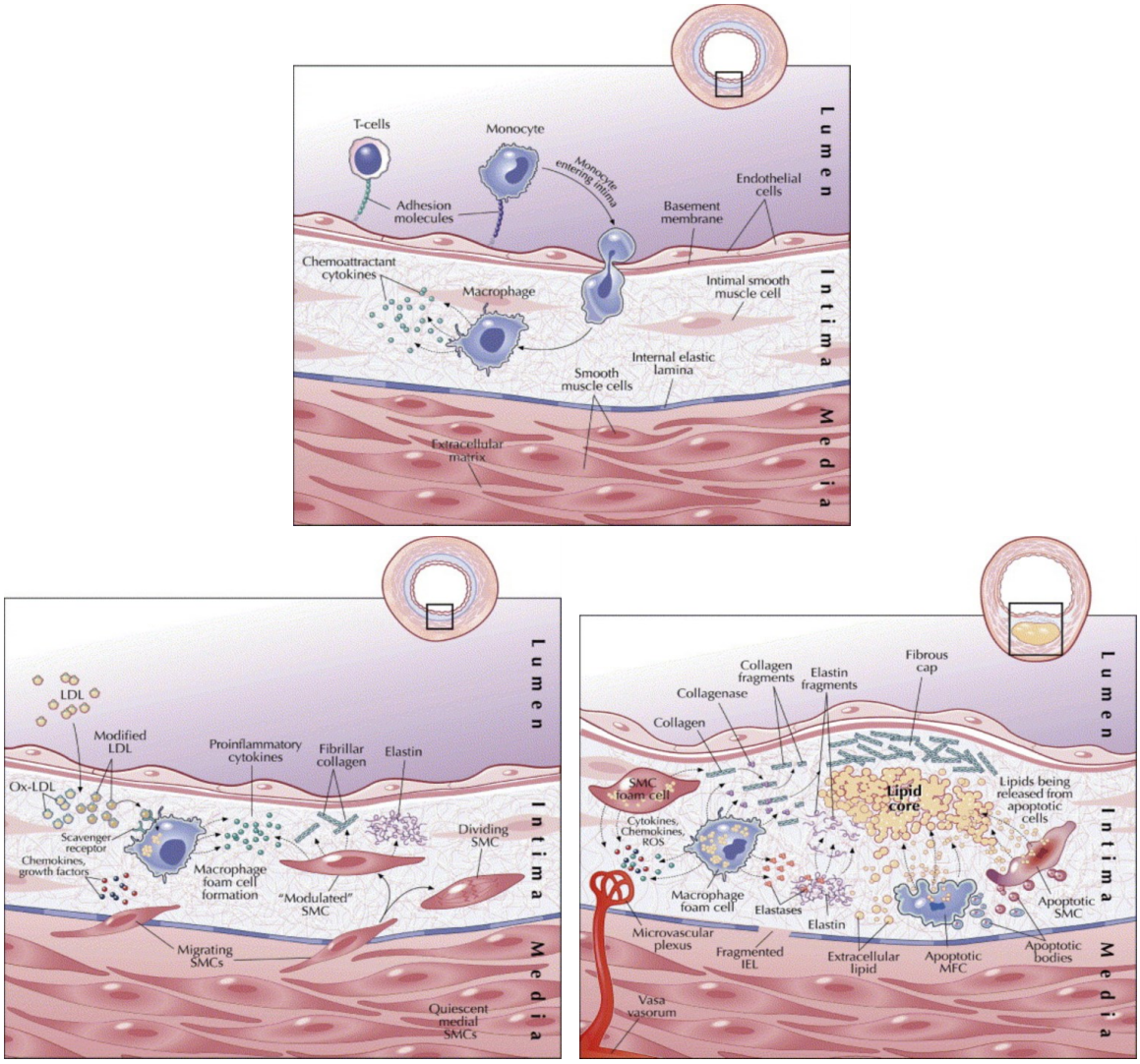

Figure 2-1. Depiction of atherosclerotic plaque initiation and progression, from Libby, *et al*. (2006).

2.5.3 Proposed Mechanisms of the Calcific Process

Currently, several mechanisms are thought to underlie vascular calcification. A range of interrelated pathways to calcification have been proposed, (84,87,88,94), including the aforementioned "passive" process; loss of inhibition to calcification; induction of bone formation (osteogenesis); and cell death (apoptosis). These pathways are briefly reviewed below so as to initiate the reader into magnesium's potential actions within these pathways. [Note: not all of these pathways may occur in both intimal and medial calcific processes. Much of the mechanistic work has been done in animal/cell models of high calcification risk — i.e., models of CKD and other disorders involving mineral imbalance.]

Via thermodynamic mechanisms, elevated calcium, phosphorous, and calcium-phosphorous products promote hydroxyapatite nucleation and crystal growth that could initiate or exacerbate vascular calcification previously initiated by any of the mechanisms described below. For example, the calcifying media including phosphorous or calcium used in many VSMC studies induces hydroxyapatite deposition and phenotypic effects characterized by changes to bone-related gene expression and proteins, including osteocalcin, osteopontin, alkaline phosphatase, matrix Gla protein, and fetuin. Interestingly, serum from CKD patients has been shown to induce osteopontin and alkaline phosphatase activity, independent of phosphate concentration (95). Therefore, calcification, at least in patients suffering from impaired renal function, is not simply a matter of phosphate levels. Another factor that appears to play a role is parathyroid hormone (PTH), which regulates calcium homeostasis. PTH prevented VSMC calcification in a dose-dependent manner by inhibiting alkaline phosphatase activity (95). Relatedly, then, elevated calcium levels are associated with altered alkaline phosphatase, with pro-mineralizing effects beyond simply raising the calcium-phosphorous product through its actions in multiple systems in VSMCs that promote susceptibility to matrix mineralization (94).

With respect to loss of inhibitors of calcification, blood vessels normally express inhibitors, notably matrix Gla protein, and the lack of these molecules has been shown to lead to spontaneous vascular calcification. In addition, decreased fetuin, another inhibitor of hydroxyapatite found in the circulation, is correlated with elevated CVD mortality in hemodialysis patients (94). Of interest is that the carboxylated form of matrix Gla protein (present in normal vasculature, thus mineralization-inhibiting) is carried in plasma by fetuin. Matrix Gla-null mice develop massive arterial calcification in the *absence* of atherosclerosis, and matrix Gla protein is expressed at loci of arterial calcification (87,95). Together, these findings may reflect the body's attempt to maintain homeostasis. When homeostatic regulation fails, matrix Gla protein expression is decreased globally before atherosclerotic or medial calcification occurs (87,95).

The presence of bone proteins such as osteopontin, osteocalcin, etc., and outright ossified tissue that is indistinguishable from bone in vascular lesions, indicate that osteogenic mechanisms are playing a role in *both* intimal and medial calcification. As mentioned earlier, osteoblast-like cells are thought to emerge from either phenotypic changes induced in VSMCs, or circulating stem cells that differentiate into osteoblast-like cells, although the latter is less likely (87). VSMCs undergo osteogenic differentiation when exposed to a variety of conditions, such as oxidative stress (exposure to TNF-alpha or oxidized LDL), stimulation by the powerful bone-morphogenetic proteins (BMPs), pyrophosphate level changes, calcifying media, etc. Interestingly, BMPs are increasingly expressed as atherosclerotic lesions evolve, not just by VSMCs, but also by endothelial cells and foam cells, thus indicating that intimal calcification is also bone-signaling related (87,94,95).

Finally, cell death provides phospholipid-rich membranous debris and apoptotic bodies that may serve to nucleate hydroxyapatite deposition. *In vitro* inhibition of apoptosis

has been shown to inhibit calcification and, similarly, stimulating apoptosis increases the rate of calcification (95). Dead cells and debris may be also enable a scaffold environment conducive to nucleation of crystals (94).

Summarizing pathways and risk factors is the following figure (from (96)):

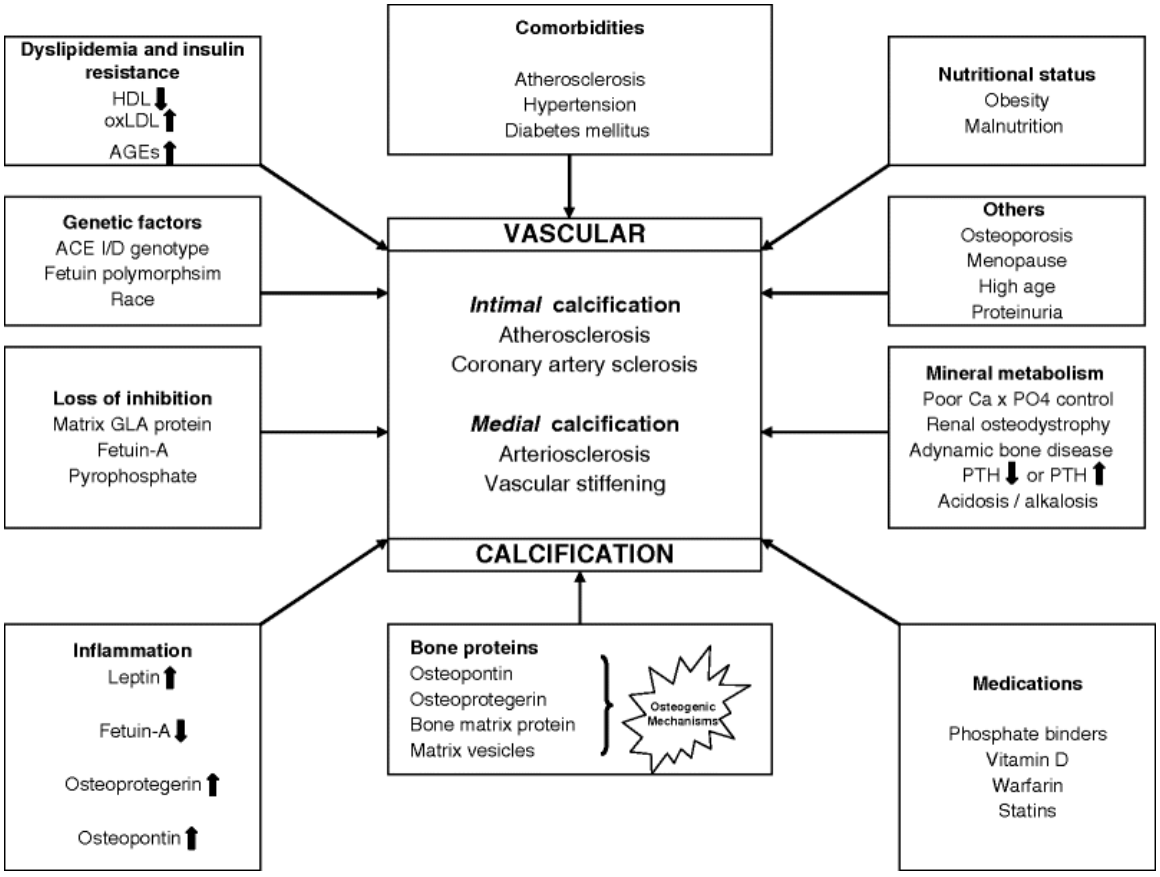

Figure 2-2. Summary of mechanisms and risk factors for calcification, from Yilmaz, *et al*. (2009).

2.5.4 Molecular and Experimental Evidence for Magnesium as Anti-Calcifying Agent

Magnesium, like calcium, is a divalent cation; magnesium is therefore a natural calcium antagonist, competing for cation transport channels and other electrochemically viable enzymatic reactions. As such, magnesium's primary role in preventing biomineralization of soft tissue may be in maintaining cellular ionic homeostasis as well as in replacing calcium

in hydroxyapatite crystals, thereby destabilizing the crystal structure (88,97) and inhibiting crystal precipitation (87).

However, as outlined above, if the molecular mechanism of calcification can be understood as more than the *passive* cellular process of hydroxyapatite deposition, but as an *active* cellular process of transdifferentiation of VSMCs into osteoblast-like cells, including the appearance of related proteins and cytokines (84,88,87), magnesium may act at a number of levels to counter calcification of vascular tissue. Experimental findings in cells and animals suggest important roles of magnesium on bone-related cell signaling, plaque formation, as well as calcification (see **Table 2-5** for supporting evidence). For example, experiments in rats show that magnesium reduces vascular calcification and osteogenic differentiation of VSMCs by increasing TRPM7 (a magnesium transporter) activity and the expression of bone-associated and mineralization-regulating proteins including osteopontin, BMP-7, and matrix Gla protein (98). Research in human osteoblast-like cells shows that high concentrations of magnesium inhibit the deposition of the mineral matrix and decrease alkaline phosphatase activity (99), while studies in human aortic VSMCs indicate that magnesium inhibits calcification and restores to normal levels osteocalcin and matrix Gla protein, and partially restores osteopontin (100). Recent findings in mice continue to lend support to a common inflammatory link between calcification and osteoporosis (101), which may also be influenced by magnesium, as low magnesium in endothelial cells has been shown to activate NFkB, increase the secretion of RANTES, interleukin (IL)-8, matrix metalloprotease (MMP)-2 and MMP-9, and up-regulate vascular cell adhesion molecule (VCAM)-1 and plasminogen activator inhibitor (PAI)-1, contributing to a pro-atherogenic state in these cells (102,103). Magnesium deficiency in animals has also been shown to increase substance P, TNF-alpha, and IL-1 beta, additionally

suggesting that low magnesium may be modulating the inflammatory response, thus priming the calcific process (104).

There is also a large body of animal evidence regarding magnesium deficiency or supplementation that suggests that magnesium may act against atherosclerotic plaque formation, with eventual downstream effects on calcification. In rabbits, inadequate magnesium intake increased atherosclerotic plaques, resulted in thicker intima, and triggered an acute elevation in C-reactive protein (105). In swine, magnesium intake deficiency concurrent with a moderately high vitamin-D3 diet showed more intimal lesions, cell degeneration, intimal thickening, and calcification (106). Magnesium deficiency in rats increased carotid artery cross-sectional area, intima-media thickness, and media-lumen ratio, with evidence that chronic deficiency was exacerbated by the effects of aging (107). Additional studies of magnesium deficiency in rats indicate induced inflammation of coronary tissue with lesions beginning as focal acute perivascular inflammation, progressing through stages of necrosis, granulomatous inflammation, and fibroblastic proliferation to scar formation (108). In addition, long-term moderate deficiency in aging rats worsened age-induced composition and structure of the aorta—aortas had thicker medial layers, increased collagen content, and reduced elastin/collagen ratios (109), while another study showed decreased aortic elastin content but higher aortic elastin calcium (110). Meanwhile, magnesium deficiency in hamsters induced endothelial cell and VSMC hyperplasia and pleomorphism in coronary arteries, chronic inflammation of the media and adventitia, and fibrinoid necrosis (111). Lesions of myocardial ischemia, distinct from lesions of myocardial necrosis and calcification, were also evident, as was calcium in VSMCs of deficient hamsters—observations consistent with the view that calcium loading of VSMCs plays a role in arteriopathy (111).

What about supplementation in animal models? Magnesium supplementation in rabbits on high cholesterol diets resulted in almost no aortic intimal atherogenesis and minimal intima-media thickening, in contrast to unsupplemented rabbits which had intimal lesions on >60% of the aortic surface (112). In another study in rabbits, supplementation reduced the area of intimal aortic plaque, interestingly without substantively affecting blood cholesterol (113). Supplementation in mice on atherogenic diets dose-dependently decreased serum total cholesterol, markers of lipid peroxidation, and aortic deposition and content of total and free cholesterol, as well as aortic cholesterol esters, also with no effect on serum triglycerides or HDL cholesterol (114). Magnesium supplementation in ApoE-null mice on a low-fat diet reduced aortic root plaque area to 66% that of animals on tap water (control) (115), while in LDLR-deficient mice fed a Western-style atherogenic diet, magnesium dose-dependently decreased atherosclerosis at the aortic sinus (116). Finally, in studies in rats with vitamin D3+nicotine-induced vascular calcification, magnesium dose-dependently reduced medial calcification in the aorta, as well as calcium content of aortic cells (117), while in rats with aortic damage induced by diabetes (e.g., thickened collagen fibers in VSMCs and foam cells), magnesium supplementation resulted in return of normal structure of thoracic aorta, as well as a return to normal levels of blood pressure and serum calcium:magnesium ratio (118).

Some experimental evidence suggests that magnesium decreases HMG-CoA reductase activity—the rate-limiting step in cholesterol biosynthesis—thus acting as a "physiological statin" (119); increases lipoprotein lipase (LPL) activity—which hydrolyzes fatty acids for transport across membranes; and increases lecithin cholesterol acyltransferase (LCAT) activity—which converts cholesterol into cholesterol esters for sequestration into HDL cholesterol particles (119,120).

In sum, the evidence from *in vitro* and animal studies suggests a role for magnesium in lipid metabolism, dyslipidemia, plaque formation, and intimal as well as medial calcification processes.

2.5.5 Epidemiological Evidence

Just one observational study has examined magnesium intake in relation to CAC in >5,000 participants 45–84 years old who were free of T2D and CVD in the Multi-Ethnic Study of Atherosclerosis (MESA) (121). In that study, CAC was defined as presence (Agatston Score >0) vs. absence, and secondarily at Agatston Score ≥100 vs. <100 (see **Chapter 3. Methods** for a discussion of the limitations of this dichotomous approach), and the authors observed no association between dietary magnesium intake and odds of CAC, but did observe 24% lower odds of carotid IMT >75th percentile (121). Importantly, the analyses were not adjusted for calcium intake (see **Section 2.5.6. Calcium in Relation to Magnesium**z).

One small pilot study in seven individuals undergoing chronic hemodialysis and with Agatston Scores >30—individuals who were at particularly high risk for rapid calcification progression—included magnesium/calcium carbonate (approximately 700 mg/day elemental magnesium and 1200 mg/day elemental calcium) supplementation in lieu of the standard (calcium-based) phosphate binder (122). The authors reported a non-significant increase in CAC of 8% over 18 months, whereas typical progression (with calcium-based binders) in this population may be as high as 50%.

No study, to our knowledge, has investigated magnesium in relation to AAC.

Four studies (123–126) have examined common sources of magnesium—coffee, whole grains, and fish—in relation to CAC with mixed results. In the Rotterdam Study of 1,540 older men and women, higher coffee intake was inversely associated with severe CAC (Agatston Score >400) in women (regardless of smoking status), and in men who smoked,

whereas a direct relationship was observed between coffee and CAC in men who did not smoke (124). Another study of coffee intake in 5115 younger adults (18–30 years old at baseline) showed no association between presence (Agatston Score >0) or progression of CAC (incident CAC at year 20 or increase in CAC score by ≥20 Agatston Score units) over 15–20 years of follow-up (123). Whole-grain intake showed no association with CAC in 5,496 MESA participants, despite significant inverse associations with obesity, IR, inflammation and elevated fasting glucose or newly diagnosed diabetes (125). Finally, researchers in Rotterdam reported that among 1,570 adults free of heart disease, those with higher fish intake had a lower prevalence of moderate CAC (Agatston Score 11–400) and borderline significant reduced prevalence of severe CAC (Agatston Score >400) compared to fish non-consumers. Intake of neither docosahexanoic acid (DHA) nor eicospentanoic acid (EPA)—the fatty acids to which fish consumption's cardiovascular benefits are often attributed—was associated with calcification (126). These findings suggest that other components of fish, such as magnesium, may have a cardioprotective effect.

Epidemiologic and clinical evidence of conditions potentially related to calcification is extensive, and implicates serum and/or dietary magnesium in metabolic syndrome (127), lipid metabolism (16,38,120,128–130), blood pressure (49,131–134), carotid IMT (121,135,136), presence of carotid plaques (135), and CVD morbidity and mortality (49,137–143).

2.5.6 Calcium in Relation to Magnesium

Excess calcium, whether dietary or supplemental, has long been considered a potential source of adverse health effects (144,145). However, observational studies of dietary calcium have generally shown a protective association, or no association, against a range of diseases, including CAC. In the Women's Health Initiative trial of estrogen, the calcium and

vitamin D sub-study's post-menopausal women receiving calcium (1000 mg/day) with vitamin D (400 IU/day) did not show higher levels of CAC than those on placebo in post-trial measurements (following 7 years of treatment) (146). (Notably, the latter study may not be as informative as one would hope, as the women measured for CAC were survivors, that is, they did not die as a result of being either in the treatment or placebo arm, and there were no pre-trial CAC measurements conducted.) A small study of calcium supplements vs. placebo in 144 elderly women indicated no differences after 4 years in the progression of CAC (147). Recent cross-sectional findings from the Framingham Heart Study support these data: in 690 women and 588 men (mean age 60 years, range 36–83), calcium intake was not associated with levels of CAC (148).

However, there is evidence suggesting that high calcium intake poses increased CVD risk. A recent meta-analysis of eight calcium supplementation trials showed that calcium supplementation alone or in conjunction with vitamin D increased risk of MI, stroke, and both combined (149). Two very recently published studies both indicate that higher calcium intake may increase risk for CVD mortality (150,151). One of these examined 61,433 Swedish women followed for a median 19 years. Women with the highest dietary calcium intake (≥1400 mg/day) had higher risk of all-cause, CVD, and ischemic, but not stroke, mortality, compared to those who consumed 600–1000 mg/day (150). In contrast, in a study of 388,229 men and women aged 50–71 years at baseline, followed for a median 12 years, supplemental calcium intake was associated with higher CVD mortality (heart disease, but not cerebrovascular disease) in men, but not in women (151). Interestingly, the authors of that study stratified by magnesium intake in subgroup analyses, and found that while subgroup trends were non-significant, higher calcium intake appeared to be more of a risk factor among those with low magnesium intake (151).

In addition, a high calcium-magnesium (Ca:Mg) intake ratio may be problematic to an individual's health. Nearly 20 years ago, Seelig (152) proposed that a dietary Ca:Mg ratio greater than 5 may increase risk for magnesium deficiency, partly because of reduced absorption but also because of increased excretion. There is some evidence that the Ca:Mg intake ratio (from food) has changed over time. A recent study indicates that the dietary ratio has been increasing in the US; the ratio was approximately 2.6 in adults 19–50 years old in 1977, and was approximately 3.1 in adults over 20 years old by 2007, reflecting a roughly 20% rise in the ratio (153). More and more foods available on the market which do not naturally contain calcium appear to be offered with supplemental calcium (e.g., orange juice), presumably additionally contributing to higher calcium intake, despite lower consumption of naturally calcium-containing foods (e.g., milk) (154). In addition, calcium supplement use has likely increased over time due to its recommended use against osteoporosis, and while supplement use helps individuals achieve the RDA in both calcium and magnesium, more Americans appear to be taking supplemental calcium than supplemental magnesium (e.g., 46% vs. 36% in the MESA cohort (21)). Further, a high Ca:Mg, whether in diet or serum, has recently been reported to be associated with colorectal and prostate cancers, respectively, both inflammatory conditions (155,156). A cross-over study of post-menopausal women indicated that moderate magnesium deprivation (107 mg/day for 3 months) resulted in increased calcium retention, exacerbating a mineral imbalance which may be a contributing factor in calcification (157).

2.5.7 Studies of Other Foods and Nutrients

B vitamins appear to have no effect on CAC or AAC, as demonstrated by a three-year double-blind, placebo-controlled supplementation trial of folic acid, B-12 and B-6 in 506 generally healthy men and women aged 40–89 years at baseline (158). A randomized, controlled trial

of vitamin K in 388 generally healthy older adults found that supplemental 500 mcg/day phylloquinone slowed CAC progression by 6% relative to placebo over three years of follow-up, but only in those participants with pre-existing CAC (Agatston Score >10), suggesting vitamin K is beneficial after calcification has already been initiated, but perhaps not before, possibly owing to its anti-ossification, but not anti-mineralization processes, *per se* (159). Several studies examining alcohol intake and calcification have resulted in conflicting reports on alcohol's role in CAC (160–165). One study of an *a priori* healthy dietary pattern in MESA in relation to markers of subclinical cardiovascular disease reported no association between a higher (i.e., healthier) pattern score and CAC (166). A data-driven approach in the same population found that a principal-components-derived pattern based on food group intake was also not associated with CAC (167).

Finally, a three-year follow-up of a one-year randomized trial of the effect of lifestyle modification (emphasizing Mediterranean diet and stress reduction) on CAC progression in 96 men and women with established heart disease (35–75 years old) found no effect on change of CAC at either year 1 or year 3 (168). The median progression was by a factor of 1.46 in the intervention group and 1.41 in the control group ($P = 0.68$). However, both groups reported improving their diets; with the intervention group reporting adherence to nutrition recommendations of 75% compared to 63% in the control group. Otherwise, no specific examination of nutrients or food groups in relation to CAC progression were undertaken as a part of that study.

With increasing recognition over the last decade of calcification measured by CT as a strong, consistent, and independent predictor of CVD morbidity and mortality, additional studies of diet and CAC, AAC, and other regions of vascular calcification — as well as calcification progression—may enhance our understanding of diet's complex role in CVD.

2.6 Summary Context for Aims

This chapter summarizes current evidence on magnesium's role in diabetes, calcification, and related traits, and presents the background needed to understand the rationale behind the three novel aims of this dissertation. **Figure 2-3** presents the aims in the broad context of what is already known, as described in this chapter.

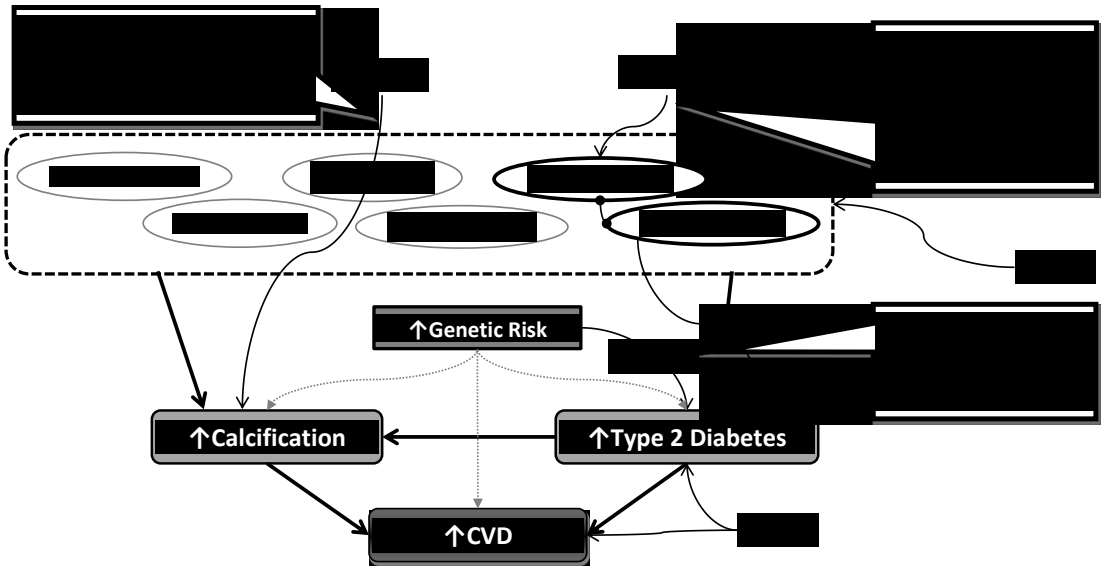

Figure 2-3. Dissertation aims in the context of high-level summary of known relationships.

As extensively discussed above, higher magnesium intake is associated with lower risk of T2D, CVD, and a variety of intermediary risk factors (e.g., oxidative stress, inflammation, hypertension, etc.) that may lie along the diet-disease trajectory. In addition, there are known genetic variants that elevate risk of CAC, T2D, and CVD. Our aims and hypotheses focus on the following unknowns: (1) whether magnesium intake interacts with genetic variants associated with higher concentrations of fasting glucose and insulin, and lower concentrations of serum magnesium, to affect concentrations of fasting glucose and insulin; if true, this research would add to our mechanistic understanding of magnesium's effects on these traits and open the door to personalized nutritional genomic guidance; (2) whether

higher magnesium intake is associated not just with lower risk of developing T2D, but also with lower risk of earlier progression from normal to metabolically impaired states (as insulin resistance and prediabetes), and from those metabolically impaired states to T2D; if true, this research would indicate that higher magnesium intake is beneficial at all stages of glucose and insulin metabolism; and (3) whether higher magnesium intake is associated with lower levels of CAC and AAC, independent of magnesium's other known associations with CVD and calcification risk factors, such as oxidative stress, inflammation, and insulin resistance; if true, this research would lead to hypotheses that magnesium has a unique role in the calcific process in addition to its known effects on other aspects of atherosclerosis.

Table 2-1. Potential Mechanisms and Experimental Evidence to Support a Benefit Effect of Magnesium in Glucose and Insulin Metabolism*

Mechanism	Type of Study and/or Model	Evidence
Effects on pancreatic beta-cell function	Rats, in vivo	Supplementation normalizes pancreatic structure (islets of Langerhans) in streptozotocin-induced diabetic rats after 8 wks of 10 g/L/d Mg-sulphate ($MgSO_4$) (note 1–3 g/L showed no substantial effect, and 50 g/L quickly resulted in rat death) (118)
	Rats, in vivo	Supplementation inhibits the inositol 1,4,5-triphosphate (IP3)-gated calcium channel—thus magnesium may be acting as a calcium antagonist (31), and as inhibitor and/or potentiator of ion balance or channel activity ultimately regulating insulin secretion (32,33)
	Rats, ex vivo and in vitro	Supplementation activates acetyl-coA carboxylase, which ultimately catalyzes the formation of long-chain fatty acids, which have a role in insulin secretion; in rat islets, acetyl-coA carboxylase activity is associated with magnesium in a dose-dependent manner (34)
	Rats, in vivo	Supplementation increases genomic transcription of pancreatic GLUT2 and insulin mRNA expression in obese Zucker fatty rats (spontaneous diabetes model animal) fed a magnesium-supplemented diet for 6 wks beginning at 6 wks old; treated animals also had lower fasting and fed-state blood glucose concentrations, better glucose disposal, (paradoxically) higher insulin and C-peptide concentrations (perhaps owing to better insulin sensitivity); at 12 wks, all 8 control animals developed diabetes, only 1 of 8 of Mg-supplemented animals did (35)
Effects on insulin signaling	Rats, in vivo	Deficiency reduces by 50% the insulin-mediated glucose disposal via reduced autophosphorylation of the beta-subunit of the insulin receptor in isolated gastrocnemius muscle and fat tissue in rats fed a magnesium-deficient diet; reducing tyrosine kinase activity of insulin receptors, while GLUT4 presence is unaffected (27)
	Humans, ex vivo, and in vitro	Low intracellular magnesium does not affect insulin-insulin receptor binding, additionally pointing to a post-binding (i.e., tyrosine kinase activation) regulatory focal point (30), and cells with lower basal intracellular magnesium concentrations are less responsive to insulin and glucose (29)
	Rats, ex vivo	Deficiency reduces insulin-stimulated (but not basal) glucose oxidation to carbon dioxide or incorporation into triglycerides when rat epididymal

Mechanism	Type of Study and/or Model	Evidence
		adipocytes are cultured in low vs. physiologic magnesium (28)
	Humans	Infusion (of magnesium, as a bolus that might occur in an emergency room setting) in healthy humans causes rapid drop in fasting serum insulin levels, accompanied by a small, transient rise in glucose, which subsequently come back to normal (169)
	Humans	Plasma (magnesium) concentrations in healthy humans determine insulin-mediated glucose disposal. Magnesium determines responsiveness to 75g glucose load and steady-state plasma insulin and glucose (SSPG) concentrations following 180-min infusion of insulin and glucose, with those with low Mg group having higher plasma glucose and insulin following the challenge , and final SSPG concentrations also higher in low Mg group (170)
Insulin as a regulatory hormone or glucose as a physiological determinant of magnesium	Rats, ex vivo	D-glucose, mannose, and glyceraldehyde, but not fructose, in rat islets induce a dose-dependent increase in Mg^{2+} uptake owing to glucose metabolism by islets, only partially owing to depolarization of cell membrane, and not merely as a consequence of insulin release (36)
	Humans and rats, ex vivo	In VSMCs and RBCs, hyperglycemia directly induces suppression of intracellular magnesium, independent of insulin action (evidence in both VSMCs and in red blood cells - where glucose transport is unaffected by insulin) (29)
	Humans, ex vivo	Insulin mediates cellular uptake of magnesium depending on the activation of the tyrosine kinase of the insulin receptor, as inhibiting the receptor via monoclonal antibody nullifies insulin's intracellular magnesium-raising effects (29)
	Mice, in vivo	High circulating insulin and/or inhibition of insulin-stimulated glucose uptake increases magnesium efflux (cellular hypomagnesaemia) from mouse skeletal muscle (29)
	Humans	High circulating insulin and hyperglycemia decreases renal magnesium reabsorption and increase excretion, inducing hypomagnesaemia (30)
	Humans, ex vivo	Incubation of lymphocytes from healthy humans in insulin increases ionized magnesium (Mg^{2+}) in a steady time-course curve; if incubated simultaneously with glucose, higher levels of glucose dampen the Mg^{2+} influx in a dose-response fashion; effect of insulin abolished by inhibiting PI3-kinase or Na-Mg transporters (171)
	Humans, ex vivo	IGF-I and insulin stimulate erythrocyte magnesium levels; but intracellular

Mechanism	Type of Study and/or Model	Evidence
		Mg insulin responses, not IGF-I responses, depend on basal magnesium levels (higher Mg has greater insulin sensitivity); IGF-I promotes insulin-induced stimulation of magnesium at doses that do not themselves raise magnesium (172)

*Abbreviations used: GLUT (2 or 4), glucose transporter; IGF, insulin growth factor; IP3, inositol 1,4,5-triphosphate; Mg, magnesium; RBC, red blood cell; RNA, ribonucleic acid; VSMC, vascular smooth muscle cell.

Table 2-2. Observational Studies of Magnesium Intake and Glucose or Insulin Metabolism, Including Type 2 Diabetes*

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
Larsson and Wolk (2007) (50)	**Meta-analysis** of 7 prospective studies 1966–2007	286,668 participants, 10,915 cases			T2D	Overall RR per 100 mg/d =0.85 (0.79–0.92)	All but one study reported an inverse relationship between intake and T2D risk. Follow-up of 4–17 yrs.
Dong et al. (2011) (56)	**Meta-analysis** of 13 prospective studies through 2011	536,318 participants and 24,516 cases			T2D	Overall RR = 0.78 (0.73–0.84) Dose-response: RR per 100 mg/d = 0.86 (0.82–0.89).	Association was not substantially modified by geographic region, follow-up length, sex, or family history of DM. Significant in overweight (≥25 kg/m2) but not normal-weight individuals, although test for interaction P=0.13. Follow-up of 4–20 yrs.
Schulze et al. (2007) (173)	**Meta-analysis** of 8 prospective studies through 2006	271,869 participants, 9192 cases			T2D	Overall RR = 0.77 (0.72–0.84)	4/8 studies not significant. Also, cereal fiber, but not fruit or vegetable fiber, was inversely associate with T2D. Follow-up of 4–16 yrs.
Hopping et al. (2010) (174)	**Prospective**, 14 yr follow-up Age: 45–75 yrs BMI: 31.6% overweight, 11.2% obese Race/ethnicity: Caucasian (39%), Japanese-American (47%), Native Hawaiian (14%) Country: US (Multi-Ethnic Cohort Study)	75,512 (52%) participants; 8587 cases	Men: ≥185.4 mg/1000 kcal/d Women: ≥200.2 mg/1000 kcal/d	Men: <129.3 mg/1000 kcal/d Women: <139.3 mg/1000 kcal/d	Incident T2D	HR Men = 0.77 (0.70–0.85), P trend <0.0001 HR Women = 0.84 (0.76–0.93), P trend = 0.0003	Stratified by age at study entry; adjusted for ethnicity, BMI, physical activity, education, energy. Further stratifications by sex and ethnicity. Fiber inversely associated with incident T2D, and Mg strongly correlated with fiber (r=0.83, P<0.001).

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
Kim et al. (2010) (48)	**Prospective**, 20 yr follow-up Age: 18–30yrs Race/ethnicity: ~50% white BMI: ~24.5 kg/m2 Country: US (CARDIA)	4497 (~50%)	201.5 mg/1000 kcal	99.9 mg/1000 kcal	Incident T2D and HOMA-IR	**T2D:** HR = 0.53 (0.32–0.86); P trend <0.01 **HOMA-IR:** −0.117 mg/L (−0.157, −0.077) lower in high vs. low consumers P trend <0.01	T2D defined as FPG ≥7.0 mmol/l or non-FPG ≥11.1 mmol/l; post-2h OGTT ≥11.1 mmol/l or HbA1C ≥6.5% or use of Rx. Adjusted for age, sex, ethnicity, study center, education, smoking status, alcohol consumption, PA, family history of DM, BMI, systolic blood pressure, total energy intake, dietary intakes of saturated fat and crude fiber. Also reported inverse associations with IL-6, CRP, and fibrinogen.
Kirii et al. (2010) (175)	**Prospective**, 5 yr follow-up Age: 40-65 yrs (mean ~53.2y) BMI: ~22.8 kg/m2 Country: Japan (Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study))	17,592 (63%) participants; 459 (48%) cases	303 mg/d	158 mg/d	Incident T2D	OR = 0.64 (0.44 to 0.94), P trend = 0.04	Adjusted for age, BMI, family history of DM, smoking status, alcohol intake, PA, consumption of green tea and coffee, and energy.
Nanri et al. (2010) (176)	**Prospective**, 5 yr follow-up Age: 45–75 yrs Country: Japan (Japan Public Health Center-based Prospective Study)	59,791 (57%) participants; 1114 cases	Men: 348 mg/d Women: 333 mg/d	Men: 213 mg/d Women: 213 mg/d	Incident T2D	NS in men or women Men: OR = 0.86 (0.63–1.16) Women: OR = 0.92 (0.66–1.28)	Age, study area, BMI, smoking status, alcohol intake, family history of DM, leisure time PA, hypertension, coffee consumption, Ca intake (mg/d), and energy intake

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
Villegas et al. (2009) (54)	**Prospective**, 7 yr follow-up Age: ~50 yrs Race/ethnicity: Chinese BMI: ~23.8 kg/m2 Country: China (Shanghai Women's Health Study)	64,191 (100%)	318.1 mg/d	213.8 mg/d	Incident T2D	HR =0.80 (0.68–0.93) P trend <0.0001	T2D defined as FPG ≥7.0 mmol/l on 2+ occasions; post-2hOGTT ≥11.1 mmol/l or use of Rx. Adjusted for age, energy intake, BMI, WHR, smoking, alcohol, physical activity, income, education, occupation, hypertension. Also investigated calcium and dairy (both also inversely related to T2D).
Schulze et al. (2007) (173)	**Prospective**, 11 yr follow-up Age: 35-65 yrs Race/ethnicity: Predominantly Caucasian BMI: ~26 kg/m2 Country: Germany (EPIC-Potsdam)	25,067 (61.3%)	377 mg/d	268 mg/d	T2D	RR = 0.90 (0.72–1.12) P trend = 0.44	Adjusted for age, sex, education, sports, cycling, occupation, smoking, alcohol, total energy intake, BMI, WC. Cereal fiber, but not fruit or vegetable fiber, was inversely associate with T2D.
He et al. (2006) (177)	**Prospective**, 15 yr follow-up Age: 18-30 yrs Race/ethnicity: 51% black, 49% white BMI: ~24 kg/m2 Country: US (CARDIA)	4637 (53.8%)	190.5 mg/1000 kcal/d	96 mg/1000 kcal/d	IFG or T2D (FG ≥6.1 mmol/l (110 mg/dL))	HR = 0.51 (0.32–0.83) P trend <0.01	Study on metabolic syndrome; inverse association with metabolic syndrome was also reported. HR adjusted for age, gender, race; education, smoking status, PA, family history of DM, alcohol consumption, BMI, quintiles of fiber, PUFA, saturated fat, carbohydrates, and energy.
Ma et al. (2006) (46)	**Prospective**, 5 yr follow-up	1036 (56%)	≥325 mg/d	<325 mg/d	IS - 12-sample insulin-	<325 mg/d, β = 0.0607/100 mg,	No association found between dairy intake and IS. Positive

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
	Age: 54.8 yrs Race/ethnicity: 26.5% African-American; 33.5% Hispanic; 40% Non-Hispanic White BMI: 28.5 kg/m2 Country: US (Insulin Resistance Atherosclerosis Study)				enhanced, intravenous glucose tolerance test	P =0.0008 ≥325 mg/d, β =-0.001/100 mg, P = 0.82	associations for both Mg and Ca. Mg intake was associated with IS in a threshold fashion - that is, <325 mg/d, the effect of more Mg was beneficial, but over 325 mg/d, there was no added benefit. Adjusted for age, sex, ethnicity, center, family history of T2D, energy intake, smoking, PA, alcohol, dietary protein, fat, fiber, whole and refined grains, vegetables, fruit, fish, meat, BMI, Ca intake.
van Dam et al. (2006) (55)	**Prospective**, 8 yr follow-up Age: ~38 yrs Race/ethnicity: African-American BMI: ~27.5 kg/m2 Country: US (Black Women's Health Study)	41,186 (100%)	244 mg/d	115 mg/d	Incident T2D	HR = 0.65 (0.54–0.78) P trend <0.0001	Adjusted for age, total energy intake, BMI, smoking, physical activity, alcohol, parental history of DM, education, coffee, sugar-sweetened soft drink intake, quintiles of processed meat and other red meat intake, and Ca intake.
Lopez-Ridaura et al. (2004) (51)	**Prospective**, 12 yr follow-up in men; 18 yr follow-up in women Age: women, ~46; men, ~54 yrs BMI: women ~24.3; men 25.3 kg/m2 Country: US (Health Professionals'	85,060 women and 42,872 men / cases: 4,085 women, 1,333 men	Women: 377 mg/d Men: 458 mg/d	Women: 217 mg/d Men: 268 mg/d	Incident T2D	Women RR = 0.66 (0.60–0.73), P trend <0.001 Men RR = 0.67 (0.56–0.80), P trend <0.001.	Adjusted for age, BMI, physical activity, family history of T2D, smoking, alcohol consumption, hypertension, and hypercholesterolemia at baseline. Still significant after additional adjustment for GL, PUFA, trans fat, cereal fiber,

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
	Follow-up Study and Nurses' Health Study)						and processed meat; and when stratified by BMI, PA, and family history of DM.
Hodge et al. (2004) (178)	**Prospective**, 4 yr follow-up Age: 40–69, mean ~54 yrs BMI: ~26 kg/m2 Country: Australia (Melbourne Collaborative Cohort Study)	31,641 participants / 365 cases	--	--	Incident T2D	OR per 500 mg/d = 0.62 (0.43–0.90) Further adjusting for BMI and WHR: OR = 0.73 (0.51–1.04), P trend = 0.07	Primary analysis was for GL. Adjusted for country of birth, PA, family history of DM, alcohol and energy intake, education, 5-yr weight change, sex, and age.
Song et al. (2004) (47)	**Prospective**, 6 yr follow-up Age: ~54 yrs BMI: ~26 kg/m2 Country: US (Women's Health Study)	39,345 (100%)	433 mg/d	255 mg/d	T2D (and FI in smaller sample)	Fully adjusted RR, high vs. low = 0.89 (P trend = 0.05). Stronger in women with BMI ≥25 kg/m2, RR = 0.78 (P trend = 0.02).	Adjusted for age, energy, BMI, smoking, physical activity, alcohol, parental history of DM. Also considered adjustment for GL, fiber, fat intake. Also conducted cross-sectional FI analysis in N=349 non-T2D women: adjusted geometric mean FI for overweight women in the lowest quartile of Mg intake: 53.5 vs. 41.5 pmol/l among those in highest quartile (P trend = 0.03).
Meyer et al. (2000) (179)	**Prospective**, 6 yr follow-up Age: ~61.5 yrs BMI: ~26.8 kg/m2 Race/ethnicity: NS Country: US	35988 (100%) participants / 1141 cases	>332 mg/d	<242 mg/d	Incident T2D	RR = 0.67 (0.55–0.82), P trend = 0.0003	Age, energy, BMI, WHR, education, pack-years of smoking, alcohol intake, and PA.

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
	(Iowa Women's Health Study)						
Kao et al. (1999) (180)	**Prospective**, 6 yrs follow-up Age: ~53 yrs Race/ethnicity: 22% black; 78% white BMI: ~28 kg/m2 Country: US (ARIC)	12,128 (~65%) participants / 1106 cases	>0.17 mg/kcal	≤0.12 mg/kcal	Incident T2D	P trend = NS	T2D defined as FPG ≥7.0 mmol/l (126 mg/dL) or non-FPG ≥11.1 mmol/l or use of Rx. Adjusted for age, sex, education, family history of DM, BMI, WHR, sports, alcohol, diuretics, dietary Ca and K.
McKeown et al. (2008) (181)	**Cross-sectional** Age: ~72 yrs (elderly) Race/ethnicity: ~95% Caucasian BMI: ~26 kg/m2 Country: US	535 (66.5%)	≥332.4 mg/d	≤215 mg/d	IFG or T2D (FG ≥5.6 mM (≥100 mg/dL))	OR = 0.41 (0.22–0.77) P trend = 0.005	Study on metabolic syndrome; inverse association with metabolic syndrome was also reported. OR adjusted for age, gender, race), education, marital status, smoking, alcohol intake, exercise, BMI, total energy intake, percentage energy of saturated fatty acid intake, lipid lowering medication use, and blood pressure medication.
Ford et al. (2007) (182)	**Cross-sectional** Age: ~43 yrs Race/ethnicity: ~75% Caucasian BMI: ~26 kg/m2 Country: US (NHANES)	7669 (~50%)	Men ≥466 mg/d Women ≥337 mg/d	Men ≤221 mg/d Women ≤164 mg/d	IFG or T2D (FG ≥5.6 mM (≥100 mg/dL))	OR = 0.85 (0.57–1.28), P trend = 0.371	Study on metabolic syndrome; inverse association with metabolic syndrome was reported. OR adjusted for age, sex, race, education, smoking status, concentration of CRP, alcohol use, physical activity, family history of early CHD, use of vitamin or supplement,

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
							history of T2D (except model for hyperglycemia), percent calories as fat (as quintiles), as carbohydrate, fiber intake, and total energy intake.
Bo et al. (2006) (183)	**Cross-sectional** Age: 45–64 yrs Race/ethnicity: Caucasian BMI: ~26.5 kg/m2 Country: Italy	1653 (~53%)	397.9 mg/d	241.2 mg/d	T2D, Metabolic syndrome, CRP, HOMA-IR, FG, FI	**OR T2D:** 4.3 in low vs. high, P trend <0.001 **OR Metabolic syndrome:** 3.1 in low vs. high, P trend <0.001 **Crude HOMA-IR:** 0.5 in low vs. 0.4 in high, P trend <0.001 **Crude FG:** 112.1 vs. 99.8 mg/dL, P trend <0.001 **Crude FI:** 1.9 vs. 1.7 uU/mL, P trend <0.001	Investigated Mg and fiber in relation to CRP, T2D, and Metabolic syndrome. Mg and fiber r=0.81. Adjusted for age, sex, BMI, smoking, alcohol, PA, energy intake, % energy from fat. Similar—possibly stronger—associations found with fiber intake. Mg associations with all but CRP were attenuated after adjusting for fiber. OR CRP ≥3 mg/L: 4.45, P trend <0.001.
Rumawas et al. (2006) (41)	**Cross-sectional** Age: ~54y Race/ethnicity: Caucasian BMI: ~27 kg/m2 Country: US (Framingham Heart Study)	2708 (54.8%)	>387.3 mg/d	<209.7 mg/d	FG, FI, 2h post-OGTT glucose and insulin and HOMA-IR	**FG:** NS **FI:** 29.9 (low) vs. 26.7 uU/mL (high), P trend <0.001 **2h post-OGTT glucose:** 104.4 (low) vs. 100.7 mg/dL (high), P trend =0.04 **2h post-OGTT insulin:** 86.4 (low) vs. 72	Adjusted for age, sex, BMI, smoking, alcohol, PA, hypertension, total energy intake.

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
						mU/mL (high), P trend <0.001 **HOMA-IR:** 7 (low) vs. 6.2 (high), P trend <0.001	
Song et al. (2005) (184)	**Cross-sectional** Age: ~52y BMI: ~26 kg/m2 Race/ethnicity: Predominantly Caucasian Country: US (Women's Health Study)	9887 (100%)	>383 mg/d	<277 mg/d	IFG or T2D (FG ≥6.1 mmol/l (110 mg/dL))	Prevalence = 5.0% (low) vs. 3.3% (high) P trend = 0.005	Study on metabolic syndrome; inverse association with metabolic syndrome was also reported. Unadjusted prevalence.
Ma et al. (1995) (49)	**Cross-sectional** Age: 45–64y BMI: NS Race/ethnicity: white and black Country: US (ARIC)	15,248	Q5 not specified, but race- and sex-specific	Q1 not specified, but race- and sex-specific	FG, FI	Mean difference, high vs. low: **FI:** white men, 13 pmol/l (p <0.001); black men, 2 pmol/l (P=0.72); white women, 12 pmol/l (P <0.001); and black women, 27 pmol/l (p <0.001) **FG** not specified	Also measured. fasting serum Mg, lipids, IMT. Adjusted for age and BMI (race-and sex-stratified)
Huerta et al. (2005) (44)	**Case-control** Age: ~13y Race/ethnicity: 32/48 Caucasian; 12/48 African-American BMI: 24 obese, 24 lean Country: US	48 (63%)	0.12 mg/kcal in obese (BMI ≥85th percentile)	vs. 0.14 mg/kcal in lean (BMI <85th percentile)	FG, HbA1c, FI, QUICKI, HOMA-IR	Dietary Mg (mg/kcal) in lower in obese compared with lean children (P = 0.001). Mg intake inversely correlated with **HOMA-IR** (r=−0.43;	Sex- and puberty-stage-matched obese cases and lean controls. Also measured cholesterol, TGs, LDL and HDL cholesterol. Also examined serum Mg; see below. Serum Mg correlated with total Mg intake (r=0.40; P = 0.005) and

Author (Year) (Ref.)	Study Design and Population	N (% female)	Mg intake (high)	Mg intake (low)	Outcome (IR or IFG or IGT or T2D)	Association	Notes
						P = 0.002) and **FI** (−0.43; P = 0.002) and positively correlated with **QUICKI** (0.43; P = 0.002) No association with markers of **IS**.	energy-adjusted Mg intake (r=0.41; P = 0.004). Also: significant association between fiber intake (g/kcal) and QUICKI (r=0.30; P = 0.036), however, after adjusting for magnesium intake (mg/kcal), no longer significant (r=0.00; P = 0.969). Association with QUICKI remained significant after adjusting for fiber intake (g/kcal) (r=0.33; P = 0.023).

*Abbreviations used: BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus; Mg, magnesium; FG, fasting glucose; FI, fasting insulin; GI, glycemic index; GL, glycemic load; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; IMT, intima-media thickness; IS, insulin sensitivity; LDL, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; PA, physical activity; PUFA, polyunsaturated fatty acid; QUICKI, quantitative insulin sensitivity check index; T2D, type 2 diabetes; TG, triglyceride.

Table 2-3. Observational Studies of Magnesium Biomarkers and Glucose or Insulin Metabolism, Including Type 2 Diabetes*

Author (Year)	Study Design and Population	N (% female)	Mg (high)	Mg (low)	Outcome(s)	Association	Notes
Guerrero-Romero et al. (2008) (185)	**Prospective**, 10 yr follow-up Age: 30–65y Race/ethnicity: Hispanic BMI: ~28.5 kg/m2 Country: Mexico (Mexican Diabetes Prevention Study)	817 participants / 276 cases(	**Serum** Mg: ≥0.74 mmol/L	vs. <0.74 mmol/L	New-onset IFG (5.6–7.0 mmol/L FG), IGT (7.8–11.1 mmol/L glucose 2-h postload), and T2D	RR IFG: 1.11 (0.5–5.1) RR IGT: 1.38 (1.1–6.3) RR IFG+IGT: 1.49 (1.1–4.9) T2D RR: 2.54 (1.1–4.1)	Adjusted for age, sex, family history of DM, WC, and HOMA-IR. Serum magnesium levels of <0.74 mmol/L defined the exposed. At baseline, 420 (51.4%) individuals had hypomagnesaemia.
Kao et al. (1999) (180)	**Prospective**, 6 yr follow-up Age: ~53 yrs Race/ethnicity: 22% black; 78% white BMI: ~28 kg/m2 Country: US (ARIC)	12,128 (~65%) participants / 1106 cases	**Serum** ≥0.95 mmol/L (1.90 mEq/L)	vs. <0.95 mmol/L	T2D	In whites, but not blacks, inversely associated with T2D risk: RR =1.76 (1.18–2.61) P trend = 0.01 (high vs. low Mg) Crude rates of 11.1 (high Mg) to 22.8 (low Mg) per 1000 PY.	T2D defined as FG ≥7.0 mmol/l (126 mg/dL) or non-FPG ≥11.1 mmol/l or use of Rx. Adjusted for age, sex, education, family history of DM, BMI, WHR, sports, alcohol, diuretics, serum Ca and K.
Randell et al. (2008) (186)	**Cross-sectional** Age: 42.7±10.5 yrs Race/ethnicity: presumably Caucasian BMI: 26.7±5.0 kg/m2 Country: Canada (Newfoundland and Labrador)	1318 (78.8%)	**Serum** Mg: 0.99 (0.93–1.85) - high quartile	vs. 0.79 (0.47–0.83) - low quartile	FG, FI, HOMA-beta, HOMA-IR	Crude, in whole population (including 54 with DM): higher **FG** (mmol/L) with **higher Mg** (5.00 vs. 5.14, P=0.04);and **higher HOMA-beta** (131 vs. 111, P=0.001) Less 54 with DM, **Mg-to-FG** r=0.150; P <0.001). In 54 with DM, strong negative correlation between Mg-FG (r=−0.328, P =	Also, in whole population (including 54 with DM): Mg directly associated with age, and serum phosphate, calcium, albumin, total cholesterol, HDL, LDL, and triglycerides. Negatively correlated with % body fat (DEXA). Essentially unadjusted correlations. Analysis not particularly sophisticated.

Author (Year)	Study Design and Population	N (% female)	Mg (high)	Mg (low)	Outcome(s)	Association	Notes
						0.02). But not with **FI** (64.4 vs. 62.4 pmol/L, P=0.30), or **HOMA-IR** (1.99 vs. 1.99, P=0.41)	
Laires et al. (2004) (187)	**Cross-sectional** healthy post-menopausal Age: 50–77 yrs Race/ethnicity: Caucasian BMI: lean (~23.3 kg/m2) vs. obese (29.5 kg/m2) Country: Portugal	74 (100%), 55 obese, 19 lean	**RBC Mg** in obese = 46.6 (12.3) **Plasma Mg** in obese = 21.3 (9.9)	RBC Mg in lean = 43.0 (19.2) Plasma Mg in lean = 19.9 (1.6)	FG, FI, HOMA-IR	**RBC Mg** correlated with FI (lean: r=0.81, P<0.001; obese: r=0.35, P = 0.01) **RBC Mg** correlated with HOMA (lean: r=0.792, P<0.001; obese: r=0.273, P=0.05)	Unexpected stronger direct correlations of FI and HOMA-IR with RBC Mg. Nothing noted for Plasma Mg; no differences between obese and lean in terms of Mg concentrations.
Ma et al. (1995) (49)	**Cross-sectional** Age: 45–64 yrs Race/ethnicity: white and black Country: US (ARIC)	15,248	**Serum** 0.8 to ~0.85 mmol/l	vs. ~0.7 mmol/l	FG, FI	Mean difference, high vs. low: **FG:** 1.46 mmol/l (P<0.01) in white men; 1.20 (P<0.01) in black men; 1.63 (P<0.01) in white women; and 2.13 (P<0.01) in black women **FI:** white men, 20 pmol/l (P<0.01); black men, 1 (P=0.89); white women, 23 (P<0.01); and black women, 31 (P<0.01).	Also measured. fasting serum Mg, lipids, IMT. Adjusted for age and BMI (race-and sex-stratified).

Author (Year)	Study Design and Population	N (% female)	Mg (high)	Mg (low)	Outcome(s)	Association	Notes
Huerta et al. (2005) (44)	**Case-control** Age: ~13 yrs Race/ethnicity: 32/48 Caucasian; 12/48 African-American BMI: 24 obese, 24 lean Country: US	48 (63%)	**Serum** Mg: 0.748 ± 0.015 mmol/L (BMI ≥85th percentile)	vs. 0.801 ± 0.012 mmol/L (BMI <85th percentile)	FG, HbA1c, FI, QUICKI, HOMA-IR	Serum Mg lower in obese (P=0.009); inversely associated with BMI (r=−0.44; P=0.002), BMI Z score (−0.42; P=0.003), % body fat (r=−0.37; P=0.009), FI (r=−0.36; P=0.011) and HOMA-IR (r=−0.35; P=0.015). Positively correlated with QUICKI (r=0.35; P=0.015).	Sex- and puberty-stage-matched obese cases and lean controls. Also measured cholesterol, TGs, LDL and HDL cholesterol. Serum Ca and K measured in 12 obese children and all lean children. No significant differences in Ca (obese: 2.36±0.02 vs. lean: 2.35±0.01 mmol/l; P=0.984) or K (obese: 4.03±0.04 vs. lean: 3.95±0.06 mmol/l; P=0.334) between groups. After adjusting for % body fat, the relationships between serum Mg and HOMA-IR (r=−0.24; P=0.104), FI (r=−0.22; P=0.124), and QUICKI (r=0.17; P=0.25) no longer significant.

*Abbreviations used: BMI, body mass index; Ca, calcium; CRP, C-reactive protein; Mg, magnesium; FG, fasting glucose; FI, fasting insulin; GI, glycemic index; GL, glycemic load; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; IFG, impaired fasting glucose; IMT, intima-media thickness; IS, insulin sensitivity; K, potassium; LDL, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; OR, odds ratio; PA, physical activity; PUFA, polyunsaturated fatty acid; PY, person-years; QUICKI, quantitative insulin sensitivity check index; RR, relative risk; T2D, type 2 diabetes; WC, waist circumference; WHR, waist-hip ratio.

Table 2-4. Trials of Magnesium and Outcomes Related to Glucose and Insulin Metabolism, Including Type 2 Diabetes*

Author (Year)	Study Design and Population	N (% female) analyzed	Mg Treatment vs. Placebo or Control	Outcome(s)	Effects	Notes
Song et al. (2006) (52)	**Meta-analysis** of 9 randomized, double-blind, controlled trials through January 2005	370 patients **with T2D**	median 360 mg/d	Glucose control as HbA1c, and FG	FG -0.56 mmol/L (-1.1, -0.01) post-Tx. No significant difference in HbA1c.	Trail duration of 4–16 wks (median 12 wks). BP and cholesterol were also assessed. No changes to SBP or DBP. Increases in HDL noted (0.08 (0.03–0.14) mmol/L)
Guerrero-Romero and Rodriguez-Moran (2011) (43)	**RCT** 12 wks Hypomagnesaemic (<0.70 mM), non-diabetic, normotensive Age: ~40 years Race/ethnicity: Hispanic BMI: ~29 kg/m2 Country: Mexico	49 Tx 48 control (~40%)	2.5 g/d $MgCl_2$ (solution) 50 mL inactive solution	**beta-cell function** (AUC of the hyperbolic model of beta-cell function), FG, FI, HOMA-beta, Belfiore Index	**HOMA-beta:** fell in placebo from 253.2 to 170.1 In Tx, **Belfiore index** improved from 0.028 to 0.038; **FG** fell from 5.0 to 4.6 mmol/L; **FI** fell from 99.6 to 87.6 pmol/L; No such changes in placebo.	Ability of beta cells to compensate for variations in insulin sensitivity. Comparison between Belfiore's and HOMA-beta indices. After supplementation, no one in treatment group was hypomagnesaemic.
Hadjistavri et al. (2010) (38)	**RCT** 12 wks Mild hypertensives Age: ~45 years Race/ethnicity: White BMI: ~28 kg/m2 Country: Greece	24 Tx 24 control (~38%)	600 mg/d Mg pidolate (solution) Lifestyle recommendations	FG, FI, OGTT-derived IS	**FG:** No change, no difference between groups. **FI:** 12.51 to 10.11 pmol/L in Tx, no change in control, P<0.05 between groups. **HOMA-IR** decreased, **Cederholm index** increased, **Matsuda index** increased, **Stumvoll index** increased, **AUC glucose** decreased, **AUC insulin** decreased in Tx, P<0.05; no changes in placebo - differences between groups were significant.	Also measured lipids. Serum Mg increased, 24h urine Mg excretion increased, total cholesterol decreased, LDL decreased, HDL increased, TGs decreased - all changes significant in treatment; no changes in control; significant differences between groups.
Lee et al.	**RCT**	75 Tx	12.2 mmol (300	Plasma FG, FI,	No differences at all between	Also measured blood

Author (Year)	Study Design and Population	N (% female) analyzed	Mg Treatment vs. Placebo or Control	Outcome(s)	Effects	Notes
(2009) (188)	12 wks Healthy, normo-magnesaemic, but overweight Age: 30-60 years Race/ethnicity: BMI: ~26 kg/m2 Country: Korea	80 control (50%)	mg) as MgO identical placebo	serum Mg, HOMA-IR	groups, not even in serum Mg.	pressure. SBP did not change, but DBP decreased in Tx. Subgroup analyses indicated responses to Mg in those with hypertension
Guerrero-Romero et al. (2004) (37)	**RCT** 12 wks Hypomagnesaemic (<0.74 mM), non-diabetic, but insulin resistant (HOMA-IR >3.0) Age: ~42.5 years Race/ethnicity: Hispanic BMI: ~29 kg/m2 Country: Mexico	30 Tx 30 control (~40%)	2.5 g/d $MgCl_2$ (solution) 50 mL inactive solution	FG, FI, HOMA-IR	**FG** (5.8 to 5.0 mmol/L), **FI** (103.2 to 70.2 mmol/L), **HOMA-IR** (4.6 to 2.6) all decreased significantly in Tx, but not in control; differences between groups were significant	Tx also significantly lowered total cholesterol and triglycerides, and raised serum Mg and HDL.
Rodriguez-Moran and Guerrero-Romero (2003) (189)	**RCT** 16 wks with T2D and hypomagnesaemic (serum Mg ≤0.74 mmol/L) Age: ~56y Race/ethnicity: Hispanic BMI: ~28 kg/m2 Country: Mexico	32 Tx 31 control	50 g $MgCl_2$ (50 ml solution) Placebo	HOMA-IR, FG, HbA1c	All P<0.05: **Serum Mg:** 0.64 to 0.74 mmol/L w no change in control **FG:** 12.8 to 8.0 mmol/L, drop in control as well, but greater in Tx **FI:** 47.4 to 67.8 mmol/L with higher rise in control **HbA1c:** 11.5 to 8.0%, drop in control, but greater in Tx **HOMA-IR:** 4.3 to 3.8 in Tx, rose from 4.7 to 5.0 in control.	Participants already being treated with glibenclamide
Chacko et al. (2010)	Double-blind, randomized **crossover**	13 (29%)	500 mg/d elemental Mg as	Insulin, FG, Serum Mg, HbA1c,	**C-peptide:** -.04 in Tx vs. +0.1 in control (p= 0.004)	Distinct up-regulation of 24 genes and down-

Author (Year)	Study Design and Population	N (% female) analyzed	Mg Treatment vs. Placebo or Control	Outcome(s)	Effects	Notes
(16)	4 wks 4 wks washout Healthy, overweight Age: 44.4y Race/ethnicity: BMI: 28.2 kg/m2		Mg citrate Placebo	inflammatory markers, etc.	**HbA1c:** +0.05 in Tx vs. -.01 in control (p =0.08) **PTH:** +8.5 in Tx vs. -5.8 in control (p =0.04). **IL-6:** +0.23 in Tx vs. -0.37 in control (p =0.03)	regulation of 36 genes, including metabolic and inflammatory pathways. Also examined TRPM6 and TRPM7. No significant changes in FG, insulin, serum Mg, Ca, leptin, lipids, CRP, TNF-alpha, sICAM-1, sVCAM-1, E-selectin.
Paolisso et al. (1994) (190)	Randomized, double-blind, **crossover** 4 wks 3 wk run-in with T2D Age: Elderly Race/ethnicity: Caucasian Country: Italy	9	15.8 mmol/d Placebo	Euglycemic hyperinsulinemic glucose clamp with simultaneous D-[3-3H]glucose infusion and indirect calorimetry	**FG:** no change. Last 60 min of glucose clamp: total body **glucose disposal** (24.5 +/- 0.4 vs. 28.2 +/- 0.7 µM/kg-min; P <0.005), and **glucose oxidation** (13.0 +/- 0.4 vs. 16.3 +/- 0.8 µM/kg-min; P<0.01) increased after Tx	Treated by diet only. No changes in endogenous glucose production, non-oxidative glucose disposal, lipid and protein oxidation, and insulin MCR. Significantly increased plasma and erythrocyte Mg.
Purvis et al. (1994) (191)	Double-blind, randomized, **crossover** 6 wks each 2 wk run-in 2 wk washout With NIDDM and hypercholesterolemia Age: 28–84 yrs, mean 53.8 yrs BMI: 21.3-50.6 kg/m2, mean 32.2 kg/m2	28 (85.7%)	384 mg/d MgCl (Slo-Mag) Placebo	FG (serum)	No change in FG.	NIDDM defined as FG > 140 mg/dL; controlled by diet or oral hypoglycemics. Hypercholeterolemia defined as serum cholesterol >200 mg/dL. Also measured blood pressure, lipids, serum and erythrocyte Mg.

Author (Year)	Study Design and Population	N (% female) analyzed	Mg Treatment vs. Placebo or Control	Outcome(s)	Effects	Notes
	Race/ethnicity: Caucasian: 42.9%; African-American: 57.1%					Only significant change was -7.4mmHg SBP. No change to others, not even Mg biomarkers.
Paolisso et al. (1992) (40)	Double-blind, randomized **crossover** 4 wks each 4 wk run-in 2 wk washout Generally healthy, non-obese Age: ~77.8y Race/ethnicity: Caucasian BMI: 24.5 kg/m2 Country: Italy	12 (50%)	4.5 g/d Mg pidolate (16.2 mmol Mg) Placebo	OGTT + clamp AIR, glucose disappearance constant and rate, net changes in total insulin response, AUC for insulin	Decreased basal plasma glucose; but no change in plasma insulin. **Higher acute and total insulin response after Tx.** Improved glucose disappearance constant. Hepatic glucose output not different, but **glucose uptake improved after Tx.**	Improved markers of plasma and erythrocyte Mg after Tx. Exact changes after Tx are difficult to deduce from text...
Paolisso et al. (1989) (*Acta Endo-crinologica* (192))	Randomized, **crossover** 4 wks 3 wk run-in 2 wk washout T2D, elderly, moderately obese No Rx during trial Age: ~67y Race/ethnicity: Caucasian BMI: ~30.5 kg/m2 Country: Italy	8 (50%)	3 g/d as Mag 2	FG, FI, IVGTT, IV-arginine test to determine alpha and beta-cell responses, AIR to glucose and arginine pulse (0-10m post-pulse), total plasma glucose, insulin AUC, glucose disappearance constant 10–60m post-pulse x 100)	**FG** fell to 8.0 mmol/L with Tx (P<0.05). Glucose pulse **AIR** and constant increased slightly following Tx (AIR: 37.1 pmol/L/10m, P<0.01; constant: 0.79%, P<0.01). Arginine pulse **AIR** increased (81 vs. 151 pmol/L/10m) and total **insulin AUC** (2.3 vs. 4.8 micromol/L/L/min, P<0.01), after Tx, significantly higher than placebo. Total plasma **glucose AUC** lower after Tx than placebo (255 vs. 178 mmol/L/m, P<0.05)	Better plasma and erythrocyte Mg content after Tx
Paolisso et	Randomized, **crossover**	8 (3/8)	2 g/d as Mag 2	FG, FI, glucose	**FG** fell from 9.0 to 8.1 mM	Better plasma and

Author (Year)	Study Design and Population	N (% female) analyzed	Mg Treatment vs. Placebo or Control	Outcome(s)	Effects	Notes
al. (1989) (*Diabetes Care* (193))	4 wks 3 wk run-in 2 wk washout with T2D, elderly, moderately obese No Rx during trial Age: ~67 yrs Race/ethnicity: Caucasian BMI: ~130% ideal BW Country: Italy		Placebo	pulse, glucose clamp, AIR (AUC 0–10m after glucose), glucose disappearance constant, 10–60m post-glucose x 100	with Tx (P<0.05). **AIR** and constant increased slightly following Tx (AIR: 4.1 mU/L/m, P<0.05; constant: 0.82%, P<0.01). **Glucose infusion** rate was significantly higher after Tx (3.6 vs. 2.9 mg/kg/m, P<0.05) vs. placebo.	erythrocyte Mg content after Tx.
Yokota et al (2004) (53)	**Uncontrolled supplementation study** 30 d with mild T2D (no insulin use) Age: 51.6 yrs BMI: 26.1 kg/m2	9 (30%)	300 mg/d as mineral water (Mag21, "bittern") n/a	Serum FI, plasma FG, HbA1c, HOMA-IR	FI and HOMA-IR decreased significantly. **FI:** 8.08 to 5.89 uU/mL; **HOMA-IR:** 2.73 to 2.05. No changes to **FG**, **HbA1c**.	Lipids and BP also measured. Serum Mg, urinary Mg excretion increased. NS good changes in HDL-C and TG. BP only changed SS in those with hypertension.
Nielsen et al. (2007) (14)	**Depletion/Repletion** Depletion: 78 d or fewer Repletion: 58 d or more Healthy, post-menopausal Age: 47–75 yrs Race/ethnicity: BMI: 28.2 kg/m2	14 (100%)	Depletion phase: 33% of Mg RDA (101 mg/2000 kcal/d) diet Repletion phase: diet plus extra 200 mg/d	Glucose tolerance (IVGTT), serum glucose and insulin	IVGTT 60m **AUC glucose** significantly higher during depletion than repletion. No change to **insulin** response.	Also examined cholesterol, heart rhythm, electrolyte metabolism. Adverse heart rhythms appeared in 4 women before d 78. Membrane Mg, total cholesterol, superoxide dismutase (SOD) decreased
Nadler et al. (1993) (57)	**Depletion** 3 wks Presumably	12 (?)	Liquid diet: 1 wk with 400 mg/d $MgCl_2$	IVGTT; FG, FI	No change to **FG** or **FI**. Bergman **insulin sensitivity** (Si) derived from IVGTT fell	Serum, urinary Mg, K, Ca; plasma aldosterone; plasma renin;

Author (Year)	Study Design and Population	N (% female) analyzed	Mg Treatment vs. Placebo or Control	Outcome(s)	Effects	Notes
	healthy/normal BMI: 120% of ideal BW		followed by 3 wks with low Mg (12 mg/d (<0.05 mmol/d)) n/a		modestly (3.69 to 2.75, P=0.03).	intracellular free Mg^{2+} intracellular free Mg^{2+} Serum Mg and intracellular Mg^{2+} fell

*Abbreviations used: AIR, acute insulin response; AUC, area under the curve; BMI, body mass index; BW, body weight; Ca, calcium; CRP, C-reactive protein; DBP, diastolic blood pressure; Mg, magnesium; FG, fasting glucose; FI, fasting insulin; GI, glycemic index; GL, glycemic load; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; IFG, impaired fasting glucose; IMT, intima-media thickness; IS, insulin sensitivity; IVGTT, intravenous glucose tolerance test; K, potassium; LDL, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; OR, odds ratio; PA, physical activity; PTH, parathyroid hormone; PUFA, polyunsaturated fatty acid; PY, person-years; QUICKI, quantitative insulin sensitivity check index; RR, relative risk; SBP, systolic blood pressure; T2D, type 2 diabetes; Tx, treatment; WC, waist circumference; WHR, waist-hip ratio.

Table 2-5. Potential Mechanisms and Experimental Evidence to Support a Role for Magnesium in Vascular Calcification and Related Phenomena*

Mechanism	Type of Study and/or Model	Evidence
Effects on lipids	Rats	Deficiency in rats induces hyperlipidemia (8-day deficient diet) characterized by postprandial accumulation of triglyceride-rich lipoproteins (194)
	Rats, in vitro	In cultured VSMCs, deficiency-induced triglyceride-rich lipoprotein accumulation is more susceptible to metal ion and cell dependent peroxidation (conjugated diene production) and affects cell growth (194)
	Humans	Deficiency in humans is associated with lower HDL and higher cholesterol and triglycerides, while supplementation is associated with reductions in triglycerides (127)
	Rats, in vivo	Deficiency decreases LPL activity, implicated in the production of HDL and the catabolism of triglyceride-rich lipoproteins (119,195)
	Rats, in vivo, in vitro, and Humans	Deficiency activates HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, and LCAT which catalyzes the formation of cholesterol esters (119,120)
	Rabbits	Supplementation in rabbits (as Mg aspartate hydrochloride) on low- or high-cholesterol diets lowered triglycerides and cholesterol (112)
	Rats	Supplementation in rats (as MgO) on 1% cholesterol diet decreased serum cholesterol (in the presence of high calcium) and lipoprotein alpha, and increased lipoprotein beta (196)
	Mice	Supplementation in ApoE-null mice (50 mg/mL as $MgSO_4$) on a low-fat diet reduced cholesterol and triglyceride levels compared to controls given tap water (115)
	Humans	Supplementation in healthy humans (as MgOH) in placebo-controlled study increased serum LCAT, HDL cholesterol and apolipoprotein A1, and decreased totol:HDL cholesterol ratio (120)
	Humans	Supplementation in humans with prior MI (as MgOH) in a placebo-controlled study significantly lowered apo-B levels and apoB/apoA1 ratios with 27% lower triglycerides; accompanied by increase in LDL size (128)
Effects on bone-related signaling molecules	Rats, ex vivo	In rat VSMCs exposed to calcification-inducing medium, co-incubation with Mg^{2+} decreased osteocalcin, BMP-2, increased osteopontin and matrix Gla protein, and activated TRPM7; notably, TRPM7 channel inhibitor recapitulated osteoblastic phenotype (98)
	Humans, ex vivo	In human aortic VSMCs exposed to calcification-inducing medium, co-incubation with Mg^{2+} restored to basal levels secretions of osteocalcin and matrix Gla protein, while ostepontin secretion was partially restored to basal levels (100)

Mechanism	Type of Study and/or Model	Evidence
	Cows, ex vivo	In bovine VSMCs exposed to calcification-inducing medium, co-incubation with Mg^{2+} decreased alkaline phosphatase activity and expression of genes associated with transdifferentiation of VSMCs into osteoblast-like cells, including *ALP*, *CBFA1*, *MSX2*, and *SOX9*, restores matrix Gla protein and BMP-2 to control levels (197)
	Rats, in vivo	In aortic ring segments of rats incubated in calcification-inducing medium, co-incubation with $MgCl_2$ reduced expression of osteopontin and osteocalcin (136)
	Rats	Deficiency in rats (50% of requirement) lowered serum PTH concentrations (198)
	Rats, in vivo	Supplementation in rats (as $MgSO_4$) in vitamin D3+nicotine-induced vascular calcification model dose-dependently reduced alkaline phosphatase levels and restored osteopontin expression to control levels (117)
	Humans	Supplementation in overweight, healthy humans (as Mg citrate) in a cross-over study increased PTH (16)
Effects on inflammation, platelet aggregation, endothelium	Humans, in vitro	Low concentration in endothelial cells activates NF-kB, increases RANTES, IL-8, MMP-2 and MMP-9 (MMPs cause plaque instability, and elevated levels of PAI-1 are pro-thrombotic) (102)
	Humans, in vitro	Low concentration inhibits endothelial cell proliferation, correlated with down-regulation of levels of CDC25B (responsible for cell cycle progression) and up-regulation of IL-1; up-regulates VCAM and PAI-1 (VCAM is responsible, at least in part, for the increased adhesion of monocytes to the endothelial cells grown in low magnesium (an early event in the formation of the atherosclerotic plaque is the adhesion of monocytes to the endothelial lining); impairs endothelial migratory response, demonstrated by cDNA array (6 genes down-regulated: c-src, ezrin, cytohesin, CD9, zyxin, and CDC25B; 2 genes up-regulated: DNA excision repair protein ERCC1 and AXL tyrosine kinase receptor) (103)
	Rats	Deficiency in rats induces activates leukocytes and macrophages and releases inflammatory cytokines and acute phase proteins (199)
	Rats	Deficiency in rats (50% of dietary requirement) increased TNF-alpha , IL-1B, and substance P in osteoclast environment (198)
	Rats, in vivo	Deficiency in rats stimulates cell proliferation, net collagen production, and superoxide generation in adult cardiac fibroblasts via elevated circulating and cardiac tissue levels of angiotensin II, and elevated plasma renin activity (199)
	Rats	Deficiency in rats led to higher neutrophil basal superoxide anion levels and circulating prostacyclin, PGE2, thromboxane A2 levels, and production of NO (199)

Mechanism	Type of Study and/or Model	Evidence
	Mice	Deficiency in mice increased circulating IL-1, IL-6, and TNF-α (199)
	Hamsters, Pigs	Deficiency in hamsters and swine decreased RBC glutathione by 62 and 45%, respectively (199)
	Humans	Depletion in humans (3 wks, <0.5 mmol/d magnesium) in healthy volunteers increased platelet aggregation (urinary thromboxane) and increased plasma adolsterone (57)
	Pigs, in vivo	Porcine aorta media exposed to venous blood of CAD patients showed inverse associations between intracellular (lymphocyte) concentration and platelet-dependent thrombosis (thrombus volume) and P-selectin expression (CD62P antigen, marker of platelet activation), but not platelet aggregation (by collagen stimulation) (200)
	Humans	Infusion in humans decreases urinary thromboxane concentration (pro-aggregatory prostaglandin) and angiotensin II-induced plasma aldosterone levels (57), as well as serum insulin levels (169)
	Humans	Supplementation in elderly diabetic humans (as Mg pidolate) in placebo-controlled study improved endothelial-dependent brachial FMD and higher serum ionized but not total serum Mg concentration (201)
	Humans	Supplementation in humans with CAD (as MgO and Mg carbonicum) in placebo-controlled study improved endothelial-dependent brachial FMD and exercise tolerance (202), but not nitroglycerine-induced (not endothelial-dependent) vasodilation, and higher sublingual epithelial Mg^{2+} concentration, with no changes to BP, BMI, glucose, or cholesterol measures
	Humans	Supplementation in overweight, healthy humans (as Mg citrate) in a cross-over study tended to decrease sICAM-1 and sVCAM-1, but increase IL-6 (16)
Effects on intima-media thickness, plaque and/or calcification	Rats, ex vivo	In rat VSMCs exposed to calcification-inducing medium, co-incubation with Mg^{2+} prevented calcification and activated TRPM7; notably, TRPM7 channel inhibitor recapitulated osteoblastic phenotype (98)
	Humans, ex vivo	In human aortic VSMCs exposed to calcification-inducing medium, co-incubation with Mg^{2+} significantly decreased calcification and improved cell viability; notably, blocking TRPM7 led to inefficiency of Mg^{2+} to prevent calcification. (100)
	Cows, ex vivo	In bovine VSMCs exposed to calcification-inducing medium, co-incubation with Mg^{2+} prevented calcium deposition and reduced cell apoptosis; in addition, higher Mg^{2+} concentrations prevented progression of already established calcification (197)
	Rats, in vivo	In aortic ring segments of rats incubated in calcification-inducing medium, co-incubation with $MgCl_2$ reduced calcium deposition (136)

Mechanism	Type of Study and/or Model	Evidence
	Pigs, in vivo	Deficiency in swine on moderately high vitamin-D3 diet showed more intimal lesions, cell degeneration, intimal thickening, and calcification (106)
	Pigs, in vivo	Deficiency in rats increased carotid artery cross-sectional area, intima-media thickness, and media-lumen ratio, with age-dependent arterial distensibility accelerated by deficiency, indicating chronic deficiency is exacerbated by the effects of aging (107)
	Rats, in vivo	Deficiency in rats induced inflammation of coronary tissue with lesions beginning as focal acute perivascular inflammation ("small, pale patches flecked with yellow in the heart muscle") and progressing through stages of necrosis, granulomatous inflammation, and fibroblastic proliferation to scar formation ("large areas of necrosis and calcification extending through the entire ventricular wall") (108); references (108,203) also summarize pre-1960s literature.
	Rats, in vivo	Long-term moderate deficiency in aging rats (vs. long-term normal diet vs. long-term supplementation as MgO) progressively increased SBP, and intra-arterial pulse pressure, and worsened age-induced composition and structure of aorta (thicker media, increased collagen content, reduced elastin/collagen ratio) compared to age-matched controls; note that long-term Mg-supplemented diet lowered BP and decreased mortality rate, without significant effect on aortic wall thickening or stiffening; interestingly, first deaths occurred earlier (wk 50, 70, and 90) and survival at end of experiment was 63%, 77%, and 90%, in deficient, control, and supplemented rats, respectively (109)
	Rats, in vivo	Deficiency in rats results in decreased aortic elastin content but higher aortic elastin calcium, suggesting altered elastase or elastase inhibitor activity may be affected by deficiency (note that initial calcium deposits appear to mainly be associated with elastic fibers, accompanied by elastin degradation and disorganization of aortic extracellular matrix) (110)
	Hamsters, in vivo	Deficiency in hamsters induced endothelial cell and VSMC hyperplasia and pleomorphism in coronary arteries, chronic inflammation of the media and adventitia, and fibrinoid necrosis; lesions of myocardial ischemia, distinct from lesions of myocardial necrosis and calcification, were evident, as were Von Kossa-positive granules in VSMCs of deficient hamsters (observations consistent with the view that Ca loading of VSMCs plays a role in arteriopathy) (111)

Mechanism	Type of Study and/or Model	Evidence
	Rabbits, in vivo	Supplementation in rabbits (as Mg aspartate hydrochloride) on 1%- or 2%-cholesterol diets showed almost no aortic intimal atherogenesis and had minimal intima-media thickening (vs. rabbits fed 2%-cholesterol diet which had intimal lesions on >60% of the aortic surface) (112)
	Mice	Supplementation in mice (as $MgCl_2$) on atherogenic diet dose-dependently decreased serum total cholesterol as well as markers of lipid peroxidation (TBARS - thiobarbituric acid reacting substances), and aortic deposition/content of total and free cholesterol, as well as aortic cholesterol esters; no effect on serum Mg, trigylcerides, or HDL cholesterol (114)
	Rabbits, in vivo	Supplementation in rabbits (as $MgSO_4$) on 1% cholesterol diets dose-dependently reduced the area of intimal aortic plaque, without substantively affecting blood cholesterol (113)
	Rabbits, in vivo	Deficiency in rabbits (equivalent to 50% of requirement) vs. supplementation as MgO, on 1% cholesterol diets had more aortic and aortic arch plaque, and 42% thicker intima than animals with adequate or high Mg intake (despite high Mg group eating more in general); note that cholesterol levels did not substantively change (105)
	Mice, in vivo	Supplementation in ApoE-null mice (50 mg/mL as $MgSO_4$) on a low-fat diet reduced aortic root plaque area to 66% that of animals on tap water (control) with a more pronounced effect in female than male mice (115)
	Mice, in vivo	Supplementation in LDLR-deficient mice (as $MgCl_2$ or $MgSO_4$) dose-dependently decreased atherosclerosis (fatty streak lesions) at the aortic sinus on animals fed a normal and western-style atherogenic diet (116)
	Rats, in vivo	Supplementation in rats (as MgO) on 1% cholesterol diet decreased sudanophilia-detected lipid deposits in heart ventricular valves and aorta, and eliminated kidney calcification induced at all levels of Ca intake (196)
	Rats, in vivo	Supplementation in rats (as $MgSO_4$) in vitamin D3+nicotine-induced vascular calcification model dose-dependently reduced medial calcification in aorta and calcium content of cells (117)
	Rats, in vivo	Supplementation in rats (at 10 g/L as $MgSO_4$) resulted in return of normal structure of thoracic aorta from diabetes-induced damage (e.g., thickened collagen fibers in VSMCs and foam cells) as well as return of BP and serum Ca:Mg ratio to normal levels (118)

Mechanism	Type of Study and/or Model	Evidence
	Humans	High serum concentration in humans in small studies of hemodialysis patients associates with lower calcification of hand arteries, carotid IMT, and mitral annular calcification (as summarized in (204))
	Humans	Supplementation in humans in small studies of hemodialysis patients associates with lower carotid IMT, and slower CAC progression (as summarized in (204))
	Humans	Lower serum concentration in humans associated with greater IMT and risk of 2 or more atherosclerotic plaques in the general Japanese population (135)
	Humans, in vivo	In humans, calcification of the carotid in patients with impaired renal function and atherosclerosis tends to consist of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) rather than whitlockite ($[Ca,Mg]_3[PO_4]_2$), which is found along with hydroxyapatite in those with normal renal function, suggesting that magnesium imbalance plays a role (205); this is supported by observations in rat models of adenine-phosphate versus calcitriol-induced chronic renal failure (206)

*Abbreviations used: BMP, bone morphogenetic protein; Ca, calcium; CAC, coronary artery calcification; CAD, coronary artery disease; FMD, flow-mediated dilation; HDL, high density lipoprotein; IMT, intima-media thickness; LCAT, lecithin-cholesterol acyl transferase; LDLR, low density lipoprotein receptor; LPL, lipoprotein lipase; Mg, magnesium; MI, myocardial infarction; NO, nitric oxide; PTH, parathyroid hormone; SBP, systolic blood pressure; TRPM. transient receptor potential cation channel, subfamily M; VSMCs, vascular smooth muscle cells.

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CHAPTER 3. METHODS

Essentially, all models are wrong, but some are useful.

George E. P. Box

in *Empirical Model-Building and Response Surfaces* (1987), page 424

co-authored with Norman R. Draper

The methods that deserve further elaboration and which are not extensively outlined in the individual manuscripts that constitute this dissertation are discussed in this chapter. Specifically addressed are:

1) justification for using the Tobit regression approach for analyzing vascular calcification data (related to aim 3); and

2) original power calculations (for each of the three aims).

3.1 Use of Tobit Regression for Calcification Data

In simplest terms, a Tobit model can basically be understood as a censored "linear" regression model. It was developed to analyze data with left- (below) or right- (above) censoring in the dependent variable. Censoring from above takes place when observations (e.g., "cases") with a value at or *above* some threshold, all take on the value of that threshold, so that a true (unobserved) value might be equal to the threshold, but it also might be higher. Similarly, in the case of censoring from below, values that fall at or *below* some threshold are censored. A familiar example of censored data might be standardized test scores. In the old high-school administered SAT, for example, it was not possible for a test-taker to receive a score below 200, although the range of all possible scores included 0–1600. Thus, if the true value, y^*, of an individual's score was anywhere from 0–200, it would have defaulted to an observed value, y, of 200. If we were trying to model predictors of SAT

scores, this value of 200 would be the threshold and would serve as an example of left-censoring. So, in the SAT example, y^*, the true values of the dependent variable, can be thought of as the propensity or capacity of students to score poorly on the SAT, but this is only realized as an actual score, y, if the scoring capacity exceeds 200 (1). (It should be noted that the author of this dissertation has no personal experience with low SAT scores.)

Given this kind of data, a researcher wanting to predict SAT scores might be tempted to conduct ordinary least squares regression with or without the scores at or below the threshold. In the former, ordinary least squares will treat the 0s as actual values and not as a lower limit. When the variable should be censored, ordinary least squares provides inconsistent estimates of the parameters, meaning that the coefficients from the analysis will not necessarily approach the "true" population parameters. The latter approach, systematically excluding individuals with scores at or below the threshold from the analysis (also called "truncated regression") results in discarding potentially many observations (loss of information). Further, the regression estimates would not apply for the whole population because they would have been derived from a non-randomly selected subsample. In other words, these estimates would be biased estimates of the true population parameters.

A censored analytic approach actually uses all the data in the dataset, unlike truncated analyses. In the case of censored data such as observed in calcification, Tobit regression also offers a way to include *all* observations — even the observations that have an Agatston Score of 0, which accounts for approximately 56% of coronary artery calcification (CAC) observations of the relevant Framingham study sample — without producing biased estimates of the population parameters. **Figures 3-1** and **3-2** are histograms depicting untransformed and natural-log (ln) transformed (Agatston Score + 1)

CAC and abdominal aortic calcification (AAC) distributions, respectively. It is clear that left censoring at 0 is a suitable approach. The figures also show that the natural-log transformation of the Agatston Score + 1 — not counting the 0 values — appears to be more normally distributed than the untransformed distribution.

In many cases, results of ordinary least squares and Tobit regressions are very similar, and coefficients (e.g., betas) are interpreted the same way, as the expected value in

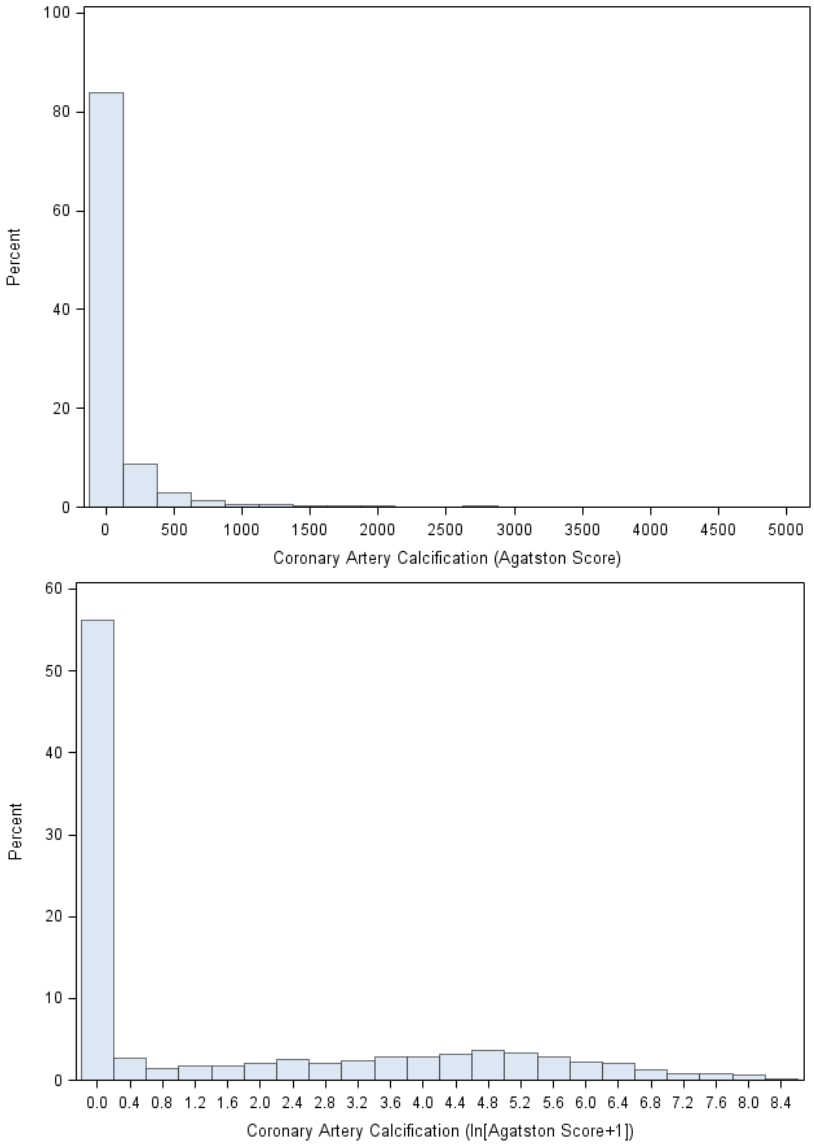

Figure 3-1. Untransformed (top) and ln-transformed distributions of CAC in the Framingham Heart Study.

the dependent variable given a change in the independent variable. For the present case, this would be the expected ln(Agatston Score +1) given a (gram) change in magnesium intake. However, as discussed above, estimates from ordinary least squares tend to be biased away from the true values of the population parameters.

A group of authors has compared Tobit regression to other commonly used regression forms in analyses of calcification data. The sample in Reilly, *et al*. in "Coronary

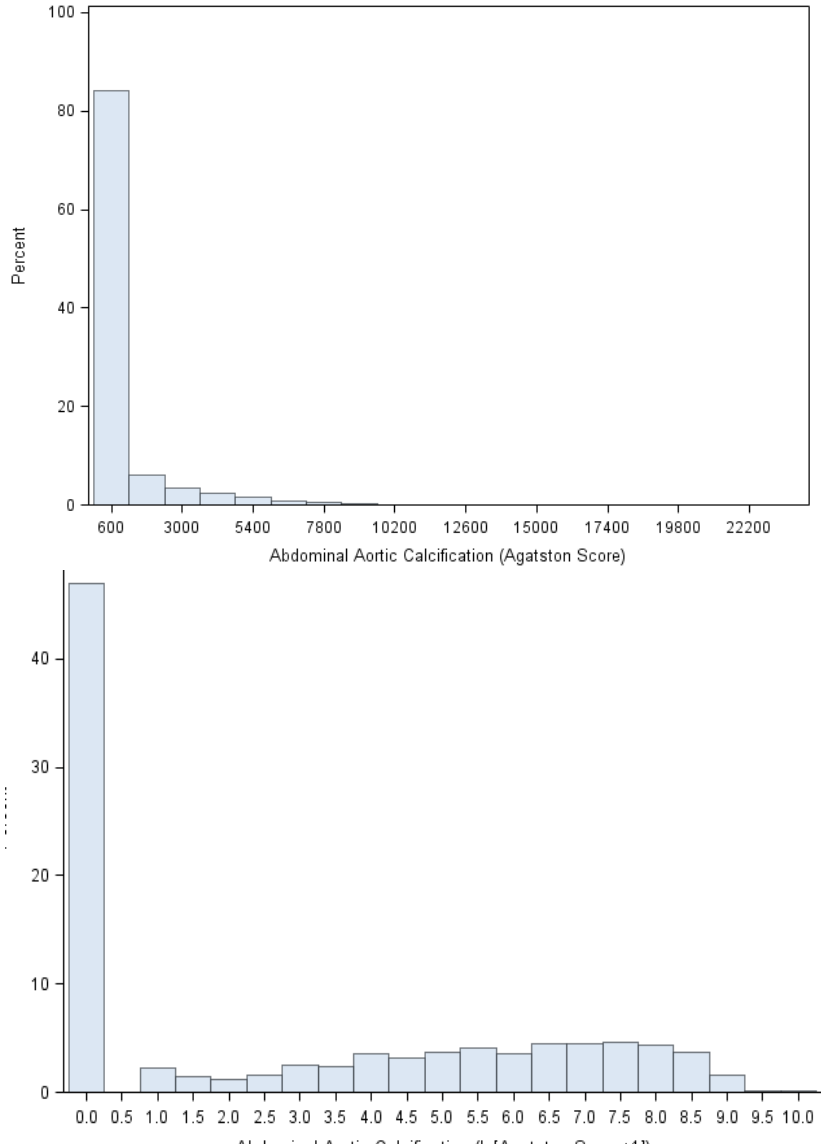

Figure 3-2. Untransformed (top) and ln-transformed distributions of AAC in the Framingham Heart Study.

artery calcification and cardiovascular risk factors: impact of the analytic approach" (2), included 914 men and women, 30–65 years old, with at least one first-degree relative with coronary artery disease, but free of overt cardiovascular disease (CVD), type 2 diabetes (T2D), hypercholesterolemia, and hypertension, and they were non-smokers. Four analytic approaches were used to estimate the cross-sectional association between CAC and established CVD risk factors: sex, age, ethnicity, smoking, family history of CVD, exercise, alcohol intake, body mass index (BMI), systolic blood pressure, total, high- and low-density lipoprotein cholesterol, triglycerides, fasting glucose and medications (including statins, beta-blockers, ACE inhibitors, aspirin, and hormone replacement therapy). The analytic approaches included:

1) ordinary least squares regression of ln(CAC), where CAC>0 (i.e., truncated regression);

2) ordinary least squares regression of ln(CAC+1), so as to include all CAC observations;

3) Tobit regression of ln(CAC+1);

4) logistic regression of CAC>0 vs. CAC=0; and

5) ordinal logistic regression of CAC in categories: 0, 1–10, 11–100, 101–400, >400.

Two models for each approach were developed, adjusted for 1) age and each risk factor separately, and 2) all risk factors in one model. The authors conducted analyses stratified by sex, in which they tested for interaction between pairs of risk factors. They also tested sex × risk factor interactions in the multivariate model when they grouped the sexes.

To compare the predictive effect of the different analytic approaches, the authors generated CAC scores for categories of age and BMI at mean values of the other risk factors, and then compared predicted values to the median CAC value for the same categories of the

factors observed in the data. They also used the AIC goodness-of-fit criterion (in which lower values are better) to select the model with the best fit to the data.

Reilly and colleagues' results indicate that the modeling approach substantially affects the identification of risk factors predictive of CAC. The ln(CAC) (approach #1) and the dichotomous logistic (approach #4) models resulted in the *fewest* statistically significant risk factors in the age-adjusted models. Furthermore, approaches #2, 3, and 5 (the ln[CAC+1], the Tobit of ln[CAC+1], and the ordinal logistic approaches) not only identified the *same* risk factors, but also had similar values for coefficients across all three approaches.

In the multivariate approach using all risk factors in the same model, the same three approaches (#2, 3, and 5) had the most consistent results. The two ln(CAC+1) models were highly consistent with each other, reflecting the earlier-mentioned observation that ordinary least squares and Tobit results are frequently very close. The authors commented: "In general, Tobit-predicted CAC values were closest to the observed CAC value when additional risk factors were tested in these analyses. AIC values were lower for Tobit regression than linear regression of ln(CAC+1) in fully-adjusted models in men ... and in women..." (2). The authors concluded that Tobit regression of ln(CAC+1) is a more "statistically appropriate" and "attractive" approach than linear regression of ln(CAC+1) given their sample.

Finally, based on their analyses, they cautioned against using dichotomous logistic regression approaches (e.g., CAC>0 vs. CAC=0) because of huge loss of information and inconsistencies they observed in terms of risk factors. This last point is particularly interesting, as the only study to date to have examined magnesium intake in relation to CAC used just such a logistic approach, and observed no association (3). The logistic approach is, however, included as a secondary analysis in the related manuscript of this dissertation for

one primary reason: it is widely used in the literature and may offer some opportunity for comparison of results across studies.

3.2 Power Calculations

3.2.1 Aim 1

Quanto (http://hydra.usc.edu/gxe/) was used to estimate required sample size based on the fasting insulin outcome with the following parameters: desired power of 80%; 2-sided alpha of 0.0018 (nominal alpha of 0.05/28 interaction tests); allele frequency ranges from 0.10 to 0.50; standard deviation of magnesium intake of 95.8 mg/day based on simple average across CHARGE cohorts; ln(fasting insulin) mean of 3.88 and standard deviation of 0.559; an estimated beta coefficient for the effect on ln(fasting insulin)of a SNP of 0.15; and an estimated beta coefficient for the effect on ln(fasting insulin) of a 1 mg/day difference in magnesium intake of 0.00055. Given the above parameters, **Figure 3-3** illustrates the

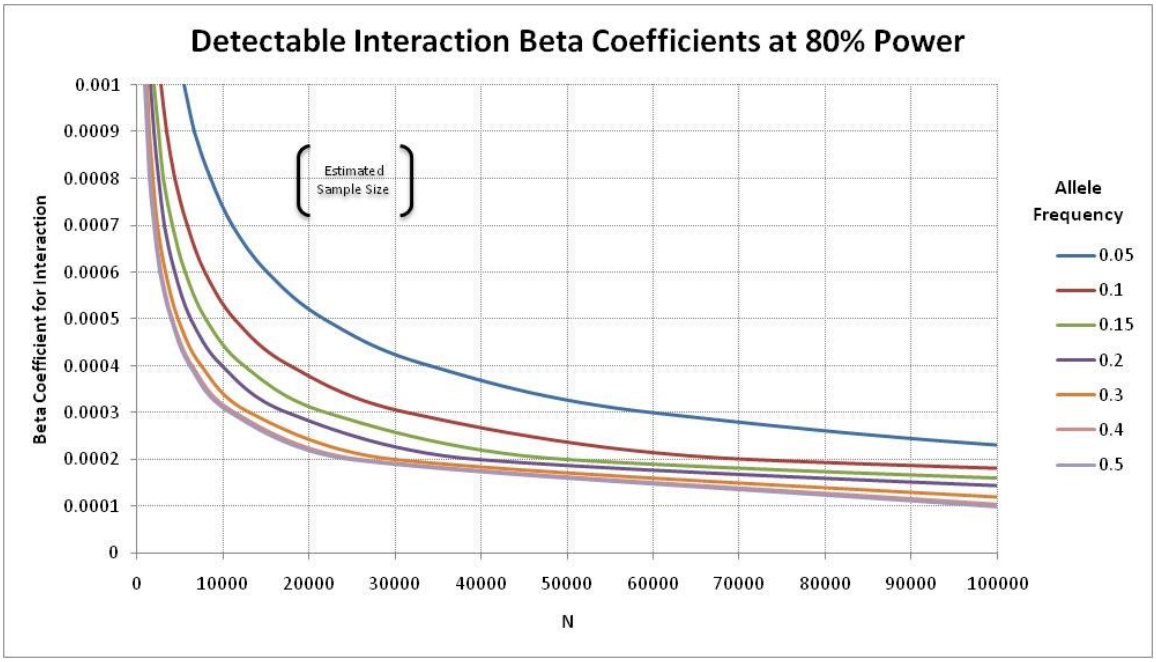

Figure 3-3. Sample sizes required to detect beta coefficients for magnesium × SNP interactions at given allele frequencies, based on magnesium intake and (ln)fasting insulin concentrations in preliminary review of meta-analysis in CHARGE cohorts.

sample size and allele frequencies needed to detect the beta coefficients for interaction indicated. For the actual analyses (see **Chapter 4**), we had two outcomes and total of 34 interaction tests; consequently we applied a Bonferroni correction of 0.05/34 interaction tests, for a final statistically significant threshold *P* value <0.0015.

By way of interpretive example, an interaction coefficient of 0.0003 in an insulin on magnesium×SNP regression would indicate that higher magnesium intake would have a weaker insulin-lowering effect in the presence of an insulin-raising allele. That is, in individuals carrying one copy of an insulin-raising allele, the lower insulin concentration observed in association with a 1 mg/day increase in magnesium intake would be reduced by 0.0003 units (that is, 0.0001 units lower insulin with each additional 1 mg/day magnesium intake instead of 0.0004 units lower without the insulin-raising allele).

3.2.2 Aim 2

Of the 3,799 participants at exam 5, we will exclude those with pre-existing T2D (diagnosed, by fasting glucose ≥126 mg/dl, or by therapy) at baseline; those missing exposure measures (i.e., missing or invalid food frequency questionnaire [FFQ], defined as FFQs which estimate intake as <600 or ≥4,000 kcal/day for women, <600 or ≥4,200 kcal/day for men, or which have ≥12 blank items); those missing outcome measures at follow-up at exam 7; and those missing covariate information.

An estimated 2,664 participants were free of diabetes at exam 5 who also had the required exposure and outcome measurements at later examinations. Although there are several outcomes in the Aim 2 analysis, power calculations are based on ability to detect differences in incident T2D rates owing to magnesium intake rather than changes in insulin resistance, as change will not be directly estimable owing to use of different assays at baseline and final exams. Therefore, if we assume that progression to diabetes within a

seven-year timeframe is 5%, at α=0.05, we will have >80% power to detect a survival difference of 3.5% or greater in the highest versus the lowest magnesium intake quartile category (approximately 500 per quartile category). Thus we will be able to detect a disease-free survival difference of 95% vs. 98.5% in lowest vs. highest quartile category of magnesium intake.

3.2.3 Aim 3

Original power calculations for this aim considered a sample of approximately 2,000 cross-sectional participants and 500 participants in each quartile category of magnesium intake. Assuming 50% of the population has prevalent CAC (Agatston Score >0), the proposed study has >80% power to detect odds of 0.70 or lower of having prevalent CAC in the highest quartile category when compared with the lowest (reference) quartile category.

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CHAPTER 4. MAGNESIUM META-ANALYSIS

Higher magnesium intake is associated with lower fasting glucose and insulin, with no evidence of interaction with select genetic loci, in a meta-analysis of 15 CHARGE consortium studies[1–2]

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[2] The full author list and affiliations are included in **Supplemental Table 4-4** at the end of this chapter. O.H.F. is the recipient of a grant from Pfizer Nutrition to establish a center for research on aging (ErasmusAGE). All other authors declare no conflict of interest.

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4.1 Abstract

Favorable associations between magnesium intake and glycemic traits, such as fasting glucose and insulin, are observed in observational and clinical studies, but whether genetic variation affects these associations is largely unknown. We hypothesized that single nucleotide polymorphisms (SNPs) associated with either glycemic traits or magnesium metabolism impact the association between magnesium intake and fasting glucose and insulin. Fifteen studies from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium provided data from up to 52,684 participants of European descent without known diabetes. In fixed-effect meta-analyses, we quantified 1) cross-sectional associations of dietary magnesium intake with fasting glucose (mmol/L) and insulin (ln-pmol/L), and 2) interactions between magnesium intake and SNPs related to fasting glucose (16 SNPs), insulin (2 SNPs), or magnesium (8 SNPs) on fasting glucose and insulin. After adjustment for age, sex, energy intake, body mass index, and behavioral risk factors, magnesium (per 50 mg/d increment) was inversely associated with fasting glucose [β (95% CI): -0.009 mmol/L (-0.013, -0.005), $P<0.0001$] and insulin [-0.020 ln-pmol/L (-0.024, -0.017), $P<0.0001$]. No magnesium-related SNP or interaction between any SNP and magnesium reached statistical significance after correction for multiple testing. However, rs2274924 in magnesium transporter-encoding *TRPM6* showed nominal association (uncorrected $P=0.03$) with glucose, and rs11558471 in *SLC30A8* and rs3740393 near *CNNM2* showed nominal interaction (uncorrected, both $P=0.02$) with magnesium on glucose. Consistent with other studies, higher magnesium intake associated with lower fasting glucose and insulin. Nominal evidence of *TRPM6* influence, and magnesium interaction with select loci, suggest further investigation is warranted.

4.2 Introduction

Magnesium is an essential mineral found in many foods; rich sources include whole grains, green leafy vegetables, coffee, and legumes. Magnesium is a critical cofactor in over 300 enzymatic reactions, including those related to energy metabolism (1). Evidence from cross-sectional and longitudinal observational studies suggests that diets higher in magnesium are associated with reduced risk of insulin resistance (2–8) and type 2 diabetes (9,10), while in intervention studies, supplemental magnesium improves measures of glucose and insulin metabolism in generally healthy adults (11,12), as well as in those with insulin resistance (13,14) and type 2 diabetes (15,16). However, little is known about potential interaction between magnesium intake and genetic variability on glycemic traits, in which genetic variants related to either magnesium transport and homeostasis, or glucose and insulin metabolism, may modify the pathways through which magnesium exerts its effects.

Single nucleotide polymorphisms (SNPs) associated with modest elevation in fasting glucose (FG) and fasting insulin (FI) levels have been identified through meta-analysis of genome-wide association studies (GWAS) (17). In addition, a GWAS meta-analysis of serum magnesium, a biomarker of magnesium status, identified six SNPs in genes linked to magnesium transport and homeostasis (18). Among these six SNPs, the C allele of rs4072037 in *MUC1*, which was associated with lower serum magnesium, was also associated with lower FG concentrations (18). Three studies have also investigated associations between magnesium-related loci in transient receptor potential cation channel, subfamily M, members 6 (*TRPM6*) or 7 (*TRPM7*) and diabetes or glycemic traits. One of these studies observed an association between carriers of the *TRPM6* rs2274924 variant and elevated total glycosylated hemoglobin and odds of gestational diabetes in 997 women following delivery (19). The loci studied in these two genes in the other two studies did not modify either disease (20,21) or glycemic traits (21); however, these were small studies,

and one included women only (20). The latter study (20) also examined interactions between magnesium intake and *TRPM6* and *TRPM7* loci on risk of type 2 diabetes, reporting increased odds of disease in women with a risk haplotype at rs3750425 and rs2274924 in *TRPM6* only when magnesium intake was <250 mg/d.

Despite plausible biological mechanisms underlying associations between magnesium and glycemic traits, such as magnesium's role as a co-factor for tyrosine kinase in the beta subunit of the insulin receptor (22,23), the interaction between dietary magnesium and glycemia-related genetic variants on glucose and insulin has yet to be examined. Further, genetic factors related to magnesium transport and homeostasis may modify associations between magnesium intake and glycemic traits. Examining interactions between dietary magnesium and these variants may enhance our understanding of type 2 diabetes etiology and pathogenesis. Therefore, we examined cross-sectional associations of dietary magnesium intake with FG and FI, associations of magnesium-related SNPs with FG and FI, and interactions between dietary magnesium intake and both magnesium-related and glycemia-related SNPs on FG and FI in meta-analyses of 15 cohort studies.

4.3 Research Design and Methods

4.3.1 Participating Cohorts

The sample for the cross-sectional meta-analyses included up to 52,684 participants of European descent from 15 cohort studies (**Table 4-1**). These studies participate in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Nutrition Working Group (24): Atherosclerosis Risk In Communities study (ARIC), Family Heart Study (FamHS), Framingham Heart Study Offspring and Generation 3 (FHS), Cardiovascular Health Study (CHS), Gene-Diet Attica Investigation on childhood obesity (GENDAI), Greek Health Randomized Aging Study (GHRAS), Gene-Lifestyle interactions And

Complex traits Involved in Elevated disease Risk (GLACIER), Health, Aging, and Body Composition study (Health ABC), Invecchiare in Chianti (InCHIANTI), Malmö Diet and Cancer Study cardiovascular cohort (Malmö), Multi-Ethnic Study of Atherosclerosis (MESA), Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS), Rotterdam Study (Rotterdam), Uppsala Longitudinal Study of Adult Men (ULSAM), and the Cardiovascular Risk in Young Finns Study (YFS). Participants provided written informed consent, and the protocol was approved by local institutional review boards. Participants within each cohort were excluded from analyses if they had diabetes, defined as diagnosed or self-reported diabetes, and/or fasting glucose ≥7 mmol/L, and/or use of diabetes medications.

4.3.2 Dietary Assessment

Dietary data was collected via food frequency questionnaire (FFQ) in 11 cohorts and via dietary recall (one cohort), food record (two cohorts), or a combination of food diary and FFQ (one cohort). Daily intakes of dietary magnesium (from food and beverage sources), kilocalories, fiber, caffeine, and alcohol were estimated for each participant (**Supplemental Table 4-1**). Using data from two of our cohorts (ARIC and FHS), we assessed the rank ordering of participants using magnesium values derived from food/beverage sources only versus food/beverage and supplemental sources. Unadjusted Spearman correlations between food/beverage magnesium and total (food/beverage and supplemental) magnesium intake were 0.93 and 0.92 in ARIC and FHS, respectively, and 0.84 and 0.83 in ARIC and FHS, respectively, after adjusting for energy intake. Ranks did not vary appreciably and supplemental sources contributed on average just 8–16 mg/d of magnesium intake above that derived from food/beverage sources. Only six of the 15 studies had supplement use information on fewer than 8,000 participants. Given these considerations, and in order to maximize sample size, we considered magnesium intake from food/beverage sources only.

4.3.3 Genotyping, Imputation, and SNP Selection

SNPs were previously directly genotyped or imputed by participating cohorts prior to inclusion in this analysis (Supplemental Table 4-1). Of the 25 SNPs included in this meta-analysis, a previous GWAS meta-analysis in the Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC)—which included data from CHS, FHS, InCHIANTI, and Rotterdam—identified 15 SNPs associated with FG, one with FI, and one with both FG and FI (17). Five SNPs were previously identified in another meta-analysis of GWAS of serum magnesium in MAGIC, including ARIC, CHS, FHS, and Rotterdam (18). The remaining three SNPs associated with magnesium transport or homeostasis selected for this meta-analysis were based on the report by Song, *et al*. (20). Not all SNPs were available in every cohort; total sample sizes for analyses vary accordingly (**Supplemental Table 4-2**).

4.3.4 FG and FI Measurement

FG (mmol/L) and FI (pmol/L) were quantified in each cohort using similar procedures (Supplemental Table 4-1). FI was natural log-transformed to reduce skewness prior to data analysis.

4.3.5 Covariate Measurement

Cohort-specific assessment methods and definitions for body mass index (BMI), education level, smoking status, and physical activity are provided in Supplemental Table 4-1.

4.3.6 Cohort-Specific Analyses

Each cohort followed a uniform analysis plan to conduct the following analyses. First, main associations between magnesium intake and FG and FI were quantified adjusting for age, sex, and energy intake (model 1); plus BMI (model 2); plus smoking, education, physical activity, and alcohol intake (model 3); plus fiber and caffeine intake (model 4). Fiber and caffeine were considered as covariates to distinguish associations of magnesium with FG and FI from those due to nutrients contained in shared food sources displaying similar

associations with glycemic traits (e.g., whole grains and coffee). Second, to verify earlier reported associations with FG (16 SNPs) and FI (2 SNPs), and to investigate associations between magnesium-related SNPs (8 SNPs) and FG and FI, cohorts regressed FG and FI on SNPs of interest using an additive genetic model (per additional outcome-raising allele) adjusted for age and sex, and where relevant, study field center and/or family or population substructure. Third, magnesium-SNP interactions were investigated by including a first-order interaction term (magnesium intake × SNP) in a model including magnesium intake, SNP, age, sex, and energy intake. Prior to meta-analysis, the beta (β) coefficients and standard errors (SE) involving magnesium intake (per 1 mg/d) reported by each cohort were multiplied by 50 to estimate the association of a 50 mg/d increment in magnesium intake, as 1 mg/d estimates were exceedingly small. Fifty milligrams of magnesium reflects intake from approximately two ounces of espresso or two slices of whole-wheat bread. Assuming a hypothesis-consistent inverse association of magnesium with FG and FI, positive interaction β coefficients indicate that the magnitude of the inverse association between magnesium intake and FG or FI is less in the presence of an FG- or FI-raising allele. That is, for individuals who carry one copy of an FG- or FI-raising allele, the lower FG or FI concentration observed in association with a 50 mg/d increment in magnesium intake would be diminished. Correspondingly, negative interaction β coefficients indicate that the magnitude of the inverse association between magnesium intake and FG or FI is greater in the presence of an FG- or FI-raising allele. That is, for individuals who carry one copy of an FG- or FI-raising allele, the lower FG or FI concentration observed in association with a 50 mg/d higher magnesium intake would be stronger.

4.3.7 Meta-Analyses

We conducted inverse variance-weighted, fixed-effect meta-analyses for 1) main associations of magnesium intake on FG and FI using STATA (version 12, Stata Corporation,

College Station, Texas); 2) main associations of SNPs with respective outcomes using METAL (www.sph.umich.edu/csg/abecasis/metal/); and 3) interactions between SNPs and magnesium intake on respective outcomes using METAL. The sample sizes for magnesium associations with FG ranged from 52,684 (model 1) to 48,588 (model 4), and with FI, they ranged from 37,640 (model 1) to 34,137 (model 4). The sample sizes for interaction analyses on FG ranged from 29,280 (rs2274924) to 52,470 (rs4607517), and on FI, they ranged from 28,851 (rs2274924) to 37,804 (rs780094). Heterogeneity across studies was tested using Cochran's *Q* statistic and quantified using the I^2 statistic (25). Approximately defined ranges for interpreting I^2 for low, moderate, substantial, and considerable heterogeneity are 0–40%, 30–60%, 50–90%, 75–100%, respectively (26). To assess potential sources of heterogeneity, we conducted meta-regression of the main magnesium association and of the interaction analyses. (For the main associations of the eight magnesium-related SNPs on FG or FI [Supplemental Table 4-2], since none [0%] to low [28%] heterogeneity was observed, meta-regressions were not conducted for these associations.) Meta-regression covariates included region (Northern Europe vs. Mediterranean vs. US), mean age of cohort (<60 vs. ≥60 yrs old), mean magnesium intake of cohort (<300 vs. ≥300 mg/d), mean BMI of cohort (<27 vs. ≥27 kg/m^2), percentage of the cohort that was female, and sample size. We also conducted sensitivity analyses to assess the influence on the meta-analyzed estimate of any single cohort study by repeating analyses removing one cohort study at a time in the associations for magnesium, magnesium-related SNPs, and magnesium-SNP interactions. Random-effects meta-analyses were conducted secondarily; results were similar to those from the fixed-effect meta-analyses. Thus we only present the results of the fixed-effect meta-analyses.

Power calculations for various magnitudes of association and sample sizes have been published elsewhere (24,27). Statistical significance was defined at an alpha level of 0.0015, based on Bonferroni correction for 34 total interaction tests.

4.4 Results

The demographic, dietary, and outcome characteristics of participants in the 15 cohort studies are provided in Table 4-1. Mediterranean cohorts tended to have the lowest overall mean dietary magnesium intake and Northern European cohorts the highest; the mean daily intake of dietary magnesium ranged from 224.7 mg/d in GENDAI (Greece) to 479.7 mg/d in YFS (Finland). Plots of mean intake across cohorts did not suggest that intake differs by either dietary assessment method or mean age (*Supplemental Figures 4-1* and *4-2*).

4.4.1 Associations of Magnesium Intake with FG and FI

After adjusting for age, sex, alcohol and energy intake, BMI, smoking, education, and physical activity (model 3), magnesium intake was inversely associated with FG and FI concentrations. Per daily 50-mg increment of dietary magnesium, FG was 0.009 mmol/L lower [β (95% CI): -0.009 mmol/L (-0.013, -0.005), *P*<0.0001] (**Table 4-2** and **Figure 4-1**) and FI was -0.020 ln-pmol/L lower [β (95% CI) -0.020 ln-pmol/L (-0.024, -0.017), *P* <0.0001] (Table 4-2 and **Figure 4-2**). After additional adjustment for caffeine and fiber intake (model 4), the association of magnesium with FG was attenuated: β (95% CI): -0.001 mmol/L (-0.006, 0.004), *P* = 0.78 (Table 4-*2*). However, magnesium intake remained significantly inversely associated with FI, although the magnitude of the association was mitigated: β (95% CI): -0.012 ln-pmol/L (-0.017, -0.007), *P* <0.0001 (Table 4-2). Results of sensitivity analyses and meta-regressions did not substantively affect our conclusions, or reveal any clear sources of heterogeneity (results not shown).

4.4.2 Associations of SNPs with FG and FI

Meta-analyzed estimates of SNP associations with FG and FI are presented in Supplemental Table 4-2. The direction and magnitude of the associations of the 16 glucose- and two insulin-related SNPs on FG or FI, respectively, were consistent with those previously reported (17). The eight SNPs related to magnesium homeostasis and transport showed no statistically significant association with either FG or FI (**Supplemental Table 4-2**). A nominally significant inverse association with FG was observed per additional G allele at rs2274924 in *TRPM6* [β (95% CI): -0.013 (-0.024, -0.001) mmol/L, $P = 0.03$]. Results of sensitivity analyses and meta-regressions did not substantively affect our conclusions, or reveal any clear sources of heterogeneity (results not shown).

4.4.3 Magnesium-SNP Interactions on FG and FI

Meta-analyzed estimates of interactions between magnesium intake and SNPs on FG and FI are presented in **Table 4-3** (with additional information in **Supplemental Figures 4-3** and **4-4**). There was no statistically significant interaction on either FG or FI, after correction for multiple testing (i.e., at $P < 0.0015$). Two nominally significant interactions were observed between SNPs and magnesium on FG. The first at rs11558471 in *SLC30A8* (previously associated with FG levels), suggested a stronger glucose-lowering association with higher magnesium intake in those with the A (risk) allele at this locus [interaction β (95% CI): -0.0045 (-0.0082, -0.0008) mmol/L per A allele and 50 mg/d increment in magnesium, $P = 0.02$]. The second at rs3740393 near *CNNM2* (previously associated with magnesium transport/homeostasis), suggested a weaker inverse association between magnesium intake and FG in those with the G (risk) allele at this locus [interaction β (95% CI): 0.0064 (0.0009, 0.0119) mmol/L per G allele with 50 mg/d higher magnesium, $P = 0.02$]. Results of sensitivity analyses and meta-regressions did not substantively affect our conclusions, or reveal any clear sources of heterogeneity (results not shown).

4.5 Discussion

In this cross-sectional meta-analysis involving more than 50,000 participants free of diabetes in 15 cohort studies from the CHARGE Consortium, we observed inverse associations between magnesium intake and FG and FI concentrations, even after adjusting for BMI and other demographic and lifestyle factors known to influence diabetes risk. After further adjusting for fiber and caffeine intake, the inverse association of magnesium with FI remained statistically significant, but not the association with FG. However, including these dietary components in the model may be an over-adjustment, reflecting common food sources, thus leading to the observed mitigated associations. Our study is among the largest, to our knowledge, to investigate these tightly controlled measures of glucose homeostasis in generally healthy populations. Our findings support those of recent meta-analyses of studies on magnesium and incident type 2 diabetes, which estimate approximately 14% reduced risk of disease per daily 100-mg increment in magnesium intake (9,10). Previous prospective cohort studies investigating whole-grain (28) and coffee (29–33) consumption on type 2 diabetes risk have observed beneficial associations with higher consumption. From a reductionist viewpoint, it remains of interest whether the whole foods themselves or their key components (e.g., magnesium or fiber in whole grains, or magnesium, caffeine, or other polyphenols in coffee) exert health benefits. Our observations suggest that the association between magnesium intake and FI is at least partly independent of other dietary constituents found in magnesium-containing foods, such as whole grains and coffee, a phenomenon previously observed in at least two smaller observational studies (2,6). In prospective studies of magnesium intake and type 2 diabetes, associations of magnesium intake do not appear to be substantially affected after accounting for fiber intake (9). However, results of previous cross-sectional studies in adults free of diabetes are inconsistent with respect to magnesium's associations with FG, irrespective of adjustment

for fiber intake (6,34,35). As blood glucose is generally under tight homeostatic control in diabetes-free populations such as those included here, our observations lend support to the hypothesis of magnesium's actions in insulin sensitivity and resistance with downstream, mitigating effects on diabetes pathogenesis (23).

Our findings on the associations of 16 glucose- and two insulin-related SNPs with FG and FI are in line with those previously reported (17). We also investigated eight magnesium-related loci in relation to FG and FI, hypothesizing that if magnesium is causally related to these traits, genes that influence magnesium transport and homeostasis might be expected to affect FG and FI. However, we found no statistically significant evidence that variation at these loci influence FG or FI. Our strongest, nominally significant association with FG was at rs2274924 (P = 0.03) in *TRPM6*, a gene encoding a magnesium-permeable epithelial channel with a critical role in magnesium reabsorption in the kidney. The missense mutation (A>G) at rs2274924 causes a Lys1584Glu amino acid change in exon 27 of the resulting channel protein. Nair, *et al*. (19) recently reported that in the presence this polymorphism, the insulin signaling cascade is unable to activate the phosphorylation of the amino acid adjacent to the substituted amino acid resulting from the polymorphism, thereby rendering the variant TRPM6 channel insensitive to the activating effects of insulin. Furthermore, increased glycosylated hemoglobin and greater risk of gestational diabetes was observed in GG homozygotes compared AA homozygotes in a cohort of 997 women (19). In contrast, rs2274924 was not associated with type 2 diabetes in one small case-control study in women (20). Consistent with other reports, we found no association of other previously studied *TRPM6* or *TRPM7* loci with either FG or FI (18,21). Taken together, it is unlikely that the most of the loci we studied implicated in magnesium transport and homeostasis are meaningfully impacting fasting measures of glucose or insulin, with the possible suggestive exception of *TRPM6* rs2274924 on glucose. Given recent evidence (19)

and our cross-sectional approach, it is possible that this locus has deleterious downstream effects on intracellular magnesium and glucose handling secondary to the variant product's reduced sensitivity to insulin. Regardless of whether these loci are themselves playing a direct role does not preclude the involvement of magnesium-dependent pathways in the regulation of glucose and insulin homeostasis.

We observed no statistically significant interactions between magnesium intake and loci on FG or FI, suggesting that magnesium's favorable associations with these traits are independent of genetic variation at the loci studied. Our strongest, albeit not statistically significant magnesium × SNP interaction on FG was at rs11558471 in *SLC30A8*, which we previously reported showed some evidence of interaction with total zinc intake on FG, although not below the multiple testing-corrected significance threshold in that study (P <0.0025) (27). The interaction we report here with magnesium was in the same direction as that reported for zinc, which may reflect shared chemical properties of zinc and magnesium cations, or reflect similar affinity for these cations by the transmembrane transporter encoded by *SLC30A8*. The second nominally significant interaction with magnesium intake on FG was at rs3740393 near *CNNM2*. The gene encodes a membrane protein required for renal magnesium handling; the G allele at this locus is associated with lower serum magnesium (18). If replicated in future studies, the interaction suggests that the magnitude of the inverse association between magnesium intake and FG is diminished in the presence of the serum magnesium-lowering G allele, versus the C allele. This interaction may plausibly indicate a higher dietary magnesium requirement in those with a propensity for lower serum magnesium in order to observe beneficial effects on fasting glucose.

To date, the only other study examining interactions between magnesium intake and loci in *TRPM6* and *TRPM7* was a small case-control study of type 2 diabetes of

predominantly Caucasian, older women followed for 10 years. The authors reported that women who were carriers of two rare alleles from non-synonymous SNPs in *TRPM6* (rs3750425 and rs2274924) had nearly five times the odds of type 2 diabetes when their magnesium intake was <250 mg/d (20). Despite our null interaction findings in relation to fasting glucose and insulin, we cannot rule out the possibility that in the presence of chronically low magnesium intake, these loci impact long-term risk of diabetes, which may not be reflected in the cross-sectional homeostatic measures analyzed in our study of individuals without known diabetes.

To our knowledge, this is among the largest observational studies to investigate magnesium intake's associations with FG and FI, and it is the largest meta-analysis investigating interactions between magnesium intake and risk loci on FG and FI. In addition to following a uniform, *a priori* analysis plan in each cohort, we used cross-cohort exposure, covariate, and outcome definitions in a consortium-based meta-analytic context that minimizes the typical recall and publication bias associated with literature-based meta-analyses (36). The favorable inverse associations we report between magnesium intake and FG and FI are consistent with other studies investigating similar relationships.

The loci for this analysis were selected *a priori* from those identified and replicated in previous GWAS meta-analyses. Inherent to the GWAS method is that emergent loci have a homogenous effect both within and across populations; that is, these are areas of no environmental interaction, despite potentially widely varying environmental exposures, such as diet (37). Gene × environment approaches such as ours that rely on prior GWAS therefore must overcome the limitations inherent to both the homogeneity and the relatively small effect sizes conveyed by these loci in order to determine whether the variants modify the effects of an environmental exposure (37). While the clinical significance of interactions even smaller than those detectable (due to power (24)) in

analyses such as ours may be limited, they nevertheless remain of considerable mechanistic interest. Despite our hypothesis, the glycemia- and magnesium-related loci we investigated may yet be implicated in pathways through which magnesium acts to ultimately affect diabetes risk. Short-term magnesium supplementation (500 mg/d) in healthy adults has been shown to up- and down-regulate over 50 genes involved in inflammatory and metabolic pathways, and magnesium regulation, as well as genomic regions with unknown function (38). Further, magnesium's interactions with loci to regulate insulin and glucose metabolism may be more evident in post-challenge measures of related traits, rather than the fasting traits used in our study (13,39,40). Therefore, our observations regarding the specific loci in the present study do not rule out the possibility that other genetic variants or genomic regions associated with glycemic traits interact with dietary magnesium.

In conclusion, our results indicate that higher dietary magnesium intake is inversely associated with FG and FI in individuals free of diabetes, generally irrespective of genetic variation at glycemia- and magnesium-related loci investigated. Nominal evidence for the influence of a *TRPM6* locus on FG, and for magnesium interaction with loci in *SLC30A8* and *CNNM2* on FG indicate that future research, including genome-wide interaction studies, is necessary and may reveal genomic regions that more strongly influence associations between magnesium intake and traits related to glucose homeostasis (37).

4.6 Acknowledgements

See Supplemental Table 4-3 for cohort study sources of support and acknowledgments. O.H.F. is the recipient of a grant from Pfizer Nutrition to establish a center for research on aging (ErasmusAGE). All other authors declare no conflict of interest. An abstract of portions of this research was previously presented at Experimental Biology 2012, April 2012, San Diego, CA (41).

Figure 4-1. Forest plot of associations between dietary magnesium (50 mg/d) and fasting glucose (mmol/L) in 15 US and European cohort studies. The estimate from each cohort study, indicated by a filled square, is adjusted for age, sex, BMI, smoking, education, physical activity, alcohol intake, energy intake, study center (in ARIC, CHS, FamHS, Health ABC, InCHIANTI, MESA), and/or family or population substructure (in CHS, FamHS, FHS, MESA, YFS). GENDAI (child/adolescent cohort) did not adjust for smoking, education, or alcohol intake, as these variables are not applicable in this study. Rotterdam did not adjust for physical activity, as this variable was not available in the study. The size of the square is proportional to the weight of the cohort study in the overall fixed-effect estimate, and the horizontal line represents the 95% CI. The overall summary estimate and its 95% CI are indicated by the open diamond.

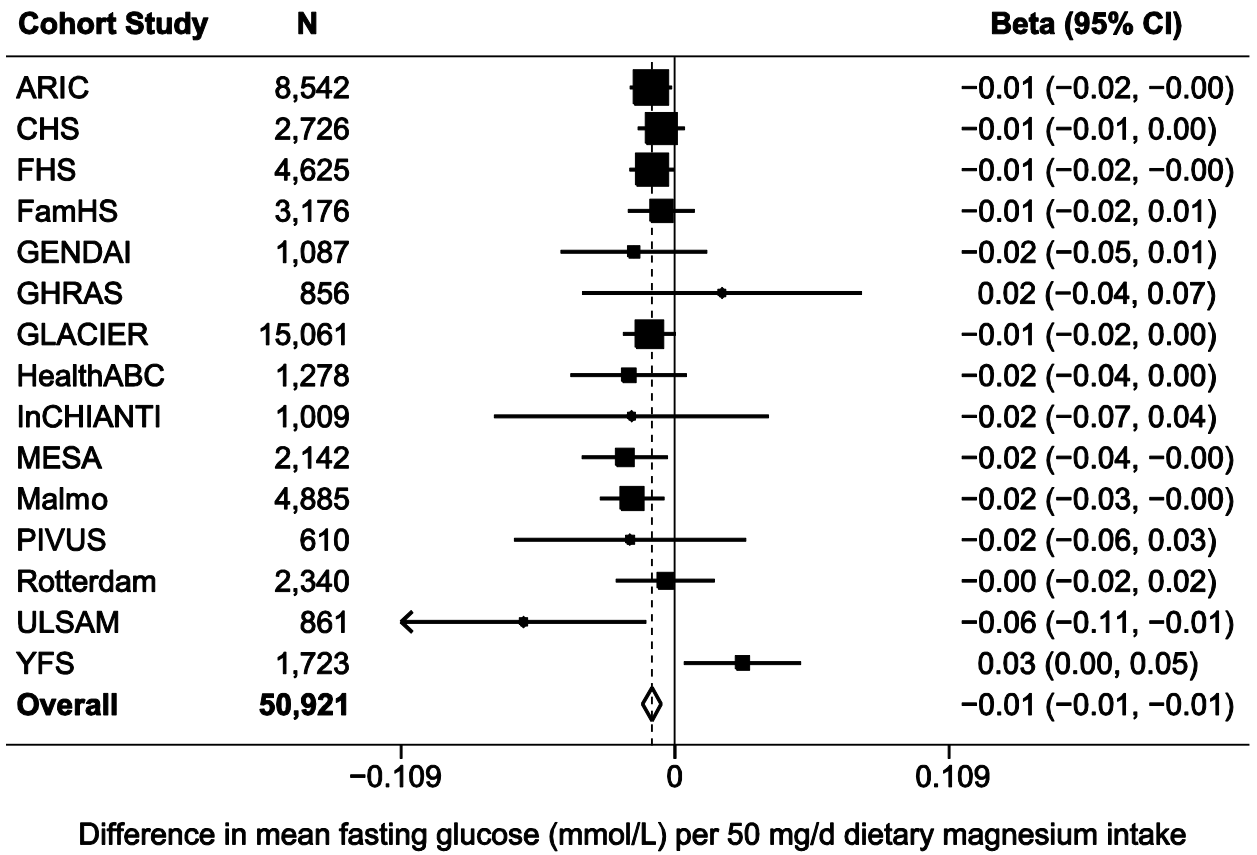

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Figure 4-2. Forest plot of associations between dietary magnesium (50 mg/d) and fasting insulin (ln-pmol/L) in 15 US and European cohort studies. The estimate from each cohort study, indicated by a filled square, is adjusted for age, sex, BMI, smoking, education, physical activity, alcohol intake, energy intake, study center (in ARIC, CHS, FamHS, Health ABC, InCHIANTI, MESA), and/or family or population substructure (in CHS, FamHS, FHS, MESA, YFS). GENDAI (child/adolescent cohort) did not adjust for smoking, education, or alcohol intake, as these variables are not applicable in this study. Rotterdam did not adjust for physical activity, as this variable was not available in the study. The size of the square is proportional to the weight of the cohort study in the overall fixed-effect estimate, and the horizontal line represents the 95% CI. The overall summary estimate and its 95% CI are indicated by the open diamond.

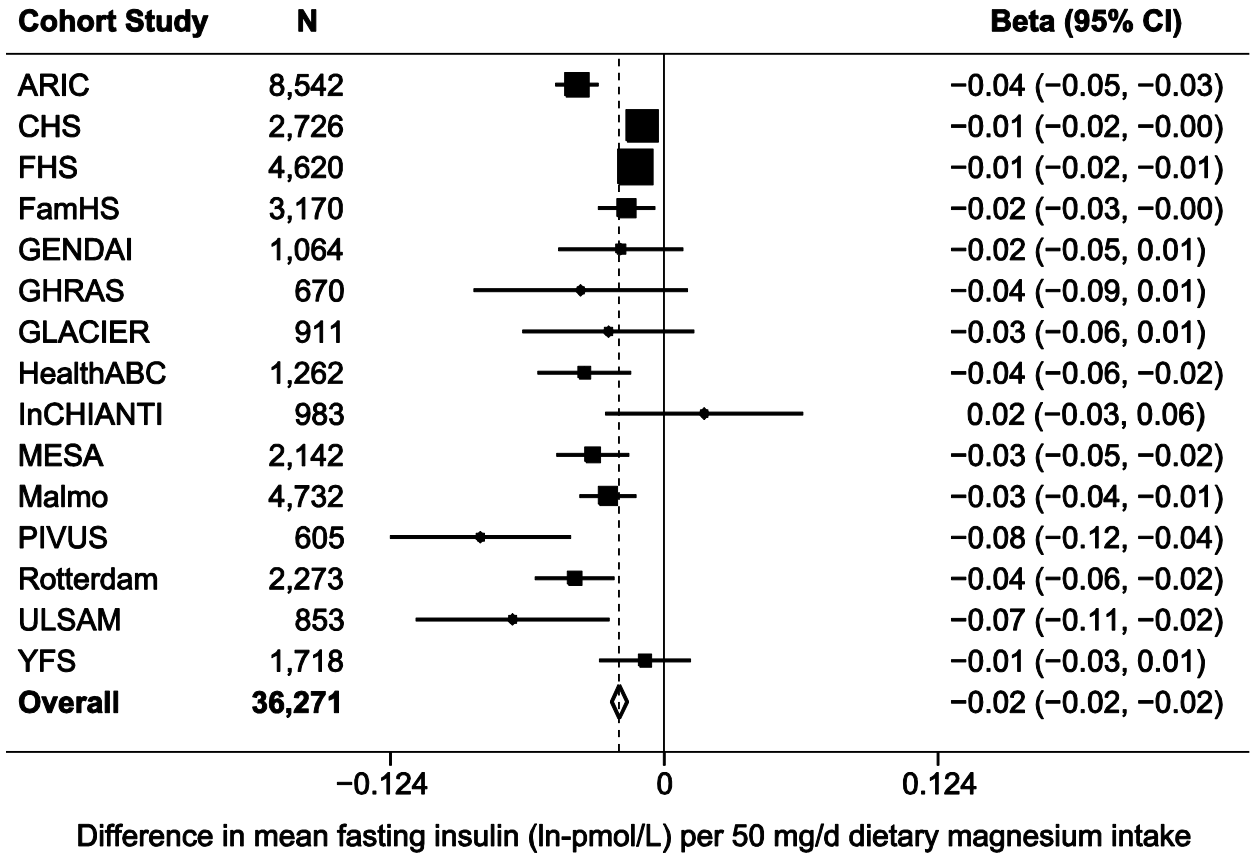

Table 4-1. Participant Characteristics of 15 US and European Cohort Studies[1]

Cohort Study (Country)	*n*[2]	Age *y*	Sex *% women*	BMI *kg/m*[2]	Fasting glucose *mmol/L*	Fasting insulin *pmol/L*[3]
Atherosclerosis Risk in Communities (ARIC) Study (US)	8951	54 ± 5.7	53.7	27 ± 4.6 (*n*=8586)	5.5 ± 0.50	58.6 ± 1.93 (16.1, 213)
Cardiovascular Health Study (CHS) (US)	2745	72 ± 5.4	62.3	26 ± 4.3 (*n*=2737)	5.5 ± 0.52	84.8 ± 1.54 (36.5, 197)
Family Heart Study (FamHS) (US)	3187 (3181)	51 ± 14	53.6	27 ± 5.3	5.2 ± 0.50	60.3 ± 1.82 (18.6, 196)
Framingham Heart Study (FHS) (US)	5743 (5435)	49 ± 14	54.9	27 ± 5.2	5.3 ± 0.50	79.8 ± 1.47 (14.9, 544)
Gene-Diet Attica Investigation on Childhood Obesity (GENDAI) (Greece)	1087 (1064)	11 ± 0.70	53.2	20 ± 3.4	4.8 ± 0.48	40.0 ± 1.72 (13.8, 116)
Greek Health Randomized Aging Study (GHRAS) (Greece)	856 (670)	72 ± 7.5	71.2	30 ± 4.8	5.8 ± 1.6	43.1 ± 1.75 (14.4, 129.10)
Gene-Lifestyle interactions And Complex traits In Elevated disease Risk (GLACIER) (Sweden)	14,940 (892)	52 ± 8.8	60.7	26 ± 4.0	5.4 ± 0.62	41.3 ± 1.90 (11.8, 144.7)
Health, Aging, and Body Composition Study (Health ABC) (US)	1281 (1256)	74 ± 2.8	50.2	26 ± 4.0	5.2 ± 0.55	45.1 ± 1.70 (15.9, 128)
Invecchiare in Chianti (Aging in the Chianti Area; InCHIANTI) (Italy)	1071 (1044)	68 ± 16	56.3	27 ± 4.1	4.8 ± 0.61	65.4 ± 1.70 (23.1, 185)
Malmö Diet and Cancer Study (Malmö) (Sweden)	4867 (4864)	58 ± 5.9	60.0	25 ± 3.8	5.5 ± 0.52	37.3 ± 1.70 (13.2, 106)
Multi-Ethnic Study of Atherosclerosis (MESA) (US)	2145	63 ± 10	52.4	28 ± 5.0	4.9 ± 0.56	32.6 ± 1.84 (9.84, 108)

Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) (Sweden)	727	70 ± 0.20	51.2	27 ± 4.1	4.9 ± 0.51	50.4 ± 1.68 (18.2, 140)
Rotterdam Study (the Netherlands)	2340 (2273)	72 ± 6.6	58.0	27 ± 3.8	5.5 ± 0.53	62.8 ± 1.68 (22.7, 174)
Uppsala Longitudinal Study of Adult Men (ULSAM) (Sweden)	927 (919)	71 ± 0.60	0	26 ± 3.2	5.4 ± 0.56	73.7 ± 1.72 (25.6, 212)
Cardiovascular Risk in Young Finns Study (YFS) (Finland)	1788 (1783)	38 ± 5.0	54.1	26 ± 4.7	5.3 ± 0.75	40.5 ± 2.16 (8.94, 183)

Table 4-1, continued	**Dietary magnesium intake** ***mg/d***	**Energy intake** ***kcal/d***	**Alcohol intake** ***g/d***	**Current smoker** ***%***	**Completed high school** ***%***
Atherosclerosis Risk in Communities (ARIC) Study (US)	260 ± 94.5	1640 ± 600	6.7 ± 1.0 (*n*=8579)	24.4 (*n*=8587)	84.1 (*n*=8585)
Cardiovascular Health Study (CHS) (US)	413 ± 145	2020 ± 650	5.8 ± 13 (*n*=2740)	11.5 (*n*=2744)	76.2 (*n*=2739)
Family Heart Study (FamHS) (US)	261 ± 95.8	1750 ± 620	6.4 ± 13	14.6	64.8
Framingham Heart Study (FHS) (US)	314 ± 115	1970 ± 660	11 ± 15	29.6	97.5
Gene-Diet Attica Investigation on Childhood Obesity (GENDAI) (Greece)	225 ± 75.6	1890 ± 600	Not applicable	Not applicable	Not applicable
Greek Health Randomized Aging Study (GHRAS) (Greece)	237 ± 63.1	2160 ± 690	45 ± 90	14.5	64.0
Gene-Lifestyle interactions And Complex traits In Elevated disease Risk (GLACIER) (Sweden)	291 ± 92.4	1720 ± 600	3.5 ± 4.5	21.6	79.1
Health, Aging, and Body Composition Study (Health ABC) (US)	294 ± 101	1810 ± 600	6.9 ± 14	6.10	89.1

Invecchiare in Chianti (Aging in the Chianti Area; InCHIANTI) (Italy)	256 ± 74.6	2010 ± 600	15 ± 21	24.3	80.4
Malmö Diet and Cancer Study (Malmö) (Sweden)	353 ± 91.3	2320 ± 670	10 ± 12	26.9	80.4
Multi-Ethnic Study of Atherosclerosis (MESA) (US)	276 ± 113	1700 ± 720	8.8 ± 16	11.0	79.3
Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) (Sweden)	317 ± 75.5	1890 ± 500	8.1 ± 7.9	10.6	44.7
Rotterdam Study (the Netherlands)	314 ± 75.5	1990 ± 510	11 ± 14	15.8	90.3
Uppsala Longitudinal Study of Adult Men (ULSAM) (Sweden)	286 ± 70.0	1750 ± 460	8.6 ± 13	20.2	41.0
Cardiovascular Risk in Young Finns Study (YFS) (Finland)	480 ± 156	2410 ± 860	13 ± 20	18.3	84.6

[1] Data are mean ± SD or percent (%) in units provided in column headings. Alcohol intake originally quantified as drinks/wk in CHS and FamHS, and as drinks/d in YFS. Conversion to g/d based on one drink containing 14 g alcohol.

[2] Maximum available observations, *n*, for interactions between magnesium intake and SNPs in glucose outcome analyses (*n* for insulin interaction analyses in parentheses). Sample sizes vary in some cohorts depending on availability of genotype information.

[3] Insulin was analyzed on the natural log scale and back-transformed to the geometric scale for presentation. Values are geometric mean ± SD (95% CI).

Table 4-2. Meta-Analyzed Associations Between Magnesium Intake and Fasting Glucose and Fasting Insulin in 15 Cohort Studies

	Association[3] of fasting glucose (mmol/L) per 50 mg/d increment in magnesium intake				Association[3] of fasting insulin (ln-pmol/L) per 50 mg/d increment in magnesium intake			
Model[1]	***n*[2]**	***β (95% CI)***	***P***	***I*[2] (95% CI)**	***n*[2]**	***β (95% CI)***	***P***	***I*[2] (95% CI)**
Model 1	52,684	-0.016 (-0.019, -0.012)	<0.0001	50 (9, 72)	37,640	-0.028 (-0.032, -0.024)	<0.0001	80 (68, 88)
Model 2	52,568	-0.013 (-0.017, -0.010)	<0.0001	20 (0, 56)	37,527	-0.023 (-0.026, -0.020)	<0.0001	81 (69, 88)
Model 3	50,921	-0.009 (-0.013, -0.005)	<0.0001	30 (0, 62)	36,271	-0.020 (-0.024, -0.017)	<0.0001	75 (59, 85)
Model 4	48,588	-0.001 (-0.006, 0.005)	0.78	30 (0, 65)	34,137	-0.012 (-0.017, -0.007)	<0.0001	46 (0, 73)

[1]Model 1 adjusted for age, sex, energy intake, study center (in ARIC, CHS, FamHS, Health ABC, InCHIANTI, MESA), and family or population substructure (in CHS, FamHS, FHS, MESA, YFS). Model 2 adjusted for model 1 plus BMI. Model 3 adjusted for model 2 plus smoking, education, physical activity, and alcohol intake. GENDAI (child/adolescent cohort) did not adjust for smoking, education, or alcohol intake, as these variables are not applicable in this cohort. Rotterdam did not adjust for physical activity, as this variable was not available in this cohort. Model 4 adjusted for model 3 plus fiber and caffeine. GHRAS, PIVUS, and ULSAM were excluded from model 4 analysis, as these cohorts did not have information on either fiber or caffeine intake.

[2]The number of independent observations in each interaction analysis.

[3]Beta coefficient and 95% confidence interval as β (95% CI).

Table 4-3. Meta-Analyzed Interactions Between Magnesium Intake and SNPs on Fasting Glucose and Fasting Insulin in 15 Cohort Studies[1]

SNP	Nearest gene	Coded allele	Other allele	Coded allele frequency	Number of cohorts	n^3	Interaction[2] between 50 mg/d increment in magnesium intake × SNP on fasting glucose (mmol/L) *β (95% CI)*	*P*	I^2 *(95% CI)*
Glucose-related SNPs									
rs10830963	*MTNR1B*	G	C	0.28	15	51,257	0.0033 (-0.0006, 0.0072)	0.10	47 (4, 71)
rs10885122	*ADRA2A*	G	T	0.89	15	52,068	-0.0013 (-0.0064, 0.0038)	0.64	15 (0, 53)
rs11071657	*C2CD4B*	A	G	0.62	15	52,098	0.0023 (-0.0012, 0.0058)	0.21	0 (0, 35)
rs11558471	*SLC30A8*	A	G	0.69	13	50,329	-0.0045 (-0.0082, -0.0008)	0.02	5 (0, 37)
rs11605924	*CRY2*	A	C	0.48	15	52,264	0.0017 (-0.0016, 0.0050)	0.30	41 (0, 68)
rs11708067	*ADCY5*	A	G	0.78	14	50,829	-0.0006 (-0.0047, 0.0035)	0.78	12 (0, 51)
rs11920090	*SLC2A2*	T	A	0.86	14	51,441	-0.0019 (-0.0066, 0.0028)	0.43	35 (0, 65)
rs174550	*FADS1*	T	C	0.67	15	52,305	-0.0022 (-0.0055, 0.0011)	0.20	27 (0, 61)
rs2191349	*DGKB-TMEM195*	T	G	0.53	15	52,241	-0.0024 (-0.0057, 0.0009)	0.14	31 (0, 63)
rs340874	*PROX1*	C	T	0.53	14	51,449	-0.0004 (-0.0037, 0.0029)	0.81	12 (0, 50)
rs4506565	*TCF7L2*	T	A	0.29	13	49,253	-0.0001 (-0.0038, 0.0036)	0.97	54 (14, 75)
rs4607517	*GCK*	A	G	0.17	15	52,470	0.0020 (-0.0023, 0.0063)	0.35	0 (0, 22)
rs560887	*G6PC2*	C	T	0.71	15	51,479	0.0026 (-0.0009, 0.0061)	0.15	0 (0, 28)
rs7034200	*GLIS3*	A	C	0.48	15	52,016	0.0026 (-0.0007, 0.0059)	0.11	5 (0, 56)
rs780094	*GCKR*	C	T	0.39	15	52,442	0.0005 (-0.0028, 0.0038)	0.74	41 (0, 68)
rs7944584	*MADD*	A	T	0.73	15	51,263	0.0017 (-0.0020, 0.0054)	0.36	46 (2, 71)
Magnesium-related SNPs									
rs11144134	*TRPM6*	T	C	0.92	10	29,978	-0.0015 (-0.0091, 0.0061)	0.70	41 (0, 72)
rs2274924	*TRPM6*	A	G	0.84	10	29,280	0.0042 (-0.0013, 0.0097)	0.12	0 (0, 46)
rs3740393	*CNNM2*	G	C	0.85	11	30,904	0.0064 (0.0009, 0.0119)	0.02	38 (0, 70)
rs3750425	*TRPM6*	G	A	0.91	10	29,978	0.0052 (-0.0017, 0.0121)	0.14	0 (0, 2)

rs4072037	*MUC1*	C	T	0.45	11	30,905	-0.0028 (-0.0069, 0.0013)	0.17	31 (0, 66)
rs6746896	*CNNM4*	A	G	0.67	11	30,210	-0.0001 (-0.0042, 0.0040)	0.95	16 (0, 56)
rs8042919	*TRPM7*	G	A	0.90	11	30,905	-0.0049 (-0.0116, 0.0018)	0.15	4 (0, 62)
rs994430	*CNNM3*	A	T	0.60	11	30,905	-0.0004 (-0.0043, 0.0035)	0.84	0 (0, 54)
							Interaction[2] between 50 mg/d increment in magnesium intake × SNP on fasting insulin (ln-pmol/L)		
Insulin-related SNPs									
rs35767	*IGF1*	G	A	0.84	15	37,485	0.0031 (-0.0020, 0.0082)	0.22	21 (0, 57)
rs780094	*GCKR*	C	T	0.58	15	37,804	-0.0028 (-0.0063, 0.0007)	0.12	36 (0, 66)
Magnesium-related SNPs									
rs11144134	*TRPM6*	T	C	0.92	10	29,549	-0.0004 (-0.0080, 0.0072)	0.94	6 (0, 65)
rs2274924	*TRPM6*	A	G	0.83	10	28,851	0.0041 (-0.0012, 0.0094)	0.13	0 (0, 60)
rs3740393	*CNNM2*	G	C	0.85	11	30,467	0.0013 (-0.0042, 0.0068)	0.64	0 (0, 49)
rs3750425	*TRPM6*	G	A	0.91	10	29,549	0.0046 (-0.0021, 0.0113)	0.17	0 (0, 58)
rs4072037	*MUC1*	C	T	0.45	11	30,468	-0.0003 (-0.0044, 0.0038)	0.88	27 (0, 64)
rs6746896	*CNNM4*	A	G	0.68	11	29,773	0.0009 (-0.0030, 0.0048)	0.65	6 (0, 63)
rs8042919	*TRPM7*	G	A	0.90	11	30,468	-0.0004 (-0.0071, 0.0063)	0.91	0 (0, 58)
rs994430	*CNNM3*	A	T	0.61	11	30,468	0.0004 (-0.0035, 0.0043)	0.84	7 (0, 63)

[1]Additive allele model, adjusted for age, sex, total energy intake, study center (in ARIC, CHS, FamHS, Health ABC, InCHIANTI, MESA), and family or population substructure (in CHS, FamHS, FHS, MESA, YFS).

[2]Interaction coefficient and 95% confidence interval as β (95% CI).

[3]The number of independent observations in each interaction analysis.

Supplemental Table 4-1. Cohort-Specific Dietary, Outcome, and Covariate Definitions*

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
ARIC	66-item, interviewer-administered, modified Willett FFQ [Willett WC, *et al. Am J Epidemiol.* 1985; 122(1):51–65. and Stevens J, *et al. Nutrition Research* 1996;16:735–745.]	Harvard	≥8-h fasting blood samples were drawn from an antecubital vein into tubes containing a serum separator gel. Serum glucose concentrations were assessed with a hexokinase/ glucose-6-phosphate dehydrogenase method.	≥8-h fasting insulin was quantified by radioimmunoassay (125Insulin Kit; Cambridge Medical Diagnosis, Billerica, MA), with a 7 pmol/L lower limit of sensitivity and 33% cross-reactivity with proinsulin.	ARIC samples were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, CA). Imputation was performed with MACH software. 11 SNPs studied in the present analyses were imputed; 6 SNPs were directly genotyped: rs340874, rs560887, rs11765, rs4506565, rs10830963, and rs7944584.	Categorized into 6 groups: grade school or none, some high school, high school graduate, vocational school, college, graduate/ professional school	Classified as current, former, never smoker or missing/ unknown	Assessed as both sport and leisure time using the Baecke questionnaire. A sports activity score and a leisure activity score ranged from low to high. Each score ranged from 1–5 in 0.25 increments.	Quantified as g/d (Association of Official Analytical Chemists [AOAC] method)	Quantified as mg/d	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$
CHS	99-item, self-administered, picture-sort version of	Harvard	≥8-h fasting glucose was quantified using a	≥8-h fasting insulin was	CHS samples were genotyped using the	Categorized into 3 groups: no high	Classified as current, former,	Derived from a questionnaire; exercise	Quantified as g/d (AOAC method)	Quantified as mg/d	Quantified as drinks/ wk (1	Calculated from measured weight

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
	National Cancer Institute FFQ [Kumanyika S, *et al. J Am Diet Assoc.* 1996;96(2):137–144.]		Kodak Ektachem 700 analyzer with reagents (Eastman Kodak, Rochester, NY). The overall CV was 1.86%, and the correlation coefficient between 169 pairs of blind replicates was 0.997.	quantified by radioimmunoassay (Coat-A-Count Insulin assay (Diagnostics Products Corp, Los Angeles, CA)	Illumina HumanCNV370-Duo BeadChip system. Imputation was performed using BIMBAM10 v0.91 with reference to HapMap CEU using release 21A, build 35 using one round of imputations and the default expectation-maximization warm-ups and runs; 12 SNPs studied in the present analyses were imputed; 4 SNPs were directly genotyped: rs340874, rs4607517, rs560887, and rs780094.	school degree, high school or vocational school degree, college degree	never smoker	intensity was classified into 3 categories: none, low/moderate, high			drink equivalent to 14 g alcohol)	(kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
FamHS	66-item, interviewer-administered, modified Willett FFQ [Willett WC, *et al. Am J Epidemiol.* 1985; 122(1):51–65. and Stein AD, *et al*. *Am J Epidemiol.* 1992; 135(6):667–677.]	Harvard	≥8-h fasting blood samples were collected, allowed to clot, centrifuged, aliquoted, and frozen at -70 degrees Celsius before shipment to a central processing laboratory. At the central processing laboratory, glucose was quantified by a thin film adaptation of a glucose oxidase enzymatic, spectrophoto metric procedure using the Vitros analyzer (Ortho Clinical Diagnostics, Rochester, NY).	≥8-h fasting insulin was quantified using the coated-tube radioimmu noassay method (Diagnostic Products Corp., Los Angeles, CA).	All participants were typed on an Illumina HumMap chip. The initial 974 were typed with 550K density; 249 were typed at 610K, and the remaining 1482 at 1M. Of these, 34 (3.3%) were excluded due to technical errors, call rates below 98%, and discrepancies between reported sex and sex-diagnostic markers. There was no significant plate-to-plate variation in allele frequencies. Imputation was performed with MACH software.	Categoriz ed into 3 groups: high school graduate or less, vocational school, college or more	Classified as current, former, never smoker or missing/ unknown	Quantified as min/d spent exercising	Quantifie d as g/d (AOAC method)	Quantifie d as mg/d	Quantifi ed as drinks/ wk (1 drink equival ent to approx-imately 14 g alcohol) and modele d as 0, 1–3, 4–7, 8–14, ≥14 drinks/ wk	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
FHS	126-item, self-administered Willett FFQ [Rimm EB, *et al. Am J Epidemiol.* 1992;135:1114–1126, 1127–1136. and Salvini S, *et al*. *Int J Epidemiol.* 1989; 18:858–867.]	USDA	≥8-h fasting plasma glucose was quantified with a hexokinase reagent kit (A-gent glucose test, Abbott Laboratories, South Pasadena, CA). Glucose assays were run in duplicate, and the intra-assay coefficient of variation ranged from 2–3%, depending on the assayed glucose concentration.	≥8-h fasting insulin concentrations were quantified in plasma using human-specific RIA at exam 7 in the Framingham Offspring Cohort and using human-specific insulin ELISA in the Framingham Generation 3 cohort (both assays from Linco Inc., St. Louis, MO).	Framingham study samples were genotyped using Affymetrix 500K (250K Nsp and 250K Sty) and MIPS 50K. Imputation was performed using MACH software. Ratio of variance of dosage to expected variance under binomial model: >0.3.	Continuous, ranging from 0–30 years of education	Classified as regular cigarette smoking in last year (yes/no)	Quantified as a weighted average of the proportion of a typical day spent sleeping and performing sedentary, slight, moderate, or heavy physical activities (expressed in metabolic equivalent units	Quantified as g/d (AOAC method)	Quantified as mg/d	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
GENDAI	Two 24-h recalls	USDA and Greek Food Composition Tables (Nutritionist Pro, v2.2, Axxya Systems-Nutritionist Pro, Stafford, TX, USA)	≥8-h fasting serum glucose concentrations were assessed using commercially available enzymatic colorimetric assays (Sigma Diagnostics, St. Louis, MO) on an automated ACE analyzer (Schiapparelli Biosystems, Inc., Fairfield, NJ).	≥8-h fasting serum insulin was quantified via immunofluorescence on an automatic AIA 600II analyzer (Tosoh Corp., Tokyo, Japan) using a commercially available kit for this purpose (ST AIA-PACK IRI, Tosoh Corp.).	Genotyping was performed using Taqman (Applied Biosystems) in accordance with the recommended protocols. The mean call rate for all SNPs was >95%.	Not applicable, all were in 5th or 6th grade of school	Not applicable	Determined from the Sallis physical activity recall checklist and quantified as m/d of physical activity	Quantified as g/d (AOAC method)	Not applicable	Not applicable	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
GHRAS	55-item, interviewer administered FFQ	USDA	≥8-h fasting serum glucose concentrations were assessed using commercially available enzymatic colorimetric assays (Sigma Diagnostics, St. Louis, MO) on an automated ACE analyzer (Schiapparelli Biosystems, Inc., Fairfield, NJ).	≥8-h fasting serum insulin was quantified via immunofluorescence on an automatic AIA 600II analyzer (Tosoh Corp., Tokyo, Japan) using a commercially available kit for this purpose (ST AIA-PACK IRI, Tosoh Corp.).	Genotyping was performed using Taqman (Applied Biosystems) in accordance with the recommended protocols. The mean call rate for all SNPs was >95%.	Categorized into 4 groups: no degree, primary degree, secondary degree, higher degree	Classified as current, former, never smoker	Metabolic equivalent units of all activities commonly performed in a week, including time spent walking, in vigorous, moderate-intensity, and in sedentary activity (sedentary work, outdoor activities, leisure time and sleep). Based on self-reported responses to the Harokopio Physical Activity Questionnaire.	Not available	Not available	Calculated as mL/d with a concen-tration of 12 g ethanol / 100 mL	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
GLACIER	66-item, self-administered FFQ [Johansson I, *et al*. *Public Health Nutr*. 2002;5(3):487–496. and Johansson G, *et al*. *Public Health Nutr*. 2001;4(4):919–927. and Wennberg M, *et al*. *Public Health Nutr*. 2009;12(9):1477–1484.]	Swedish National Food Administration Database	≥8-h fasting plasma glucose was assayed using fresh capillary plasma on a benchtop analyzer (Reflotron; Boehringer Mannheim, Mannheim, Germany). A threshold glucose value of ≤1 mmol/L was used to exclude potentially spurious values.	≥8-h fasting serum insulin was quantified on a Roche Modular E170 analyzer (Diagnostics GmbH, Mannheim, Germany) with limits of detection of 2.6–24.9 mIU/L.	DNA was extracted at the Medical Biobank in Umeå from peripheral white blood cells. Genomic DNA samples were subsequently diluted to 4 ng/μL. Genotyping was undertaken using Sequenom iPLEX platform (Sequenom Inc., CA, USA), in accordance with the recommended protocols. Approximately 10% duplicate samples were included for the assessment of genotyping concordance. The mean concordance was 99.3% and the mean success rate was 98.4%.	Categorized into 3 groups: total of 6–7 years of compulsory school education, total 12–13 years of education (school + college), school + college + university	Classified as current, former, never smoker	Based on a questionnaire response and classified into 2 exercise frequency categories (never/ infrequently vs. medium/high levels)	Quantified as g/d (AOAC method)	Quantified as mg/d from coffee, tea, and soda	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
Health ABC	108-item, interviewer-administered Block FFQ [Houston, *et al. Am J Clin Nutr*. 2008; 87(1):150–155.]	Block Dietary Data Systems	≥8-h fasting plasma glucose was quantified by an automated glucose oxidase reaction (YSI 2300, Yellow Springs, OH).	≥8-h fasting plasma insulin was assayed with a microparticle enzyme immunoassay (Abbott IMx, Abbott Laboratories, South Pasadena, CA).	Genotyping was performed by the Center for Inherited Disease Research using the Illumina Human1M-Duo BeadChip system. Samples were excluded from the dataset for sample failure, genotypic sex mismatch, and first-degree relative of an included individual based on genotype data. Imputation was based on HapMap CEU (r22, build 36) using MACH software (v 1.0.16); 11 SNPs in the present analyses were imputed; 6 were directly genotyped: rs340874, rs780094, rs560887, rs4607517, rs11558471, and rs35767.	Categorized into 3 groups: <high school degree, high school degree, postsecondary degree	Classified as current, former, never smoker	Physical activity over previous 7 d assessed by interviewer-administered questionnaire. Time spent on a variety of leisure and moderate- and high-intensity exercise activities obtained in addition to intensity level of activity. Approximate metabolic equivalent unit values assigned to each activity category to calculate weekly energy expenditure (kcal/ kg body weight) which was multiplied by each participants' weight, for kcal/wk.	Quantified as g/d of total dietary fiber	Not available	Quantified as g/d	Calculated from measured weight (kg) and height (m^2), at baseline

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
InCHIANTI	236-item, interviewer-administered FFQ [Bartali, *et al*. *Arch Gerontol Geriatr*. 2004;38: 51–60. and Pisani, et al. Int J Epidemiol. 1997; 26:152–160.]	Italian Food Composition Database for Epidemio-logical Studies	≥8-h fasting blood glucose was determined by an enzymatic colorimetric assay using a modified glucose oxidase-peroxidase method (Roche Diagnostics GmbH, Mannheim, Germany) and a Roche-Hitachi 917 analyzer.	Fasting plasma fasting insulin concentrations were determined with a double-antibody, solid-phase radioimmunoassay (intra-assay CV: 3.1 ± 0.3%) (Sorin Biomedica, Milan, Italy).	Genotyping was conducted using Illumina 550K. Samples QC: call rate filter was set at >98.5%; sex misspecification. SNPs QC: MAF >1%; HWE >10^{-4}; call rate >99%. Imputation was made using MACH software. Ratio of variance of dosage to expected variance under binomial model: >0.3, MAF >1%.	Categorized into 3 groups: elementary, secondary, or undocu-mented; high school or professional school degree; university degree or higher	Classified as current, former, never smoker	Activity in prior 12 mo assessed through a standard interview-administered questionnaire. Based on responses to 7 questions, activity was collapsed: sedentary (inactivity or light-intensity <1 h/wk); light activity (2–4 h/wk); moderate-high activity (light at least 5 h/wk or moderate >1–2 h/wk)	Quantified as g/d	Quantified as servings/d of coffee, cappuc-cino, latte, or tea (1 cup of coffee contains approx-imately 100 mg caffeine).	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
Malmö	7-d food diary combined with a 168-item FFQ followed by a 1-h diet interview [Elmstahl, *et al*. *Eur J Clin Nutr.* 1996; 50:134–142. and Elmstahl, *et al. Eur J Clin Nutr*. 1996; 50:143–151. and Riboli, *et al. Int J Epidemiol*. 1997;26 Suppl 1:S161–173. and Callmer, *et al. J Intern Med*. 1993; 233:53–57.]	Swedish National Food Administration Database	Fasting glucose was quantified in overnight fasting whole blood samples by a hexokinase-glucose-6-phosphate dehydrogenase method. Blood glucose was converted to plasma glucose using a correction factor of 1.13.	Fasting insulin was quantified by non-specific radioimmunoassay in overnight fasting blood samples.	SNPs were genotyped using either the iPLEX Sequenom MassARRAY platform (GCK, TCF7L2, FADS1) or allelic discrimination (GCKR) on an ABI 7900 instrument (Applied Biosystems). All genotyped SNPs had a genotyping call rate >95% (mean 97.8%) and a HWE p <0.10.	Categorized into 5 groups: elementary, primary and secondary, upper secondary, further education without a degree, university degree	Classified as current, former, never smoker	Leisure-time physical activity was obtained from a list of 18 different activities. The duration of each activity was multiplied by an intensity factor creating a score. The score was divided into six categories.	Quantified as g/d (AOAC method)	No information on caffeine. Coffee intake was used, as g/d. De-caffeinated coffee is very rare in Sweden.	Quantified as g/d (over 7 consec-utive days)	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
MESA	120-item, self-administered, modified-Block FFQ [Mayer-Davis E, *et al*. *Ann Epidemiol.* 1999;9:314–324. and Nettleton JA, *et al*. *Br J Nutr.* 2009;102: 1220–1227.]	Nutrition Data Systems for Research (NDS-R) software database	≥8-h fasting serum glucose was quantified by rate reflectance spectrophotometry using thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN).	≥8-h fasting insulin was quantified by raidoimmunoassay (Linco Human Insulin Specific RIA Kit; Linco Research, Inc., St. Charles, MO) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN).	MESA participants were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, CA); for the current meta-analysis only self-reported Caucasian participants were analyzed. IMPUTE version 2.1.0 was used to perform imputation for the MESA SHARe Caucasian participants (chromosomes 1–22) using HapMap Phase I and II CEU as the reference panel (release 24, build 36 (dbSNP b126)).	Categorized into 3 groups: high school not completed; high school completed, some college or technical school certificate; completion or Associate's degree, Bachelor's degree or higher	Classified as current, former, never smoker	Quantified as metabolic equivalent-minutes in sedentary leisure activity and active leisure activity (included walking, conditioning, doing sport/dance)	Quantified as g/d (AOAC method)	Quantified as mg/d	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
PIVUS	7 one-d food records [Becker W, Lennernäs MM, Gustafsson I-B, *et al*. *Food intake. The Sixth Nordic Conference in Nutrition.* Göteborg, Sweden, 1996.]	Swedish National Food Administration Database	Reference method at Uppsala University Hospital	Enzymatic-immunological assay at Uppsala University Hospital	SNPs were genotyped by multiplex mini-sequencing (fluorescent single base extension) using the SNPstream system (Beckman Coulter). SNPstream UHT: ultra-high throughput SNP genotyping for pharmacogenomics and drug discovery. (*Biotechniques* 2002;30:S70–77). SNP QC filters: MAF>5%; HWE $<10^{-3}$; call rate >93%. Average SNP call rate: 0.97777335625. Sample QC filters: call rate >95%. Average sample call rate: 0.97629158.	Categorized into 3 groups: elementary school only, secondary school only, college or graduate degree	Classified as current smoker (yes/no)	Leisure time physical activity was assessed using a questionnaire and participants were classified into four categories and referred to as sedentary, moderate, regular and athletic physical activity	Not available	Not available	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
Rotterdam	Two-step procedure: (1) A simple self-administered questionnaire was first completed at home, only questions were asked about which food items were consumed; no questions about portion sizes (or frequency) were asked during this step. (2) A subsequent interviewer-administered FFQ [Klipstein-Grobusch K, *et al*. *Eur J Clin Nutr.* 1998;52(8):588–596]	NEVO Dutch Food Composition Table	In 1997–1999 (approximately 6 years after the baseline exam where FFQ data were gathered), ≥8-h fasting blood collected and glucose was quantified enzymatically using the hexokinase method (Boehringer Mannheim, Mannheim, Germany).	In 1997–1999 (approximately 6 years after the baseline exam where FFQ data were gathered), ≥8-h fasting blood samples were drawn and stored at -80 degrees Celsius. In 2008, insulin concentrations were quantified on a Modular Analytics E170 analyzer, using a Cobas Roche electrochemi-luminescence immunoassay (12017547122).	Genotyping was conducted using the Illumina 550K array among participants of self-reported European descent, and succeeded in 6,240 participants (sample call rate 97.5%). We excluded participants for excess autosomal heterozygosity, mismatch between called and phenotypic gender, or being outliers identified by the IBS clustering analysis.	Categorized into 4 groups: completed primary education; lower vocational training or general education; intermediate vocational training or intermediate and higher general education; higher vocational training, college, or university	Classified as current, former, never smoker	Not available	Total dietary fiber, quantified as g/d	Quantified as mg/d	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
ULSAM	7 one-d food records [Becker W, Lennernäs MM, Gustafsson I-B, *et al*. *Food intake. The Sixth Nordic Conference in Nutrition.* Göteborg, Sweden, 1996.]	Swedish National Food Administration Database	≥8-h fasting venous plasma samples were obtained from the antecubital vein. Glucose was quantified by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany). The intra-individual CV for fasting plasma glucose was 3.2%.	≥8-h fasting plasma insulin was assayed using an enzymatic-immunological assay (Enzymmun, Boehringer Mannheim, Mannheim, Germany) performed in an ES300 automatic analyzer (Boehringer Mannheim) and the concentrations were originally given in mU/L.	SNPs were genotyped by multiplex mini-sequencing (fluorescent single base extension) using the SNPstream system (Beckman Coulter). SNPstream UHT: ultra-high throughput SNP genotyping for pharmacogenomics and drug discovery. (*Biotechniques* 2002;30:S70–77). SNP QC filters: MAF >5%; HWE $<10^{-3}$; call rate >93%. Average SNP call rate: 0.97777335625. Sample QC filters: call rate >95%. Average sample call rate: 0.97629158.	Categorized into 3 groups: elementary school only, secondary school only, college or graduate degree	Classified as current smoker (yes/no)	Leisure time physical activity was assessed using a questionnaire and participants were classified into four categories and referred to as sedentary, moderate, regular, and athletic physical activity	Not available	Not available	Quantified as g/d	Calculated from measured weight (kg) / height $(m)^2$

Cohort Study	Dietary assessment method	Nutrient data-base	Fasting glucose	Fasting insulin	GWAS/ genotyping	Education	Smoking status	Physical activity	Fiber intake	Caffeine intake	Alcohol intake	Body mass index
YFS	131-item FFQ [Paalanen L, *et al*. *J Clin Epid.* 2006;59(9):994–1001.]	Finnish food composition database	≥8-h fasting glucose concentrations were analyzed enzymatically (Olympus Diagnostica GmbH, Hamburg, Germany).	≥8-h fasting serum insulin was quantified by microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot, South Pasadena, CA).	Genotyping was performed at the Sanger Institute (UK) using the custom-built Illumina BeadChip Human670K. Genotypes were called using Illumina's clustering algorithm. SNPs that were present on HapMap and that passed quality control measures were used for imputation with MACH version 1.0.	Continuous, as sum of all education years (elementary through graduate, as applicable)	Classified into current or former smoker vs. never smoker	Frequency of moderate to intense exercise in leisure time (6 categories): never, 1 time/mo, 1 time/wk, 2–3 times/wk, 4–6 times/wk, daily	Total fiber, quantified as g/d	From coffee only in servings/d, quantified as mg/d caffeine (1 cup of coffee contains 100 mg caffeine)	Quantified as drinks/d (1 drink equivalent to approx-imately 14 g alcohol)	Calculated from measured weight (kg) / height $(m)^2$

*AOAC, Association of Official Analytical Chemists; ARIC, Atherosclerosis Risk in Communities Study; CHS, Cardiovascular Health Study; FamHS, Family Heart Study; FFQ, food frequency questionnaire; FG, fasting glucose; FHS, Framingham Heart Study; FI, fasting insulin; GENDAI, Gene-Diet Attica Investigation on Childhood Obesity; GHRAS, Greek Health Randomized Aging Study, GLACIER, Gene-Lifestyle interactions And Complex traits Involved in Elevated disease Risk; GWAS, genome-wide association study; Health ABC, Health, Aging, and Body Composition Study; InCHIANTI, Invecchiare in Chianti; MESA, Multi-Ethnic Study of Atherosclerosis; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; SNP, single nucleotide polymorphism; ULSAM, Uppsala Longitudinal Study of Adult Men; YFS, Cardiovascular Risk in Young Finns Study.

Supplemental Table 4-2. Meta-Analyzed Associations of SNPs on Fasting Glucose (mmol/L) and Fasting Insulin (ln-pmol/L)*

SNP	Nearest Gene	Coded / Other Allele	*n*	Coded Allele Frequency	β	SE	*P*	I^2 (95% CI)
Outcome is FASTING GLUCOSE								
Glucose-related SNPs								
rs10830963	*MTNR1B*	G/C	51,735	0.28	0.083	0.004	5.4E-99	54 (18 to 74)
rs10885122	*ADRA2A*	G/T	52,546	0.88	0.022	0.005	2.9E-05	0 (0 to 35)
rs11071657	*C2CD4B*	A/G	52,576	0.61	0.009	0.004	0.01	15 (0 to 53)
rs11558471	*SLC30A8*	A/G	50,807	0.69	0.035	0.004	1.7E-19	0 (0 to 50)
rs11605924	*CRY2*	A/C	52,742	0.48	0.023	0.003	1.6E-11	0 (0 to 52)
rs11708067	*ADCY5*	A/G	51,172	0.78	0.026	0.004	1.7E-10	30 (0 to 63)
rs11920090	*SLC2A2*	T/A	51,784	0.86	0.031	0.005	5.8E-10	28 (0 to 62)
rs174550	*FADS1*	T/C	52,783	0.67	0.017	0.004	2.5E-06	0 (0 to 13)
rs2191349	*DGKB-TMEM195*	T/G	52,719	0.53	0.029	0.003	2.7E-17	0 (0 to 51)
rs340874	*PROX1*	C/T	51,792	0.53	0.020	0.004	9.7E-09	42 (0 to 69)
rs4506565	*TCF7L2*	T/A	49,731	0.29	0.025	0.004	5.2E-11	0 (0 to 39)
rs4607517	*GCK*	A/G	52,948	0.17	0.063	0.005	1.1E-44	0 (0 to 33)
rs560887	*G6PC2*	C/T	51,957	0.70	0.076	0.004	5.7E-95	0 (0 to 25)
rs7034200	*GLIS3*	A/C	52,494	0.48	0.018	0.003	9.3E-08	0 (0 to 13)
rs780094	*GCKR*	C/T	52,920	0.61	0.031	0.004	8.5E-19	20 (0 to 57)
rs7944584	*MADD*	A/T	51,741	0.73	0.024	0.004	6.0E-10	6 (0 to 42)
Magnesium-related SNPs								
rs11144134	*TRPM6*	T/C	30,432	0.92	0.007	0.008	0.39	0 (0 to 55)
rs2274924	*TRPM6*	A/G	29,734	0.84	-0.013	0.006	0.03	0 (0 to 58)
rs3740393	*CNNM2*	G/C	31,382	0.85	-0.003	0.006	0.63	0 (0 to 41)
rs3750425	*TRPM6*	C/T	30,432	0.91	-0.014	0.008	0.07	0 (0 to 62)
rs4072037	*MUC1*	C/T	31,383	0.45	-0.005	0.004	0.29	0 (0 to 11)
rs6746896	*CNNM4*	A/G	30,688	0.67	0.004	0.005	0.44	0 (0 to 48)

SNP	Nearest Gene	Coded / Other Allele	*n*	Coded Allele Frequency	β	SE	*P*	I^2 (95% CI)
rs8042919	*TRPM7*	G/A	31,383	0.90	0.001	0.007	0.92	28 (0 to 65)
rs994430	*CNNM3*	A/T	31,383	0.60	0.003	0.004	0.54	0 (0 to 49)
Outcome is FASTING INSULIN								
Insulin-related SNPs								
rs35767	*IGF1*	G/A	37,862	0.84	0.016	0.005	0.003	20 (0 to 56)
rs780094	*GCKR*	C/T	38,181	0.59	0.028	0.004	4.3E-13	20 (0 to 56)
Magnesium-related SNPs								
rs11144134	*TRPM6*	T/C	29,902	0.92	0.010	0.009	0.26	0 (0 to 18)
rs2274924	*TRPM6*	A/G	29,204	0.83	0.003	0.006	0.61	0 (0 to 23)
rs3740393	*CNNM2*	G/C	30,844	0.85	-0.003	0.006	0.60	0 (0 to 39)
rs3750425	*TRPM6*	C/T	29,902	0.91	0.0004	0.008	0.95	0 (0 to 37)
rs4072037	*MUC1*	C/T	30,845	0.45	-0.003	0.005	0.59	0 (0 to 36)
rs6746896	*CNNM4*	A/G	30,150	0.68	0.002	0.005	0.60	0 (0 to 58)
rs8042919	*TRPM7*	G/A	30,845	0.90	-0.005	0.007	0.50	0 (0 to 55)
rs994430	*CNNM3*	A/T	30,845	0.61	0.0003	0.004	0.94	0 (0 to 48)

*In an additive allele model, adjusted for age, sex, study center (in ARIC, CHS, FamHS, Health ABC, InCHIANTI, MESA), and family or population substructure (in CHS, FamHS, FHS, MESA, YFS). β represents the change in fasting glucose (mmol/L) or fasting insulin (ln-pmol/L) per each additional coded allele. I^2 represents the heterogeneity statistic, presented as %.

Supplemental Table 4-3. Cohort Study Acknowledgements*

Cohort Study	Country	Description and Acknowledgements	Consortium Membership(s)
ARIC	US	The **A**therosclerosis **R**isk **I**n **C**ommunities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. Dr. Nettleton is supported by a K01 from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (5K01DK082729-04). [representing authors: JAN, KEN, JSP, WHLK]	CHARGE, MAGIC
CHS	US	The **C**ardiovascular **H**ealth **S**tudy research was supported by NHLBI contracts N01-HC-85239, N01-HC-85079 through N01-HC-85086; N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133, HHSN268201200036C and NHLBI grants HL080295, HL087652, HL105756 with additional contribution from NINDS. Additional support was provided through AG-023629, AG-15928, AG-20098, and AG-027058 from the NIA. See also http://www.chs-nhlbi.org/pi.htm. DNA handling and genotyping was supported in part by National Center of Advancing Translational Technologies CTSI grant UL1TR000124 and National Institute of Diabetes and Digestive and Kidney Diseases grant DK063491 to the Southern California Diabetes Endocrinology Research Center. [representing authors: RNL, DM, LD, KM, DSS]	CHARGE, MAGIC
FamHS	US	The **Fam**ily **H**eart **S**tudy work was supported in part by NIH R01-HL-087700 and R01-HL-088215 (Michael A. Province, PI) from NHLBI; and R01-DK-8925601 and R01-DK-075681 (Ingrid B. Borecki, PI) from NIDDK. The investigators thank the staff and participants of the Family Heart Study for their important contributions. [representing authors: MKW, IBB]	MAGIC

Cohort Study	Country	Description and Acknowledgements	Consortium Membership(s)
FHS	US	The Framingham Offspring Study (Exam 7) and Framingham Third Generation Study (Exam 1) analyses were conducted in part using data and resources from the **F**ramingham **H**eart **S**tudy of the National Heart, Lung, and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the Framingham investigators participating in the SNP Health Association Resource (SHARe) project. This work was partially supported by the National Heart, Lung, and Blood Institute's Framingham Heart Study (contract no. N01-HC-25195) and its contract with Affymetrix, Inc for genotyping services (contract no. N02-HL-6-4278). A portion of this research utilized the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. Also supported by National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) R01 DK078616 and NIDDK K24 DK080140 to Drs. Meigs., Dr. McKeown is supported by the USDA agreement no. 58-1950-7-707. AH is supported by an American Heart Association Predoctoral Fellowship. [representing authors: AH, JSN, PFJ, JBM, NMM, LAC]	CHARGE, MAGIC
GENDAI	Greece	The **Gen**e-**D**iet **A**ttica **I**nvestigation on childhood obesity thank all the field investigators for samples and data collection and all the children, their parents and the elderly people for participation in the study. Diabetes UK (grant RD08/0003704). The research of Inga Prokopenko is funded through the European Community's Seventh Framework Programme (FP7/2007-2013), ENGAGE project, grant agreement HEALTH-F4-2007-201413. The GENDAI cohort was supported by a research grant from Coca Cola Hellas. [representing authors: IN, GVD, MY, CP, CJG, IP, NH, MIM]	MAGIC
GHRAS	Greece	The **G**reek **H**ealth **R**andomized **A**ging **S**tudy would like to thank all the field investigators for samples and data collection and all the children, their parents and the elderly people for participation in the study. Diabetes UK (grant RD08/0003704). The research of Inga Prokopenko is funded in part through the European Community's Seventh Framework Programme (FP7/2007-2013), ENGAGE project, grant agreement HEALTH-F4-2007-201413. [representing authors: SK, GVD, EG, CJG, IP, NH, MIM]	MAGIC

Cohort Study	Country	Description and Acknowledgements	Consortium Membership(s)
GLACIER	Sweden	The **G**ene-**L**ifestyle interactions **A**nd **C**omplex traits **I**nvolved in **E**levated Disease **R**isk study is nested within the Northern Swedish Health and Disease Study cohort and the Västerbotten Intervention Programme (VIP). We are indebted to the study participants who dedicated their time and samples to these studies. We also thank the VIP and Umeå Medical Biobank staff for biomedical data collection and preparation. We specifically thank John Hutiainen, Åsa Ågren and Sara Nilsson (Umeå Medical Biobank) for data organization, Kerstin Enqvist and Thore Johansson (Västerbottens County Council) for expert technical assistance with DNA preparation, and David Hunter, Patrice Soule and Hardeep Ranu (Harvard School of Public Health) for expert assistance with planning and undertaking genotyping of GLACIER samples. The GLACIER Study was funded by project grants from Novo Nordisk (PWF), the Swedish Heart-Lung Foundation (PWF), the Swedish Diabetes Association (to PWF), Påhlssons Foundation (PWF), the Swedish Research Council (PWF), Umeå University Career Development Award (PWF), and The Heart Foundation of Northern Sweden (PWF). FR was supported by a post-doctoral stipend from the Swedish Heart-Lung Foundation. [representing authors: FR, GH, IJ, FBH, PWF]	
Health ABC	US	The **Health**, **A**ging and **B**ody **C**omposition study was supported in part by the Intramural Research Program of the NIH, National Institute on Aging contracts N01AG62101, N01AG62103, and N01AG62106. The genome-wide association study was funded by NIA grant R01 AG032098 to Wake Forest University Health Sciences and genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C. [representing authors: DKH, KKL, YL, SBK]	
InCHIANTI	Italy	**In**vecchiare in **Chianti** (aging in the Chianti area, **InCHIANTI**) study investigators thank the Intramural Research Program of the NIH, National Institute on Aging who are responsible for the InCHIANTI samples. Investigators also thank the InCHIANTI participants. The InCHIANTI study baseline (1998–2000) was supported as a “targeted project” (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the US National Institute on Aging (contracts 263 MD 9164 and 263 MD 821336). [representing authors: TT, SB, LF]	MAGIC

Cohort Study	Country	Description and Acknowledgements	Consortium Membership(s)
Malmö	Sweden	The **Malmö** Diet & Cancer Study was initiated and planned in collaboration with the International Agency for Research on Cancer, the Swedish Cancer Society, and Swedish Medical Research Council and the Faculty of Medicine Lund University, Sweden. The study is also funded by Region Skåne, City of Malmö, Påhlsson Foundation and the Swedish Heart and Lung Foundation. [representing authors: ES, MO-M]	
MESA	US	The **M**ulti-**E**thnic **S**tudy of **A**therosclerosis and MESA SHARe project are conducted and supported by contracts N01-HC-95159 through N01-HC-95169 and RR-024156 from the National Heart, Lung, and Blood Institute (NHLBI). Funding for MESA SHARe genotyping was provided by NHLBI Contract N02-HL-6-4278. The authors thank the participants of the MESA study, the Coordinating Center, MESA investigators, and study staff for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org. [representing authors: AM, JAN, JIR]	CHARGE, MAGIC
PIVUS	Sweden	The participants in the **P**rospective **I**nvestigation of the **V**asculature in **U**ppsala **S**eniors were randomly sampled from all men and women at age 70 living in Uppsala County in 2001 (www.medsci.uu.se/PIVUS). Of the 2025 individuals invited, 1016 participated. The participants underwent a medical examination including a detailed questionnaire on lifestyle and socioeconomic factors, fasting blood sampling, blood pressure measurement and anthropometric measurements, as previously described.18 Blood and plasma samples have been frozen until analysis, and blood tests performed include a wide variety of traditional and more recent CVD risk factors, along with DNA extraction. In addition, the individuals have also undergone extensive phenotyping including whole body MRI, echocardiography, endothelial function measurements, carotid ultrasound, DXA, and spirometry. Genotyping was performed by the SNP&SEQ Technology Platform in Uppsala (www.genotyping.se). We thank Tomas Axelsson, Ann-Christine Wiman and Caisa Pöntinen for their excellent assistance with genotyping. The SNP Technology Platform is supported by Uppsala University, Uppsala University Hospital and the Swedish Research Council for Infrastructures. E.I. is supported by grants from the Swedish Research Council, the Swedish Heart-Lung Foundation, the Swedish Foundation for Strategic Research, and the Royal Swedish Academy of Science. [representing authors: AG, UR, ACS, LL, EI]	MAGIC

Cohort Study	Country	Description and Acknowledgements	Consortium Membership(s)
Rotterdam	The Netherlands	The generation and management of GWAS genotype data for the **Rotterdam Study** is supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project nr. 050-060-810. We thank Pascal Arp, Mila Jhamai, Marijn Verkerk, Lizbeth Herrera and Marjolein Peters for their help in creating the GWAS database, and Karol Estrada and Maksim V. Struchalin for their support in creation and analysis of imputed data. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists. [representing authors: FJAvR, MCZ, AGU, AH, OHF, JCMW]	CHARGE, MAGIC
ULSAM	Sweden	Subjects born between 1920 and 1924 in Uppsala, Sweden were invited to participate at age 50 in the the **U**ppsala **L**ongitudinal **S**tudy of **A**dult **M**en, that was started in 1970. Subjects were reinvestigated at the ages of 60, 70, 77, 82 and 88 years.19 Blood samples for DNA extraction and main cardiovascular risk factors were available from the 70-years old investigation (n=1,146 with DNA and data on CHD risk factors). The participants have undergone extensive phenotyping at repeated time points, including for example euglycemic clamps, oral glucose tolerance tests, echocardiography, 24-h ambulatory blood pressure measurement, and a range of biomarkers. Genotyping was performed by the SNP&SEQ Technology Platform in Uppsala (www.genotyping.se). We thank Tomas Axelsson, Ann-Christine Wiman and Caisa Pöntinen for their excellent assistance with genotyping. The SNP Technology Platform is supported by Uppsala University, Uppsala University Hospital and the Swedish Research Council for Infrastructures. E.I. is supported by grants from the Swedish Research Council, the Swedish Heart-Lung Foundation, the Swedish Foundation for Strategic Research, and the Royal Swedish Academy of Science. [representing authors: AG, UR, ACS, LL, EI]	MAGIC

Cohort Study	Country	Description and Acknowledgements	Consortium Membership(s)
YFS	Finland	The Cardiovascular Risk in **Y**oung **F**inns **S**tudy has been financially supported by the Academy of Finland: grants 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi), the Social Insurance Institution of Finland, Kuopio, Tampere and Turku University Hospital Medical Funds (grant 9M048 for TL), Juho Vainio Foundation, Paavo Nurmi Foundation, Finnish Foundation of Cardiovascular Research and Finnish Cultural Foundation, Tampere Tuberculosis Foundation and Emil Aaltonen Foundation (TL). The expert technical assistance in the statistical analyses by Irina Lisinen and Ville Aalto are gratefully acknowledged. [representing authors: TL, OTR, JV, VM, MK]	

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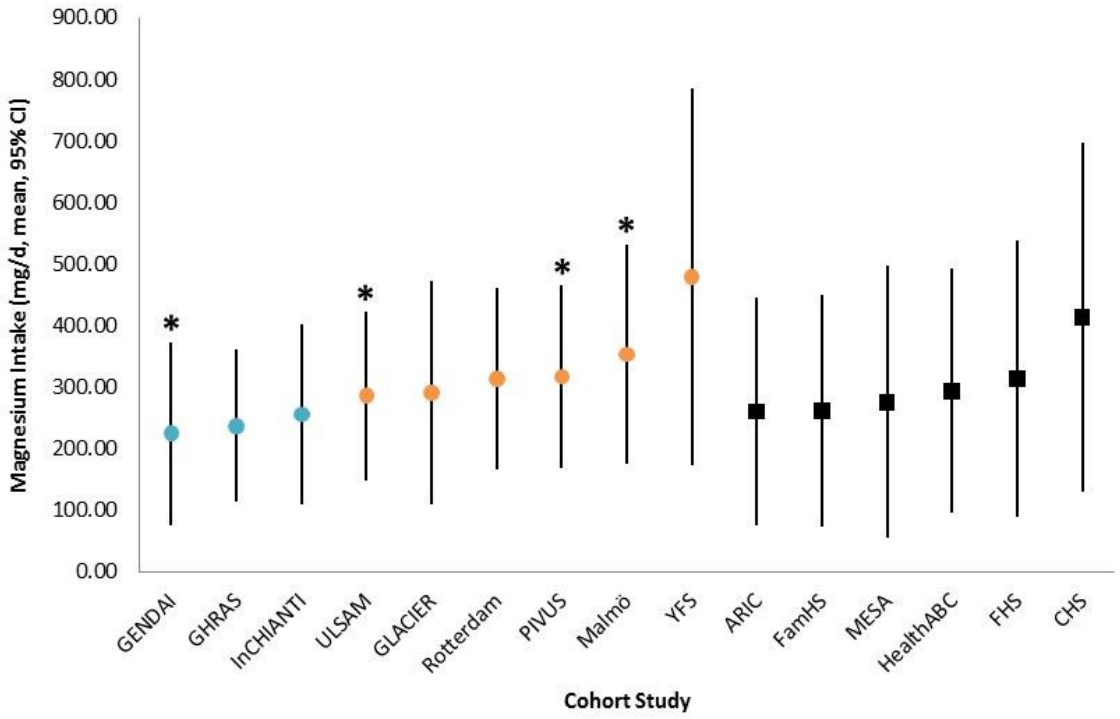
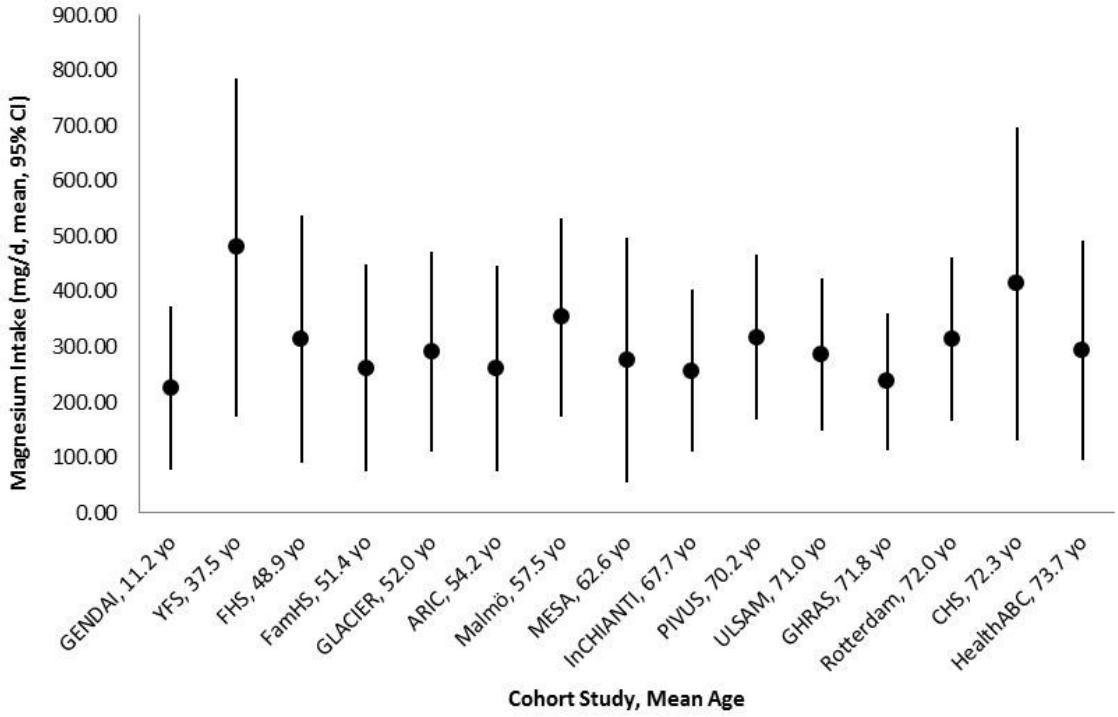
Rotterdam	M. Carola Zillikens, MD, PhD	MCZ	Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), Leiden, the Netherlands; Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands
Rotterdam	André G. Uitterlinden, PhD	AGU	Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), Leiden, the Netherlands; Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

Cohort Study	Author Name	Initials	Institutional Affiliation(s)
Rotterdam	Albert Hofman, MD, PhD	AH	Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), Leiden, the Netherlands
Rotterdam	Oscar H. Franco, MD, PhD	OHF	Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), Leiden, the Netherlands
Rotterdam	Jacqueline C.M. Witteman, PhD	JCMW	Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), Leiden, the Netherlands
PIVUS/ULSAM	Andrea Ganna, MSc	AG	Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
PIVUS/ULSAM	Ulf Riserus, MD, PhD	UR	Department of Public Health and Caring Sciences, Clinical Nutrition and Metabolism, Uppsala University, Uppsala, Sweden
PIVUS/ULSAM	Ann-Christine Syvänen, PhD	ACS	Department of Medical Sciences, Molecular Medicine and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
PIVUS/ULSAM	Lars Lind, MD, PhD	LL	Department of Medical Sciences, Cardiovascular Epidemiology, Uppsala University, Uppsala, Sweden
PIVUS/ULSAM	Erik Ingelsson, MD, PhD, FAHA	EI	Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
YFS	Terho Lehtimäki, MD, PhD	TL	Fimlab Laboratories and University of Tampere, School of Medicine, and Tampere University Hospital, Tampere, Finland
YFS	Olli T. Raitakari, MD, PhD	OTR	Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital and Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland
YFS	Jorma Viikari, MD, PhD	JV	Department of Medicine, University of Turku and Turku University Hospital, Turku, Finland
YFS	Vera Mikkilä, PhD	VM	Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland
YFS	Mika Kähönen, MD, PhD	MK	Department of Clinical Physiology, Tampere University Hospital and University of Tampere, Tampere, Finland

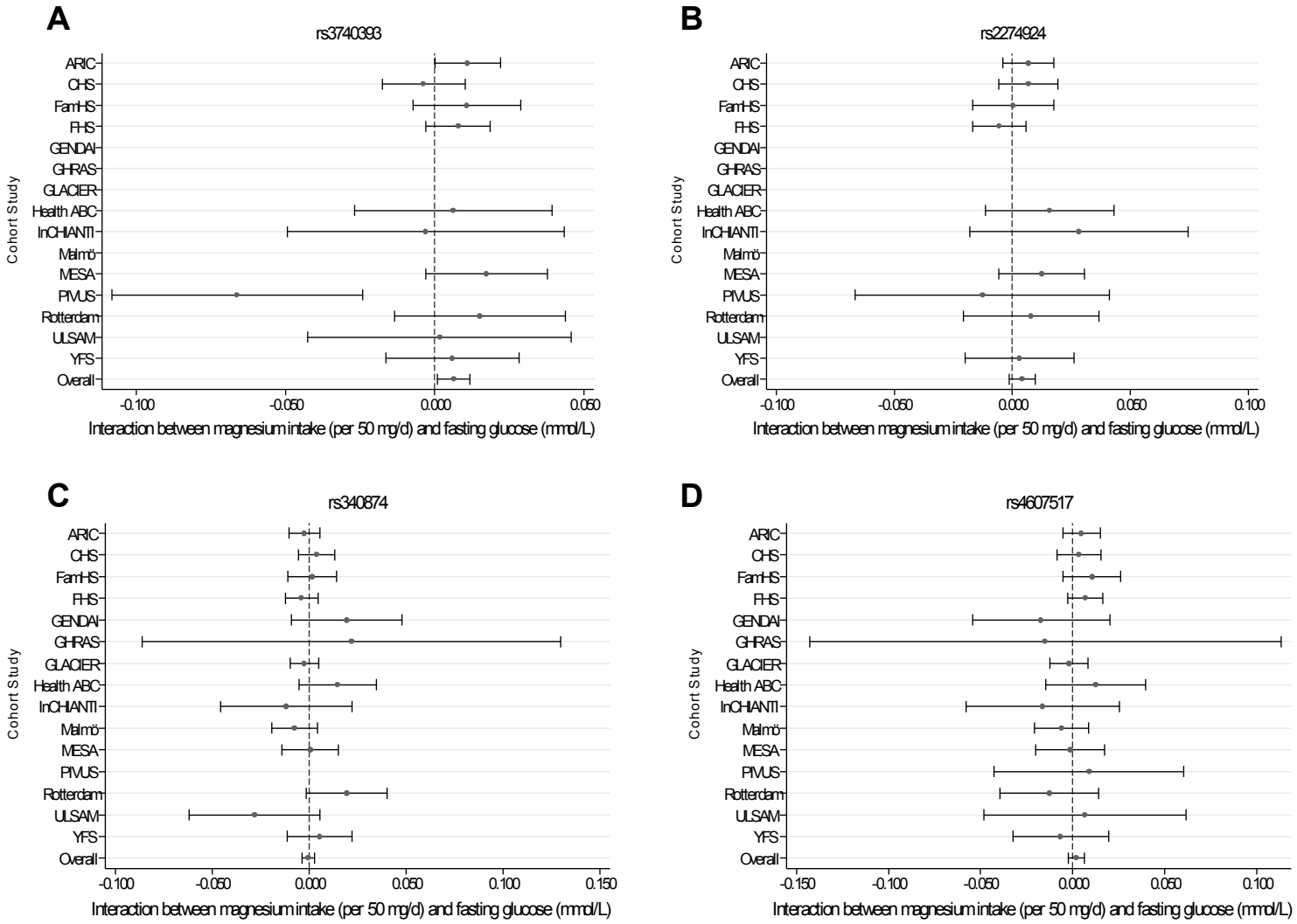
Supplemental Figure 4-1. Mean and 95% confidence interval of dietary magnesium intake (mg/d). Values are shown by region in order of ascending intake: Mediterranean cohort studies = aqua circles; Northern European cohort studies = orange circles; North American cohort studies = black squares. All cohort studies estimated intake using food frequency questionnaire, except where starred (*): GENDAI = 24-h dietary recall; Malmö = food diary and FFQ; PIVUS and ULSAM = dietary record.

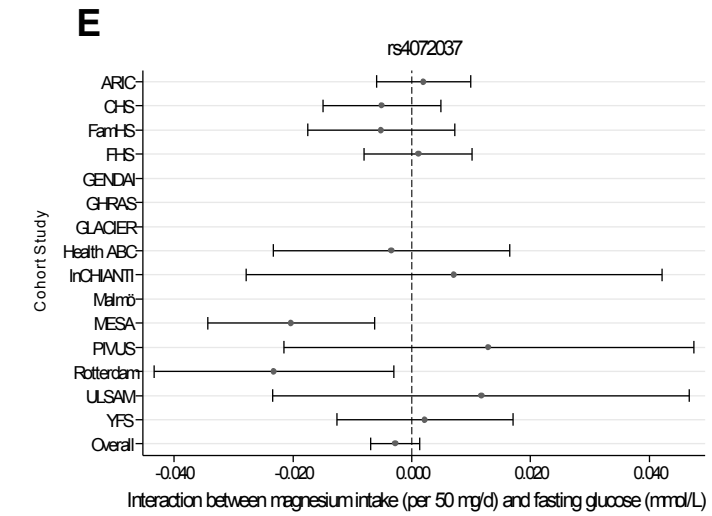


Supplemental Figure 4-2. Mean and 95% confidence interval of dietary magnesium intake (mg/d) in 15 CHARGE cohorts by ascending mean age of each cohort study.



Supplemental Figure 4-3. Forest Plots of Cohort-Specific and Meta-Analyzed Magnesium × SNP Interactions on Fasting Glucose (24 plots, A–X)

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Malmö
MESA
PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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-0.020
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0.020
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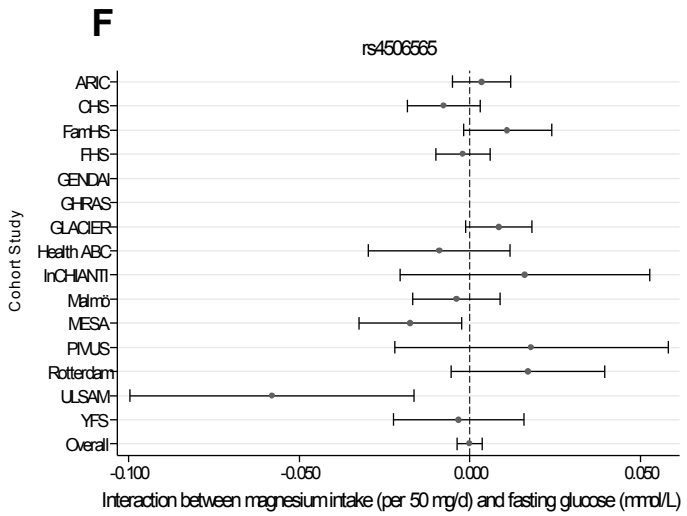
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
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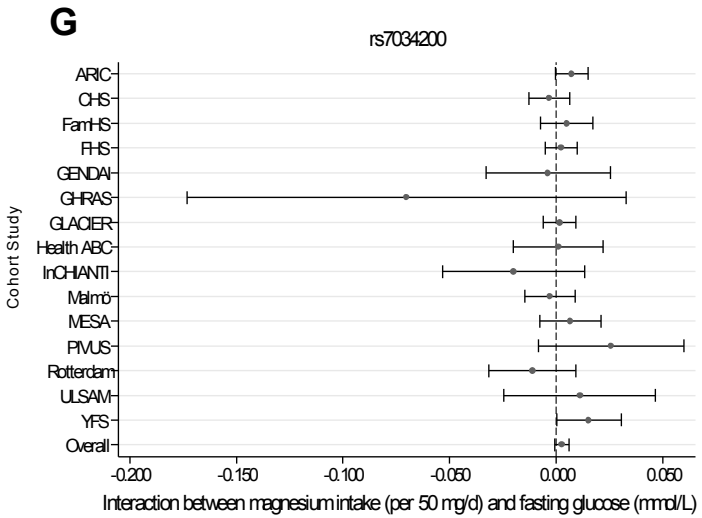
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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Rotterdam
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YFS
Overall
Cohort Study
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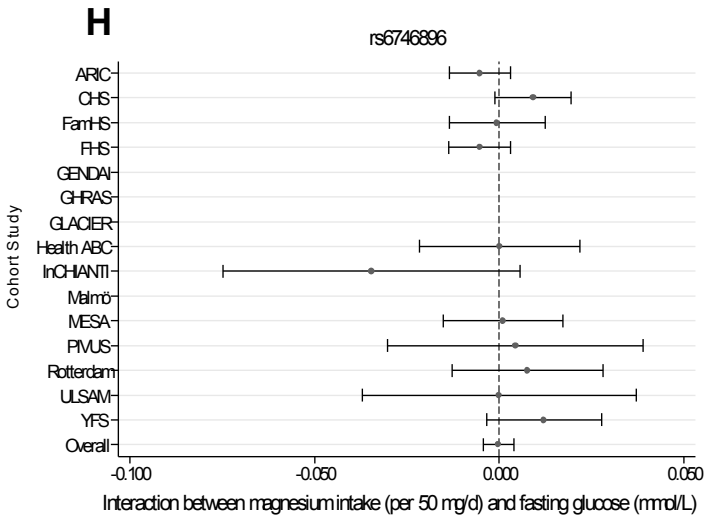
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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Malmö
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PIVUS
Rotterdam
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Cohort Study
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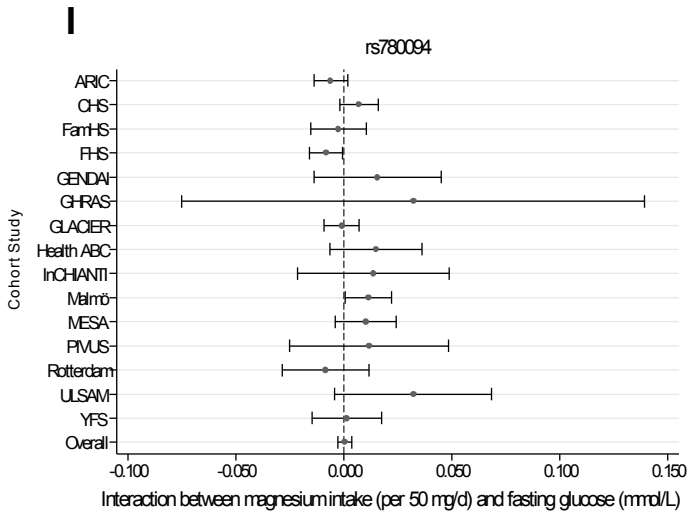
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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YFS
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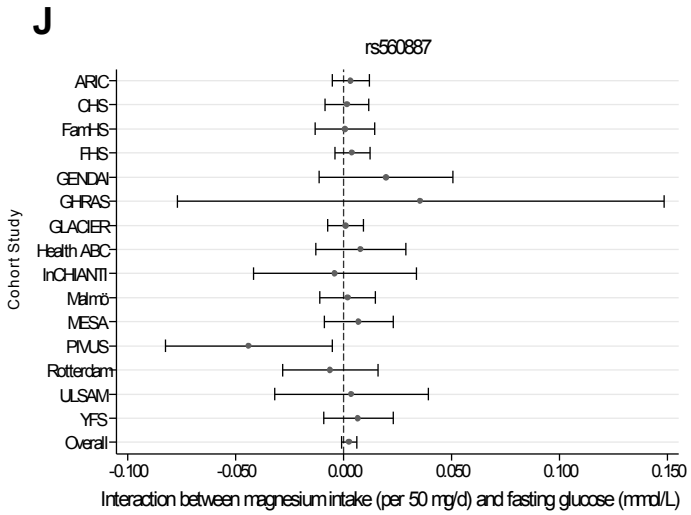
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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Cohort Study
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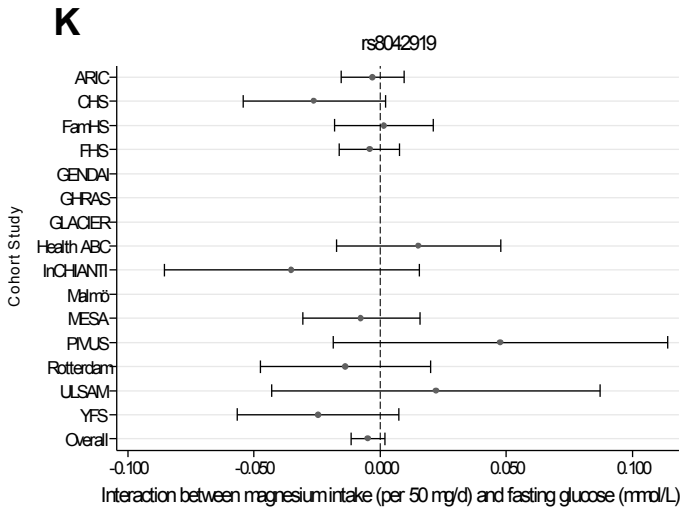
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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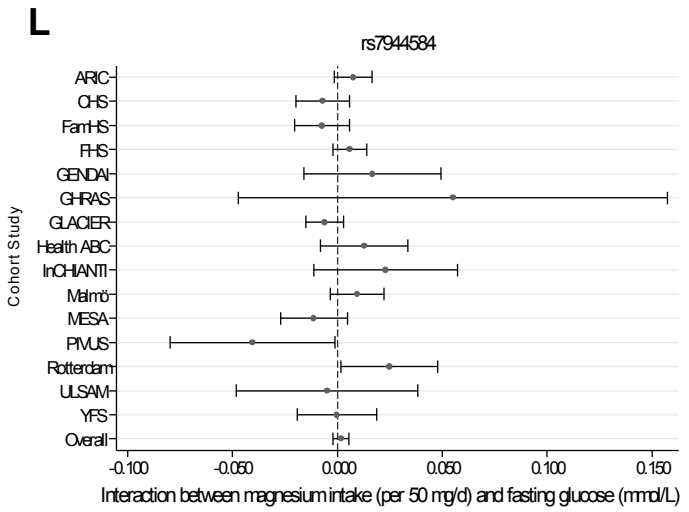
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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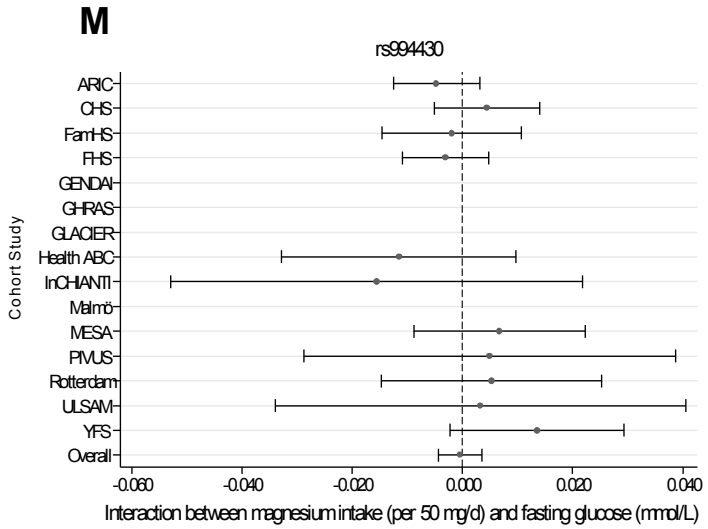
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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Overall
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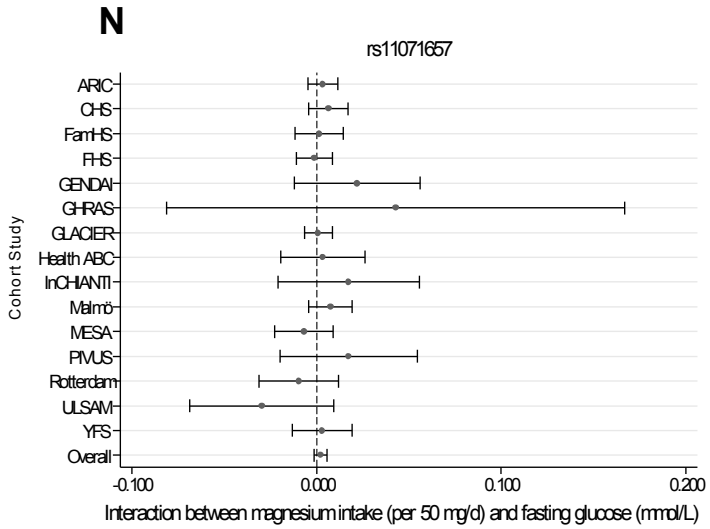
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
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YFS
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Cohort Study
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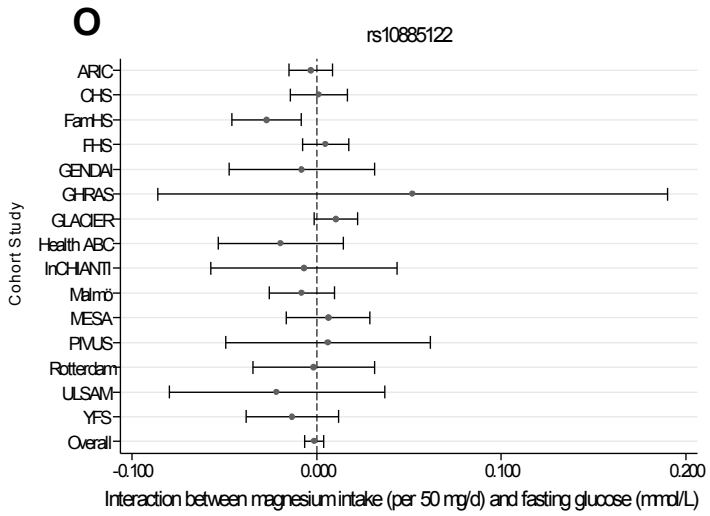
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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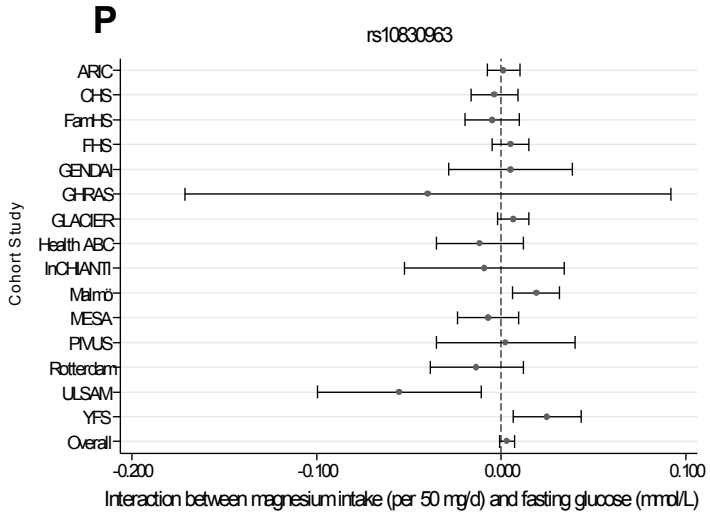
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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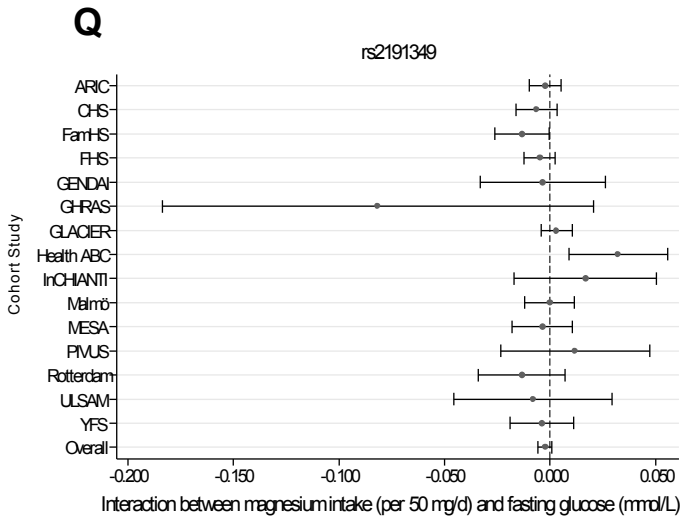
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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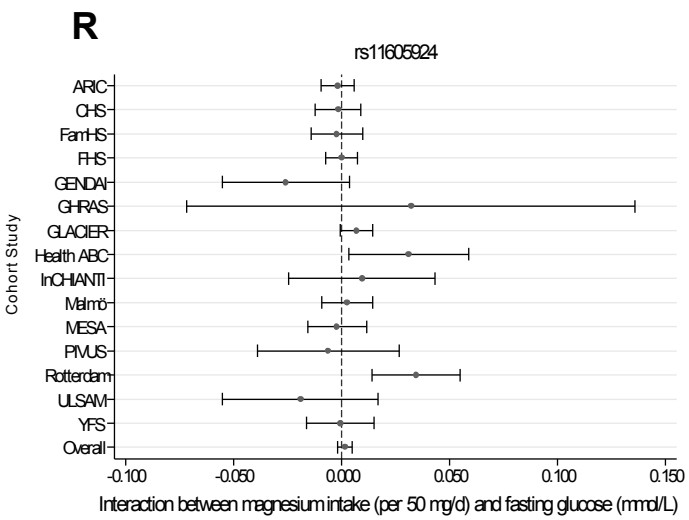
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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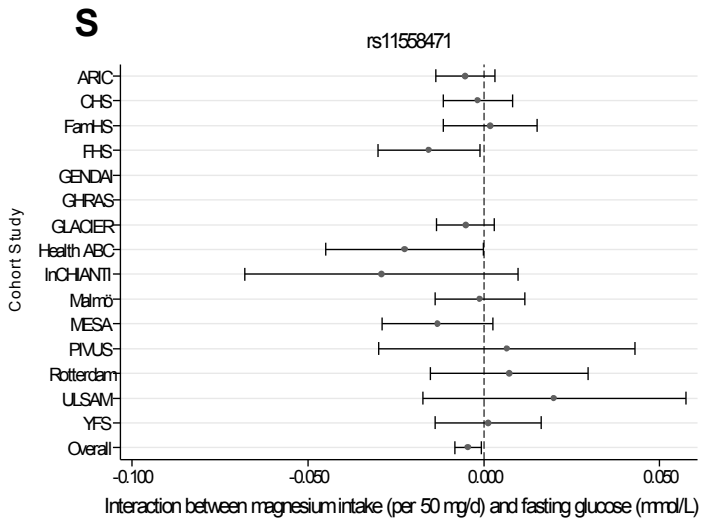
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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Malmö
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PIVUS
Rotterdam
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Cohort Study
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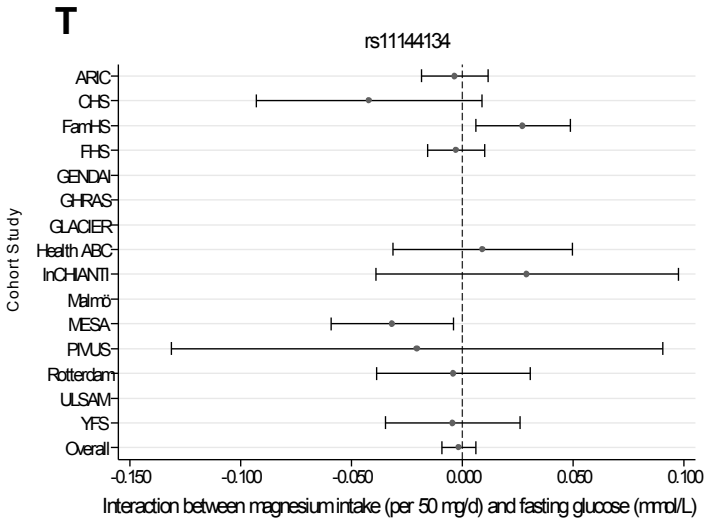
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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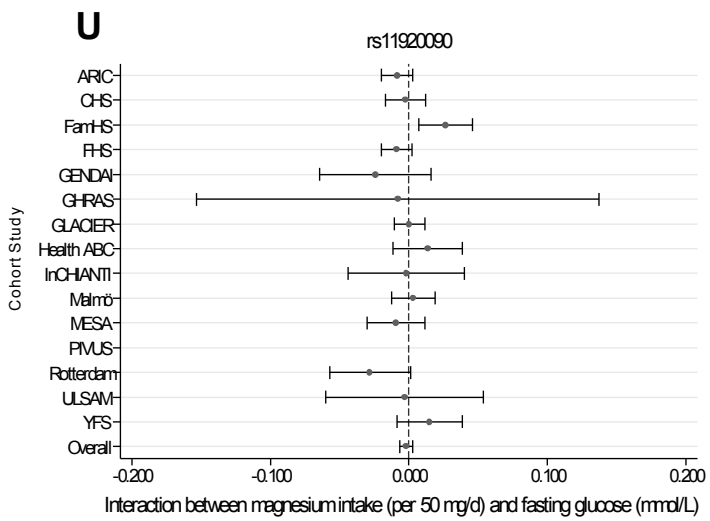
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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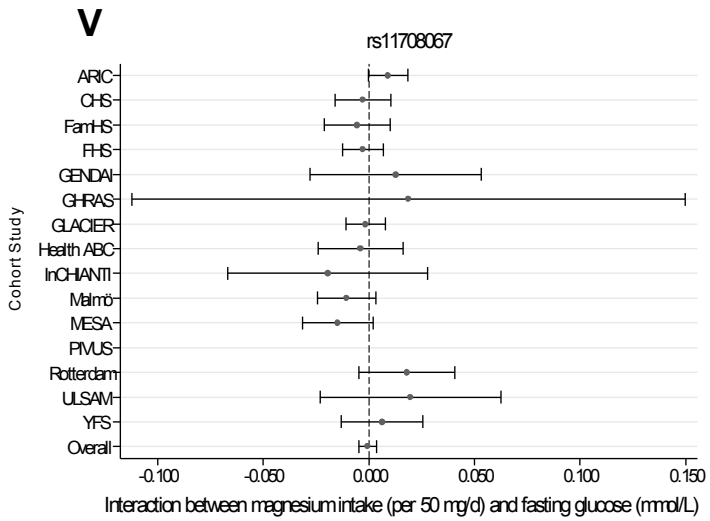
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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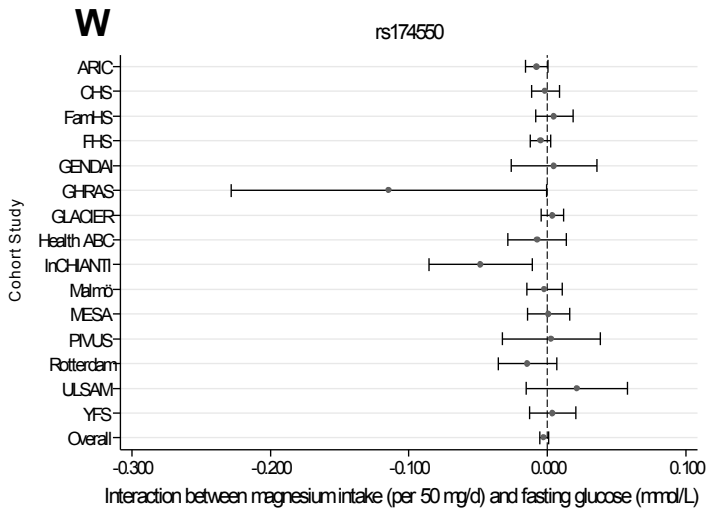
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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Rotterdam
ULSAM
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Overall
Cohort Study
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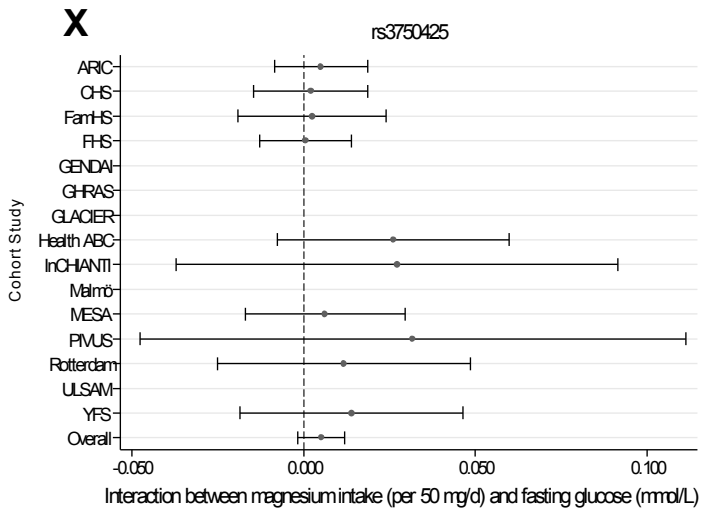
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)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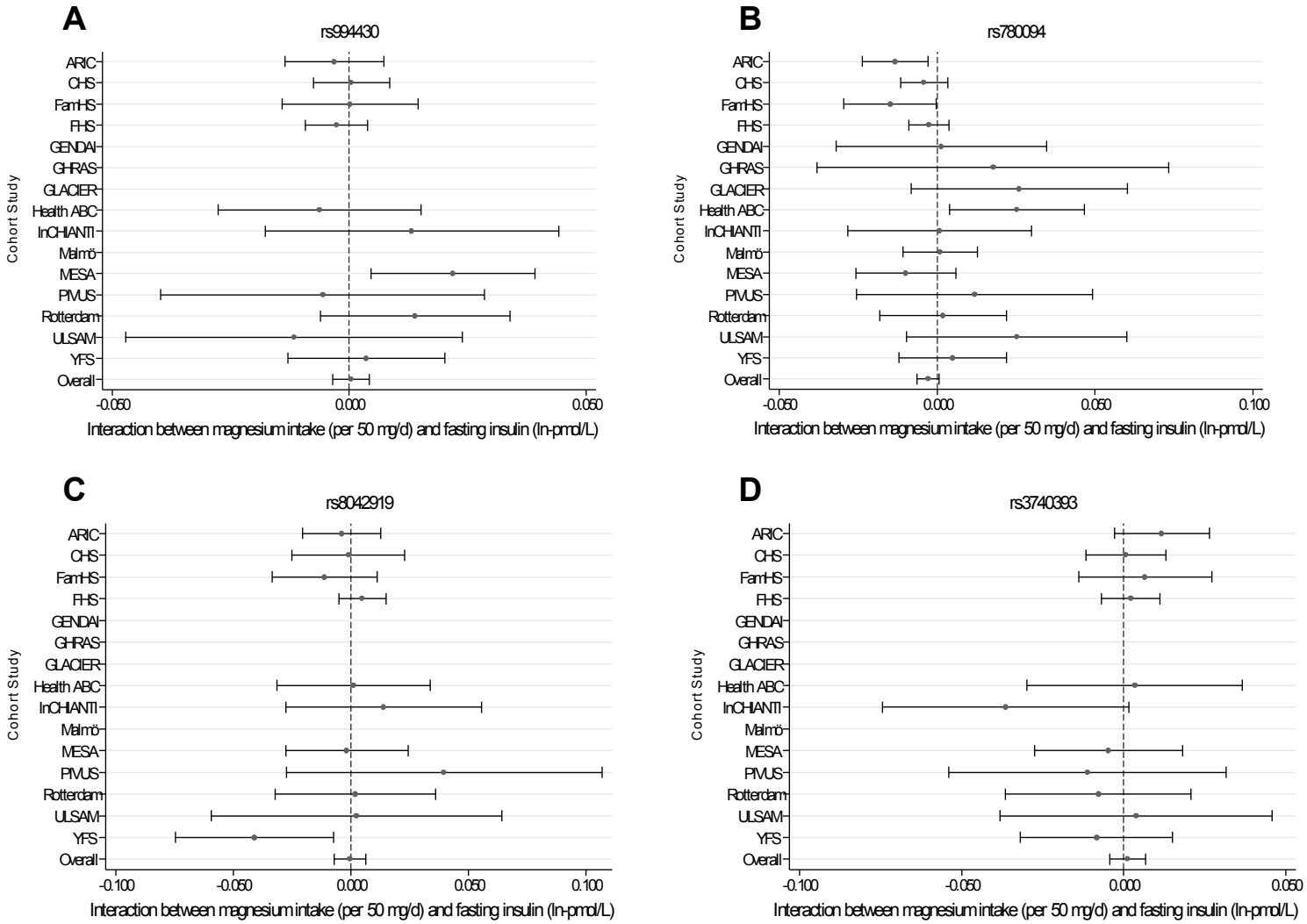
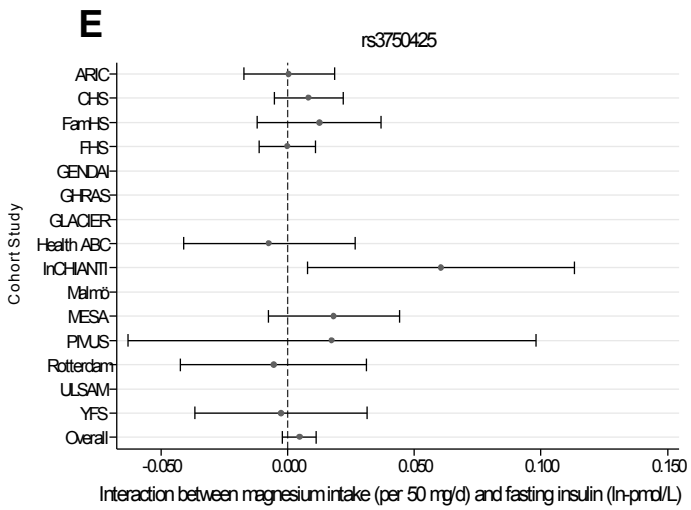
Malmö
MESA
PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
-0.050
0.000
0.050
0.100
Interaction between magnesium intake (per 50 mg/d) and fasting glucose (mmol/L)

Supplemental Figure 4-4. Forest Plots of Cohort-Specific and Meta-Analyzed Magnesium × SNP Interactions on Fasting Insulin (10 plots, A–J)

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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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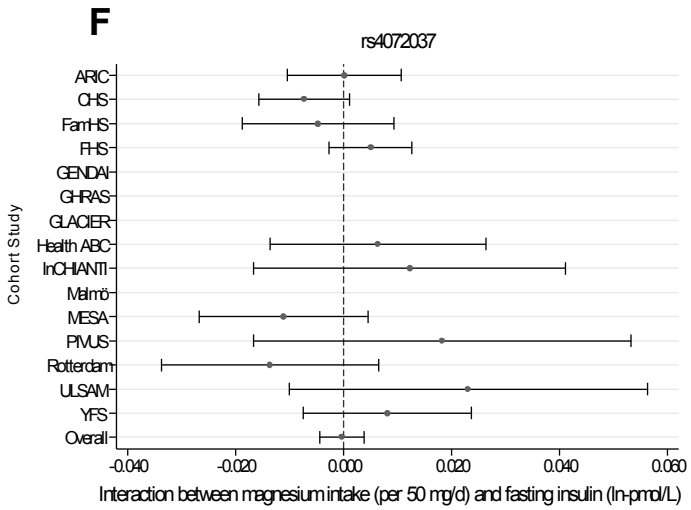
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PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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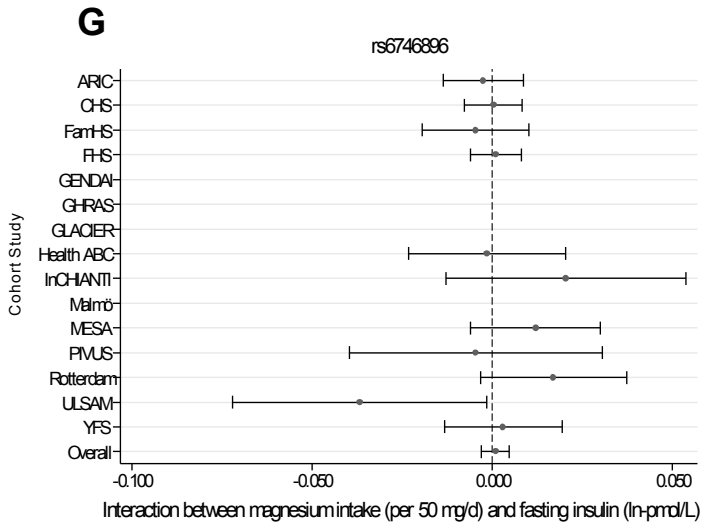
Interaction between magnesium intake (per 50 mg/d) and fasting insulin (ln-pmol/L)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Malmö
MESA
PIVUS
Rotterdam
ULSAM
YFS
Overall
Cohort Study
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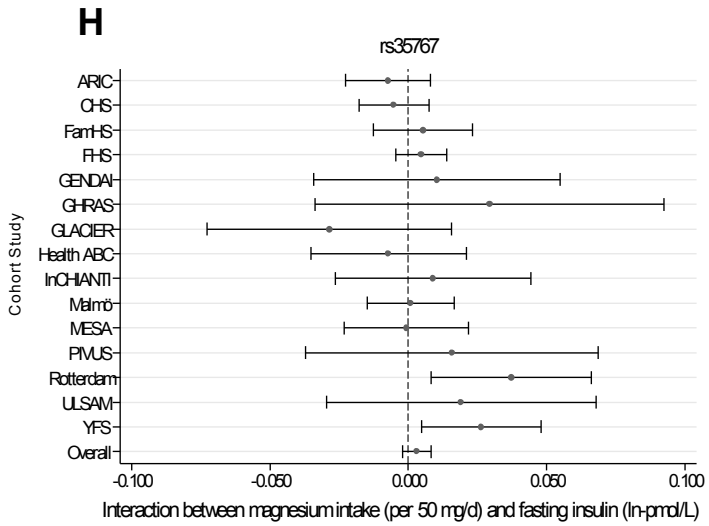
Interaction between magnesium intake (per 50 mg/d) and fasting insulin (ln-pmol/L)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PIVUS
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ULSAM
YFS
Overall
Cohort Study
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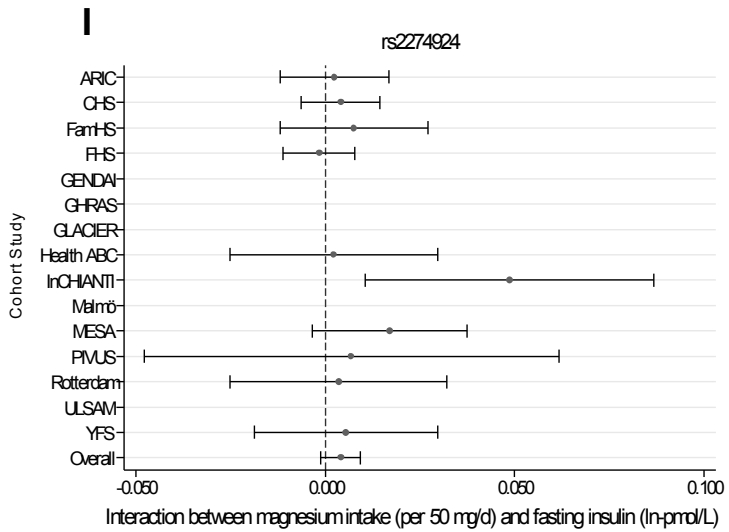
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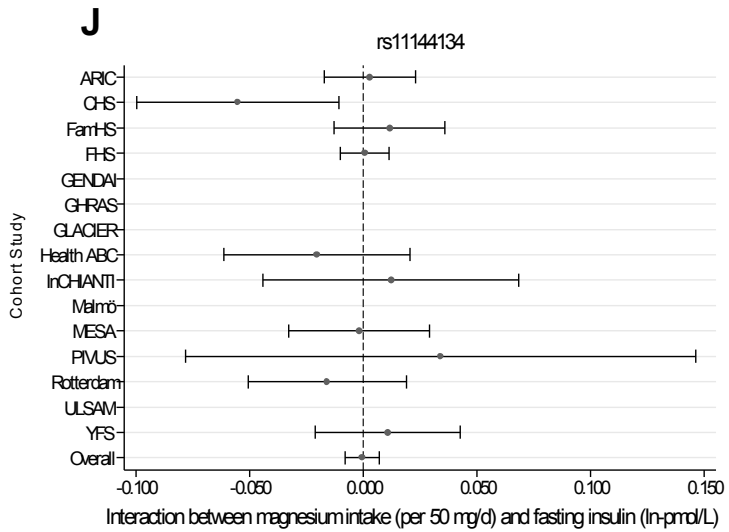
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Interaction between magnesium intake (per 50 mg/d) and fasting insulin (ln-pmol/L)

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CHAPTER 5. MAGNESIUM AND GLUCOSE AND INSULIN METABOLISM

Higher magnesium intake reduces risk of impaired glucose and insulin metabolism, and progression from prediabetes to diabetes in middle-aged Americans

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5.1 Abstract

Background: Magnesium has a beneficial role in glucose and insulin homeostasis and metabolism. Few long-term studies have examined relationships between magnesium intake and progression from normal to prediabetic states, or from prediabetic states to type 2 diabetes (T2D).

Objective: To examine the prospective association between magnesium intake and development of glucose impairment and insulin resistance, and the progression from these metabolically impaired states to incident T2D using data from a community-dwelling cohort of 2,582 men and women aged 26–81 y over an average 7-y follow-up period.

Design: Magnesium intake, assessed by food frequency questionnaire, and risk of incident "metabolic impairment," defined as impaired fasting glucose (IFG; fasting glucose ≥5.6 but <7.0 mmol/L), impaired glucose tolerance (IGT; post-OGTT glucose ≥7.8 but <11.1 mmol/L), insulin resistance (≥90th percentile of homeostasis model assessment of insulin resistance, HOMA-IR), *or* hyperinsulinemia (≥90th percentile of fasting insulin), was estimated among those with normal baseline status, and risk of incident T2D was estimated among those metabolically impaired at baseline. In addition, we examined magnesium intake in relation to 7-yr changes in fasting and post-OGTT glucose and insulin, HOMA-IR, and insulin sensitivity, as Gutt's Insulin Sensitivity $\text{Index}_{0,120}$ (ISI), in those free of T2D at baseline and follow-up.

Results: After adjusting for age, sex, and energy intake, those with the highest magnesium intake had 37% lower risk of incident metabolic impairment (*P* trend = 0.02), compared to those with the lowest intake. Those with baseline metabolic impairment and the highest magnesium intake had 32% lower risk of incident T2D (*P* trend = 0.05) than those with the lowest intake. Additionally adjusting for risk factors and dietary fiber attenuated

magnesium's associations in the baseline normal population, but did not substantially affect associations in the metabolically impaired population. Higher magnesium intake also tended to be associated with lower follow-up fasting glucose and HOMA-IR, but not fasting insulin, 2-hr OGTT values, or Gutt's $ISI_{0,120}$.

Conclusion: Our findings support a beneficial role for higher magnesium intake in offsetting risk of developing T2D among those at high risk. However, magnesium's associations with long-term changes in non-steady state (i.e., dynamic) measures, such post-OGTT and derived values deserve further research.

5.2 Introduction

Prediabetes and diabetes affected an estimated 45% of US adults in 2010 (1). Diabetes significantly raises risk of heart disease and stroke morbidity and mortality, and is the leading cause of adult blindness and kidney failure. An estimated $174 billion in indirect and direct medical costs are attributable annually to diabetes (1). Diet modification is recommended as an important prevention strategy at any stage of progression from health to overt type 2 diabetes (T2D) (2). Prospective studies (3–5) have shown that individuals with higher magnesium intake are 10–47% less likely to develop T2D. However, only 50% of Americans one year or older achieve the Recommended Dietary Allowance (RDA) for magnesium, which is 400–420 mg/day for adult men, and 300–310 mg/day for adult women (6,7).

A body of clinical evidence (8–13) supports a role for magnesium supplementation in glucose and insulin metabolism. A meta-analysis of nine magnesium supplement trials in those with T2D found that a median magnesium dose of 360 mg/d was associated with significantly lower post-intervention fasting glucose (FG) in the treatment groups, suggesting improved glucose control (11). A recent small randomized, placebo-controlled trial in obese, non-diabetic, insulin resistant individuals demonstrated that treatment with 365 mg/d of magnesium for six months significantly lowered FG, fasting insulin (FI), and insulin resistance (IR), and improved insulin sensitivity (12). Three-month supplementation with magnesium in individuals with other risk factors, such as mild hypertension or hypomagnesaemia, has also been found to improve insulin sensitivity and pancreatic beta-cell function (8–10). Finally, low-magnesium diets given to otherwise healthy individuals have been shown to impair insulin sensitivity after just three wks (13).

Few prospective studies have evaluated magnesium intake in relation to various stages of progression of disordered glucose and insulin metabolism, i.e., from normal to impaired states including prediabetes and insulin resistance, over the long term (i.e., >5 yrs), even though these states are significant risk factors for T2D as well as for cardiovascular disease (14–16). In addition, few prospective studies have examined magnesium's associations with long-term progression from baseline impaired states to T2D (3,17). One study of magnesium intake in US adults estimated that the optimal magnesium intake level in relation to insulin sensitivity measured 5 yrs later was at least 325 mg/d (17); and another study in US adults reported lower long-term IR with higher magnesium intake (3).

In the present analysis, we evaluated the longitudinal association between magnesium intake and incidence of metabolic impairment, defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), insulin resistance (IR), or hyperinsulinemia, in otherwise healthy individuals, and to incident T2D in those with baseline metabolic impairment, to assess whether magnesium intake may have differing associations at different stages of underlying metabolic impairment.

5.3 Methods

5.3.1 *Study Sample*

The National Heart, Lung, and Blood Institute's Framingham Heart Study (FHS) Offspring cohort is a community-based, longitudinal study of cardiovascular disease that began in 1971 whose participants are among the offspring of the Original FHS cohort (18). In the fifth examination cycle (1991–1995) of the Offspring cohort, 3,799 participants underwent a standard medical examination, including laboratory and anthropometric measurements, as well as dietary assessment. Participants were followed from baseline at the fifth through

seventh (1998–2001) examinations. Individuals were excluded if they had a history of T2D or were identified as having T2D at the baseline examination (*n*=400); if they had invalid dietary data at baseline (*n*=326); if they were missing necessary covariates (*n*=109); if they were not present at the final follow-up examination (*n*=329, 135 of whom were lost-to-follow-up owing to death); or if they had invalid or missing dietary data at follow-up (*n*=53). The final sample size for the primary analysis was 2,582 participants.

A 2-hour 75-g oral glucose tolerance test (OGTT) was administered to all participants at exam 5 and in a subset of participants at exam 7 who had undergone OGTT at exam 5, based on glucose tolerance status at exam 5 (sex block-randomly selected from 5 quintile strata of fasting glucose), as described in detail elsewhere. A total of 863 participants had follow-up OGTT measures available for the present analysis.

The original data collection protocols were approved by the Institutional Review Board at Boston University Medical Center, and written informed consent was obtained from all participants. The present study protocol was reviewed by the Tufts Medical Center and Tufts University Health Sciences Institutional Review Board.

5.3.2 Dietary Assessment

The Harvard semi-quantitative, 126-item food frequency questionnaire (FFQ) was used to assess dietary intake (19). FFQs were mailed to participants prior to each exam, and participants were instructed to bring the completed FFQ with them to their exam appointment. The FFQ included a list of foods together with a standard serving size and 9 consumption frequency categories ranging from “never, or less than once per month” to “6+ per day.” The FFQ allowed for notation of foods usually consumed that were not otherwise listed in the FFQ. Participants were asked to report consumption of each food item over the previous yr. Invalid FFQs were defined as those which estimated daily caloric intake as

<600 kcal/day, or ≥4,000 kcal/day for women, ≥4,200 kcal/day for men, or those which had ≥12 blank items. The relative validity of the FFQ for energy-adjusted magnesium intake has been previously reported (19–21), and shows reasonable correlation with estimates from dietary records (r = 0.67–0.71) (19).

To account for long-term dietary exposure and to reduce within-person variability, intake of nutrients are presented as mean intake obtained from the dietary data of the fifth (baseline), sixth, and/or seventh examinations, as follows. For those with incident T2D, intake of nutrients and energy were averaged across the dietary data from the fifth examination up to but not including the examination at which T2D incidence was ascertained. For those without incident T2D, intake was averaged across all exams (5, 6, and/or 7) for which dietary data was available.

5.3.3 Outcome Measures and Definitions

Fasting plasma glucose (FG) and 2-hr OGTT glucose were measured in fresh specimens with a hexokinase reagent kit (A-Gent glucose test; Abbot, South Pasadena, CA). At baseline (exam 5), fasting plasma insulin (FI) and 2-hr OGTT insulin were measured using Coat-A-Count total insulin radioimmunoassay (RIA) (Diagnostic Products Corp., Los Angeles, CA) while at exam 7, FI and 2-hr OGTT insulin were measured using a different assay, the human-specific insulin RIA (Linco Research Inc., St. Charles, MO). Owing to the different assays used to measure insulin between exams 5 and 7, a calibration study was conducted using FI in frozen plasma samples from 87 participants. These samples from exam 5 were re-analyzed ≈9 yrs later using the human-specific insulin RIA assay and a regression equation was derived to calibrate total insulin RIA measures at exam 5 to human-specific RIA-equivalent values. The calibrated measures were used in the present analysis.

We defined metabolic impairment or T2D based in part on impaired glucose criteria from the American Diabetes Association (ADA) (22), in addition to impaired insulin criteria, as summarized in **Supplemental Table 1**. Participants were classified as having T2D if they had a diagnosis of T2D, reported use of an oral hypoglycemic drug or insulin, or had FG ≥7.0 mmol/L (126 mg/dL) or 2-hr OGTT glucose ≥11.1 mmol/l (200 mg/dL). Metabolic impairment was defined has having one or more of the following: IFG, IGT, IR, or hyperinsulinemia, per criteria that follow. Participants were classified as having normal fasting glucose (NFG) if they had FG <5.6 mmol/L (100 mg/dL). IFG was classified as FG ≥5.6 but <7.0 mmol/L (100–126 mg/dL). Normal glucose tolerance (NGT) was classified as 2-hr OGTT glucose <7.8 mmol/L (140 mg/dL). IGT was classified as 2-hr OGTT glucose ≥7.8 but <11.1 mmol/L (140–200 mg/dL). HOMA-IR, a measure of hepatic IR, was calculated as FI (μU/mL) × FG (mmol/L) / 22.5 (23). IR was defined as HOMA-IR ≥90th percentile. Hyperinsulinemia was defined as FI ≥90th percentile. Gutt's Insulin Sensitivity Index$_{0,120}$ (ISI), a measure of peripheral tissue insulin sensitivity, was calculated as ISI = (m/mean plasma glucose)/log(mean serum insulin), where the glucose uptake rate in peripheral tissues (m) = (75000 mg + [FG (mg/dL) – 2-hr OGTT glucose (mg/dL)] × 0.19 × weight (kg)] / 120 min; mean plasma glucose = mean of FG (mmol/L) and 2-hr OGTT glucose (mmol/L); and mean serum insulin = mean of serum FI (μU/L) and 2-hr OGTT serum insulin (μU/L) (24).

5.3.4 Covariates

Potential confounders of the relationship between diet and progression to metabolic impairment or T2D were considered as covariates. Covariates were assessed at baseline as follows: age (yrs), body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference (cm) was measured at the umbilicus

with the participant standing. Parental history of diabetes was based on self-reported history in one or both natural parents. Blood pressure (BP) was measured twice by a physician and averaged to calculate the systolic and diastolic BP (mmHg). Hypertension (yes/no) was defined as BP of ≥130/85 mmHg or undergoing treatment for hypertension. Information on regular smoking during the year prior to the baseline examination (yes/no) was assessed via questionnaire. Physical activity was quantified as a continuous score based on activity levels as well as intensities of these activities, as previously described (25).

5.3.5 Statistical Analyses

We generated energy-adjusted quintile categories of averaged magnesium intake. Participant characteristics adjusted for age, sex, and energy (in the case of foods and nutrients) are presented across quintile categories. Tests for linear trend across increasing categories of intake were performed by assigning the median value of intake within each category and treating these as a continuous variable.

Because we sought to characterize magnesium's associations with progression from normal to metabolic impairment, we assessed the association of magnesium intake with: 1) incident metabolic impairment (defined as having IFG, IGT, IR, *or* hyperinsulinemia), among participants with normal status (NFG, NGT, no IR, *and* normoinsulinemia) at baseline; and 2) incident T2D among participants who had baseline metabolic impairment, as defined above. Because there were few cases of incident T2D among those with normal baseline status (n=25), these cases were incorporated into our definition of incident metabolic impairment. In secondary, sensitivity analyses, we redefined metabolic impairment by use of the ADA prediabetes criteria of IFG and IGT *only*, as these are frequently used in other clinical and research contexts. This redefinition also allowed us to examine whether excluding IR and hyperinsulinemia from the definition of metabolic impairment impacts

magnesium's associations with incident impairment. Relative risks (RR) and 95% confidence intervals (95%CI) across quintile categories of magnesium intake were estimated from multivariable logistic regression analyses (PROC GENMOD) for incident metabolic impairment or T2D. *P* for trend was estimated using the median value in each category of intake as a continuous exposure.

In secondary analyses in participants without incident T2D, we assessed the association between magnesium intake and change in continuous measures of FG, FI, HOMA-IR, 2-hr OGTT glucose and insulin, and ISI over 7 yrs. Change was modeled in each case as the final measure adjusted for the baseline measure. For these continuous outcomes, we estimated least squares adjusted means of values in each quintile category of energy-adjusted magnesium intake (PROC GLM). *P* for trend was estimated using the median value in each category of intake as a continuous exposure. Natural-logged values were used for FI, HOMA-IR, and 2-hr OGTT insulin, which were back-transformed to geometric means for presentation.

For all outcomes, the initial analysis was adjusted for age, sex, and energy intake (model 1). Model 2 was adjusted as for model 1, plus parental history of diabetes, BMI, physical activity, smoking status, alcohol intake, and hypertension. In model 3, we further adjusted for dietary fiber. Additional adjustment for caffeine did not change the results and therefore we do not include those results. Dietary fiber and caffeine were initially chosen because they represent surrogates of non-magnesium constituents of commonly consumed magnesium-containing foods (i.e., whole grains and coffee), which themselves have been associated with lower risk of T2D (5,26–28). Adjusting for these dietary variables allows us to at least partially distinguish the associations of magnesium from the associations of the foods themselves, their constituents (e.g., phytochemicals), or from health behaviors

associated with these nutrients (e.g., higher fiber may also be a surrogate for a healthy lifestyle).

Finally, we separately tested for statistical interaction between magnesium and age, sex, and BMI, in the final models of dichotomous outcomes using cross-product terms. No interaction was statistically significant (all P >0.1). Substituting waist circumference for BMI, or including waist circumference or change in weight between baseline and follow-up did not substantively alter the results.

All analyses were conducted in SAS (version 9.3, SAS Institute, Cary, North Carolina). Statistical significance was set at the 0.05 level. All tests were two-tailed.

5.4 Results

Baseline clinical and dietary characteristics of 2,582 participants are presented across quartile categories of energy-adjusted magnesium intake in **Table 1**. The average age of the population was 54 yrs, 55% were women, 42% were overweight, and 21% were obese. Average magnesium intake was 308 mg/d, which parallels intake reported in other US adult populations (29). Approximately 50% of women and 75% of men reported magnesium intake below the RDA. In analyses of trend from lowest to highest quartile category of magnesium intake, those in the highest category were more likely to be female, older, and have lower BMI. They were less likely to have hypertension or to have smoked regularly in the prior year. Intake of energy and most other nutrients increased along with increasing magnesium intake, except for alcohol. Baseline characteristics of almost all glucose and insulin parameters tended to be lower in participants with higher magnesium intake. Magnesium intake was moderately correlated with dietary fiber (r=0.67, P <0.001), but not with caffeine (r=0.03, P=0.08).

5.4.1 Incident Metabolic Impairment among Those with Normal Status at Baseline

Among the 1,654 (64.1%) participants without metabolic impairment at baseline, there were 307 (18.6%) cases of incident metabolic impairment, of which 25 were cases of incident T2D over an average 6.9 years of follow-up. Risk of incident metabolic impairment and T2D in those with normal status at baseline, according to magnesium intake, are presented in **Table 2**. In the basic model, adjusted for age, sex, and energy intake, higher magnesium intake was associated with 37% lower risk of incident metabolic impairment (Q1 (reference) vs. Q5 RR [95%CI] 0.63 [0.45–0.87], *P* trend = 0.02), which was attenuated after adjusting for risk factors (*P* trend = 0.08), and further attenuated after adjusting for dietary fiber (*P* trend = 0.26).

5.4.2 Incident T2D among Metabolically Impaired at Baseline

Among the 928 (35.9%) participants impaired at baseline, there were 154 (16.6%) cases of incident T2D over an average 6.9 years of follow-up. In the basic model, adjusted for age, sex, and energy intake, higher magnesium intake was associated with 32% lower risk of incident T2D (0.68 [0.41–1.12], *P* trend = 0.05) (**Table 2**). The trend was attenuated after adjusting for risk factors (*P* trend = 0.18), but further adjusting for fiber intake de-attenuated the estimate such that the final estimate was 38% lower risk in the highest compared to the lowest category of magnesium intake (0.62 [0.35–1.10)], *P* trend = 0.05).

In the total study population, there were 179 (6.9%) incident cases of T2D over an average 6.9 years of follow-up. In fully adjusted models, higher magnesium intake was associated with 51% lower risk of incident T2D (0.49 [0.27–0.88], *P* trend = 0.01) (**Table 2**).

5.4.3 Secondary Analyses

In secondary analyses, IR and hyperinsulinemia were excluded from the working definition of baseline or incident metabolic impairment and, as such, more closely aligned with ADA prediabetes criteria (IGT and/or IFG). Prevalence of baseline metabolic impairment, as a percentage of the total sample, decreased from 35.9% to 31.4%, and incident metabolic impairment, as a percentage of those who were normal at baseline, also decreased from 18.6% to 17.2%.

Results of these analyses were similar to those using the primary definition. Among those with normal status at baseline when impairment was defined by IGT and/or IFG, higher magnesium intake was not associated with risk of incident metabolic impairment after adjusting for age, sex, and energy intake (*P* trend = 0.12) (**Supplemental Table 2**). However, among those initially impaired at baseline, trends for lower risk of incident T2D across increasing quintile categories of magnesium showed associations similar to those observed when the definition of metabolic impairment included insulin-based criteria: in the fully adjusted models, those with the highest magnesium intake had 44% lower risk of developing T2D compared to the lowest magnesium intake [RR 0.56 (0.32–0.99], *P* trend = 0.02).

5.4.4 Linear Outcomes in the Total Sample

Adjusted means of various measures of glucose and insulin homeostasis and metabolism at exam 7, in those without incident T2D, are presented in **Table 3**. In basic models adjusted for age, sex, energy intake, and the corresponding baseline measure, there were significant inverse trends with higher magnesium intake and subsequent FG (Q1 vs. Q5: 5.42 vs. 5.32 mmol/L, *P* trend = 0.003) and HOMA-IR (3.08 vs. 2.89, *P* trend = 0.05). However, all trends

were attenuated after additionally adjusting the risk factor model (model 2) for dietary fiber (model 3).

5.5 Discussion

Our results support previously reported longitudinal associations between higher magnesium intake and lower risk of T2D (4,5). Over 7 yrs of follow-up, higher magnesium intake appeared to partially offset risk of developing metabolic impairment in those with normal baseline glucose and insulin homeostasis. In addition, in those with baseline metabolic impairment, magnesium intake was also associated with lower risk of T2D. Interestingly, magnesium's associations with incident impairment were stronger when the definition of metabolic impairment included hyperinsulinemia and IR, than when they included hyperglycemia or impaired glycemic response alone. This is intriguing, since elevated insulin or insulin resistance are etiological predecessors of chronically elevated fasting glucose concentrations (14), perhaps indicating that magnesium intake is more important to maintaining long-term healthy insulin metabolism. This is supported by our observation that those with the highest magnesium intake had, on average, 6% lower HOMA-IR after 7 yrs than those with the lowest magnesium intake, after adjusting for risk factors. However, as our results indicate, once metabolic impairment had taken hold, magnesium intake seemed to be associated with lower risk of T2D, regardless of whether baseline metabolic impairment was defined by both glucose and insulin criteria, or glucose criteria alone.

Our observation of lower risk of T2D with higher magnesium intake is one that is fairly well-established in the magnesium literature (3–5,30,31). In addition, several clinical studies of magnesium supplementation in those with and without diabetes indicate that magnesium supplementation can improve glycemic control, insulin sensitivity, and beta-cell

function (8–10,12,32). However, the duration of these clinical studies have been relatively short (≤6 months) and most of the observational studies of magnesium intake in relation to insulin homeostasis or metabolism have been cross-sectional (31,33–35). As such, there is a relative dearth of knowledge on the long-term impact of magnesium intake on insulin metabolism.

Our results related to HOMA-IR are consistent with another study in younger American adults (18–30 yo at baseline) evaluating magnesium intake against repeated measures of HOMA-IR over 20 yrs, in which a significant inverse association was observed between insulin resistance and magnesium intake, after adjusting for risk factors similar to those adjusted for in the present analysis (3). While our follow-up was shorter, our population was older, and we excluded those with incident T2D in our analyses, we nevertheless also observed an inverse trend between higher magnesium intake and long-term HOMA-IR. However, this association did not persist after adjustment for dietary fiber. One other prospective study examined magnesium intake and insulin sensitivity in 1,036 US adults (56.4% women) participating in the Insulin Resistance Atherosclerosis Study (IRAS) (17). In this study, a threshold effect of magnesium intake (at 325 mg/d) was observed in relation to insulin sensitivity, derived from intravenous glucose tolerance test (17). Progressively poorer 5-yr insulin sensitivity was observed below that threshold, with no evidence for improvement of sensitivity above that threshold.

Magnesium's associations with insulin sensitivity are supported by experimental evidence in animals fed magnesium-deficient diets, in which insulin sensitivity of peripheral tissue decreases via reduced autophosphorylation of tyrosine kinase, a component of the beta subunit of the insulin receptor for which magnesium is a co-factor (36). In addition, hypomagnesaemia is thought to deleteriously impact the proliferation and mass of beta-

cells, thus affecting insulin production (37,38). Insulin itself may also be a regulating magnesium metabolism, as prolonged high concentrations of circulating insulin, such as those known to occur in insulin resistance, induce increases in renal magnesium excretion, thus perpetuating a deleterious cycle (38).

While we observed that higher magnesium intake was inversely associated with long-term change in fasting glucose and insulin resistance in those without incident T2D (associations which were attenuated after adjustment for fiber intake), we did not observe statistically significant trends of magnesium intake with fasting insulin, glucose clearance or insulin metabolism (as post-OGTT measures), or insulin sensitivity (as ISI), although we had >80% power to observe, for example, the observed difference in 2-hr OGTT glucose between extreme quintiles. However, our findings are consistent with a recent 6-month trial in non-diabetic, insulin resistant individuals which demonstrated that treatment with 365 mg/d of magnesium results in significantly lowered FG, HOMA-IR, and improved insulin sensitivity (Matsuda index, but not Gutt's ISI)—with no effect on 2-hr OGTT glucose or insulin, and only marginal effects on FI (12). It may be that Gutt's ISI, measured both in the trial and in the present analysis with null results, is measuring peripheral insulin resistance, where as other insulin-related measures, such as HOMA-IR or the Matsuda index, reflect hepatic insulin resistance (12).

We included fiber as a potential confounder owing to the body of literature on fiber's protective effects against T2D (5), and to the fact that magnesium and fiber share common dietary sources such as whole grains and vegetables. Interestingly, including fiber in our models (model 3) had differential effects on magnesium's T2D-risk-lowering associations, depending on whether the population was initially normal or impaired. In those with normal baseline status, fiber attenuated the observed associations of magnesium

on risk of metabolic impairment, suggesting that magnesium intake is not acting independently of the effects of fiber in those who are initially healthy. However, in those with impaired baseline status, fiber de-attenuated the association of magnesium, suggesting that higher magnesium intake may be more important to those with existing metabolic impairment, irrespective of fiber intake. Of note is that there was no interaction between magnesium intake and fiber, or between magnesium intake and impairment status. Fiber intake was only approximately 0.5 g/d higher, and magnesium intake approximately 8 mg/d higher, on average, in those with normal vs. impaired status at baseline.

Our study has several strengths. We benefitted from a large sample size in a well-characterized community-based population cohort with repeated dietary measures (up to 3) for estimation of magnesium intake over 7 yrs. Incident metabolic impairment and diabetes were classified based on fasting and post-OGTT measures, rather than relying on self-report alone. This study also has several limitations. First, different insulin assays were used at exam 5 and exam 7. Although we calibrated fasting insulin values at exam 5 to those at exam 7, no calibration was possible for post-OGTT insulin. Therefore, the null findings observed between magnesium intake and fasting and OGTT insulin and ISI may be a partial result of this. Second, higher magnesium intake may also be reflective of better health consciousness, a confounder which we may have inadequately controlled for despite adjusting for fiber intake and physical activity, surrogate markers of a healthy lifestyle. However, the attenuation by fiber intake of our estimates may also represent an over-adjustment of the model, owing to magnesium and fiber's shared food sources (namely, whole grains). Finally, the generalizability of our findings may be limited, as ours was a relatively homogenous, middle-aged Caucasian population of primarily European descent.

In conclusion, higher magnesium intake appears to lower risk of progressing to diabetes among those with the highest risk of doing so—namely, those with insulin resistance or prediabetes. Higher magnesium intake in those with low risk of diabetes may also lower risk of progressing to prediabetes and diabetes; however these associations were not separable from the putative benefits of a fiber-rich diet. These findings support a role for higher magnesium intake—or at the very least, intake meeting the RDA—in those at high risk of developing diabetes.

5.6 Acknowledgments

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Table 5-1. Baseline Characteristics in Those Free of T2D

	Quintile Category of Energy-Adjusted Averaged Magnesium Intake					
	1	**2**	**3**	**4**	**5**	
N	516	517	516	517	516	
Median, mg/d	236	272	299	332	395	
Range, mg/d	101–258	258–286	286–314	314–355	355–651	
Characteristic*						***P* linear trend**
Age, yrs	53.0 (0.4)	53.6 (0.4)	53.5 (0.4)	54.0 (0.4)	55.2 (0.4)	0.003
Sex, % female	44 (2)	52 (2)	53 (2)	59 (2)	68 (2)	<0.001
BMI, kg/m2	27.7 (0.2)	27.1 (0.2)	26.9 (0.2)	26.7 (0.2)	26.6 (0.2)	0.003
Current smoker, %	23 (2)	19 (2)	22 (2)	18 (2)	10 (2)	<0.001
Waist circumference, cm	93.1 (0.5)	91.2 (0.5)	91.2 (0.5)	90.5 (0.5)	90.3 (0.5)	0.002
Hypertensive, %	49 (2)	45 (2)	41 (2)	41 (2)	41 (2)	0.02
Physical activity score	34.8 (0.3)	34.9 (0.3)	34.4 (0.3)	35.1 (0.3)	34.9 (0.3)	0.37
Dietary Characteristics						
Magnesium, mg/d	231.7 (2.0)	272.3 (2.0)	292.1 (2.0)	321.6 (2.0)	378.7 (2.1)	<0.001
Alcohol, g/d	10.6 (0.7)	11.0 (0.7)	11.2 (0.7)	11.4 (0.7)	9.4 (0.7)	0.23
Fiber, g/d	13.7 (0.2)	16.4 (0.2)	17.4 (0.2)	19.1 (0.2)	22.8 (0.2)	<0.001
Caffeine, mg/d	253.0 (9.7)	281.2 (9.6)	298.9 (9.6)	335.5 (9.6)	302.5 (9.7)	<0.001
Energy, kcal/d	1959 (26)	1741 (26)	1792 (26)	1822 (26)	2020 (26)	<0.001
Glucose and Insulin Characteristics						
Fasting glucose, mmol/L	5.28 (0.02)	5.27 (0.02)	5.26 (0.02)	5.22 (0.02)	5.19 (0.02)	0.01
Fasting insulin, pmol/L †	208.5 (1.0)	200.3 (1.0)	200.3 (1.0)	196.4 (1.0)	192.5 (1.0)	<0.001
HOMA-IR †	7.03 (1.01)	6.75 (1.01)	6.69 (1.01)	6.55 (1.01)	6.36 (1.01)	<0.001
2-hr OGTT glucose, mmol/L	6.07 (0.07)	5.89 (0.07)	5.73 (0.07)	5.73 (0.07)	5.75 (0.07)	<0.001
2-hr OGTT insulin, pmol/L †	595.9 (1.0)	550.0 (1.0)	523.2 (1.0)	523.2 (1.0)	507.8 (1.0)	<0.001
Gutt's ISI	25.4 (0.3)	26.2 (0.3)	26.9 (0.3)	26.8 (0.3)	27.1 (0.3)	<0.001
NFG, %	71 (2)	70 (2)	74 (2)	76 (2)	77 (2)	0.02
NGT, %	85 (1)	88 (1)	90 (1)	90 (1)	88 (1)	0.08
Fasting insulin >90th percentile, %	16 (1)	8 (1)	11 (1)	7 (1)	8 (1)	<0.001
HOMA-IR >90th percentile, %	15 (1)	8 (1)	11 (1)	7 (1)	7 (1)	<0.001

Abbreviations: BMI, body mass index; HOMA-IR, homoestasis model assessment of insulin resistance; ISI, Gutt's insulin sensitivity index$_{0–120}$; NFG, normal fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; T2D, type 2 diabetes.

* Characteristics are age- and sex-adjusted, except for age and sex, which are mutually adjusted. Dietary characteristics, except energy, are further adjusted for energy. Data are mean (SD) or %, unless otherwise indicated.
† Analyzed in the natural-log scale and back-transformed to geometric mean (geometric SD) for presentation.
‡ Normal fasting glucose defined as fasting plasma glucose <5.6 mmol/L (100 mg/dL). Normal glucose tolerance defined as 2-hr OGTT glucose <7.8 mmol/L (140 mg/dL).

Table 5-2. Relative Risk of Progression from Normal to Metabolically Impaired (IFG, IGT, Insulin Resistant, or Hyperinsulinemic) and Metabolically Impaired to T2D, by Quintile Categories of Energy-Adjusted Magnesium Intake.*

	Quintile Category of Energy-Adjusted Averaged Magnesium Intake†					
Median intake, mg/d	**1** 235	**2** 272	**3** 298	**4** 331	**5** 395	*P* linear trend
Incident metabolic impairment (or T2D) among unimpaired at baseline						
Total, *n*	298	319	325	356	356	1654
Cases, *n*	71	56	59	72	49	307
Model 1‡	1 *(Ref)*	0.79 (0.58–1.08)	0.81 (0.60–1.10)	0.93 (0.70–1.24)	0.63 (0.45–0.87)	0.02
Model 2	1 *(Ref)*	0.78 (0.57–1.07)	0.87 (0.64–1.17)	0.95 (0.72–1.26)	0.68 (0.49–0.94)	0.08
Model 3	1 *(Ref)*	0.79 (0.57–1.08)	0.88 (0.64–1.22)	0.97 (0.71–1.33)	0.70 (0.47–1.06)	0.26
Incident T2D among metabolically impaired at baseline						
Total, *n*	218	198	191	161	160	928
Cases, *n*	39	40	31	24	20	154
Model 1‡	1 *(Ref)*	1.15 (0.77–1.71)	0.92 (0.60–1.41)	0.83 (0.52–1.32)	0.68 (0.41–1.12)	0.05
Model 2	1 *(Ref)*	1.22 (0.83–1.80)	1.00 (0.66–1.53)	0.94 (0.59–1.49)	0.77 (0.47–1.27)	0.18
Model 3	1 *(Ref)*	1.14 (0.77–1.70)	0.90 (0.58–1.41)	0.82 (0.49–1.36)	0.62 (0.35–1.10)	0.05
Incident T2D in total population						
Total, *n*	516	517	516	517	516	2582
Cases, *n*	49	46	33	29	22	179
Model 1‡	1 *(Ref)*	0.98 (0.66–1.44)	0.70 (0.46–1.07)	0.62 (0.40–0.97)	0.47 (0.28–0.76)	0.0004
Model 2	1 *(Ref)*	1.02 (0.70–1.50)	0.84 (0.55–1.27)	0.75 (0.49–1.16)	0.59 (0.36–0.96)	0.01
Model 3	1 *(Ref)*	0.97 (0.66–1.43)	0.77 (0.49–1.20)	0.68 (0.42–1.09)	0.49 (0.27–0.88)	0.01

Abbreviations: BMI, body mass index; FG, fasting plasma glucose; FI, fasting plasma insulin; HOMA-IR, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test; T2D, type 2 diabetes.

* Baseline "normal" defined as FG <5.6 mmol/L (100 mg/dL), 2-hr OGTT glucose <7.8 mmol/L (126 mg/dL), HOMA-IR <90th percentile, *and* FI <90th percentile. Baseline and incident "metabolic impairment" defined as FG ≥5.6 and <7.0 mmol/L (100–125 mg/dL) *or* 2-hr OGTT glucose ≥7.8 and <11 mmol/L (140–199

mg/dL) *or* HOMA-IR ≥90th percentile *or* FI ≥90th percentile. T2D defined as FG ≥7.0 mmol/L (126 mg/dL), 2-hr OGTT ≥11 mmol/L (200 mg/dL), or use of insulin or oral hypoglycemics.

† As median value in each quintile of energy-adjusted magnesium intake (mg/d).

‡ Model 1 adjusted for age (y), sex (male vs. female), and energy intake (kcal/d). Model 2 was adjusted as for model 1, plus parental history of diabetes (yes vs. no), BMI (kg/m^2), physical activity score, smoking status (current vs. non-smoker), alcohol intake (g/d), and hypertension (yes vs. no). Model 3 adjusted as for model 2, plus dietary fiber (g/d).

Table 5-3. Adjusted Means of Measures of Glucose and Insulin Parameters by Quintile Categories of Energy-Adjusted Magnesium Intake over 7-yrs of Follow-Up in Participants without Incident T2D.

	Quintile Category of Energy-Adjusted Averaged Magnesium Intake†					
Median,	**1**	**2**	**3**	**4**	**5**	
mg/d	236	272	299	332	395	*P* linear trend
Fasting glucose (mmol/L), *n*=2312						
1*	5.42 (0.02)	5.38 (0.02)	5.38 (0.02)	5.39 (0.02)	5.32 (0.02)	0.003
2	5.40 (0.02)	5.38 (0.02)	5.38 (0.02)	5.39 (0.02)	5.33 (0.02)	0.02
3	5.40 (0.02)	5.38 (0.02)	5.38 (0.02)	5.40 (0.02)	5.34 (0.02)	0.17
Fasting insulin (pmol/L), *n*=2185 †						
1*	89.14 (1.02)	88.11 (1.02)	86.99 (1.02)	88.16 (1.02)	85.20 (1.02)	0.13
2	88.79 (1.02)	88.18 (1.02)	87.21 (1.02)	88.18 (1.02)	85.23 (1.02)	0.15
3	88.30 (1.02)	88.00 (1.02)	87.17 (1.02)	88.32 (1.02)	85.74 (1.02)	0.41
HOMA-IR, *n*=2185 †						
1*	3.09 (1.02)	3.01 (1.02)	2.98 (1.02)	3.02 (1.02)	2.89 (1.02)	0.04
2	3.07 (1.02)	3.02 (1.02)	2.99 (1.02)	3.02 (1.02)	2.89 (1.02)	0.05
3	3.05 (1.02)	3.01 (1.02)	2.98 (1.02)	3.03 (1.02)	2.91 (1.02)	0.26
2-hr OGTT glucose (mmol/L), *n*=863						
1*	6.75 (0.11)	6.35 (0.12)	6.63 (0.11)	6.75 (0.11)	6.43 (0.11)	0.27
2	6.69 (0.11)	6.36 (0.12)	6.61 (0.11)	6.78 (0.11)	6.46 (0.11)	0.64
3	6.64 (0.12)	6.34 (0.12)	6.61 (0.11)	6.80 (0.11)	6.51 (0.12)	0.79
2-hr OGTT insulin (pmol/L), *n*=837 †						
1*	383.84 (1.05)	347.90 (1.05)	368.35 (1.04)	381.10 (1.04)	338.50 (1.04)	0.14
2	380.67 (1.05)	348.71 (1.05)	365.32 (1.04)	387.28 (1.04)	337.95 (1.04)	0.19
3	378.40 (1.05)	348.08 (1.05)	365.11 (1.04)	387.75 (1.04)	340.08 (1.05)	0.40
Gutt's ISI, *n*=837						
1*	22.47 (0.40)	23.69 (0.41)	22.87 (0.39)	22.41 (0.39)	23.58 (0.38)	0.26
2	22.67 (0.40)	23.69 (0.40)	22.94 (0.38)	22.26 (0.39)	23.49 (0.38)	0.57
3	22.80 (0.44)	23.73 (0.41)	22.96 (0.38)	22.23 (0.39)	23.34 (0.42)	0.95

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; T2D, type 2 diabetes.

*Model 1 adjusted for corresponding baseline measure, age (y), sex (male vs. female), and energy intake (kcal/d). Model 2 adjusted as for model 1, plus parental history of diabetes (yes vs. no), BMI (kg/m^2), physical activity score, smoking status (current vs. non-smoker), alcohol intake (g/d), and hypertension (yes vs. no). Model 3 adjusted as for model 2, plus dietary fiber (g/d).
† Analyzed in the natural-log scale and back-transformed to geometric mean (geometric SE) for presentation.

Supplemental Table 5-1. Criteria Underlying Definitions of Metabolic Impairment.

	Defining Criteria		
Indicator	**Normal**	**Impaired**	**T2D***
Fasting glucose	<5.6 mmol/L (100 mg/dL)	≥5.6 but <7.0 mmol/L (100–126 mg/dL)	≥7.0 mmol/L (126 mg/dL)
2-hr OGTT glucose	<7.8 mmol/L (140 mg/dL)	≥7.8 but <11.1 mmol/L (140–200 mg/dL)	≥11.1 mmol/L (200 mg/dL)
Fasting insulin	<90th percentile†	≥90th percentile	--
HOMA-IR	<90th percentile‡	≥90th percentile	--

Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test; T2D, type 2 diabetes.

* Or by reported diagnosis of T2D, or reported use of an oral hypoglycemic drug or insulin.

† In this sample, baseline cut-point of 41.0; incident cut-point of 25.0.

‡ In this sample, baseline cut-point of 10.1; incident cut-point of 6.7.

Supplemental Table 5-2. Relative Risk of Progression from Normal to Metabolically Impaired (IFG or IGT) or Metabolically Impaired to T2D by ADA Criteria, by Quintile Categories of Energy-Adjusted Magnesium Intake.*

	Quintile Category of Energy-Adjusted Averaged Magnesium Intake†					
	1	**2**	**3**	**4**	**5**	
Median intake, mg/d	235	272	298	331	395	*P* linear trend
Incident metabolic impairment among unimpaired at baseline						
Total, *n*	333	337	355	372	374	1771
Cases, *n*	72	50	58	75	49	304
Model 1‡	1 *(Ref)*	0.75 (0.54–1.04)	0.81 (0.59–1.11)	1.04 (0.78–1.38)	0.67 (0.48–0.93)	0.12
Model 2	1 *(Ref)*	0.77 (0.55–1.06)	0.85 (0.63–1.15)	1.07 (0.81–1.41)	0.74 (0.53–1.02)	0.33
Model 3	1 *(Ref)*	0.78 (0.56–1.08)	0.87 (0.63–1.21)	1.11 (0.82–1.51)	0.78 (0.52–1.18)	0.71
Incident T2D among metabolically impaired at baseline						
Total, *n*	183	180	161	145	142	811
Cases, *n*	38	39	30	21	19	147
Model 1‡	1 *(Ref)*	1.06 (0.71–1.57)	0.91 (0.59–1.39)	0.70 (0.43–1.14)	0.63 (0.38–1.05)	0.02
Model 2	1 *(Ref)*	1.12 (0.76–1.67)	1.03 (0.67–1.57)	0.84 (0.52–1.35)	0.71 (0.43–1.18)	0.09
Model 3	1 *(Ref)*	1.05 (0.70–1.56)	0.93 (0.60–1.44)	0.73 (0.43–1.22)	0.56 (0.32–0.99)	0.02

Abbreviations: ADA, American Diabetes Association; BMI, body mass index; FG, fasting plasma glucose; OGTT, oral glucose tolerance test; T2D, type 2 diabetes.
* Baseline "normal" defined as FG <5.6 mmol/L (100 mg/dL) *and* 2-hr OGTT glucose <7.8 mmol/L (126 mg/dL). Baseline and incident "metabolic impairment" defined as FG ≥5.6 and <7.0 mmol/L (100–125 mg/dL) *or* 2-hr OGTT glucose ≥7.8 and <11 mmol/L (140–199 mg/dL). T2D defined as FG ≥7.0 mmol/L (126 mg/dL), 2-hr OGTT ≥11 mmol/L (200 mg/dL), or use of insulin or oral hypoglycemics.
† As median value in each quintile of energy-adjusted magnesium intake (mg/d).
‡ Model 1 adjusted for age (y), sex (male vs. female), and energy intake (kcal/d). Model 2 was adjusted as for model 1, plus parental history of diabetes (yes vs. no), BMI (kg/m^2), physical activity score, smoking status (current vs. non-smoker), alcohol intake (g/d), and hypertension (yes vs. no). Model 3 adjusted as for model 2, plus dietary fiber (g/d).

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CHAPTER 6. MAGNESIUM AND CALCIFICATION

Magnesium intake is inversely associated with coronary artery calcification in the Framingham Heart Study

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6.1 Abstract

Background: Dietary magnesium may prevent vascular calcification, including calcification within atherosclerotic plaques underlying cardiovascular disease (CVD).

Objective: We sought to examine whether higher magnesium intake was associated with calcification in the coronary arteries (CAC) and abdominal aorta (AAC).

Design: We examined cross-sectional associations of magnesium intake, ascertained by food frequency questionnaire, on CAC and AAC in men and women (*n*=2,695, 53±11 y) free of CVD who participated in the Framingham Heart Study Multi-Detector Computed Tomography (MDCT) Sub-study. Calcium measures from CT scans were scored using a modified Agatston Score (AS), and natural-log transformed for use in Tobit models adjusted for age, body mass index, major CVD risk factors and treatment for CVD risk factors, as well as intakes of calcium, vitamins D and K, saturated fat, fiber, alcohol, and energy, and hormone replacement therapy use and menopausal status in women. Secondary analyses of CAC and AAC outcomes as cut-points (AS >0 and AS ≥90th percentile for age and sex of a healthy reference population), as well as sex-specific analyses, were also conducted.

Results: In fully adjusted models, a 50-mg/d increment in magnesium intake was associated with 22% lower CAC (P <0.001) and 12% lower AAC (P = 0.07). Controlling for additional confounders did not alter the findings. Secondary analyses of the CAC and AAC cut-points yielded similar results: compared to those in the lowest category of magnesium intake, those in the highest category had 58%, 37%, and 34% lower odds of having any prevalent CAC (P trend <0.001), high CAC (P trend = 0.01), or any prevalent AAC (P trend = 0.01), respectively. Odds of high AAC in the highest category of magnesium intake were 30% lower compared to the lowest intake category, but the linear trend was non-significant (P trend = 0.10). Stronger inverse associations were observed in women than in men.

Conclusions: This cross-sectional study using data from an established cohort of community-dwelling participants free of CVD indicates that magnesium intake is significantly inversely associated with CAC, with suggestive evidence that magnesium may have a protective role in inhibiting calcification initiation. Stronger associations were observed between magnesium and CAC, rather than AAC, suggesting that magnesium's specificity to the coronary arteries warrant further investigation.

6.2 Introduction

Coronary artery calcification (CAC) (1–3) and abdominal aortic calcification (AAC)(3–5) are measures of advanced atherosclerosis that predict cardiovascular disease (CVD) morbidity and mortality independently of traditional CVD risk factors. In recent studies, CAC in particular has been shown to discriminate and reclassify future risk for clinical coronary events (6). Dietary magnesium, which impacts many aspects of cardiovascular health (7,8) but is under-consumed in the US (9), may also play a key role in vascular calcification. A protective role of dietary magnesium in calcification may also underlie previous observations of lower risk of stroke (10,11), sudden cardiac death, non-fatal myocardial infarction (MI), and fatal coronary heart disease (CHD) (12–15) in those with higher intake.

In vitro (16–23) and animal (23–28) studies suggest a plausible biological role for magnesium in preventing or reversing plaque formation and calcification, and magnesium may directly inhibit hydroxyapatite and crystal precipitation (29–31). In humans with chronic kidney disease (CKD), end-stage renal disease (ESRD), or on hemodialysis—patients who are known to exhibit accelerated calcification—inverse associations have been reported between serum magnesium and calcification in various vascular beds (31) and with related measures of atherosclerosis or arteriosclerosis, such as carotid intima media thickness (IMT) and pulse-wave velocity (PWV) (19). In healthy populations, observational studies have also found serum magnesium to be inversely associated with IMT, presence of atherosclerotic plaques, and progression of atherosclerosis (32,33).

However, serum magnesium only poorly correlates with magnesium intake (34). Magnesium intake has not been extensively studied in relation to CAC in generally healthy humans, and to our knowledge, no study has examined the association between magnesium intake and AAC. One small pilot study of supplemental magnesium (as buffer) in high-risk hemodialysis patients

reported favorable CAC-slowing effects (35). Several observational studies have examined the relationship between magnesium-rich food sources and CAC: higher intakes of chocolate (36) and fish (37), but not whole grains (38), have been inversely associated with CAC, and inverse associations were observed between coffee intake in older (39), but not younger adults (40). Only one study has examined dietary magnesium in association with CAC in a generally healthy population, observing no association (41). However, that study did not consider the potential confounding effects of calcium intake. Magnesium may be acting as a calcium antagonist (42), very high calcium intake can interfere with magnesium absorption (43), and moderate magnesium deprivation leads to calcium retention (44), all of which may also have implications in vascular calcification. Therefore, we tested the hypothesis that higher magnesium intake is associated with lower levels of calcification of the coronary arteries and abdominal aorta in generally healthy individuals, by cross-sectionally assessing magnesium intake with CAC and AAC as continuous measures in a community-dwelling population free of clinically apparent CVD.

6.3 Methods

6.3.1 *Study Population*

The National Heart, Lung, and Blood Institute's Framingham Heart Study is a longitudinal, community-based, observational study that began in 1948 in Framingham, Massachusetts. The children, and their spouses, of the original cohort participants (second generation, "Offspring") were enrolled between 1971 and 1975, and have returned for follow-up examination following standardized protocols approximately every four years (45). The third-generation cohort ("Gen3") was recruited between 2002 and 2005, and includes 4,095 children of the Offspring (46). The present study includes dietary and risk factor data collected from participants who attended Offspring exam 7 (1998–2001, n=3,539) or Gen3 exam 1 (2002–2005, n=4,095), and who

participated in exam 1 (2002–2005) of the ongoing Multi-Detector Computed Tomography (MDCT) sub-study. Recruitment details for the MDCT sub-study have been previously described (47). In brief, 3,529 Offspring and Gen3 participants located in the greater New England area underwent MDCT scanning. Men were ≥35 years old, women were ≥40 years old and not pregnant, and all participants weighed ≤350 pounds due to scanner limitations (47).

We excluded participants from this analysis if they were missing or had an uninterpretable CT scans (*n*=278); had clinically apparent CVD (*n*=136), defined as coronary artery bypass graft, valve replacement, percutaneous coronary stent placement, pacemaker, stroke, congestive heart failure, MI, or coronary insufficiency identified or occurring prior to the date of the clinic exam (47); were missing or had invalid dietary information (*n*=172, reporting <600 or ≥4,000 kcal/d for women, <600 or ≥4,200 kcal/d for men, or those who had ≥12 blank items); reported extreme values of magnesium or calcium intake (*n*=48, with intake values in the 0.5th or 99.5th percentile); or were missing complete covariate information (*n*=200, covariates defined below). After exclusions, 2,695 participants remained in the present analyses.

The original data collection protocols were approved by the Institutional Review Boards at Boston University and Massachusetts General Hospital, and written, informed consent was obtained from all participants. The present study protocol was reviewed by the Tufts Medical Center and Tufts University Health Sciences Institutional Review Board.

6.3.2 Dietary Assessment

The Harvard semi-quantitative, 126-item food frequency questionnaire (FFQ) was used to assess dietary intake (48). FFQs were mailed to participants prior to each exam, and participants were instructed to bring the completed FFQ with them to their exam appointment. The FFQ included a list of foods together with a standard serving size and 9 consumption frequency categories ranging from “never, or less than once per month” to “6+ per day.” The FFQ allowed for notation of foods

usually consumed that were not otherwise listed in the FFQ. Participants were asked to report consumption of each food item over the previous year. Foods, beverages, and supplements contributing to nutrient intake were estimated from percent contributions of each item to total intake of a given nutrient. The relative validity of the FFQ for energy-adjusted magnesium intake and calcium intake has been previously reported (48–50), and show reasonable correlation with estimates from dietary records (0.67–0.71 for magnesium, 0.48–0.61 for calcium) (48).

6.3.3 Outcome Measures

CAC and AAC were quantified from CT scans using a modified Agatston Score (AS), as previously described (47,51). In brief, each participant underwent 8-slice MDCT scanning consisting of two chest scans and one abdominal scan (Lightspeed Ultra, General Electric Medical Systems, Milwaukee, WI) during a single end-inspiratory breath hold. For CAC, 48 contiguous 2.5-mm-thick slices were acquired in each scan. For AAC, the top of the S1 vertebral body selected as the most caudal extent of the abdominal volume to be imaged, and 30 contiguous 5-mm-thick slices were acquired to 15 cm above S1. Scans were read and calcium measurements were obtained independently by experienced readers using an offline workstation (Acquarius, Terarecon, San Matteo, CA). A calcified lesion was defined as an area of ≥3 connected pixels with CT attenuation of >130 Hounsfield units. AS was calculated by multiplying the area of each lesion with a weighted attenuation score dependent on the maximal attenuation within the lesion. We defined high CAC (47) and high AAC (51) according to previously defined age- and sex-specific 90th-percentile cut points relative to a healthy reference sample of the Framingham Heart Study.

6.3.4 Covariates

Covariate information was assessed at baseline examination from medical history, physical examination, or laboratory assessment, as follows. From medical history interviews, information was obtained related to each participant's age, smoking status, menopausal status, physical activity,

aspirin use, treatment for hyperlipidemia (e.g., niacin, fibrates, statins), osteoporosis (e.g., calcitonin preparations, selective estrogen receptor modulators, and other drugs affecting bone structure and mineralization including bisphosphonates, bisphosphonate combinations, and bone morphogenetic proteins), hypertension or CVD prevention (e.g., ACE inhibitors, nitroglycerin, calcium-channel blockers, beta blockers), or diabetes (oral hypoglycemics or insulin), menopausal status, and use of estrogen or other hormone replacement therapy (HRT) in women. Age was calculated as the difference between the participant's date of attendance at the MDCT exam and the date of birth. In women, menopausal status was defined as >1 year cessation of menses. Physical activity was calculated as the sum of reported hours per day typically spent in moderate-intensity (e.g., golfing, gardening, light housework) or high-intensity (e.g., heavy yard work, jogging, swimming) activities, based on metabolic equivalent task values. Height to the nearest 0.25 inch and weight to the nearest 0.25 lb were measured with the participant standing, with shoes off, wearing only a hospital gown. Body mass index (BMI, kg/m^2) was calculated as weight in kilograms divided by height in meters squared. Blood pressure (mmHg) was measured to the nearest 2 mmHg with a mercury-column sphygmomanometer on the left arm after the subject had been seated quietly for five minutes. Two readings were obtained by a physician and averaged to calculate the systolic and diastolic blood pressures. Total cholesterol (mg/dL) was measured enzymatically and the HDL-c fraction (mg/dL) was measured after the precipitation of low density lipoprotein cholesterol and very-low-density lipoprotein cholesterol with dextran sulfate magnesium. Fasting plasma glucose (mg/dL) was measured in fresh specimens with a hexokinase reagent kit quantified with a hexokinase reagent kit (A-gent glucose test, Abbott Laboratories, South Pasadena, CA). Fasting plasma insulin (pmol/L) was measured using human-specific RIA in the Offspring and ELISA in the Gen3 (both assays from Linco Inc., St. Louis, MO; calibrated to each other for analysis). Type 2 diabetes was defined as fasting blood glucose ≥126 mg/dL or use of oral hypoglycemics or insulin. Serum C-reactive protein

(CRP, mg/L) was measured by particle enhanced immunonephelometry using high sensitivity CRP reagent (Dade Behring, Newark, DE). Serum creatinine was measured using the modified Jaffe method and the simplified Modification of Diet in Renal Disease study equation was used to estimate the glomerular filtration rate (GFR, mL/min/1.73 m^2) (52).

6.3.5 Statistical Analyses

All nutrients were energy-adjusted using the residual method (53,54). Major food group contributors to total magnesium intake in each sex were calculated based on percent contribution to total intake of each food group. Partial Pearson correlation coefficients were calculated between total intakes of select nutrients.

Quartile categories of energy-adjusted magnesium intake were created to present participant characteristics and for use in regression analyses. Linear trends in the means or percentages of age- and sex-adjusted (age-, sex-, and energy-adjusted, for nutrients) participant characteristics across quartile categories were assessed using the median intake value in each quartile category.

We used natural logarithmic (ln)-transformed values of CAC and AAC, after adding 1 to each score, owing to a large number of zero values and to reduce skewness. For our primary tests of associations between ln(CAC+1) or ln(AAC+1) and magnesium intake, we conducted Tobit regression analyses (PROC LIFEREG, with a censored threshold of zero CAC or AAC) using continuous intake as the exposure. Tobit regression is an appropriate model for calcification data which typically violates the assumptions of linear regression, owing to the absence of detectable calcification (i.e., AS = 0) in a large proportion of a given cohort (55). We present beta coefficients and standard errors (SE) per 50 mg/d of magnesium intake. For analyses of quartile categories, we present adjusted means and SEs of ln(CAC+1) or ln(AAC+1) from ordinary least squares regression (PROC GLM), and *P* values for linear trend across median values of each quartile category of

magnesium intake. The lowest quartile category of magnesium intake was used as the reference category.

Regression models included known or potential confounders as follows: model 1 was adjusted for age (yrs), sex, exam cycle, energy intake (kcal/d), and calcium intake (mg/d). Model 2 was adjusted as for model 1, plus known CVD risk factors, which may also be mediators of the diet-calcification relationship, including BMI (kg/m^2), smoking status (never, former, or current), total-to-HDL cholesterol ratio, fasting insulin (ln-pmol/L), systolic blood pressure (mmHg), use of estrogen or HRT and menopausal status (in women, both yes or no), and treatment for hypertension or CVD prevention, hyperlipidemia, or diabetes (all yes or no), and alcohol intake (g/d). Model 3, the fully adjusted model, was adjusted as for model 2 covariates, plus dietary factors frequently associated with CVD or implicated in calcification, including intakes of fiber (g/d), saturated fat (g/d), vitamin D (IU/d), and vitamin K (mcg/d). Further adjustment of model 3 for CRP (mg/L), regular aspirin use (yes or no), GFR (mL/min/1.73 m^2), physical activity (hrs/d), treatment for osteoporosis (yes or no), or AAC (as ln[AS+1], in CAC analysis only), did not substantively alter results [data not shown]. In addition, analyses to account for familial correlations did not materially alter the results; we therefore present results unadjusted for these relationships. To assess potential effect modification between magnesium and calcium, sex, BMI, and age, we tested for statistical interaction using separate cross-product terms in Tobit regression analyses. As there were no statistically significant interactions (all P >0.05), interaction terms were removed but covariates were retained in the models. However, owing to known differences in CAC distributions between sexes (47), we repeated analyses in men and women separately in exploratory analyses using sex-specific quartile categories of energy-adjusted magnesium intake, and present these in supplemental tables.

In secondary analyses, we estimated odds of having any CAC or AAC (AS 0 vs. >0), and high CAC or AAC (AS <90th vs. ≥90th percentile for age and sex relative to a healthy reference population (47,51)) using median values of quartile categories of magnesium intake in logistic regressions (PROC LOGISTIC). We present odds ratios (OR) and 95% confidence intervals (95% CI) in each quartile category, and *P* values for linear trend across quartile categories. The lowest quartile category of magnesium intake was used as the reference category.

All analyses were conducted in SAS 9.3 (SAS Institute, Cary, North Carolina). A two-sided *P* value <0.05 was considered statistically significant, since our primary outcomes—the continuous measures of CAC and AAC—are correlated.

6.4 Results

Average adjusted magnesium intake among study participants was 338 mg/day, which is slightly higher than estimated intake in similar US populations (56). The top five food groups contributing to magnesium intake in women and men were grains (18.4 and 19.3%, respectively), dairy (11.5 and 10.4%, respectively), fruit (10.3 and 9.2%, respectively), legumes (7.3 and 7.4%, respectively), and vegetables (7.1 and 6.5%, respectively). Supplemental sources of magnesium only contributed 6.4% and 4.6% of total magnesium intake in women and men, respectively. The highest nutrient correlations were between intakes of dietary magnesium and fiber (r = 0.77 in women, 0.71 in men), likely owing to shared whole grain sources. Total magnesium intake was only moderately correlated with total calcium intake (r = 0.44 in women, 0.40 in men).

Clinical and dietary characteristics of participants across quartile categories of energy-adjusted magnesium intake are shown in **Table 1**. In analyses of trend from lowest to highest quartile category of magnesium intake, those in the highest category were more likely to be female, older, have lower BMI, diastolic blood pressure, total cholesterol, total:HDL cholesterol ratio, fasting

insulin, and CRP. They were also more likely to use lipid-lowering treatment and aspirin, and less likely to have smoked regularly in the prior year. As expected, intake of energy and most other nutrients increased along with increasing magnesium intake. Saturated fat and alcohol intake, however, were lowest among those in the highest category of magnesium intake.

6.4.1 Primary Analyses

CAC was present (AS >0) in 43.7% of the study population (33.7% of women and 53.7% of men). AAC was more prevalent: 52.9% of the population had some detectable AAC (AS >0), and prevalence was similar between the sexes (50.9% of women and 55.3% of men). Of those with prevalent AAC, 65.3% had detectable CAC (55.3% of women and 74.3% of men with prevalent AAC, had detectable CAC). Of those with high AAC (AS ≥90th percentile for age and sex), 33.7% had high CAC (32.3% of women and 34.3% of men with high AAC also had high CAC).

In fully adjusted models, higher magnesium intake was associated with 22% lower CAC (β [SE]: -0.25 [0.07] ln(CAC+1) per 50 mg/d magnesium, P <0.001) (**Table 6-2**). Magnesium intake was not significantly associated with lower AAC (-0.13 [0.08] ln(AAC+1) per 50 mg/d magnesium, P = 0.07). In sex-specific exploratory analyses, the inverse associations appeared to be stronger in women than in men for both continuous outcomes (**Supplemental Table 6-1**). The trends across quartile categories of magnesium intake for the means of ln(AS+1) for CAC and AAC are consistent with the Tobit regression results (**Table 6-3** and **Figure 6-1** for pooled analyses, and **Supplemental Table 6-2** and **Supplemental Figure 6-1** for sex-specific analyses).

6.4.2 Secondary Analyses

We examined magnesium's associations with odds of having any calcification (AS >0) and odds of having high calcification (AS ≥90th percentile for age and sex) at either site (**Table 6-4** and **Figure 6–2**). In fully adjusted models, compared to those in the lowest quartile category of magnesium intake, those in the highest category had 58% lower odds of any CAC (OR [95% CI] Q1 vs. Q4: 0.42

[0.29–0.62], *P* trend <0.001), 37% lower odds of high CAC (OR [95% CI] Q1 vs. Q4: 0.63 [0.40–0.98], *P* trend = 0.01), and 34% lower odds of any AAC (OR [95% CI] Q1 vs. Q4: 0.66 [0.44–0.98], *P* trend = 0.01). The linear trend across increasing quartile categories of intake for lower odds of having high AAC was not statistically significant (OR [95% CI] Q1 vs. Q4: 0.70 [0.47–1.05], *P* trend = 0.10). Sex-specific exploratory analyses resulted in similar, statistically significant associations for odds of any CAC between men and women. For odds of high CAC, any AAC, and high AAC, linear trends were not statistically significant in either sex (**Supplemental Table 6-3** and **Supplemental Figure 6-2** and **6-3**).

6.5 Discussion

The main finding of this cross-sectional study in individuals free of clinically apparent CVD is that higher magnesium intake is associated with lower levels of CAC, a sensitive, discriminating measure of subclinical CVD and overall burden of atherosclerosis. Those with the highest magnesium intake had approximately half the odds of having *any* detectable CAC, compared to those with the lowest intake, which suggests magnesium intake may have a protective role in inhibiting calcification initiation. This relationship persisted for odds of having high levels of CAC in women, but not in men, perhaps indicating that consuming higher levels of magnesium may also be beneficial for women with comorbidities predisposing them to very high levels of coronary calcification.

To date, only one cross-sectional analysis, conducted in the Multi-Ethnic Study of Atherosclerosis (MESA), has examined magnesium intake in relation to CAC. In contrast to our observation of an inverse relationship between magnesium intake and CAC, no association was observed in the MESA study; however, magnesium intake was associated with lower odds of high common carotid IMT (41), an indicator of atherosclerotic disease moderately correlated with CAC (57). The major differences between our analysis and the MESA investigation is that the MESA

study examined a population of multiple races/ethnicities, unadjusted for calcium intake, only using cut-points of CAC (>0 or >100) rather than also evaluating it as a continuous measure.

Several studies have examined common sources of magnesium—chocolate (36), coffee (39,40), fish (37), and whole grains (38)—in relation to CAC, with mixed results. In the Family Heart Study, chocolate consumption was inversely associated with odds of CAC (AS >100) in a dose-response manner (36). In the Rotterdam Study, higher coffee intake was inversely associated with severe CAC in older women, but not in older men (39). Another study of coffee intake in a younger population (18–30 y) showed no association between coffee and presence or progression of CAC over 15–20 y of follow-up (40). Whole-grain intake showed no association with CAC in a MESA study, despite significant inverse associations with other CVD risk factors (38). Finally, researchers in Rotterdam reported that those with higher fish intake had a lower prevalence of moderate CAC (AS 11–400) and borderline significant reduced prevalence of severe CAC (AS >400) compared to fish non-consumers. Interestingly, intake of neither docosahexanoic acid (DHA) nor eicospentanoic acid (EPA)—the fatty acids to which fish consumption's cardiovascular benefits are often attributed—was associated with calcification in the above-mentioned study (37). These findings prompted the authors to suggest that other components of fish—including magnesium—may have a calcification-protective effect. A small pilot study in patients with ESRD undergoing chronic hemodialysis—at particularly high risk for rapid calcification progression—reported a non-significant progression of CAC of just 8% over 18 months using a magnesium/calcium carbonate (approximately 700 mg/day elemental magnesium and 1200 mg/day elemental calcium) binder in lieu of the standard calcium-based phosphate binder (35). Typical progression in this population may be as high as 50%.

The observed associations with CAC were significant after further adjusting for a range of cardiometabolic risk factors and potential mediators, as well as AAC levels, suggesting that

magnesium may be acting in the coronary arteries over and above its other known anti-inflammatory, anti-hypertensive, and lipid-lowering functions to affect calcification (8,58). Magnesium deficiencies have been associated with CVD and a range of related disorders, from hypertension and endothelial dysfunction to vascular damage of the heart (7,8,58). Observational studies in humans indicate higher magnesium intake is associated with lower levels of CRP and other inflammatory markers (41,59–61). In addition, animal studies (24–27) and clinical evidence in humans (62–64) point to a role for magnesium in modulating lipid metabolism. Trials of magnesium supplementation in individuals with mild hypertension (62), ischemic heart disease (63), and diabetes (64) have reported lower triglycerides and very low density lipoprotein concentrations, and higher HDL cholesterol following magnesium treatment.

To our knowledge, ours is the first study to examine magnesium intake in relation to AAC, which, like CAC, is an independent predictor of CVD morbidity and mortality (3–5). Despite some pathological differences (65), CAC and AAC are thought to be similar phenomena, and presence of AAC is a good predictor of CAC (e.g., in the present study, of women and men with prevalent AAC, 55.3 and 74.3%, respectively, also had detectable CAC).

Interestingly, our associations between magnesium intake and CAC were stronger and more consistent than they were for AAC. We are unable to explain the differing magnitudes of magnesium's associations between CAC and AAC, apart from a potential primary role of magnesium in unknown processes more predominantly associated with, and therefore specific to, atherosclerotic calcification of the coronary arteries. We had hypothesized that magnesium intake would have similar associations with calcification at both sites. One of magnesium's putative roles in preventing biomineralization of extra-skeletal tissue is its inhibition of hydroxyapatite formation, in which magnesium destabilizes the crystal structure and inhibits precipitation (19,29–31). In addition, magnesium has been shown to inhibit osteogenic differentiation of vascular smooth

muscle cells (VSMCs) (17,18,21) and increase the expression of calcification-inhibiting proteins including osteopontin and matrix Gla protein, while decreasing expression of osteocalcin and BMP-2 activity (18). In cells exposed to calcifying media, even small amounts of magnesium can decrease alkaline phosphatase activity, reverse other effects of magnesium-deficient states, and prevent cell apoptosis (17).

Risk factor differences between CAC and AAC have been previously observed (66), which may contribute to some of the differences we found with respect to magnesium's associations. Our observation regarding sex differences in magnesium's associations with AAC is particularly interesting since, in contrast to CAC, sex is not as strong a risk factor for AAC (66). We considered some possible reasons for the differences between magnesium's associations with CAC and AAC. For example, since current CT imaging technology does not differentiate between medial and intimal calcification (67), we could not rule out the presence of medial calcification as a possible explanation for some of the differing associations. Medial calcification, which occurs in conditions of long-standing mineral and metabolic imbalances (diabetes, impaired renal function, and CKD/ESRD), is thought to be rare in the coronary arteries (68), but it may be more prevalent in the abdominal aorta in the presence of mild metabolic or mineral derangement and not just overt clinical diagnoses. However, none of our results materially changed after excluding participants with prevalent diabetes (5% of study sample) or impaired kidney function (GFR <60 mL/min/1.73 m^2, 2% of study sample) (data not shown), and fasting insulin, GFR, and diabetes treatment were otherwise controlled for. It seems unlikely, then, that the differences we observed between magnesium's associations with CAC and AAC are attributable to differing types of calcification at the two sites. Therefore, these discrepancies, as well as the role of magnesium in processes specific to atherosclerotic calcification in the coronary arteries, deserve further investigation.

There are several limitations to the present study. First, as a cross-sectional study, we cannot infer a temporal relationship from the observed associations. While the associations of magnesium with CAC and AAC observed in our study have plausible biological underpinnings, the mechanisms underlying these relationships remain elusive. High magnesium intake may be a surrogate marker of a healthy lifestyle. However, we attempted to account for other nutrients and characteristics associated with a healthy lifestyle or implicated in CVD or calcification, including fiber, saturated fat, and vitamins K and D, as well as other lifestyle characteristics. Nevertheless, residual confounding may yet be a factor. Finally, our study sample was predominantly Caucasian of European descent; thus our observations may not be generalizable to other races/ethnicities.

6.6 Summary and Conclusions

In this study, we observed strong, favorable associations between higher magnesium intake and lower calcification of the coronary arteries, an important, discriminating measure of subclinical atherosclerotic burden that has been shown to reclassify risk of CVD morbidity and mortality. We may therefore consider the lowering of coronary artery calcification as another potential physiological mechanism through which dietary magnesium mitigates risk of stroke, non-fatal myocardial infarction, sudden cardiac death, and fatal coronary heart disease. While any recommendations emerging from cross-sectional studies should be made with appropriate caution, our observations, in line with a wide body of literature supporting magnesium's heart health benefits, suggest that diets rich in magnesium, including green leafy vegetables, whole grains, nuts, and dark chocolate, may provide protection against atherosclerosis. As this is among the first studies to broadly examine these associations, and the mechanisms underlying these relationships are not perfectly understood, further prospective research is required to elucidate magnesium's relationships with these and other sites of vascular calcification, as well as the possible benefits of

magnesium supplementation in inhibiting onset and progression of atherosclerosis and calcification.

6.7 Acknowledgments

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Table 6-1. Participant Characteristics across Quartile Categories of Energy-Adjusted Magnesium Intake*

	Energy-Adjusted Magnesium Intake				
Quartile category	Q1	Q2	Q3	Q4	
n	673	674	674	674	
Median intake, mg/d	258.8	303.6	351.1	427.4	
Intake range, mg/d	159.8–283.9	284.0–325.4	325.5–383.6	383.9–669.4	**P trend**
General Characteristics					
Age at CT exam, yrs	51.4 (0.4)	52.9 (0.4)	52.4 (0.4)	54.1 (0.4)	<0.001
Sex, % female	35.0 (2.0)	49.0 (2.0)	55.0 (2.0)	59.0 (2.0)	<0.001
Body mass index, kg/m^2	28.6 (0.2)	28.0 (0.2)	27.7 (0.2)	27.3 (0.2)	<0.001
Physical activity, hrs/d	4.9 (0.1)	4.5 (0.1)	4.7 (0.1)	4.8 (0.1)	0.19
Current smoker, %	17.0 (1.0)	13.0 (1.0)	11.0 (1.0)	7.0 (1.0)	<0.001
Current HRT use, % of women	18 (2)	21 (2)	20 (2)	23 (2)	0.50
Post-menopausal, % of women	49 (2)	52 (2)	49 (2)	55 (2)	0.09
Clinical and Laboratory Characteristics					
Systolic BP, mmHg	122.9 (0.6)	122.4 (0.6)	121.3 (0.6)	120.8 (0.6)	0.05
Diastolic BP, mmHg	77.2 (0.4)	76.5 (0.3)	76.0 (0.3)	75.7 (0.4)	0.02
Anti-hypertensive Rx, %	18.0 (1.0)	16.0 (1.0)	16.0 (1.0)	17.0 (1.0)	0.88
Total cholesterol, mg/dL	201.1 (1.4)	198.3 (1.3)	196.8 (1.3)	193.8 (1.3)	0.002
HDL cholesterol, mg/dL	52.1 (0.6)	53.7 (0.5)	53.2 (0.5)	53.9 (0.6)	0.09
Total:HDL cholesterol	4.2 (0.1)	4.0 (0.1)	4.0 (0.1)	3.9 (0.1)	<0.001
Lipid-lowering Rx, %	9.0 (1.0)	14.0 (1.0)	12.0 (1.0)	15.0 (1.0)	0.008
Fasting glucose, mg/dL	99.0 (0.8)	98.8 (0.8)	98.9 (0.8)	97.8 (0.8)	0.64
Fasting insulin, pmol/L †	85.6 (1.0)	82.3 (1.0)	79.8 (1.0)	79.0 (1.0)	0.001
Diabetes Rx, %	1.0 (1.0)	2.0 (1.0)	2.0 (1.0)	3.0 (1.0)	0.45
CVD prevention Rx, %	13.0 (1.0)	13.0 (1.0)	13.0 (1.0)	13.0 (1.0)	0.97
Aspirin use, %	13 (1)	16 (1)	20 (1)	22 (1)	<0.001

Osteoporosis Rx, %	4.0 (1.0)	3.0 (1.0)	3.0 (1.0)	4.0 (1.0)	0.64
C-reactive protein, mg/L	3.3 (0.2)	3.1 (0.2)	2.7 (0.2)	2.6 (0.2)	0.01
GFR, mL/min/1.73 m^2	93.9 (0.7)	94.0 (0.7)	94.1 (0.7)	94.1 (0.7)	0.99
Dietary Characteristics					
Magnesium, total, mg/d	250.9 (1.3)	302.4 (1.3)	350.3 (1.3)	442.7 (1.3)	<0.001
From diet, mg/d	250.8 (1.6)	300.5 (1.6)	334.4 (1.5)	380.0 (1.6)	<0.001
From supplements, mg/d	1.8 (1.3)	3.6 (1.3)	17.6 (1.3)	64.4 (1.3)	<0.001
Calcium, total, mg/d	803.7 (15.2)	947.1 (15.1)	1078.5 (15.0)	1279.0 (15.2)	<0.001
From diet, mg/d	696.6 (10.9)	813.5 (10.8)	886.0 (10.7)	929.1 (10.8)	<0.001
From supplements, mg/d	112.1 (11.9)	138.5 (11.8)	197.4 (11.7)	354.8 (11.9)	<0.001
Energy, kcal/d	2036.2 (23.9)	1829.6 (23.5)	1948.7 (23.6)	2092.0 (23.7)	<0.001
Vitamin K, mcg/d	109.2 (4.7)	145.9 (4.6)	185.4 (4.6)	236.2 (4.7)	<0.001
Vitamin D, IU/d	246.5 (10.0)	345.4 (9.9)	427.7 (9.9)	599.7 (10.0)	<0.001
Saturated fat, g/d	27.3 (0.2)	25.9 (0.2)	24.1 (0.2)	21.8 (0.2)	<0.001
Fiber, g/d	14.2 (0.2)	17.2 (0.2)	20.0 (0.2)	23.7 (0.2)	<0.001
Alcohol, g/d	11.8 (0.6)	11.2 (0.5)	10.4 (0.5)	9.5 (0.6)	0.01
Multivitamin use, %	27 (2)	39 (2)	52 (2)	79 (2)	<0.001

Abbreviations: BP, blood pressure; CT, computed tomography; CVD, cardiovascular disease; GFR, estimated glomerular filtration rate; HDL, high density lipoprotein; Rx, use of medication/treatment.

* All characteristics are age- and sex-adjusted, except for age and sex, which are mutually adjusted. Dietary characteristics are also energy-adjusted.

† Analyzed in the natural logarithm scale and back-transformed. Geometric mean (geometric SD) shown.

Table 6-2. Association (β [SE]) of Magnesium Intake (per 50 mg/d increment) with Coronary Artery and Abdominal Aortic Calcification

		Association of Magnesium Intake (per 50 mg/d increment)		
Model*	*n*	β	SE	*P* value
CAC as ln(AS+1)				
Model 1	2695	-0.18	0.06	0.001
Model 2		-0.13	0.05	0.011
Model 3		-0.25	0.07	<0.001
AAC as ln(AS+1)				
Model 1	2681	-0.19	0.06	0.001
Model 2		-0.09	0.06	0.09
Model 3		-0.13	0.08	0.07

Abbreviations: AAC, abdominal aortic calcification; AS, Agatston Score; CAC, coronary artery calcification; HDL, high density lipoprotein; SE, standard error.
* Model 1 adjusted for calcium and energy intake, age, sex, and exam cycle. Model 2 adjusted as for model 1, plus body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and alcohol intake. Model 3 adjusted as for model 2, plus intake of vitamins K and D, saturated fat, and fiber. For AAC, *n*=2681.

Table 6-3. Adjusted Means (SE) of Coronary Artery and Abdominal Aortic Calcification in Quartile Categories of Energy-Adjusted Magnesium Intake

	Energy-Adjusted Magnesium Intake				
Quartile category	Q1	Q2	Q3	Q4	
n	673	674	674	674	
Median, mg/d	258.8	303.6	351.1	427.4	*P* linear
Range, mg/d	159.8–283.9	284.0–325.4	325.5–383.6	383.9–669.4	trend
CAC as ln(AS+1)					
Model 1*	1.78 (0.07)	1.86 (0.07)	1.74 (0.07)	1.52 (0.07)	0.004
Model 2	1.77 (0.07)	1.85 (0.07)	1.75 (0.07)	1.52 (0.07)	0.006
Model 3	1.85 (0.08)	1.88 (0.07)	1.74 (0.07)	1.43 (0.08)	0.0005
AAC as ln(AS+1)					
Model 1*	3.21 (0.09)	3.04 (0.09)	2.77 (0.09)	2.77 (0.09)	0.001
Model 2	3.10 (0.09)	3.06 (0.08)	2.81 (0.08)	2.83 (0.09)	0.01
Model 3	3.13 (0.10)	3.07 (0.08)	2.80 (0.08)	2.80 (0.10)	0.02

Abbreviations: AAC, abdominal aortic calcification; AS, Agatston Score; CAC, coronary artery calcification; HDL, high density lipoprotein; SE, standard error.
* Model 1 adjusted for calcium and energy intake, age, sex, and exam cycle. Model 2 adjusted as for model 1, plus body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and alcohol intake. Model 3 adjusted as for model 2, plus intake of vitamins K and D, saturated fat, and fiber. For AAC, *n*=2681.

Table 6-4. Odds Ratios (95%CI) of Coronary Artery and Abdominal Aortic Calcification in Quartile Categories of Energy-Adjusted Magnesium Intake

	Energy-Adjusted Magnesium Intake				
Quartile category	Q1	Q2	Q3	Q4	
n	673	674	674	674	
Median, mg/d	258.8	303.6	351.1	427.4	*P* linear
Range, mg/d	159.8–283.9	284.0–325.4	325.5–383.6	383.9–669.4	trend
CAC (*>0)*					
Model 1	1 (Ref)	0.95 (0.73 – 1.23)	0.79 (0.60 – 1.03)	0.60 (0.45 – 0.81)	<0.001
Model 2	1 (Ref)	0.93 (0.71 – 1.23)	0.79 (0.60 – 1.05)	0.58 (0.43 – 0.79)	<0.001
Model 3	1 (Ref)	0.84 (0.63 – 1.11)	0.65 (0.47 – 0.89)	0.42 (0.29 – 0.62)	<0.001
CAC *(≥90th %ile)*					
Model 1	1 (Ref)	1.20 (0.89 – 1.62)	0.94 (0.68 – 1.29)	0.71 (0.50 – 1.02)	0.02
Model 2	1 (Ref)	1.26 (0.92 – 1.72)	0.99 (0.71 – 1.38)	0.71 (0.49 – 1.03)	0.02
Model 3	1 (Ref)	1.20 (0.87 – 1.64)	0.92 (0.64 – 1.32)	0.63 (0.40 – 0.98)	0.01
AAC *(>0)*					
Model 1	1 (Ref)	0.99 (0.75 – 1.30)	0.71 (0.54 – 0.94)	0.70 (0.51 – 0.95)	0.01
Model 2	1 (Ref)	1.07 (0.80 – 1.44)	0.76 (0.56 – 1.02)	0.77 (0.55 – 1.06)	0.03
Model 3	1 (Ref)	1.02 (0.75 – 1.37)	0.69 (0.49 – 0.95)	0.66 (0.44 – 0.98)	0.01
AAC *(≥90th %ile)*					
Model 1	1 (Ref)	0.75 (0.57 – 0.99)	0.62 (0.46 – 0.83)	0.73 (0.54 – 0.99)	0.05
Model 2	1 (Ref)	0.80 (0.59 – 1.08)	0.65 (0.48 – 0.90)	0.79 (0.57 – 1.10)	0.17
Model 3	1 (Ref)	0.76 (0.56 – 1.04)	0.61 (0.43 – 0.86)	0.70 (0.47 – 1.05)	0.10

Abbreviations: AAC, abdominal aortic calcification; AS, Agatston Score; CAC, coronary artery calcification; CI, confidence interval; HDL, high density lipoprotein.
* Model 1 adjusted for calcium and energy intake, age, sex, and exam cycle. Model 2 adjusted as for model 1, plus body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and alcohol intake. Model 3 adjusted as for model 2, plus intake of vitamins K and D, saturated fat, and fiber. For AAC, *n*=2681.

Figure 6-1. Mean (± standard error) of coronary artery calcification (closed circles) and abdominal aortic calcification (open circles) (as ln[Agatston Score+1]) according to median values of energy-adjusted magnesium intake in quartile categories in 2695 participants of the Framingham Heart Study. Values are adjusted for age, sex, exam cycle, calcium and energy intake, body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, use of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and intake of alcohol, vitamins K and D, saturated fat, and fiber.

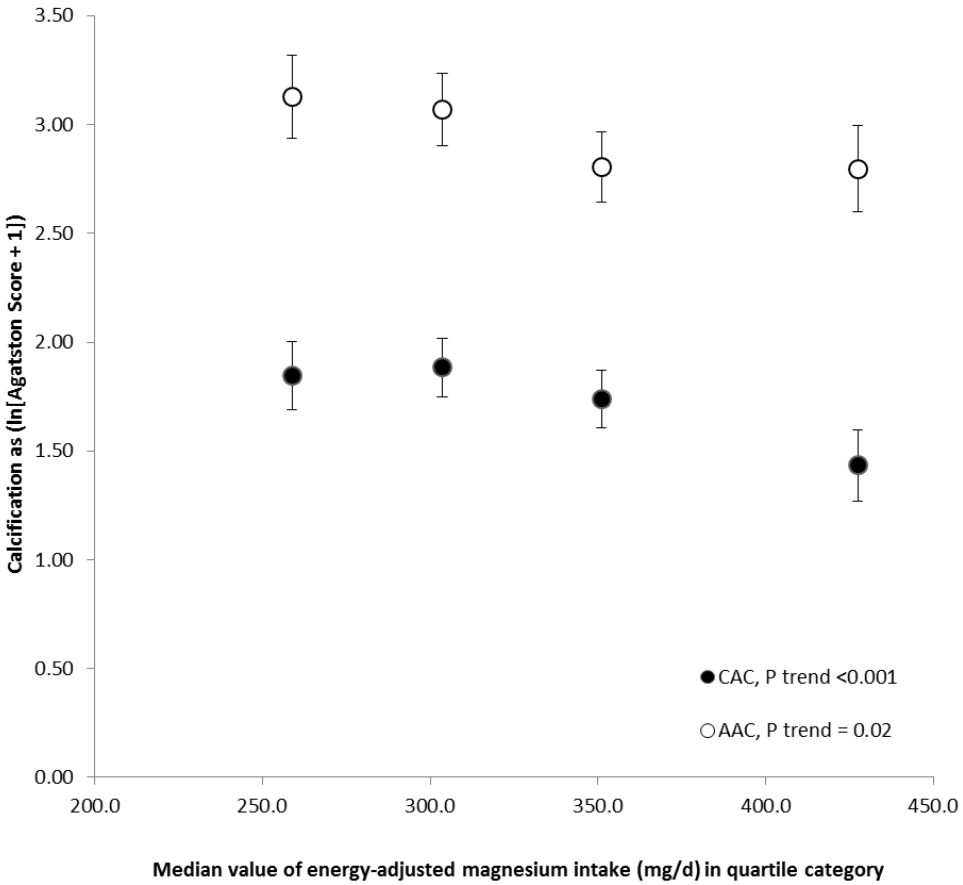
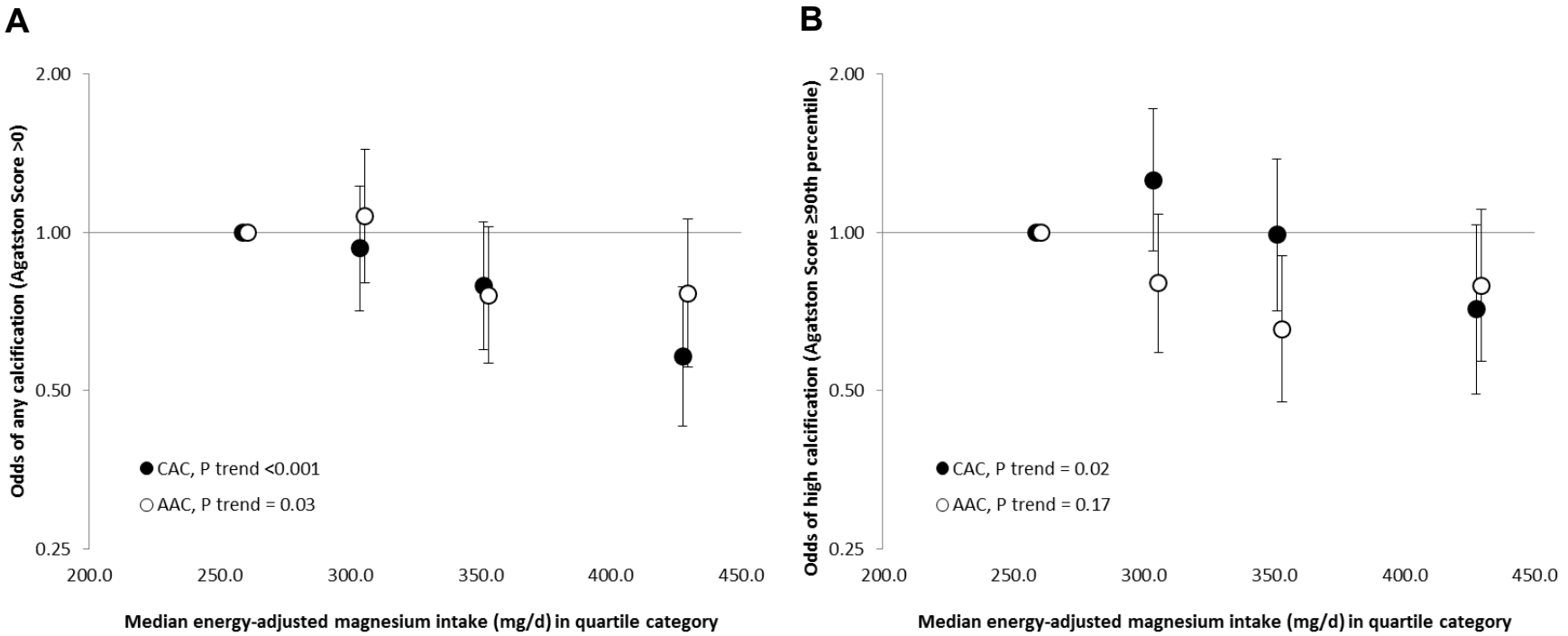


Figure 6-2. Odds ratios (95% confidence interval) of any (Agatston Score >0) [**A**] or high (Agatston Score ≥90th percentile for age and sex in a healthy referent population) [**B**] coronary artery calcification (CAC, closed circles) or abdominal aortic calcification (AAC, open circles) according to median values of energy-adjusted magnesium intake (mg/d) in quartile categories in 2695 participants of the Framingham Heart Study. Odds ratios are adjusted for age, sex, exam cycle, calcium and energy intake, body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, use of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and intake of alcohol, vitamins K and D, saturated fat, and fiber.



Supplemental Table 6-1. Association (β [SE]) of Magnesium Intake (per 50 mg/d increment) with Coronary Artery and Abdominal Aortic Calcification, in Women and Men

	Association of Magnesium Intake (per 50 mg/d increment)							
	Women				Men			
Model*	***n***	**β**	**SE**	**P value**	***n***	**β**	**SE**	**P value**
CAC as ln(AS+1)								
Model 1	1338	-0.19	0.09	0.03	1357	-0.12	0.07	0.07
Model 2		-0.20	0.09	0.02		-0.06	0.06	0.36
Model 3		-0.31	0.12	0.01		-0.21	0.09	0.02
AAC as ln(AS+1)								
Model 1	1332	-0.17	0.09	0.06	1349	-0.15	0.07	0.04
Model 2		-0.14	0.09	0.11		-0.04	0.07	0.62
Model 3		-0.20	0.11	0.08		-0.08	0.10	0.45

Abbreviations: AAC, abdominal aortic calcification; AS, Agatston Score; CAC, coronary artery calcification; HDL, high density lipoprotein; SE, standard error.
* Model 1 adjusted for calcium and energy intake, age, and exam cycle. Model 2 adjusted as for model 1, plus body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and alcohol intake. Model 3 adjusted as for model 2, plus intake of vitamins K and D, saturated fat, and fiber. Note that tests for magnesium × sex interactions were not statistically significant: *P* for magnesium × sex cross-product term in model 3 sex-pooled analysis was 0.98 and 0.78 for CAC and AAC, respectively.

Supplemental Table 6-2. Adjusted Means (SE) of Coronary Artery and Abdominal Aortic Calcification in Energy-Adjusted Quartile Categories of Magnesium Intake, in Women and Men

	Energy-Adjusted Magnesium Intake									
	Women					Men				
Quartile	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
n	333	333	334	333		341	341	341	341	
Median (range), mg/d	257.5 (149.6–280.8)	299.9 (281.0–322.9)	348.2 (323.2–377.9)	420.6 (378.3–611.8)	*P* linear trend	266.8 (173.5–289.6)	310.0 (289.6–328.8)	353.3 (329.0–381.7)	430.4 (381.9–748.7)	*P* linear trend
CAC as ln(AS+1)										
Model 1*	1.33 (0.09)	1.37 (0.09)	1.21 (0.09)	1.00 (0.09)	0.01	2.18 (0.11)	2.40 (0.11)	2.28 (0.11)	2.02 (0.11)	0.17
Model 2	1.36 (0.09)	1.37 (0.09)	1.20 (0.09)	0.98 (0.09)	0.001	2.14 (0.11)	2.38 (0.10)	2.31 (0.10)	2.04 (0.11)	0.28
Model 3	1.46 (0.10)	1.40 (0.09)	1.17 (0.09)	0.88 (0.10)	0.0001	2.22 (0.12)	2.41 (0.10)	2.30 (0.10)	1.95 (0.13)	0.08
AAC as ln(AS+1)										
Model 1*	3.18 (0.14)	3.02 (0.13)	2.66 (0.13)	2.83 (0.14)	0.05	3.11 (0.13)	3.17 (0.12)	2.86 (0.12)	2.88 (0.13)	0.10
Model 2	3.18 (0.13)	2.99 (0.12)	2.66 (0.12)	2.86 (0.13)	0.06	3.00 (0.12)	3.14 (0.11)	2.95 (0.11)	2.93 (0.12)	0.42
Model 3	3.23 (0.14)	3.00 (0.12)	2.65 (0.12)	2.81 (0.14)	0.07	3.02 (0.13)	3.14 (0.12)	2.94 (0.11)	2.91 (0.14)	0.42

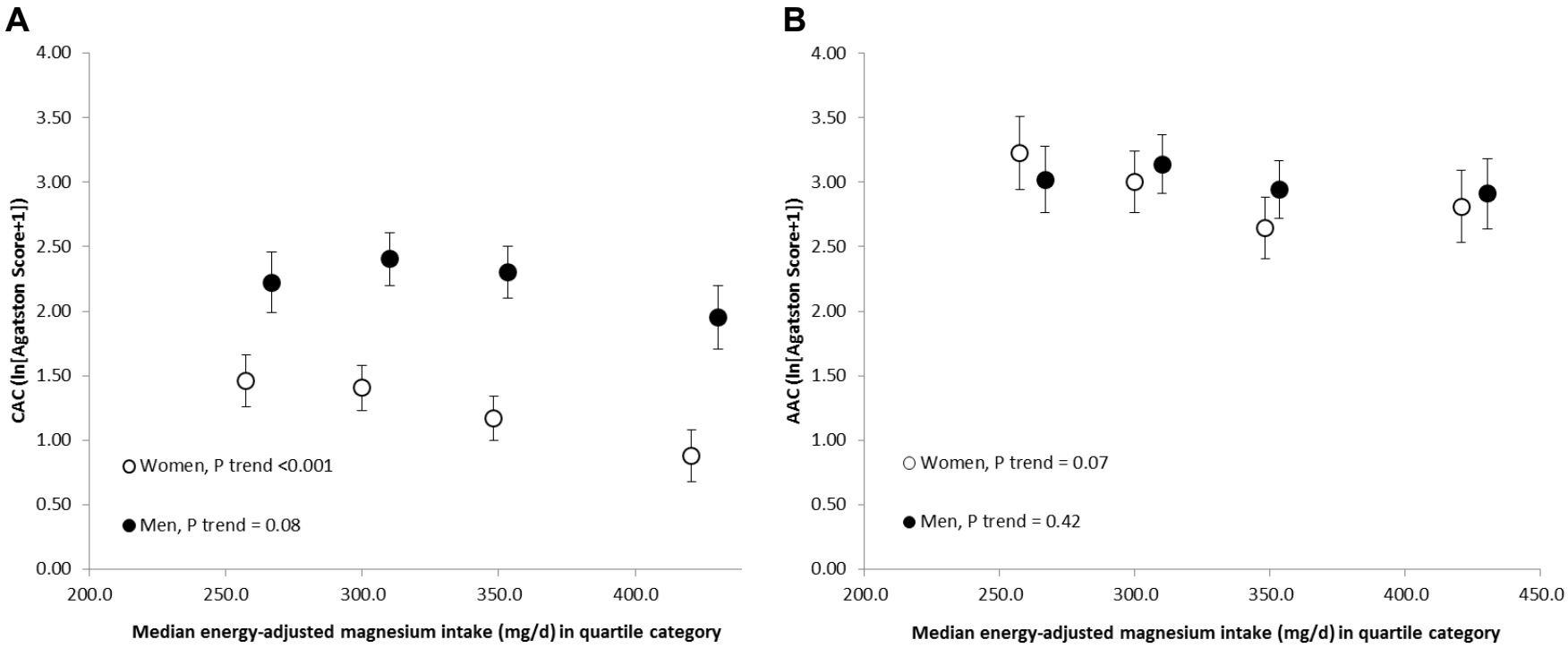
Abbreviations: AAC, abdominal aortic calcification; AS, Agatston Score; CAC, coronary artery calcification; HDL, high density lipoprotein; SE, standard error.
* Model 1 adjusted for calcium and energy intake, age, and exam cycle. Model 2 adjusted as for model 1, plus body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and alcohol intake. Model 3 adjusted as for model 2, plus intake of vitamins K and D, saturated fat, and fiber. For AAC, *n*=1332 women and 1349 men.

Supplemental Table 6-3. Odds Ratios (95%CI) of Coronary Artery and Abdominal Aortic Calcification across Quartile Categories of Magnesium Intake, in Women and Men

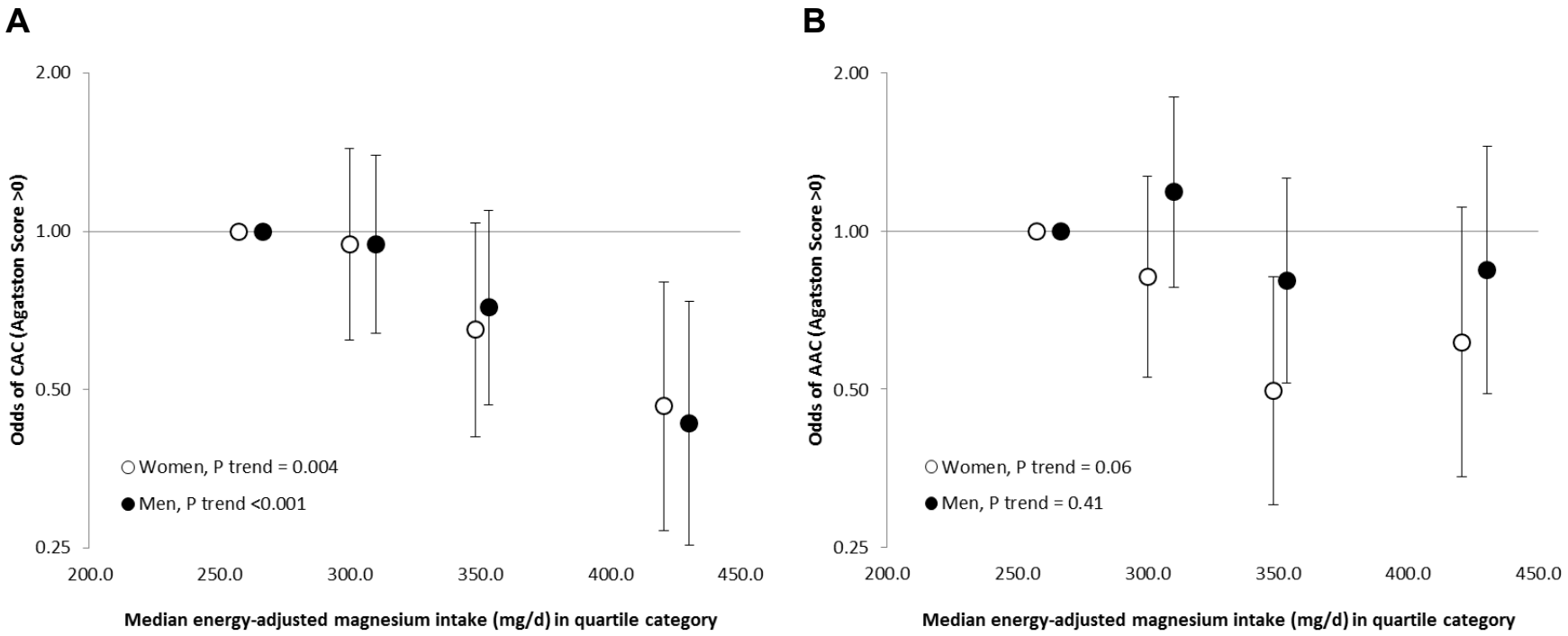
	Energy-Adjusted Magnesium Intake									
	Women					Men				
Quartile	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
n	333	333	334	333		341	341	341	341	
Median (range), mg/d	257.5 (149.6–280.8)	299.9 (281.0–322.9)	348.2 (323.2–377.9)	420.6 (378.3–611.8)	*P* linear trend	266.8 (173.5–289.6)	310.0 (289.6–328.8)	353.3 (329.0–381.7)	430.4 (381.9–748.7)	*P* linear trend
CAC >0										
Model 1*	1 (Ref)	1.08 (0.73–1.60)	0.79 (0.52–1.18)	0.63 (0.41–0.97)	0.01	1 (Ref)	1.18 (0.82–1.70)	0.99 (0.69–1.44)	0.73 (0.49–1.07)	0.05
Model 2	1 (Ref)	1.02 (0.68–1.54)	0.74 (0.49–1.13)	0.55 (0.35–0.86)	0.003	1 (Ref)	1.10 (0.76–1.60)	0.93 (0.64–1.36)	0.69 (0.46–1.03)	0.04
Model 3	1 (Ref)	0.95 (0.62–1.44)	0.65 (0.41–1.04)	0.47 (0.27–0.80)	0.002	1 (Ref)	0.95 (0.64–1.40)	0.72 (0.47–1.10)	0.43 (0.25–0.74)	0.001
CAC ≥90th										
Model 1*	1 (Ref)	1.06 (0.68–1.63)	0.81 (0.51–1.29)	0.66 (0.39–1.10)	0.06	1 (Ref)	1.54 (1.01–2.34)	1.09 (0.69–1.70)	1.02 (0.63–1.64)	0.56
Model 2	1 (Ref)	0.98 (0.62–1.55)	0.76 (0.47–1.23)	0.60 (0.35–1.02)	0.04	1 (Ref)	1.51 (0.98–2.33)	1.11 (0.70–1.77)	0.97 (0.59–1.60)	0.47
Model 3	1 (Ref)	0.95 (0.59–1.52)	0.73 (0.42–1.25)	0.57 (0.30–1.08)	0.06	1 (Ref)	1.51 (0.96–2.35)	1.12 (0.68–1.85)	0.97 (0.53–1.76)	0.56
AAC >0										
Model 1*	1 (Ref)	0.95 (0.64–1.41)	0.59 (0.39–0.90)	0.73 (0.47–1.14)	0.08	1 (Ref)	1.29 (0.88–1.90)	0.85 (0.57–1.25)	0.91 (0.60–1.37)	0.30
Model 2	1 (Ref)	0.89 (0.58–1.35)	0.56 (0.36–0.88)	0.72 (0.45–1.16)	0.11	1 (Ref)	1.23 (0.82–1.85)	0.86 (0.57–1.30)	0.93 (0.60–1.43)	0.42
Model 3	1 (Ref)	0.82 (0.53–1.27)	0.50 (0.30–0.82)	0.61 (0.34–1.11)	0.09	1 (Ref)	1.19 (0.78–1.80)	0.81 (0.51–1.26)	0.84 (0.49–1.45)	0.33
AAC ≥90th										
Model 1*	1 (Ref)	1.03 (0.70–1.50)	0.68 (0.45–1.03)	0.80 (0.52–1.23)	0.16	1 (Ref)	0.91 (0.62–1.35)	0.54 (0.35–0.83)	0.93 (0.61–1.41)	0.50
Model 2	1 (Ref)	0.93 (0.61–1.41)	0.62 (0.39–0.97)	0.75 (0.47–1.20)	0.14	1 (Ref)	0.90 (0.59–1.37)	0.58 (0.37–0.92)	0.96 (0.61–1.51)	0.71
Model 3	1 (Ref)	0.87 (0.56–1.35)	0.58 (0.35–0.95)	0.68 (0.39–1.21)	0.15	1 (Ref)	0.83 (0.54–1.28)	0.50 (0.30–0.82)	0.74 (0.42–1.31)	0.22

Abbreviations: AAC, abdominal aortic calcification; AS, Agatston Score; CAC, coronary artery calcification; CI, confidence interval; HDL, high density lipoprotein.
* Model 1 adjusted for calcium and energy intake, age, and exam cycle. Model 2 adjusted as for model 1, plus body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and alcohol intake. Model 3 adjusted as for model 2, plus intake of vitamins K and D, saturated fat, and fiber. For AAC, *n*=1332 women and 1349 men. AAC, abdominal aortic calcification; CAC, coronary artery calcification.

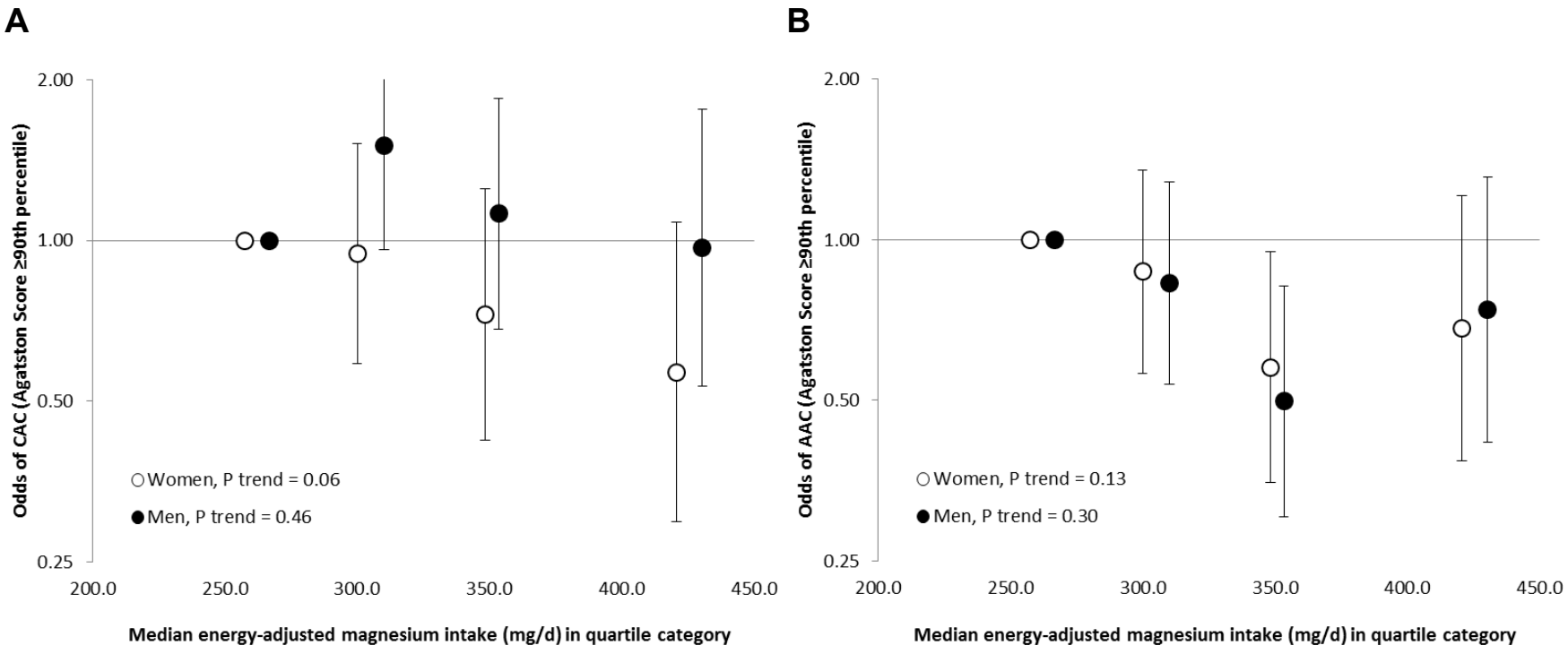
Supplemental Figure 6-1. Mean (± standard error) of coronary artery calcification [A] and abdominal aortic calcification [B] (as ln[Agatston Score+1]) according to median values of energy-adjusted magnesium intake (mg/d) in quartile categories in women (open circles) and men (closed circles) of the Framingham Heart Study. Values are adjusted for age, exam cycle, calcium and energy intake, body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, use of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and intake of alcohol, vitamins K and D, saturated fat, and fiber.



Supplemental Figure 6-2. Odds ratios (95% confidence interval) of any (Agatston Score >0) coronary artery calcification (CAC) [**A**] and abdominal aortic calcification (AAC) [**B**] according to median values of energy-adjusted magnesium intake (mg/d) in quartile categories in women (open circles) and men (closed circles) of the Framingham Heart Study. Odds ratios are adjusted for age, sex (in pooled analysis), exam cycle, calcium and energy intake, body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, use of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and intake of alcohol, vitamins K and D, saturated fat, and fiber. Quartile category 1–4 (Q1–Q4) median values of magnesium intake (mg/d) in men: 266.8, 310.0, 353.3, and 430.4; and in women: 257.5, 299.9, 348.2, and 420.6.



Supplemental Figure 6-3. Odds ratios (95% confidence interval) of high (Agatston Score ≥90th percentile for age and sex in a healthy referent population) coronary artery calcification (CAC) [**A**] and abdominal aortic calcification (AAC) [**B**] according to median values of energy-adjusted magnesium intake (mg/d) in quartile categories in women (open circles) and men (closed circles) of the Framingham Heart Study. Odds ratios are adjusted for age, exam cycle, calcium and energy intake, body mass index, smoking status, systolic blood pressure, insulin, total:HDL cholesterol, use of hormone replacement therapy (women only), menopausal status (women only), treatment for hyperlipidemia, hypertension or cardiovascular disease prevention, or diabetes, and intake of alcohol, vitamins K and D, saturated fat, and fiber. Quartile category 1–4 (Q1–Q4) median values of magnesium intake (mg/d) in men: 266.8, 310.0, 353.3, and 430.4; and in women: 257.5, 299.9, 348.2, and 420.6.



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CHAPTER 7. SUMMARY AND DISCUSSION

The strongest arguments prove nothing so long as conclusions are not verified by experience.

Roger Bacon
in *Opus Tertium* (*circa* 1267)

7.1 Summary

The results of this dissertation show that higher dietary magnesium is beneficially associated with subclinical risk factors—the early warning signs—for diabetes and cardiovascular disease. In the first manuscript, we showed that higher magnesium intake was inversely associated with concentrations of fasting glucose and insulin in individuals without diabetes, and in a novel finding, this was true generally irrespective of genetic variation that predisposes individuals to higher risk of impaired fasting glucose, insulin, or lower levels of serum magnesium.

Adding to these findings are the results of our second manuscript in which we showed that in initially healthy people, higher magnesium intake is associated with lower risk of metabolic dysfunction (i.e., prediabetes, insulin resistance, or hyperinsulinemia), and in initially metabolically impaired people, higher magnesium intake is associated with lower risk of progressing to overt diabetes. These findings contribute to what is currently known about the benefits of magnesium supplementation on glycemic control from short-term clinical trials in healthy and metabolically impaired individuals (1–6), by providing a longer-term picture of magnesium's potential benefits in protecting against progression from healthy to impaired states many years later.

Finally, in our third manuscript, we showed in a cross-sectional study that higher magnesium intake is associated with lower levels of coronary artery calcification—a direct marker of coronary atherosclerosis that is a powerful risk factor for predicting heart disease as well as cardiac events, like heart attack and stroke (7). We also showed that magnesium has a weaker relationship with abdominal aortic calcification than with coronary artery calcification, with pronounced differences between men and women. This was only the second study to have examined the relationship of magnesium intake and coronary artery calcification, and the first to examine magnesium in relation to abdominal aortic calcification. From an analytic standpoint, it was the first to have considered the potentially confounding role of calcium intake, and the results were consistent with the potential underlying mechanism of calcium and magnesium antagonism. This may have implications for those who are taking calcium supplements—which many women do in later years to maintain bone health; whether concurrent magnesium supplementation is required to offset risk remains a matter of debate.

7.2 Public Health Relevancy

Prediabetes and diabetes affected over 45% of US adults in 2010, reflecting a rise from preceding decades, and costing an estimated $174 billion in medical expenditures and lost productivity in 2007 alone (8,9). Heart disease, for which diabetes is a significant risk factor, will affect an expected 40.8% of the US population by 2030 (9). By then, heart disease will cost Americans approximately $1.48 trillion per year (9). With its aging population, the US faces an almost unprecedented growth of chronic disease burden and associated costs. As chronic diseases such as diabetes and heart disease are predominantly driven by poor lifestyle choices, it comes as no surprise that over $43 billion annually in medical costs and lost productivity associated with these conditions are attributable to poor

nutrition (9). Diet modification is therefore recommended as an important prevention strategy at every point along the health-disease continuum (9–11).

In the various US and European populations studied in this dissertation, magnesium intake has been, on average, below US Recommended Dietary Allowance (RDA) levels of 400–420 mg/day for adult men, and 300–310 mg/day for adult women (see, for example, **Supplemental Figure 4-2**, in **Chapter 4**) (12). According to the USDA, only 50% of Americans one year or older achieve the RDA for magnesium, with more pronounced inadequate intake in adolescents and in adults over 70 years old (13). Data from the Framingham Heart Study Offspring and Third Generation participants used in this dissertation parallel national estimates: approximately 50% of women and 75% of men reported not meeting the RDA for magnesium. In national data, magnesium-containing supplements help 50% of those taking them meet their magnesium needs, while those not supplementing are considerably worse off, with only 15.7% meeting the RDA from diet alone (14). The chronic under-consumption of this important dietary mineral has clear ramifications—as shown in this research—in poor outcomes related to diabetes and heart disease. In addition, evidence of a dose-response for calcification in both men and women, and in the total population for lower risk of progression to metabolic impairment or T2D, suggest that amounts meeting and even exceeding the RDA may be of incremental benefit.

Magnesium is one of the nutrients that has been considered a candidate for "micronutrient triage" theory, in which a nutrient, when scarce in the body (owing to higher excretion or dietary inadequacy), is directed to its most vital roles first, leaving important but secondary functions to suffer, such that DNA damage and late onset (age-related) disease are the inevitable consequences of chronic under-nutrition, even in the absence of overt malnutrition (15). Considering this theory, then, magnesium, when chronically under-consumed, would be pulled either from bone (where the body stores 50% of its magnesium

(16,17)) or from intracellular stores in order to continuously maintain adequate energy metabolism, since presumably its primary role is as a cofactor for adenosine triphosphatase (ATPase). If magnesium is not available for secondary functions—which may include inhibiting soft tissue calcification, maintaining beta-cell function, acting as a cofactor for insulin receptors, lipid metabolism, anti-oxidant capacity, maintaining bone, etc.—then the tissues that rely on these functions begin to deteriorate. The end result is what has, in the past at least, been referred to as age-related chronic disease: osteoporosis, hardening of the arteries, diabetes, etc.

7.3 Future Directions

The most obvious need emerging from the present research is for confirmation of the presently observed associations of magnesium intake and coronary artery calcification in other cohort settings of multiple races/ethnicities, followed by the analysis of magnesium intake in relation to additional calcification sites, namely the cardiac valves as well as thoracic aorta. These cross-sectional studies would need follow-up in longitudinal studies of magnesium intake on progression of calcification, before moving into possible randomized, controlled trials of magnesium intake for calcification. Thus far, a single, small pilot trial in a very high risk population of chronic hemodialysis patients reported considerably less progression of calcification after 18 months of supplementation with 258 mg/day elemental magnesium (just 8% progression versus typical 50% progression in this population) (18). Notably, reports of adverse effects from trials of magnesium supplementation (in doses as high as, for example, 576 mg/day as magnesium oxide or 678 mg/day as magnesium hydroxide) for various conditions are rare, although mild diarrhea and gastrointestinal discomfort (as the very first signs of possible toxicity) have been variably reported (12). Otherwise, magnesium toxicity is generally reported only in cases of “large” pharmacologic

overdose (usually of self-administered magnesium-containing laxatives or antacids, or as infusions in clinical settings) (12). Currently, the Institute of Medicine has set the Tolerable Upper Intake Level (UL) of 350 mg/day (elemental) magnesium from supplements alone, as no adverse health effects are known to occur due to magnesium obtained from diet (12). Therefore, based on current knowledge of adverse events from previous trials and as well as the UL, the putative benefits of a well-designed magnesium supplementation trial of doses at or moderately exceeding 350 mg/day would seem to outweigh known risks. (Note that special populations — e.g., those with renal disease, certain mineral imbalance disorders or other health conditions — may need medical advice and monitoring prior to taking magnesium, or, indeed any supplement.)

Mechanistically, given the associations observed in this study, the known relationships between osteoporosis and increasing arterial calcification (19), and magnesium's role in bone density (20,21), it would seem that magnesium may be one of the key players at the intersection of bone loss and concurrent arterial calcification. Therefore, magnesium's dual role in these co-occurring conditions also deserves further investigation.

With respect to magnesium's role in diabetes and related conditions, despite the observational studies to date (see **Table 2-2** in **Chapter 2**) which would seem to render obvious the need for long-term supplementation trials, the number of randomized, double-blind, parallel-arm, placebo-controlled trials of magnesium are relatively few. The trials to date (see **Table 2-4** in **Chapter 2**), both randomized and cross-over, have generally been small (<100 participants or fewer per arm) and of short duration (<6 months of supplementation). Unfortunately, most long-term vitamin/mineral supplementation trials (almost regardless of nutrient) have met with considerable controversy, and the costs associated with such trials are increasingly prohibitive. Therefore, even in the absence of long-term trials, clinicians treating patients with prediabetes or diabetes would benefit

from recommendations to follow a magnesium-rich diet (containing leafy greens, nuts, whole grains, legumes, moderate amounts of dark chocolate and coffee), and such a diet would deliver other essential nutrients, dietary fiber, antioxidants, and polyphenols. Alternatively, if adherence to a healthy diet is unlikely, a variety of magnesium supplement forms and preparations are inexpensive, widely available, and with high bioavailability (e.g., effervescent magnesium oxide, magnesium citrate, magnesium aspartate, etc. (22–24)).

In terms of genetic aspects, much remains to be discovered about the myriad ways magnesium may be interacting with the genome and implications for personalized nutritional guidelines. Just one small cross-over study to date has examined up- and down-regulation of genes and protein expression as a result of supplementation (25). Of the 60 regions in which expression was modified, over half were of unknown function. There is a clear need for in-depth work to uncover functions of these regions as well as magnesium's role within them. Further research is also needed to elucidate magnesium's biological mechanisms in glucose and insulin homeostasis and response. Currently underway is a genome-wide interaction study designed to evaluate magnesium intake's genome-wide associations on the outcomes of fasting glucose and insulin. This endeavor, involving >20 cohort studies, is currently being co-led by the author of this dissertation, and will hopefully yield greater insight to magnesium's genetic interactions, as well as its role in glucose and insulin metabolism.

Finally, readers of this dissertation are by now well aware that magnesium is chronically under-consumed, despite its wide availability in foods, and despite substantial evidence indicating its importance in a range of cardiometabolic conditions. Therefore, this author strongly advocates magnesium-rich diets, and where this is unfeasible or unlikely, magnesium supplements in readily absorbable forms and preparations.

7.4 Literature Cited

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Table A-1 Table of Single Nucleotide Polymorphisms for Aim 1

SNP	Nearest Gene	Coded/ Other Allele	Coded Allele Freq.[a]	Justification *(derived from NCBI Entrez Gene and dbSNP databases, UniProtKB database, and cited articles)*
				Fasting Glucose[b]
rs560887	*G6PC2*	C/T	0.70-0.71	Glucose transport and sensing. Encodes an enzyme that catalyzes the hydrolysis of glucose-6-phosphate (G6P), terminal step in gluconeogenesis and glucogenolysis, allowing glucose into bloodstream - exists in liver and pancreatic islets, with tissue-specific variants and levels of expression. *A specific role for Mg is unclear.*
rs10830963	*MTNR1B*	G/C	0.27-0.29	Circadian rhythm regulation. Encodes melatonin receptor 1B, primarily expressed in islet beta cells, which is a high affinity receptor for melatonin, and may affect circadian rhythm releases of insulin and glucose levels. The receptor is a G-protein coupled receptor, on which subsequent activation of the cAMP is dependent. cAMP activation is dependent on Mg-ATP. *A specific up/downstream role for Mg is unclear.*
rs4607517	*GCK*	A/G	0.18-0.19	Signal transduction. Encodes glucokinase, which functions as a glucose sensor in pancreatic beta cells and regulates glucose metabolism. Mg participates as an Mg-ATP complex in a reaction catalyzed by glucokinase, coupled to an NADP+-dependent G6P dehyrodgenase reaction. Activated glucokinase is related to the concentration of Mg. Glucose-dependent interactions of glucokinase and glucokinase regulatory protein require ATP.
rs2191349	*DGKB/ TMEM195*	T/G	0.54-0.56	Signal transduction. Encodes a catalytic domain of diacylglycerol kinase, catalyzes the phosphorylation of the intracellular concentration of second messenger diacylglycerol. In islets, glucose increases diacylglycerol, later potentiating insulin secretion. Binds 1 Mg ion per subunit; Mg appears to have a structural role and is required for catalytic activity.
rs780094	*GCKR*	C/T	0.55-0.60	Signal transduction. Encodes glucokinase regulatory protein, regulating glycolysis in liver hepatocytes and also, but less so, in pancreatic beta cells. Mg participates as an Mg-ATP complex in a reaction catalyzed by glucokinase, coupled to an NADP+-dependent G6P dehyrodgenase reaction. Activated glucokinase is related to the concentration of Mg. Glucose-dependent interactions of glucokinase and glucokinase regulatory protein require ATP.

SNP	Nearest Gene	Coded/ Other Allele	Coded Allele Freq.[a]	Justification *(derived from NCBI Entrez Gene and dbSNP databases, UniProtKB database, and cited articles)*
rs11708067	*ADCY5*	A/G	0.78-0.80	Signal transduction. Encodes adenylate cyclase 5, which catalyzes generation of cAMP, which downstream induces transcription of proinsulin gene and stimulates insulin secretory processes. Adenylate cyclases bind 2 Mg ions per subunit.
rs7944584	*MADD*	A/T	0.72-0.73	Cell proliferation and development. Encodes mitogen-activated protein kinase (MAPK) to activate MAPK, which is implicated in proliferation of beta-cells induced by glucagon-like peptide 1. *A specific role for Mg is unclear.*
rs11605924	*CRY2*	A/C	0.45-0.47	Circadian rhythm regulation. Encodes cryptochrome 2, a component of the mammalian circadian pacemaker. *A specific role for Mg is unclear.*
rs10885122	*ADRA2A*	G/T	0.88-0.90	Signal transduction. Encodes alpha-2 adrenergic receptor, regulating neurotransmitter release from sympathetic nerves and neurons in the CNS. Member of the G protein-coupled receptor superfamily. *A specific role for Mg is unclear*, however, beta-2 adrenergic agonists stimulate large Mg efflux (10-15% of total cell Mg) from perfused heart and isolated myocytes.[126]
rs174550	*FADS1*	T/C	0.67-0.68	Signal transduction. Encodes fatty acid desaturase 1, which catalyzes biosynthesis of highly unsaturated FAs from essential PUFAs. Certain FAs augment glucose-mediated insulin release. *A specific role for Mg is unclear, unless these desaturases require ATP.*
rs340874	*PROX1*	C/T	0.52-0.53	Cell proliferation and development. Encodes Prospero homeobox protein 1, plays a role in transcription and transcription regulation, with potential role in beta-cell development. *A specific role for Mg is unclear, but many DNA replication and transcription proteins require Mg.*
rs11920090	*SLC2A2*	T/A	0.86-0.87	Signal transduction. Encodes GLUT2 transporter, mediates bidirectional glucose transport into/out of beta cells and triggers glucose-mediated insulin secretory cascade. Low affinity for glucose, so might be more of a glucose sensor. *No specific role for Mg*; however a study in Zucker (diabetic fatty model) rats on a Mg-supplemented diet, showed that insulin, C-peptide, pancreatic GLUT2 and insulin mRNA expression were all higher in the supplemented rats, and only 1/8 of them developed diabetes (whereas all control rats did).[72]
rs7034200	*GLIS3*	A/C	0.49-0.50	Cell proliferation and development. Encodes the GLIS3 transcription factor (zinc finger protein) that

SNP	Nearest Gene	Coded/ Other Allele	Coded Allele Freq.[a]	Justification *(derived from NCBI Entrez Gene and dbSNP databases, UniProtKB database, and cited articles)*
				activates and represses transcription and participates in pancreatic beta cell development. *A specific role for Mg is unclear, but many DNA replication and transcription proteins require Mg.*
rs11558471	*SLC30A8*	A/G	0.68-0.74	Encodes a zinc efflux transporter involved in zinc accumulation in intracellular vesicles. Only highly expressed in islets of Langerhans. Colocalizes with insulin in insulin-secreting beta cell-line (INS-1) cells. *A specific role for Mg is unclear.*
rs11071657	*FAM148B*	A/G	0.63	Also known as *C2CD48*. Encodes nuclear localized factor 2 (NLF2), expressed in endothelial cells and up-regulated by pro-inflammatory cytokines (e.g., IL-8, TNF-alpha). Highly expressed in the pancreas. Relationship with glucose homeostasis is unclear. *A specific role for Mg is unclear.*
rs4506565	*TCF7L2*	T/A	0.31-0.32	Encodes high mobility group (HMG) box-containing transcription factor (TCF-4) that plays a role in the Wnt signaling pathway — implicated in vascular remodeling and regulation of smooth muscle cell proliferation and endothelial cell growth. Interestingly, rs7903146 variant significantly associated with coronary stenoses in diabetic individuals, with significantly greater effect in diabetic individuals.[127] *A specific role for Mg is unclear.*
				Fasting Insulin[b]
rs780094	*GCKR*	C/T	0.55-0.60	Signal transduction. Encodes glucokinase regulatory protein, regulating glycolysis in liver hepatocytes and also, but less so, in pancreatic beta cells. Mg participates as an Mg-ATP complex in a reaction catalyzed by glucokinase, coupled to an NADP+-dependent G6P dehyrodgenase reaction. Activated glucokinase is related to the concentration of Mg. Glucose-dependent interactions of glucokinase and glucokinase regulatory protein require ATP.
rs35767	*IGF1*	G/A	0.84-0.85	Associated with HOMA-IR (and FG). Encodes insulin-like growth factor 1. May be regulator of glycogen synthesis in osteoblasts. Stimulates glucose transport in osteoblastic cells, stimulates glycogen and DNA synthesis and enhances glucose uptake. Produced mainly by liver. Potent activator of AKT signaling pathway, which stimulates Akt2 to help translocate (with insulin) GLUT4) to plasma membrane. IGF-1 administered to T2D patients improves IS and reduces need for exogenous insulin to maintain glucose homeostasis. Higher

SNP	Nearest Gene	Coded/ Other Allele	Coded Allele Freq.[a]	**Justification** *(derived from NCBI Entrez Gene and dbSNP databases, UniProtKB database, and cited articles)*
				levels may be associated with lower serum Mg in vitamin-D insufficient women. One study in Mg-deficient Sprague-Dawley rats showed lower degree of translocated GLUT4 after 4 weeks.[128]
				Biological[c]
rs3750425	*TRPM6*	C/T	0.91-0.92	Crucial for Mg homeostasis. Encodes essential ion channel and serine/threonine-protein kinase. Role in epithelial Mg transport and active Mg absorption in gut and kidney. Other SNP (see below) related to FG and BMD.
rs2274924	*TRPM6*	A/G	0.83-0.84	Crucial for Mg homeostasis. Encodes essential ion channel and serine/threonine-protein kinase. Role in epithelial Mg transport and active Mg absorption in gut and kidney. Other SNP (see below) related to FG and BMD.
rs8042919	*TRPM7*	G/A	0.89	Crucial for Mg homeostasis. Encodes essential ion channel and serine/threonine-protein kinase. Divalent cation channel for both calcium and magnesium. Regulates anoxic neuronal cell death. May be involved in adjusting plasma membrane divalent cation fluxes. *A specific role for this gene in relation to FG or FI is unclear.*
rs4072037	*MUC1*	C/T	0.42-0.46	Associated with serum Mg, FG, BMD. Encodes mucin-1, which has cell adhesion properties. May provide a protective layer on epithelial cells against bacterial and enzyme attack. One subunit involved in cell signaling via Erk, SRC, and NFkB pathways. In activated T cells, influences Ras/MAPK pathway. Expressed on apical surface of epithelial cells (esp. airway, breast, uterus). *A specific role for this gene in relation to FG or FI is unclear.*
rs11144134	*TRPM6*	T/C	0.91-0.92	Crucial for Mg homeostasis and associated with FG, BMD. Encodes essential ion channel and serine/threonine-protein kinase. Role in epithelial Mg transport and active Mg absorption in gut and kidney.
rs3740393	*CNNM2*	G/C	0.85-0.86	Regulator of Mg homeostasis. Associated with serum Mg. Encodes ACDP2, member of the ancient conserved domain proteins family. Widely expressed in human tissue, with highest levels in brain, kidney, placenta. Transports divalent metal cations (Mg preferentially). Defects associated with hypomagnesemia due to defective tubular reabsorption. *A specific role for this gene in relation to FG or FI is unclear.*
rs994430	*CNNM3*	A/T	0.59-0.62	Regulator of Mg homeostasis. Associated with serum Mg. Widely expressed, but moreso in heart

SNP	Nearest Gene	Coded/ Other Allele	Coded Allele Freq.[a]	Justification *(derived from NCBI Entrez Gene and dbSNP databases, UniProtKB database, and cited articles)*
				and spleen. *A specific role for this gene in relation to FG or FI is unclear.*
rs6746896	*CNNM4*	A/G	0.66-0.71	Regulator of Mg homeostasis. Associated with serum Mg. Interacts with COX11 (metal ion chaperone); may play a role in biomineralization. *A specific role for this gene in relation to FG or FI is unclear.*

a. Derived from preliminary Cohorts for Heart and Aging Research in Genomic Epidemiology cohort data or dbGaP European descent/Caucasian.
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c. Derived from Meyer TE, Verwoert GC, Hwang S-J, et al*. Genome-Wide Association Studies of Serum Magnesium, Potassium, and Sodium Concentrations Identify Six Loci Influencing Serum Magnesium Levels Visscher PM, ed.* PLoS Genet*. 2010;6(8):e1001045; and Song Y, Hsu Y-H, Niu T,* et al*. Common genetic variants of the ion channel transient receptor potential membrane melastatin 6 and 7 (TRPM6 and TRPM7), magnesium intake, and risk of type 2 diabetes in women.* BMC Med Genet. *2009;10(1):4.*