MAGNESIUM AND CARDIOVASCULAR DISEASE

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MAGNESIUM AND CARDIOVASCULAR DISEASE

Magnesium has been associated with many health conditions. Many studies have shown inconsistent results on the relationship between magnesium (both serum and dietary) and risk of CVD related outcomes.

The aim of this thesis was to investigate the association of serum magnesium concentration with risk of CVD related incidence and mortality.

The subjects for this study were 2540 eastern Finnish men who participated in the baseline study of Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) and whose information regarding serum magnesium concentration was obtained. KIHD is an ongoing population based prospective follow up study. The baseline study involved two cohorts who were followed up. The first cohort enrolled men aged 54 years from 1984 to 1986 (n =1166) and the second cohort enrolled men aged 42, 48, 54 and 60 years from 1986 to 1989 (n =1516). Serum magnesium concentration and other factors were assessed at the baseline. All the cardiovascular events were ascertained from the medical records of hospitals and death certificates. *Cox* regression analysis was used to calculate the hazard ratio of all the outcome variables.

Before adjusting for confounders, the risk of fatal CVD, CHD and SCD decreased with a unit increase in serum magnesium concentration. But after multivariable adjustment for potential confounders only the risk of fatal CVD and CHD decreased by 9% (*HR*: 0.91; 95% CI: 0.85, 0.96), and 9% (*HR*: 0.91; 95% CI: 0.85, 0.98) respectively with a unit increase in serum magnesium concentration. Similarly, when serum magnesium concentration was modeled into fourths by quartiles, the findings showed decrease in risk of CVD, CHD mortality in all quartiles when compared to the lowest serum magnesium concentration. However there was not much difference in the *HR* among the fourths. The multivariable adjusted *HR*, comparing the highest quartile with the lowest quartile of serum magnesium concentration were 0.0.74 (95% CI: 0.57, 0.95) for CVD death, 0.72 (95% CI: 0.53, 0.97) for CHD death. However the incidence of CVD, CHD, AMI and stroke (also ischemic and hemorrhagic) were not found to be associated with baseline serum magnesium concentration.

In conclusion serum magnesium concentration was found to be inversely associated with fatal CVD, CHD in this large cohort prospective study of men. SCD was significantly inversely associated with total serum magnesium concentration only when it was not adjusted for confounders. Thus low serum magnesium is a stronger risk factor for fatal than non fatal CVD outcomes.

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Abbreviations:

AMI: Acute myocardial infarction
ATP: Adenosine triphosphate
BP: Blood pressure
CHD: Coronary heart disease
CVD: Cardiovascular disease
DBP: Diastolic blood pressure
DNA: Deoxyribonucleic acid
GI: Gastrointestinal
ICD: International Classification of Disease
IHD: Ischemic heart disease
KIHD: Kuopio Ischemic Heart Disease Risk Factor Study
Mg: Magnesium
MI: Myocardial infarction
OR: Odds ratio
RNA: Ribonucleic acid
SBP: Systolic blood pressure
SCD or SCA: Sudden cardiac death or sudden cardiac arrest
SD: Standard deviation

1 INTRODUCTION

Cardiovascular diseases (CVD) are one of the leading causes of death worldwide, explaining 17.3 million deaths (30% of all deaths) worldwide in the year 2008. Of these total deaths, 7.3 million were because of coronary heart disease and 6.2 million were due to stroke (WHO 2011). According to Statistics Finland, diseases due to the circulatory system were the leading cause of death in both men and women in Finland in the year 2012 (causes of death 2012, Statistics Finland). Ischemic heart disease is the main cause of death in Finns among the circulatory diseases. However there has been decrease in the trend from 47 to 39 percent in the past twenty years (causes of death 2012, Statistics Finland). Behavioral life style factors which include unhealthy diet, physical inactivity, and harmful use of alcohol and tobacco are associated with the occurrence of cardiovascular diseases (WHO 2011).

Several epidemiological studies suggest that diet plays important role in determining CVD. Increase in intake of salt, saturated fat and trans fat have shown increased risk of CVD whereas increase in intake of fruits and vegetables, polyunsaturated fat, monounsaturated fat, fiber have shown protective effect (O Flaherty *et al*. 2012; Scarborough *et al*. 2011). There was a decline in the incidence and mortality of CHD in Finland during the period 1982 to 1987, which was due to the changes in the major risk factors (Laatikainen *et al*. 2005). Another study done in United Kingdom has shown that changing the dietary pattern that is slight decrease in the consumption of harmful macronutrients and increasing the consumption of fruits and vegetables could reduce the total CVD death by approximately 20% (O Flaherty *et al*. 2012). Mediterranean dietary pattern has shown protective effect on cardiovascular disease and many cancers (Panico *et al*. 2014).

In recent period the intake of micronutrient is decreasing. The consumption of micronutrients like potassium, calcium and magnesium is found below the recommended value in many of the studies. Thus increase in intake of vegetables, fruits, and dairy should be recommended (Gallaghar *et al*. 2014; Mirzaeian *et al*. 2013). Along with all other nutrients present in diet, the importance of magnesium intake has also been studied and understood. Many studies have shown association between dietary magnesium intake and serum magnesium and the incidence

of CVD whereas some studies have failed to prove this. Thus the effect of magnesium on CVD morbidity and mortality is unclear (Eliat-Adar *et al*. 2013).

This study shows us the role of magnesium in the occurrence of outcome variables CVD, CVD death, AMI, CHD, CHD death, stroke, ischemic stroke, and hemorrhagic stroke, SCD or SCA. Thus from this study we will know if increasing or decreasing magnesium has protective effect on various cardiovascular events and death.

2 LITERATURE REVIEW

2.1 Magnesium

Magnesium is the fourth most common cation found in the body and the second most common intracellular cation. It is important to perform many physiological roles for different body functions like enzyme function, direct enzyme activation including many ATP generating reactions, membrane function, structural function which includes synthesis of RNA, DNA and protein, and acts as calcium antagonist in muscle contraction (Jahnen-Dechent and Ketteler. 2012). It also has important role in cell cycle, mitochondrial integrity, modulating ion transport (Romani 2011; Swaminathan 2003). The binding of magnesium with important intracellular anionic –liganda especially ATP and the competition with calcium for binding sites on protein membranes are the most important role of magnesium that help to perform those function (Swaminathan,2003). It also acts as a cofactor for more than 300 enzymatic reactions that includes energy metabolism and nucleic acid synthesis and cell replication. It is also important for normal neurological and muscular function, and cardiac excitability. A study done in human volunteers showed that consuming magnesium deficient diet resulted in negative magnesium balance but did not affect the serum magnesium concentration. This also affected the calcium, potassium and cholesterol metabolism (Nielsen 2004).

2.2 Body content and distribution of magnesium

Adult human body contains approximately 1mol (21-28g) Mg. Of total magnesium content in human body, less than 1% is found in serum and red blood cells. About 53% that is one half of the magnesium is found between bones, 27% in intracellular compartment of muscles, and 19% in soft tissues, 0.5%in erythrocyte and 0.3% in serum (Fawcett 1999). Bone magnesium can be used in the state of magnesium deficiency. It stays in equilibrium with ionized magnesium of extra cellular fluid and antagonizes calcium during muscle contraction (de Baaij *et al*. 2012). The availability of magnesium in bone decreases with age and therefore might not be completely available when there is magnesium deficiency (Maguire and Cowan 2002). Serum magnesium is present in three different states; free, complexes to anions and bound to

protein. Approximately 62% of the plasma magnesium is found circulating in ionized state, approximately 33% are protein bound mostly to albumin and approximately 5% are complexes to anions, citrates and phosphate (Fawcett *et al*. 1999; Elin 1987). However ionized magnesium is the one which is most involved in biological activity. The reference value for magnesium concentration in blood plasma ranges from 0.65 to 1.05 mmol/l for adults (Saris *et al*. 2000) and for ionized magnesium reference value ranges from 0.53 to 0.67 mmol in normal healthy people (Altura *et al*. 1991).

2.3 Magnesium intake

Magnesium concentration depends on the magnesium intake from food and drinking water. Whole seeds, unmilled grains, green leafy vegetables (rich in magnesium containing chlorophyll) legumes and nuts are the most important sources of dietary magnesium. Meat, fish, fruits are also good sources of magnesium. Drinking water especially hard water is also one of the sources of magnesium which might account for almost 10% of daily magnesium intake. The absorption of magnesium is influenced by various dietary factors either promoting or inhibiting the absorption. Absorption of magnesium can be inhibited by phytate, fibre, alcohol or excess of calcium and phosphate. Processing and refining of food leads to loss of magnesium content in food (Saris *et al*. 2000; Shils *et al*. 2006). The effect of vitamin D in magnesium absorption is still unclear. Some studies have shown that vitamin D and its active metabolites increases intestinal magnesium absorption in normal human beings and also in patients with chronic renal failure (Shils *et al*. 2006). The normal serum magnesium concentration or (Mg^{2+}) ranges between 0.75 and 0.95mmol/l (Weisinger and Bellorin-Font 1998).

The recommended dietary allowance of magnesium for male is 350 mg per day and for female it is 280 mg per day. But the requirement for female during pregnancy is increased to 355 mg per day. Several studies have shown that nowadays magnesium intake in Western countries is below the recommended value (Saris *et al*. 2000).

2.4 Magnesium homeostasis

Magnesium homeostasis is maintained by intestine, the bone and the kidney. In brief magnesium is absorbed through gut, stored in bones and excreted through kidney if excess. Intestinal absorption of magnesium was inversely related to magnesium intake in a healthy volunteer which ranged from 65% at low intake and 11% at high intake (Fine *et al*. 1991). Homeostasis of magnesium depends on its intake, efficiency of intestinal and renal absorption and also excretion and many other relevant factors like hormones. Two pathways that is paracellular and trans cellular pathways are involved in the absorption of Mg^{2+}. Paracellular pathway which is a passive mechanism absorbs Mg^{2+} through small spaces between epithelial. The trans cellular pathway involves movement of Mg^{2+} to the blood through the interior of epithelial cell. Around 30 to 40% of normal dietary intake of magnesium is absorbed through intestine. Jejunum and ileum are the important sites where magnesium absorption takes place. After 1 hour of ingestion the absorption begins and continues for 2 to 8 hours. After 12 hours the ingested material reaches large bowel in human where little or no absorption takes place (Shils *et al*. 2006; de Baaij *et al*. 2012).

Kidney plays an important role in regulating magnesium homeostasis especially in filtration and reabsorption process. Of the total magnesium present in the body 10 % (approx 100mmol or 2400mg) is filtered through glomeruli daily in normal healthy human. Of filtered magnesium only 5% is excreted through urine. 90 to 95% of filtered magnesium is reabsorbed in different segments of nephron. Approximately 10-25% of magnesium is reabsorbed via paracellular transport in proximal tubule. The major site where 65 to 75% of the magnesium is reabsorbed is the thick ascending loop of henle by the same paracellular mechanism. Only less amount of magnesium 5 to 10% is reabsorbed in distal convoluted tubule by active transcellular pathway (Dai *et al*. 2001; de Baaij *et al*. 2012).

2.5 Hypomagnesia

The level of magnesium in our body might not always be the same. Hypomagnesia indicates depletion of body magnesium. It is defined as hypomagnesia when the serum magnesium is

less than 1.8mg/dl (<0.74mmol/l). Most of the cases of hypomagnesia are asymptomatic. Symptomatic cases are seen only when serum magnesium falls below 1.2mg/dl (Assadi 2010). Magnesium deficiency or hypo magnesia can occur due to various reasons and mechanisms. Some of the reasons for magnesium deficiency are redistribution of magnesium, reduction in dietary intake and intestinal absorption, renal loss, endocrine causes, diabetes mellitus, alcohol, drugs (Fawcett *et al*. 1999; Swaminathan 2003).

2.5.1 Causes of hypomagnesia

Hypomagnesia due to redistribution: Hypomagnesia is seen when the magnesium from extracellular fluid moves to cells and bones in refeeding syndrome, during correction of acidosis, and in hungry bone syndrome which is seen in patient with diffuse osteoblastic metastases (Swaninathan 2003).

Gastrointestinal causes: The absorption of magnesium might reduce in cases of chronic diarrhea, malabsorption syndrome and after extensive bowel resection (Swaninathan 2003).

Renal loss: Urinary Mg^{2+} loss might be because of the defect in renal tubular magnesium reabsorption. It might be also because of reduction in sodium reabsorption as proximal tubular magnesium reabsorption is proportional to sodium and calcium reabsorption (Swaminathan 2003; Weisenger *et al*. 1998). Excess renal excretion can be another cause of magnesium depletion. Use of diuretics can result in magnesium wasting, loop of henle being the major site for magnesium reabsorption (Shils *et al*. 2006).

Many congenital disorders affecting renal tubular reabsorption of magnesium has been described. Some of them are barters syndrome, Gitelmans syndrome, Familial hypomagnesia, Antenatal Bartters syndrome (Assadi 2010; Swaninathan 2003; De Franciso and Rodriguez 2013).

Diabetes: Among all the disorders due to magnesium deficiency diabetes mellitus is the most common. Renal magnesium wasting and osmotic diuresis generated by hyperglycosuria are

the causes of magnesium depletion in diabetes patient. Depletion in magnesium also affects the insulin secretion and insulin resistance. This may be due to abnormal glucose metabolism as Mg is involved in this cycle. It may also affect the activity of tyrosine kinase at the insulin receptor (Takaya *et al*. 2004). Fasting blood glucose, glycated hemoglobin, albumin excretion and the duration of diabetes are correlated with magnesium deficiency (Corsonello *et al*. 2000). Hypomagnesia is also associated with type 2 diabetes mortality (Curiel-Garcia *et al*. 2008; Lecube *et al*. 2012; S P *et al*.2013; Dasgupta *et al*. 2012).

Alcohol: Hypomagnesia is found in chronic alcoholism with an incidence rate of 29.8%. The reason for hypomagnesia among alcoholic may be due to poor nutritional intake, diarrhea, vomiting, malabsorption as a result of chronic pancreatitis or liver disease, vitamin D deficiency, decrease renal tubular dysfunction, and the property of ethanol that alters ionic permeability (Stasiukyniene 2002; Romani 2008).

Hypo magnesia may further lead to various diseases like diabetes, cardiovascular disease, and osteoporosis. The most common manifestation of hypo magnesia is cardiac arrhythmias, hypocalcaemia and blood pressure (Swaminathan 2003).

2.6 Hyper magnesia

Hypermagnesia, the excess of magnesium in body may be the result of high intake of magnesium salt or magnesium containing drugs which is mostly seen in people with renal failure or reduced renal function. Occurrence of hyper magnesia is very rare but it may results in various neuromuscular, cardiovascular manifestation and hypocalcaemia. Higher level of magnesium also leads to cardio toxicity (Swaminathan 2003).

Chronic kidney disease or end stage kidney disease is the only strong clinical predictor for hyper magnesia and net positive magnesium balance. Dialysis patients have higher magnesium level (Spiegel 2011).

2.7 Measurement of magnesium

Total body magnesium cannot be measured by a single test. Different measurement techniques are available to measure the magnesium level in various parts of human body (Jahnen-Dechent and Ketteler 2012):

a) Concentrations in serum, red blood cell, blood mononuclear cells or muscles help to estimate the tissue magnesium level.

b) Isotopic analysis and evaluation from renal mg excretion and retention helps in metabolic assessment of Mg^{2+} balance.

c) Fluorescent probes, nuclear magnetic resonance spectroscopy, or ion selective electrodes can be used to determine Mg^{2+} level.

Assessing total serum magnesium concentration is the easiest, practicable, and inexpensive method of measuring magnesium and its changes in status. However it does not correlate accurately with total body magnesium content (Jahnen-Dechent and Ketteler 2012). Serum magnesium is more preferred than plasma magnesium because additive such as anticoagulant may bind with plasma Mg^{2+} affecting the examination (Noronha and Matuschak 2002). Serum magnesium concentration can also be taken as a good way of determining magnesium status because they correlate with ionized (Huijgen *et al*. 1998; Koch *et al*. 2002) and intracellular magnesium (Huijgen *et al*. 1998).

2.8 Clinical manifestation

2.8.1 Magnesium and cardiovascular manifestation

Magnesium depletion causes various cardiac manifestation, among which cardiac arrhythmia is an important complication where both extracellular and /or intracellular magnesium has been implicated. Prolonged QTc interval is found to be associated magnesium deficiency (Seelig 1969) and also magnesium supplementation has shown to reduce QTc interval in patient with normal serum magnesium (Krasner *et al*. 2002). The competition between Mg^{2+} and Ca^{2+} to bind in the same site of myocardial contractile protein may be the reason for the

affect of Mg^{2+} and calcium ($Ca^{2+)}$ on myocardial contractility (Chakraborti *et al*. 2002). A decrease in myocardial Mg^{2+} decreases intracellular potassium (K^{+}), which results in less negative resting membrane potential. This leads to prolonged QTc interval and increase the risk of ventricular arrhythmia (Chakraborti *et al*. 2002).

Neuroprotective and vasodilatory effect of magnesium can improve the clinical outcome of patient suffering from subarachnoid hemorrhage and also can reduce the chance of cerebral ischemia after subarachnoid hemorrhage (Van den Bergh *et al*. 2004).

Increase in magnesium intake was found to be inversely associated with coronary artery calcification and abdominal aortic calcification. The association was stronger in female than in male. Thus magnesium might have role in preventing stroke and fatal coronary heart disease (Hruby *et al*. 2014).

2.9 Magnesium and cardiovascular disease

According to Japan collaborative cohort (JACC) study, dietary intake of magnesium was inversely associated with mortality from hemorrhagic stroke in men and with total and ischemic stroke, coronary heart disease, heart failure in women aged 40-79 years (Zhang *et al*. 2012).

A recent prospective study conducted in Spain among the Mediterranean population who were at risk of developing CVD, showed that the increase in magnesium intake lowers the risk of cardiovascular events and death, also after adjusting for various confounders (Guasch-Ferre *et al*. 2014). Similarly a prospective cohort study in men showed no relation between magnesium intake and all cause CVD and cancer mortality (Kaluza *et al*. 2010). Serum magnesium was not found to be associated with subsequent development of hypertension, CVD or all-cause mortality in the Framingham heart study offspring cohort (Khan *et al*. 2010). In another prospective study of men highest serum magnesium concentration was found to reduce the risk of cardiovascular mortality by 40% (Leone *et al*. 2006).

There are many risk factors that affect the association of serum and dietary magnesium with cardiovascular events. A study done in India among people with coronary artery disease showed that various cardiovascular risk factors are significantly correlated with serum magnesium and dietary magnesium intake. Risk factors like hypertension, diabetes, smoking, dyslipidemia was found to be inversely correlated with magnesium level. And also most of the people having these risk factors were having low dietary and serum magnesium level compared to patient without the risk factors (Mahalle *et al*. 2012). Atherosclerosis Risk in communities study done among black and white population showed that mean magnesium level is significantly lower in people having diabetes, hypertension and CVD compared to disease free population even after adjusting for age and BMI. In contrast mean dietary magnesium intake was generally found to be low in disease free population. Further there was seen an inverse correlation between magnesium level (serum and dietary) and few selected variables such as systolic blood pressure, HDL cholesterol, fasting insulin, smoking (Ma *et al*. 1995). In a study done among haemodialysis patient, the risk factors for CVD like age, body

mass index, systolic blood pressure before dialysis, serum lipid and calcium profile were not found to be correlated with serum magnesium level (Khatami *et al*. 2013). Thus magnesium can be one of the related causes to CVD.

In a prospective cohort study done among male high serum magnesium value was found to decrease the risk of cardiovascular mortality by 40%. It remained the same even after adjusting the nutrient intake (Leone *et al*. 2006). Another study conducted in male population also revealed that lower magnesium concentration is associated with increased risk of all-cause mortality and cardiovascular mortality even after adjustment for cardiovascular risk factors and drug therapy and left ventricular mass in the baseline (Reffelman *et al*. 2011). In a study done among hemodialysis patient low serum magnesium was found to be independent predictor for CVD mortality (Sakaguchi *et al*. 2014). In another study done among population of ambulatory heart failure, it was seen that the cardiovascular mortality is a bit more in patient with low serum magnesium (Adampoulos *et al*. 2009).

2.10 Magnesium and coronary heart disease

Increase in magnesium intake among men was associated with modest lower risk CHD compared to the lowest quartile after adjusting for its confounders (Al-Delaimy *et al*. 2004). Similarly, a case control study conducted in Serbia, explained that intake of foods high in magnesium level (cereals, legumes and vegetables) was significantly less in subjects with coronary heart disease compared to control groups, showing an inverse association of magnesium intake with risk of CHD (Stevanovic *et al*. 2011). In a nurses' health study conducted to examine the association of plasma and dietary magnesium with the risk of coronary heart disease, higher magnesium intake was not found to be associated with decrease risk of total CHD after adjusting for various known risk factors like alcohol intake, diabetes, hypertension, omega 3 fats, calcium and many others. But the risk of fatal CHD was decreased by 36% in the highest quartile when compared to the lowest quartile of magnesium intake. Regarding the plasma magnesium, no linear inverse association was seen after adjusting for various potential confounders (Chiuve *et al*. 2013). Plasma magnesium was not found to be associated with IHD but urinary magnesium excretion was found to be associated with IHD in

a nonlinear trend (Joosten *et al*. 2013). There was slight difference in the mean concentration of ionized magnesium in patient with stable coronary disease and healthy population but it was not found to be statistically significant. The difference was a bit more in patient having both arterial hypertension and coronary artery disease compared to healthy population which was also not statistically significant (Kozielec *et al*. 2005).

A study conducted among US adults showed that the serum magnesium is more strongly associated with mortality of all cause and ischemic heart disease compared to ischemic incidence. Thus increase in serum magnesium defines significant lower in risk of ischemic and all-cause mortality (Ford 1999). In ARIC study lowest risk for CHD incidence among both men and women was found in the highest quartile of serum magnesium concentration compared to the lowest quartile after adjusting for its confounders (Liao *et al*. 1998).

2.11 Magnesium and stroke

In a study done among health professionals it was found that magnesium was inversely associated with the risk of stroke when adjusted for age but was not the same when further adjusted for potassium and calcium which had positive correlation with the magnesium (Ascherio *et al*. 1998). There was not any association between dietary intake of magnesium and total stroke, intracerebral hemorrhage, or subarachnoid hemorrhage after adjustment for other risk factors in a study done among Swedish female (Larsson et la. 2011). Similarly another study done among Finnish male smokers showed that the high magnesium intake was significantly associated with the reduction in risk of cerebral infarction also after adjusting for other potential confounders. But there was not found any association between subarachnoid and intracerebral hemorrhage though the risk of getting subarachnoid hemorrhage was found very high in third (1.64, 95% CI: 1.01, 2.64) and fourth quartile (1.83, 95% CI: 1.15, 2.93) (Larsson *et al*. 2008). In a trail done among adults having aneurysmal subarachnoid hemorrhage mean serum magnesium concentration was higher in group receiving magnesium sulfate ($MgSO_{4)}$ than in placebo (receiving saline) group. There was seen worse clinical outcome in the highest plasma concentration of magnesium sulfate ($MgSO_{4)}$ group and in the third and fourth quartile in the placebo group. However there was association between good

clinical outcome and third and fourth quartile of serum magnesium in the placebo group. Thus this study did not provide satisfactory result to say increasing serum magnesium reduces the risk of subarachnoid hemorrhage (Wong *et al*. 2010). In another clinical trial, patient presenting within 12 hours of clinically diagnosed acute middle cerebral artery stroke were randomized to magnesium sulfate ($MgSO_{4)}$ and placebo group. Magnesium level started to rise over 24 hour after the infusion and remained significantly high at 48 hours. There was seen fewer cases of death and disability in magnesium treated patient (30%) than placebo group (40%) at 3 months (Muir *et al*. 1995).

A case control study done in China showed that the mean dietary intake of magnesium was lower in cases (patient with ischemic stroke) than in controls (patient without ischemic stroke). There was a decrease in risk of stroke with an increase in dietary magnesium level, with the lowest risk in the highest quartile of magnesium intake. There was a similar relation also with dietary calcium level (Liang *et al*. 2011). In a cohort study done to know the relationship between tap water hardness and risk of stroke mortality and IHD mortality, there was not found any association between them among both men and women. When the analysis was restricted to the subject with 20% lowest dietary intake, there was seen a protective effect of tap water hardness in the fourth quartile compared to the lowest for stroke among men but there was not seen any association with IHD mortality (Leurs *et al*. 2010). Also dietary intake of magnesium from water has shown protective effect on the risk of cerebrovascular disease (Yang 1998). Arthrosclerosis risk in communities study cohort showed that the inverse association of ischemic stroke with dietary magnesium intake is weaker than that with serum magnesium level. But when other risk factors like systolic blood pressure, diabetes mellitus and use of anti hypertensive medication was added the association with serum magnesium reached to a no significant level. Having both dietary and serum magnesium greater than the median value was associated with 36% lower risk of ischemic stroke (Ohira *et al*. 2009). Although magnesium intake had modest effect on risk of ischemic stroke, the inverse association was no longer significant when adjusted for other cardiovascular risk factors, including hypertension in middle aged American women (Iso *et al*. 1999). The other dietary minerals like potassium and calcium can also affect the association between magnesium intake and incident of ischemic stroke and CHD (Iso *et al*. 1999; Al-Delaimy *et al*. 2004).

2.12 Magnesium and AMI

A cohort study in women showed no significant association between magnesium intake and the incidence of total cardiovascular disease, coronary heart disease, non-fatal MI or stroke. However lower risk of total and ischemic stroke was associated with high intake of magnesium (Song *et al*. 2005). Low serum magnesium was associated with the increase risk of atrial fibrillation. The risk of atrial fibrillation increased by 54% in the lowest quartile compared to the highest quartile of serum magnesium (Khan *et al*. 2013).

Also environmental risk factors can explain the variation in the occurrence of CHD among different places. An ecologic population based study done in Finland among men aged 35-74 years suggested that there was a relationship between hardness of water and occurrence of acute myocardial infarction. It had shown that the increment in magnesium level in ground water decreased the risk of AMI. It also suggested that the increase in Ca:Mg ratio increased the risk of AMI as both of these elements were found to be highly correlated. Similarly when the age was standardized the risk of AMI was highest in the lowest quartile of magnesium and was lowest in the highest quartile of magnesium (Kousa *et al*. 2006). Whereas another similar study done in Taiwan based on ground water magnesium level did not show protective effect on the risk of death from AMI with *OR* of 1 (95% CI: 0.93, 1.08) and 1.09 (95% CI: 0.99, 1.19) respectively, for the two higher magnesium level after adjusting for calcium (Yang *et al*. 2006). Serum magnesium concentration was found to be lower in south Indian male population having AMI than in the controls. And also there was significant correlation between serum magnesium level and other cardiac biomarkers (Ramasamy *et al*. 2013).

A clinical trial, done among patients with suspected acute myocardial infarction did not show significant difference in the incidence of rhythm disturbance for ventricular fibrillation, ventricular tachycardia, supraventricular tachycardia or heart block of any degree among placebo and $MgSO_4$ receiving group. Sinus bradycardia was more observed in $MgSO_4$ group. Thus $MgSO_4$ did not show significant effect on arrhythmia in AMI (Roffe *et al*. 1994). In another clinical trial study serum magnesium was found to be lower in patients with AMI.

There was seen significant lower incidence of arrhythmia in patients receiving magnesium compared to placebo group (Gyamlani *et al*. 2000).

A study done in male Wistar rats showed that when $MgSO_4$ was infused intravenously before reperfusion there was significant reduction in formation of thrombus in vivo (Ravn *et al*. 1997).

2.13 Magnesium and sudden cardiac death

In a prospective cohort study conducted among women, the relative risk of sudden cardiac death was lower in highest quartile of dietary and plasma magnesium. But the association was seen stronger in highest plasma magnesium level (*RR*: 0.23, 95% CI: 0.09, 0.60) with a significant linear trend (Chiuve *et al*. 2011). ARIC study conducted in middle aged biracial population showed that the risk of sudden cardiac death was decreasing in a linear trend from the lowest serum magnesium level to the highest serum magnesium level. But when it was adjusted for diabetes, hypertension and use of diuretics the association was weakened showing slightly less protective effect (Peacock *et al*. 2010).

In a meta-analysis of prospective cohort study magnesium intake was found to be inversely associated with ischemic stroke but not with intracerebral hemorrhage or subarachnoid hemorrhage (Larson *et al*. 2012).

According to the study done in animal, inadequate intake of magnesium resulted in development of increase atherosclerotic plaque in rabbits (King *et al*. 2009). In a study conducted in rats, higher serum cholesterol and total phospholipid was found among magnesium deficient rats than in controls (Cunnane *et al*. 1985).

3 AIM OF THE STUDY

The aim of this thesis is to explore the association of magnesium with cardiovascular events among men from Eastern Finland.

The specific aims of the study are to:

- To assess the association between magnesium and risk of nonfatal events, namely CVD, AMI, CHD, stroke, ischemic stroke, hemorrhagic stroke in a population based KIHD study conducted in 1984 to 2011.
- To assess the association between magnesium and risk of fatal events, namely CVD death, CHD death and SCD or SCA in a population based KIHD study conducted in 1984 to 2011.

4 METHODS

4.1 Study population

This study used the data from Kuopio Ischemic Heart Disease Risk Factor Study. The KIHD study is an ongoing population based cohort study being conducted in Eastern Finland to investigate the risk factors for cardiovascular disease and other chronic disease in middle aged men and women.

A baseline study was conducted between March 1984 to December 1989 which included 3235 men (of which 82.9% participated) living in Kuopio and neighboring rural communities. The baseline study was conducted in two cohorts. The first cohort carried out between 1984 to 1986 enrolled 1,166 men, aged 54 years and the second cohort carried out between 1986 to 1989 enrolled 1516 men aged 42, 48, 54 or 60 years.

This baseline study was followed by 11 year follow up study which included 854 men from second cohort and 920 randomly selected post-menopausal women aged 53-73 years from the same area. The study further proceeded to 20 year follow up. In order to assess the dietary

intake of nutrients, four day food records were maintained during the baseline and 11 year follow up examination. This current study includes 2540 men from the baseline whose serum magnesium level has been determined.

4.2 Ethical consideration

KIHD study has been approved by the ethical committee of University of Eastern Finland and also the study subjects gave an informed consent.

4.3 Data collection

There was three set of self-administered questionnaire that were emailed to the participants before they came for health checkups. The first questionnaire included the information regarding demographic characteristics, major life events, leisure time activities, family life, socioeconomic background, childhood circumstances and health behavior such as smoking, physical activity, alcohol consumption, health status, drug use. The other two questionnaires were related to psychosocial wellbeing. The participants were called for a health examination where they were also interviewed by a research nurse for some of the questions in the questionnaire. Blood sample was taken at the same time.

4.4 Measurements

4.4.1 Blood sample

The blood samples were collected from subjects between 8 and 10 am on Tuesday, Wednesday or Thursday at the baseline examination. The subjects were abstained from taking alcohol for 3 days, smoking and eating for 12 hours prior to the sample collection. Further description regarding the measurement of serum lipids (Salonen *et al*. 1991) and lipoproteins, blood glucose, blood hemoglobin, ferritin, copper, has already been explained in the previous studies (Salonen *et al*. 1992; Kurl *et al*. 2006). Diabetes was either defined as self-reported or fasting blood glucose ≥8.0 mmol/l.

4.4.2 Dietary intake

Dietary intake of food was assessed with the help of well instructed and interview checked 4 day food record during the baseline examination by household measure. Data on calcium, fiber, alcohol intake and energy was obtained from 4 day food record. A software named Nutrica version 2.5 was used to calculate the nutrients intake. The software mainly uses the Finnish values for the nutrients in the composition of food, taking into account the loss of vitamins during food preparation. It's software with a database on 1300 food items and dishes and 30 nutrients. A research institution named Research Center of the Social Insurance Institution of Finland has developed this software (Voutilainen *et al*. 2001).

All the dietary nutrients were adjusted for energy intake using the residual methods. The energy adjustment was done in order to balance the differences in the energy requirements among individuals (Voutilainen *et al*. 2001).

4.4.3 Assessment of serum magnesium

The venous blood samples were collected into trace-element and centrifuged at 1000g for 10 minutes. All the precautions were taken to prevent the sample from contamination during collection and laboratory work. The polyethylene tubes used in the analysis were washed with 5% nitric acid and rinsed with deionized water. Samples were stored at -20 centigrade until the assay. The method used to measure serum magnesium in this study was atomic absorption spectrometry (Perkin Elmer Zeeman 5000, Perkin Elmer, Norwalk, CT, USA) where acetylene –air (1:4) flame technique was used. Serum magnesium was diluted in a ratio of 1:50 with distilled water. The wavelengths were 185.2nm for magnesium. The between-run CV% for the method is 2.4 (37 assays). (Saaranen *et al*. 1987)

4.4.4 Assessment of other covariates

The data on age, education, and current number of cigarettes, cigars or a pipe smoked daily, time period of regular smoking (years), history of CVD, presence of hypertension, and use of

antihypertensive drugs, use of diuretics and aspirin medication was obtained using self-administered questionnaire. Re interviews were conducted again by a physician to collect the information on medical history. Family history of CVD was considered as positive if biological mother, father, brother and sister of participant had CVD history.

A subject was defined as smoker if he had ever smoked cigarettes, cigars or a pipe on a regular basis or within the past 30 days (Salonen *et al*. 1992).

Resting blood pressure was measured by a nurse on the first examination day with a random zero sphygmomanometer. And the mean of all 6 measurements (3 supine, 1 standing, 2 sitting) was used as the final mean systolic and diastolic blood pressure (Salonen *et al*. 1992).

Physical activity was assessed from 12 months history using leisure time physical activity questionnaire which had the information on frequency, average duration and intensity, for each activity performed. Mean intensity of physical activity was expressed in MET (Lakka *et al*. 1994).

Energy expenditure (kcal/week) was measured for each physical activity by multiplying the metabolic index of activity (in metabolic equivalent *hour/week) by body weight in kilograms (Lakka *et al*. 1994).

Body mass index was calculated as the ratio of weight in kilograms divided by the square of heights in meters (kg/m^2).

Serum fatty acids were measured with capillary gas chromatography (Virtanen *et al*. 2005).

4.5 Follow up ascertainment

Information on stroke, AMI and CHD events between the periods 1984 to 1992 were collected through FINMONICA register. It’s the Finnish version of WHO MONICA project (Multinational Monitoring of Trends and Determinants in Cardiovascular Diseases).

Information on other cardiovascular outcome events throughout and information on AMI, stroke and CHD later from 1992 to 2011 were obtained from the National Hospital Discharge Register data by computer linkage and death certificates registers using Finnish personal identification code. In a manner similar to FINMONICA criteria, information on diagnosis was collected from hospital and was classified by a neurologist and cardiologist. However the FINMONICA register data were rechecked annually with the data obtained from hospital register and death register. There were no losses to follow up. The events were coded according to tenth ICD codes (Kurl *et al*. 2006). The tenth ICD codes for all outcomes are as follow: Stroke (I60 –I64), ischemic stroke (I63) and hemorrhagic stroke (I60-I61) CVD (I00-I99), CHD (I20-I25), AMI (I20-I22), cardiac arrest (I460, I490). The codes for deaths were the same.

All the events of stroke, ischemic and hemorrhagic stroke that occurred from the study entry to 2004 were included. Whereas all the events of CVD, CVD death, CHD, CHD death, AMI, SCD or SCA that occurred from the study entry until 2011 were included in this study.

4.6 Statistical analysis:

The descriptive data were presented in the form of mean and standard deviation for continuous variables and in numbers and percentages for categorical variables. The background characteristics were presented according to the quartiles of serum magnesium level. Serum magnesium concentration was categorized into fourths Q1, Q2, Q3 and Q4 by three quartiles in an ascending order moving from the lowest (Q1) serum magnesium concentration to the highest (Q4) serum magnesium concentration.

Our aim in this study was to find the association between exposure (magnesium) and outcome (cardiovascular events) keeping in consideration all the confounding factors (risk factors).

List of dependent variables, outcome variables and possible confounders used in this study

DependentVariable(Exposure)	**Outcome variables**	**Possible confounders**
Serum magnesium concentration (mg/l)	CVD	Age (years)
	CVD death	Years of education
Dietary magnesium intake (mg/d)	AMI	BMI ($kg/m^{2)}$
	CHD	Energy (KJ/d)
	CHD death	Energy expenditure (Kcal/d)
	SCD or SCA	Alcohol/week (g/d)
	Stroke	Mean SBP (mmHg)
	Ischemic stroke	Mean DBP (mmHg)
	Hemorrhagic stroke	LDL–chol (mmol/l)
		HDL– chol (mmol/l)
		Calcium (mg/d)
		Total serum n-3 (% of energy intake)
		Fiber (g/day)
		DU-k (mmol/d)
		Smoking (yes, no)
		Diabetes (yes, no)
		CVD in family (yes, no)
		Magnesium supplementation (yes, no)
		Elevated BP (yes, no)
		Aspirin medication (yes, no)
		Diuretics (yes, no)
		Date of examination, year

Following the model of causal pathway between magnesium and cardiovascular events, analyses were done to check if the criteria for confounding were met or not. The criteria needed to be fulfilled to be a confounder is that the possible confounders has to be significantly associated with the outcome independent of exposure; possible confounders has

to be associated with the exposure and it shouldn't be in the causal pathway between exposure and outcome.

Firstly, in order to check the association between exposure and confounder, spearman correlation for the continuous variable and independent sample student *T-test* for categorical variables were used. The *P*-values ≤0.1 were taken as the rule to select the variable into further analysis.

Secondly, in order to check the association between each confounders and different outcomes, univariate survival analysis was conducted separately for each of them, predicting if the variable was a risk factor for the disease or not. *P*-values ≤ 0.1 were taken as the rule to select the variable into further analysis.

For each outcome events separately, we took those variables as the potential confounding variables which showed significant association with both exposure and that particular outcome. Variables that were found to be the potential confounders were not the same for all outcome events.

Further we calculated hazard ratio (95% CI) of all CVD events (CVD, CVD death, CHD, CHD death, AMI, stroke, ischemic stroke, hemorrhagic stroke, SCD or SCA by fourths of serum magnesium, adjusting for all the potential confounders using multivariate *Cox* proportional analysis. The two sided *P*-values of ≤0.05 were considered as statistically significant for the analysis.

All the statistical analyses were performed using statistical software SPSS version 19.

5 RESULTS

5.1 Dietary magnesium intake

Table 1: One way ANOVA showing mean, SD and post hoc analysis of dependent variable (serum magnesium) for each category of dietary magnesium.

Dietary magnesium	**Mean±SD**	**Post hoc**
Q1 (<372.23 mg/d)	19.84±1.63	1 (ref)
Q2(372.27-412.59 mg/d)	19.99±1.54	-0.16 (0.69)
Q3 (412.63-457.37 mg/d)	19.76±1.52	0.08 (0.36)
Q4 (>457.42 mg/d)	19.79±1.54	0.04 (0.63)

Mean ±SD, Mean difference (*P*-value)

Q1, Q2, Q3, Q4: fourths of dietary magnesium

There was seen statistical significant difference between groups as determined by one way ANOVA (*P*-value*:* 0.034). Further post hoc analysis showed that there was statistically significant difference in the mean of serum magnesium concentration only when the third (19.76) and fourth (19.79) level of dietary magnesium was compared to the second quartile (19.99). But there was not seen any statistical significant differences in the mean serum magnesium concentration between all other groups of dietary magnesium when compared to the first group respectively. Since there was not much change in the mean serum magnesium concentration in the various levels of dietary magnesium we decided to do the analysis only as per the serum magnesium categories. Also inconsistency between serum and dietary estimations, and serum measurement being considered as more reliable indicator of body magnesium status became the reason to drop dietary magnesium from further analysis.

5.2 Serum magnesium concentration

There was seen statistically significant difference in the mean serum magnesium concentration based on absence or presence of CVD mortality (*P*-value: 0.013) and CHD mortality (*P*-value: 0.041). The mean level of serum magnesium concentration was higher in men without the event CVD and CHD mortality. But there was not any statistically significant difference in the mean serum magnesium concentration based on the presence or absence of CVD, AMI, stroke, ischemic stroke, and hemorrhagic stroke, SCD or SCA. But lower serum magnesium is associated with the occurrence of fatal events.

Mean dietary magnesium intake was not the same for men based on the absence or presence SCD or SCA and non fatal CVD.

5.3 Analysis of background characteristics

Table 2: Baseline characteristics among male in KIHD study by quartiles of serum magnesium

Characteristic	**Q1**	**Q2**	**Q3**	**Q4**
Rangeof magnesium,mg/l	<18.85	18.86–19.85	19.86–20.87	>20.88
N	636	635	634	635
Age, year	53.07±5.45	52.46±5.66	53.12±4.90	53.57±4.12
Years of education	8.41±3.33	8.89±3.50	8.42±3.13	8.79±3.68
BMI, kg/m^2	27.10±3.69	26.71±3.52	26.95±3.49	26.84±3.49
Energy, KJ	9935.01±2747.64	9864.78±2661.94	9911.03±2592.71	9722.11±2460.27
Energy expenditure, kcal/d	135.68±163.79	152.20±202.73	136.82±168.18	135.12±156.09
Alcohol/week, g/d	81.78±162.59	80.44±157.49	71.26±102.04	65.50±108.94
Mean SBP, mmHg	134.37±17.04	133.79±16.46	134.57±16.73	133.70±17.35
Mean DBP, mmHg	88.88±10.88	88.42±10.22	89.15±10.26	88.52±10.71
LDL-chol, mmol/l	3.92±1.02	3.99±1.03	4.06±0.95	4.19±1.01
HDL-chol, mmol/l	1.30±0.33	1.29±0.29	1.28±0.28	1.29±0.29
Calcium, mg/d	1168.83±518.95	1162.62±495.70	1204.05±499.70	1168.41±487.85
Total serum n-3, %	5.47±1.65	5.44±1.63	5.33±1.54	5.32±1.49
Fiber, g/d	25.53±9.21	25.45±8.94	25.13±8.65	24.32±8.21
DU-K, mmol/d	85.85±30.01	85.36±26.67	87.30±27.56	85.61±25.05
Smokers (yes, no)	204 (32.1)	191 (30.1)	208 (32.8)	198 (31.2)
Diabetes (yes, no)	68 (10.7)	35 (5.5)	24 (3.8)	22 (3.5)
Elevated BP (yes, no)	388 (61.0)	373 (58.7)	401 (63.2)	369 (58.1)
CVD in family (yes, no)	513 (80.7)	533 (83.9)	518 (81.7)	521 (82.0)
CVD history (yes, no)	250 (39.3)	219 (34.5)	223 (35.2)	263 (41.4)
Magnesium supplementation (yes, no)	-	-	2 (3)	2 (3)
Aspirin medication (yes, no)	42 (6.6)	53 (8.3)	39 (6.2)	23 (3.6)
Diuretics (yes, no)	48 (7.5)	28 (4.4)	40 (6.3)	53 (8.3)
Marital status				
Married/living as couple	541 (85.1)	554 (87.2)	554 (87.5)	551 (86.6)
Single	42 (6.6)	43 (6.8)	40 (6.3)	40 (6.5)
divorced/widow	53 (8.3)	38 (6.0)	39 (6.2)	44 (6.9)

Mean±SD, frequency (%)

The detailed information regarding the distribution of characteristics in this population across the quartiles of serum magnesium is described in Table 1. Men with higher serum magnesium concentration intended to have lesser mean alcohol consumption, fiber intake, diabetes prevalence, and more LDL cholesterol level. There was not found any constant increase or decrease in the value of all other characteristics with increasing serum magnesium concentration. There was not much difference in the mean age, BMI, mean systolic and diastolic blood pressure across the quartiles of serum magnesium concentration.

Only 2 respondents each in the thirds and fourths of serum magnesium concentration were using magnesium supplementation. More than 80% of the respondents in each quartile of serum magnesium concentration had a familial history of cardiovascular disease.

5.4 Analysis of exposure and possible confounders

Table 3: Spearman correlation between exposure (serum magnesium) and possible confounders

Variables	**correlation coefficient**	**Sig (*P*-value)**
Age, years	0.010	0.622
Years of education	0.009	0.666
Body mass index, kg/m^2	-0.014	0.471
Energy, KJ	-0.014	0.486
Energy expenditure, Kcal/d	0.019	0.347
Alcohol /week, g/d	**-0.045**	**0.025**
Mean systolic BP, mmHg	-0.019	0.330
Mean diastolic BP, mmHg	-0.001	0.941
LDL-chol, mmol/l	**0.100**	**≤0.001**
HDL-Chol, mmol/l	-0.004	0.859
Calcium, mg/d	0.021	0.298
Total serum n-3, %	**-0.037**	**0.068**
Fiber, g/d	**-0.041**	**0.038**
DU-k, (l)mmol/d	0.029	0.209
Date of examination, year	**-0.187**	**≤0.001**

A spearman correlation was done to understand the relationship between the exposure (serum magnesium) and the possible confounding variables those were continuous. *P*-values less than or equals to 0.1 were taken as rule to considered the variable to be statistically significant for further analysis. Only few variables were found to be statistically significantly correlated with serum magnesium concentration and they were total serum n-3, LDL cholesterol, fiber, alcohol and date of examination. Among them LDL cholesterol was strongly positively correlated with Serum magnesium concentration(r=0.100, *P*-value ≤0.001) and date of examination year was strongly negatively correlated with serum magnesium concentration.

For every increase in year there was a decrease in unit serum magnesium concentration by 18.7%. The other three variables alcohol, fiber and total serum n-3 showed significant negative correlation that is they were inversely proportionate to serum magnesium.

Table 4: Independent sample *T*- test between exposure (serum magnesium) and possible confounders calculating the mean difference

Variables (n)	**Mean diffference (*P*-value)**	
magnesium supplementation		
No (2536)	19.84	**(0.005)**
Yes (4)	22.05	
Smokers		
No (1739)	19.87	(0.253)
Yes (801)	19.79	
Diabetes		
No (2391)	19.89	**(≤0.001)**
Yes (149)	19.07	
Elevated blood pressure		
No (1009)	19.90	(0.15)
Yes (1531)	19.81	
CVD in family		
No (455)	19.79	(0.38)
Yes (2085)	19.86	
Diuretics		
No (2371)	19.84	(0.73)
Yes (169)	19.89	
Aspirin medication		
No (2383)	19.86	**(0.006)**
Yes (157)	19.51	
Marital status		
1 (2200)	19.86	(0.344) (between groups)
2 (165)	19.81	
3 (174)	19.68	

The distribution of serum magnesium mg/l was not the same across categories of magnesium supplementation, diabetes, and aspirin medication ($P = \leq 0.05$) whereas it was same across smokers, people with elevated blood pressure, having CVD history in family, diuretics and different marital status. Thus magnesium supplementation, diabetes and aspirin medication were significantly associated with or were responsible for change in serum magnesium level.

5.5 Analysis of association between possible confounders and outcome events

The results of univariate *cox* proportional hazard analysis showing hazard ratio for all the outcome variables according to each possible confounders are shown in Table 5.

Variables like body mass index, mean systolic and diastolic blood pressure, elevated blood pressure were found to be strongly associated with all the outcome variables showing slight increase in risk of getting the outcome when there was a unit increase in those variables.

Diabetes was also found to be strongly significantly associated with all outcome variables except for hemorrhagic stroke. The risk of occurrence of all outcome variables except hemorrhagic stroke was more than 200% among people with diabetes.

Magnesium supplementation, total serum n-3, energy consumed, energy expenditure, potassium did not show significant association with any of the outcome variables.

Table 5: Univariate hazard ratio (95% CI) of non fatal AMI, CVD, CHD, stroke, ischemic and hemorrhagic stroke and fatal CVD, CHD, SCD24 or cardiac arrest according to all the possible confounders

Variables	**CVD**	**CVD death**	**AMI**	**CHD**	**CHD death**	**SCD**	**Stroke**	**Ischemic stroke**	**Hemorrhagic stroke**
Age ,years	1.06(1.05,1.07)	1.10(1.08,1.13)	1.07(1.05,1.08)	1.07(1.06,1.09)	1.12(1.09,1.15)	1.09(1.06,1.13)	1.07(1.04,1.11)	1.09(1.05,1.12)	1.00(0.95,1.06)
Alcohol/week, g/d	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(0.99,1.00)	1.00(0.99,1.00)	1.00(0.99,1.00)
LDL-chol, mmol/l	1.15(1.10,1.21)	1.16(1.07,1.26)	1.34(1.25,1.43)	1.29(1.22,1.38)	1.24(1.12,1.37)	1.22(1.08,1.38)	1.07(0.95,1.22)	1.12(0.98,1.29)	0.92(0.69,1.23)
HDL-chol, mmol/l	0.65(0.55,0.78)	0.56(0.40,0.77)	0.41(0.32,0.54)	0.44(0.35,0.55)	0.45(0.31,0.68)	0.47(0.29,0.75)	0.81(0.52,1.27)	0.72(0.43,1.19)	1.08(0.42,2.80)
Years of education	0.95(0.93,0.96)	0.92(0.89,0.94)	0.93(0.91,0.95)	0.94(0.92,0.96)	0.91(0.87,0.94)	0.93(0.89,0.97)	0.89(0.85, 0.94)	0.900.85,0.95)	0.92(0.83,1.02)
energy, KJ/d	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.009)
Body mass index, kg/m2	1.06(1.04,1.07)	1.09(1.06,1.11)	1.07(1.04,1.09)	1.05(1.04,1.07)	1.09(1.06,1.12)	1.10(1.06,1.13)	1.08(1.05,1.12)	1.091.06,1.14)	1.07(0.99,1.15)
Mean systolic blood pressure, mmHg	1.01(1.01,1.01)	1.02(1.01,1.02)	1.01(1.02,1.02)	1.01(1.01,1.01)	1.02(1.01,1.02)	1.02(1.01,1.02)	1.021.01,1.03)	1.021.01,1.03)	1.03(1.01,1.04)
Mean diastolic blood pressure, mmHg	1.02(1.01,1.02)	1.02(1.01,1.03)	1.01(1.04,1.02)	1.01(1.00,1.02)	1.01(1.00,1.02)	1.01(1.00,1.03)	1.03(1.02,1.04)	1.03(1.02,1.04)	1.04(1.01,1.07)
DU-K (l) mmol/d	0.99(0.99,1.00)	1.00(0.99,1.00)	0.99(0.99,1.00)	0.99(0.99,1.00)	1.00(0.10,1.01)	1.00(0.99,1.01)	0.99(0.99,1.01)	0.99(0.99,1.00)	0.99(0.97,1.02)
calcium,mg/d	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1(1.00,1.00)	1(0.99,1.00)	1.00(0.99,1.00)	1.00(1.00,1.00)	1.00(1.00,1.00)	1.00(0.99,1.00)

Table 5: Univariate hazard ratio (95% CI) of non fatal AMI, CVD, CHD, stroke, ischemic and hemorrhagic stroke and fatal CVD, CHD, SCD24 or cardiac arrest according to all the possible confounders

Variables	CVD	CVD death	AMI	CHD	CHD death	SCD	Stroke	Ischemic stroke	Hemorrhagic stroke
Fiber , g/d	0.99(0.98,0.99)	0.98(0.97,0.99)	0.99(0.98,0.99)	0.99(0.98,0.99)	0.98(0.97,0.99)	0.99(0.97,1.00)	0.98(0.96,0.99)	0.98(0.97,1.00)	0.95(0.92,0.99)
Energy expenditure ,Kcal/d	1.00(1.00,1.00)	1.00(0.99,1.00)	1.00(0.99,1.00)	1.00(1.00,1.00)	1.00(0.10,1.00)	0.99(0.10,1.00)	1.00(0.99,1.00)	1.00(0.99,1.00)	1.00(0.99,1.00)
Total serum n-3 , %	0.99(0.96,1.03)	0.96(0.90,1.01)	0.99(0.94,1.04)	0.99(0.95,1.03)	0.97(0.90,1.05)	0.97(0.89,1.06)	0.960.88,1.05)	0.98(0.89,1.08)	0.86(0.67,1.08)
magnesium supplement	1.58(0.51,4.91)	1.50(0.211,10.70)	0.90(0.13, 6.42)	1.42(0.35,5.69)	2.24(0.31,16.01)	3.15(0.44,22.55)	0.05(0.00,501072.31)	0.05(0.00,1807947.25)	0.05(0.00,4.27)
Date of examination ,years	0.96(0.93,0.99)	0.94(0.89,0.99)	0.92(0.88,0.96)	0.93(0.90,0.97)	0.94(0.87,1.00)	0.95(0.87,1.02)	0.98(0.90,1.06)	0.98(0.89,1.07)	0.97(0.81,1.16)
Smoking	1.29(1.16,1.44)	1.96(1.67,2.34)	1.49(1.29,1.74)	1.29(1.13,1.49)	1.94(1.56,2.41)	2.01(1.55,2.61)	1.24(0.94,1.64)	1.15(0.84,1.57)	1.69(0.94,3.02)
Diabetes	2.07(1.71,2.51)	3.21(2.47,4.18)	3.00(2.38,3.78)	2.60(2.08,3.25)	3.65(2.69,4.95)	3.07(2.09,4.50)	2.781.87,4.13)	3.11(2.04,4.74)	1.76(0.63,4.91)
elevated blood pressure	1.66(1.50,1.85)	2.56(2.07,3.18)	1.82(1.55, 2.14)	1.91(1.66,2.20)	2.53(1.95,3.28)	3.12(2.24,4.35)	1.89(1.41,2.53)	1.95(1.40,2.71)	1.82(0.96,3.45)
CVD in family	1.29(1.12,1.47)	1.13(0.89,1.44)	1.25(1.02, 1.53)	1.43(1.19,1.72)	1.07(0.80,1.43)	1.18(0.83,1.69)	1.37(0.94,2.00)	1.19(0.79,1.78)	2.36(0.85,6.56)
Diuretics	2.05(1.72,2.44)	2.03(1.53,2.68)	2.08(1.65,2.64)	2.27(1.84,2.79)	1.98(1.40,2.78)	1.91(1.26,2.89)	1.57(1.00,2.47)	1.79(1.12,2.89)	0.68(1.65,2.80)
Aspirin medication	1.39(1.14,1.70)	1.46(1.05,2.03)	1.41(1.08, 1.86)	1.66(1.32,2.11)	1.52(1.03,2.26)	1.51(0.95,2.42)	1.37(0.84,2.25)	1.14(0.82,2.44)	1.13(0.35,3.63)

5.6 Association of serum magnesium with all CVD outcomes

5.6.1 Association with CVD and CVD death

From the previous analysis LDL, fiber, aspirin medication, date of examination, alcohol intake, diabetes were found to be the potential confounders for CVD incidence and CVD mortality. Based on this result multivariate *Cox* hazard analysis was done for both CVD and CVD death adjusting for potential confounders.

Table 6: Hazard ratio of CVD incidence (n =1582) by total serum magnesium concentration, both adjusted and unadjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*-value**
Serum magnesium (mg/l)	0.98 (0.95, 1.02)	0.36
Diabetes (yes, no)	2.07 (1.71, 2.51)	<0.001
LDL-chol (mmol/l)	1.15 (1.10, 1.21)	< 0.001
Fiber (g/d)	0.99 (0.98, 0.10)	0.002
Aspirin medication (yes, no)	1.41 (1.16, 1.72)	0.001
Date of examination (year)	0.97 (0.94, 1.00)	0.11
Alcohol /week (g/d)	1.00 (1.00, 1.00)	0.16
Unadjusted serum magnesium (mg/l)	0.99 (0.95, 1.02)	0.41

Table 7: Hazard ratio of CVD death (n = 491) by total serum magnesium concentration, both adjusted and unadjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	0.91 (0.85, 0.96)	0.001
Diabetes (yes, no)	2.98 (2.27, 3.89)	<0.001
LDL-chol (mmol/l)	1.16 (1.07, 1.27)	0.001
Fiber (g/d)	0.98 (0.97, 0.99)	0.005
Aspirin medication (yes, no)	1.48 (1.06, 2.07)	0.02
Date of examination (year)	0.94 (0.88, 0.10)	0.04
Alcohol /week (g/d)	1.00 (1.00, 1.00)	<0.001
Unadjusted serum magnesium (mg/l)	0.89 (0.84, 0.94)	<0.001

Q1, Q2, Q3, Q4: fourths of serum magnesium concentration

We found that with an increase in a unit serum magnesium concentration, risk of CVD death decreased by 9% (*HR*: 0.91, 95% CI: 0.85, 0.96) after adjusting for all other confounders. The result was almost the same also for unadjusted serum magnesium concentration.

Both adjusted and unadjusted serum magnesium concentration were not found to have association with CVD incidence. However diabetes was strongly associated with both CVD incidence and death.

Table 8: Hazard ratio of CVD incidence (n = 1582) by quartiles of serum magnesium concentration, adjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*- value**
Q1(<18.85 mg/l)	1 (ref)	
Q2 (18.86-19.85 mg/l)	0.89 (0.77, 1.03)	0.11
Q3 (19.86-20.87 mg/l)	0.99 (0.86, 1.14)	0.89
Q4 (>20.88 mg/l)	0.96 (0.83, 1.10)	0.54
Diabetes (yes, no)	2.08 (1.71, 2.52)	<0.001
LDL-chol (mmol/l)	1.15 (1.10, 1.21)	<0.001
Fiber (g/d)	0.99 (0.98, 0.10)	0.002
Aspirin medication (yes, no)	1.41 (1.16, 1.72)	0.001
Date of examination (year)	0.98 (0.95, 1.00)	0.16
Alcohol /week (g/d)	1.00 (1.00, 1.00)	0.15

Q1, Q2, Q3, Q4: fourths of serum magnesium concentration

Table 9: Hazard ratio of CVD death (n = 491) by quartiles of serum magnesium concentration, adjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*- value**
Q1(<18.85mg/l)	1 (ref)	
Q2 (18.86-19.85mg/l)	0.74 (0.57, 0.94)	0.02
Q3 (19.86-20.87mg/l)	0.75 (0.59, 0.97)	0.03
Q4 (>20.88mg/l)	0.74 (0.57, 0.95)	0.02
Diabetes (yes, no)	3.02 (2.31, 3.95)	<0.001
LDL-chol (mmol/l)	1.16 (1.06, 1.26)	0.001
Fiber (g/d)	0.98 (0.97, 0.99)	0.005
Aspirin medication (yes, no)	1.51 (1.08, 2.11)	0.01
Date of examination (year)	0.95 (0.89, 1.00)	0.06
Alcohol /week (g/d)	1.00 (1.00, 1.00)	<0.001

Q1, Q2, Q3, Q4: fourths of serum magnesium concentration

All quartiles of serum magnesium concentration were significantly inversely associated with CVD death. Men in the highest quartile of serum magnesium concentration had the lowest risk (*HR*: 0.74, 95% CI: 0.57, 0.95) of CVD death compared to the men in the lowest quartile. LDL, fiber, aspirin medication, date of examination, diabetes were all found to be associated with CVD mortality.

After adjusting for confounders, quartiles of serum magnesium were not associated with the risk of CVD incidence.

5.6.2 Association with CHD incidence and mortality

From the above analysis LDL, aspirin medications, date of examination year, diabetes were found to be the confounders of both CHD incidence and CHD death. Whereas fiber was found to be the confounding variable for only CHD incidence and alcohol intake was found to be the confounding variable for CHD death

Table 10: Hazard ratio of CHD incidence (n = 930) by serum magnesium concentration, both adjusted and unadjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	1.00 (0.96, 1.04)	0.95
Diabetes (yes, no)	2.65 (2.12, 3.33)	<0.001
LDL-Chol (mmol/l)	1.28 (1.21, 1.37)	<0.001
Aspirin medication (yes, no)	1.74 (1.37, 2.21)	<0.001
Fiber (g/d)	0.99 (0.98, 1.00)	0.06
Date of examination (year)	0.96 (0.92, 1.00)	0.06
Unadjusted serum magnesium (mg/l)	1 (0.96, 1.04)	0.94

Table 11: Hazard ratio of CHD death (n = 332) by serum magnesium concentration, both adjusted and unadjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	0.91 (0.85, 0.98)	0.01
Diabetes (yes, no)	3.43 (2.51, 4.69)	<0.001
LDL-Chol (mmol/l)	1.25 (1.13, 1.39)	<0.001
Aspirin medication (yes, no)	1.57 (1.05, 2.33)	0.03
Alcohol intake (g/d)	1.00 (1.00, 1.00)	0.003
Date of examination (year)	0.95 (0.88, 1.02)	0.13
Unadjusted serum magnesium (mg/l)	0.89 (0.83, 0.95)	0.001

Total serum magnesium concentration was strongly associated with CHD mortality but was not found to be associated with CHD incidence after adjusting for potential confounders. The findings were similar also for unadjusted serum magnesium concentration. Increase in serum magnesium concentration by 1 unit, decreased the risk of CHD death by 9% after adjusting for all the potential confounders. All other confounders except date of examination were found to be strongly associated with CHD incidence and death.

Table 12: Hazard ratio for CHD incidence (n = 930) by quartiles of serum magnesium, adjusted for confounders

Variables (unit)	***HR* (95%CI)**	***P*- value**
Q1 (<18.85mg/l)	1 (ref)	
Q2 (18.86-19.85mg/l)	0.86 (0.72, 1.04)	0.13
Q3 (19.86-20.87mg/l)	1.06 (0.88, 1.27)	0.55
Q4 (>20.88mg/l)	0.95 (0.79, 1.14)	0.59
Diabetes (yes, no)	2.65 (2.11, 3.32)	<0.001
LDL-Chol (mmol/l)	1.29 (1.21, 1.37)	<0.001
Aspirin medication (yes, no)	1.74 (1.37, 2.21)	<0.001
Date of examination (year)	0.96 (0.92, 1.00)	0.08
Fiber (g/d)	0.99 (0.98, 1.00)	0.06

Q1, Q2, Q3, Q4: fourths of serum magnesium concentration

Table 13: Hazard ratio for CHD death (n = 332) by quartiles of serum magnesium, adjusted for confounders

Variables (unit)	***HR* (95%CI)**	***P*- value**
Q1 (<18.85mg/l)	1	
Q2 (18.86-19.85mg/l)	0.71(0.53, 0.96)	0.03
Q3 (19.86-20.87mg/l)	0.70 (0.52, 0.95)	0.02
Q4 (>20.88mg/l)	0.72 (0.53, 0.97)	0.03
Diabetes (yes, no)	3.44 (2.52, 4.70)	<0.001
LDL-Chol (mmol/l)	1.25 (1.13, 1.39)	<0.001
Aspirin medication (yes, no)	1.6 (1.07, 2.37)	0.02
Alcohol intake/week (g/d)	1.00 (1.00, 1.00)	0.001
Date of examination (year)	0.95 (0.88, 1.02)	0.17

Q1, Q2, Q3, Q4: fourths of serum magnesium concentration

All the fourths of serum magnesium concentration were strongly associated with CHD death after adjusting for all the confounders. However the risk of death due to CHD did not vary much among the quartiles of serum magnesium concentration. Fourths of serum magnesium concentration were not found to be associated with CHD incidence.

5.6.3 Association with AMI

From the previous analysis LDL cholesterol, fiber, aspirin medication, date of examination year, and diabetes were derived as the confounders for AMI.

Table 14: Hazard ratio of AMI (n = 732) by total serum magnesium concentration, both adjusted and unadjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	1.00 (0.95, 1.04)	0.88
Diabetes (yes, no)	3.1 (2.45, 3.92)	<0.001
LDL-chol (mmol/l)	1.33 (1.24, 1.43)	<0.001
Fibre (g/d)	0.99 (0.98, 1.00)	0.03
Aspirin medication (yes, no)	1.48 (1.12, 1.95)	0.005
Date of examination (year)	0.96 (0.92, 1.01)	0.09
Unadjusted serum magnesium (mg/l)	0.99 (0.95, 1.04)	0.83

Total serum magnesium concentration when unadjusted was not found to be associated with risk of AMI. The result was same also when adjusted for its confounders. Instead all other confounders except date of examination were found to be strongly associated with AMI.

Table 15: Hazard ratio of AMI (n = 732) by quartiles of serum magnesium concentration, adjusted for confounders

Variables (unit)	***HR* (95%CI)**	***P*- value**
Q1 (<18.85mg/l)	1 (ref)	
Q2 (18.86-19.85mg/l)	0.79 (0.64, 0.98)	0.03
Q3 (19.86-20.87mg/l)	1.01 (0.83, 1.24)	0.90
Q4 (>20.88mg/l)	0.96 (0.78, 1.18)	0.69
Diabetes (yes, no)	3.07 (2..42, 3.88)	<0.001
LDL-chol (mmol/l)	1.33 (1.24, 1.43)	<0.001
Fiber (g/d)	0.99 (0.98, 1.0)	0.03
Aspirin medication (yes, no)	1.49 (1.13, 1.96)	0.005
Date of examination (year)	0.96 (0.92, 1.01)	0.13

Q1, Q2, Q3, Q4: fourths of serum magnesium concentration

Serum magnesium only in the seconds, comparing to the lowest quartile was associated with risk of AMI. There was 21% decrease in the risk of AMI among men belonging to second quartile than in the lowest quartile of serum magnesium concentration after adjusting for fiber, aspirin medication and date of examination year, diabetes. Adjusted LDL (*HR*: 1.33; 95% CI: 1.24, 1.43), diabetes (*HR*: 3.07, 95% CI: 2.42, 3.88) and aspirin medication (*HR*: 1.49, 95% CI: 1.13, 1.96) were found to increase the risk of occurrence of AMI.

5.6.4 Association with SCD or SCA

LDL, fiber, aspirin medication, alcohol intake, diabetes were found to be the potential confounders for sudden cardiac death or sudden cardiac arrest from the various analyses done above.

Table 16: Hazard ratio SCD24 or cardiac arrest (n = 232) by total serum magnesium concentration, both adjusted and unadjusted for confounders

Variables	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	0.91 (0.84, 0.99)	0.13
Diabetes (yes, no)	3.00 (2.03, 4.44)	<0.00
LDL-chol (mmol/l)	1.24 (1.09, 1.39)	<0.001
Fiber (g/d)	0.99 (0.97, 1.01)	0.31
Aspirin medication (yes, no)	1.46 (0.91, 2.34)	0.08
Alcohol /week (g/d)	1.00 (1.00, 1.00)	0.01
Unadjusted serum magnesium (mg/l)	0.92 (0.84, 0.99)	0.03

Increase in serum magnesium concentration by 1 unit decreased the risk of SCD by 8% when it was not adjusted for it confounder. After further adjustment, total serum magnesium concentration was not found to be associated with SCD24. Also LDL and diabetes were significant risk factors for SCD whereas alcohol intake neither increased nor decreased the risk of SCD.

Table 17: Hazard ratio SCD24 or cardiac arrest (n = 232) by quartiles of serum magnesium concentration, adjusted for confounders

Variables (unit)	***HR* (95% CI)**	***P*- value**
Q1 (<18.85mg/l)	1 (ref)	
Q2 (18.86-19.85mg/l)	0.69 (0.47, 0.99)	0.05
Q3 (19.86-20.87mg/l)	0.82 (0.58, 1.17)	0.28
Q4 (>20.88mg/l)	0.76 (0.53, 1.09)	0.13
Diabetes (yes, no)	2.99 (2.02, 4.42)	<0.001
LDL-chol (mmol/l)	1.24 (1.10, 1.10)	<0.001
Fiber (g/d)	0.99 (0.97, 1.01)	0.31
Aspirin medication (yes, no)	1.54 (0.96, 2.46)	0.07
Alcohol /week (g/d)	1.00 (1.00, 1.00)	0.004

Q1, Q2, Q3, Q4: fourths of serum magnesium concentration

Adjusted multivariate *cox* proportional hazard showed that men in the second quartile (0.69, 95% CI: 0.47, 0.99) of serum magnesium concentration had significant lower risk of SCD compared to the lowest quartile of serum magnesium concentration.

5.6.5 Associations with stroke, ischemic stroke and hemorrhagic stroke

From the above analysis we found that only diabetes was significantly associated with both serum magnesium and outcome variable stroke and ischemic stroke, i.e only diabetes was found as the confounder for both ischemic and hemorrhagic stroke. Fiber was found to be the potential confounder for hemorrhagic stroke.

Table 18: Hazard ratio of stroke (n = 226) by total serum magnesium concentration, both adjusted and unadjusted for confounder

Stroke	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	0.96 (0.88, 1.04)	0.341
Diabetes (yes, no)	2.69 (1.8, 4.02)	<0.001
Unadjusted serum magnesium (mg/l)	0.93 (0.86, 1.02)	0.11

Table 19: Hazard ratio of ischemic stroke (n = 182) by total serum magnesium concentration, both adjusted and unadjusted for confounder

Ischemic stroke	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	0.97 (0.89, 1.07)	0.56
Diabetes (yes, no)	3.04 (1.97, 4.67)	<0.001
Unadjusted serum magnesium (mg/l)	0.94 (0.86, 1.02)	0.22

Table 20: Hazard ratio of hemorrhagic stroke (n = 47) by total serum magnesium concentration, adjusted for confounder

Hemorrhagic stroke	***HR* (95% CI)**	***P*- value**
Serum magnesium (mg/l)	0.90 (0.75, 1.08)	0.28
Fiber (g/d)	0.95 (0.92, 0.99)	0.01
Unadjusted serum magnesium (mg/l)	0.91 (0.76, 1.09)	0.31

There was not found any significant association between total serum magnesium concentration (both adjusted and unadjusted) and the occurrence of stroke, ischemic stroke, and hemorrhagic stroke (Table.18, 19, 20). However the respective confounding variable fiber was found to be associated for slight decrease in the risk of hemorrhagic stroke and diabetes was found to be associated with increase in risk of stroke and ischemic stroke after adjustment of serum magnesium

Table 21: Hazard ratio of stroke (n = 226) by quartiles of serum magnesium, adjusted for confounder

Variables (unit)	***HR* (95% CI)**	***P*- value**
Q1(<18.85mg/l)	1 (ref)	
Q2 (18.86-19.85mg/l)	0.89 (0.62, 1.29)	0.55
Q3 (19.86-20.87mg/l)	1.08 (0.75, 1.55)	0.67
Q4 (>20.88mg/l)	0.86 (0.59, 1.25)	0.43
Diabetes (yes, no)	2.75 (1.84, 4.11)	<0.001

Table 22: Hazard ratio of ischemic stroke (n = 182) by quartiles of serum magnesium, adjusted for confounder

Variables (unit)	***HR* (95% CI)**	***P*- value**
Q1(<18.85mg/l)	1 (ref)	
Q2 (18.86-19.85mg/l)	1.0 (0.66, 1.50)	0.99
Q3 (19.86-20.87mg/l)	1.08 (0.72, 1.62)	0.72
Q4 (>20.88mg/l)	0.88 (0.58, 1.35)	0.57
Diabetes (yes, no)	3.09 (2.01, 4.75)	<0.001

Table 23: Hazard ratio of hemorrhagic stroke (n = 47) by quartiles of serum magnesium, adjusted for confounder

Variables (unit)	***HR* (95% CI)**	***P*- value**
Q1(<18.85mg/l)	1 (ref)	
Q2 (18.86-19.85mg/l)	0.78 (0.34, 1.81)	0.56
Q3 (19.86-20.87mg/l)	1.18 (0.55, 2.52)	0.67
Q4 (>20.88mg/l)	0.75 (0.32, 1.74)	0.50
Fiber (g/d)	0.95 (0.91, 0.99)	0.01

There was not any association between quartiles of serum magnesium concentration and stroke, ischemic stroke and hemorrhagic stroke after adjusting for confounders.

6 DISCUSSION

In this prospective cohort of Finnish men, change in total serum magnesium concentration by a unit, was associated with lower risk of CVD, CHD and SCD mortality when unadjusted. But after adjusting for the potential confounders, total serum magnesium concentration was found to be associated only with CVD and CHD mortality. When compared to the quartiles of serum magnesium concentration, highest serum magnesium concentration was very strongly associated with the lower risk of CVD and CHD mortality. Men with highest serum magnesium concentration had 26% lower risk of CVD death and 28% lower risk of CHD death after adjusting for its potential confounders. Total serum magnesium concentration was not associated with non-fatal CVD, CHD and AMI after controlling for their risk factors. But there was found significant decrease in risk of non-fatal AMI and fatal SCD in the second quartile compared to the lowest quartile of serum magnesium concentration. Serum magnesium concentration was not found to be associated with stroke, ischemic stroke, and hemorrhagic stroke at any condition.

There are conflicting results in many previous studies regarding the association between magnesium and all the outcome events. There are many studies based on dietary magnesium intake which has either gone against or in a support of our study findings (Guasch-Ferre *et al*. 2014; Stevanovic *et al*. 2011; Al-Delaimy *et al*. 2004). These studies based on dietary measures might be influenced by residual confounding or recall bias. And also diet or food rich in magnesium may contain other nutrient factors which might have influenced the CVD outcomes and its risk factors. There are only few studies which have examined the association between serum magnesium and CVD events. Similar to our result serum magnesium was not found to be associated with CVD (Khan *et al*. 2010). A prospective study showed no significant association with serum magnesium concentration and stroke after adjusting for potential confounders (Ohira *et al*. 2009). Consistent with other studies result there was not found any association between serum magnesium concentration and stroke after adjusting for potential confounders. In our study there was seen significant lower risk of SCD when unadjusted but when it was adjusted for confounders there was not seen any association between total serum magnesium concentration and SCD. When divided into fourths there was

seen a significant lowest risk of SCD in the second lowest quartile of serum magnesium concentration. Though the result supported the finding of other studies that serum magnesium is associated with lower risk of SCD it did not support other studies finding stating that there is the lowest risk of SCD in the highest serum magnesium concentration (Chiuve *et al*. 2011; peacock *et al*. 2010). Similar to other studies serum magnesium concentration was strongly associated with fatal CHD but not with CHD incidence (Ford 1999; Chiuve *et al*. 2013). In contrast few studies have shown no effect of magnesium on CHD. Similar to serum magnesium concentration many studies showed the association between dietary intake of magnesium and risk of cardiovascular events (Lian *et al*. 2011; Al-Delaimy *et al*. 2004).

Magnesium is an intracellular cation, so serum magnesium may not represent the total body magnesium stores (Jahnen-Dechent and Ketteler 2012). However measurement of serum magnesium is the easiest, cheapest and most common laboratory measurement and also serum magnesium concentration can be correlated with the intracellular magnesium.

Magnesium affects the movement of sodium, potassium and calcium (Swaminathan 2003), which may lead to affect in the cardiovascular system resulting in increased risk. However in our study there was not seen any significant correlation between serum magnesium concentration and calcium and potassium. Magnesium and many CVD outcomes were found to be associated with hypertension, diabetes, and alcohol (Ma *et al*. 1995; Romani 2008). Thus one of the important finding of this study was the exploration of factors that might affect the association between magnesium and cardiovascular events. We found that alcohol intake, fiber, aspirin medication, date of examination, diabetes were statistically affecting both the serum magnesium concentration and CVD mortality. Diabetes was one of the variables which was found to be strongly associated with the change in magnesium level as renal magnesium wasting and osmotic diuresis causes magnesium depletion. In contrast magnesium depletion also affects insulin secretion and insulin resistance (Takaya *et al.* 2004). Also diabetes independently was a very strong factor predicting almost 200% increased risk of CVD outcomes except hemorrhagic stroke. However even after adjusting for these potential confounders, serum magnesium remained strongly associated with CVD mortality. There was no association between total serum magnesium concentration and CVD after adjustment for all

the biomarkers. These results suggest that magnesium can protect fatal CVD. Clinical trials also had shown both protective and no effect of magnesium infusion in patient to prevent from arrhythmias (Roffe *et al*.1994; Gyamlani *et al*. 2000). Thus further clinical trials are needed to know antithrombotic effect of magnesium to prevent AMI.

7 STRENGTHS AND LIMITATIONS OF THE STUDY

The strength of this study is that it is a prospective population based study that includes reliable sample size at baseline and follow up. We had reliable definitions of all the outcome variables as they were based on ICD-10 diagnoses. The outcomes were based on the diagnosis available from the National Hospital Discharge Registry, the National Causes of Death Registry and FINMONICA registry which are very reliable sources of information. The level of magnesium was based on the measurement of serum magnesium concentration present in the body. The method used to measure serum magnesium concentration was atomic absorption spectrometry. Hence it has high level of accuracy.

The study also has few limitations like the study only includes male population so it cannot be generalized to women. Serum magnesium represents very small part of total body magnesium so it may not reflect if there is really a deficiency of magnesium in our body which is increasing or decreasing the risk of CVD events.

8 CONCLUSION

In conclusion there was strong relation between serum magnesium concentration and CVD, CHD mortality. Increase in serum magnesium concentration decreased the occurrence of fatal CVD and CHD. Total serum magnesium concentration was found to be associated with SCD only when it was unadjusted. However total serum magnesium concentrations was not found to be associated with incidence of CVD, CHD, AMI and stroke risk both before and after adjusting for its potential confounders. But serum magnesium concentration when divided into

fourths showed significant decrease in risk in the second quartile of AMI incidence and SCD and in all fourths of fatal CVD and CHD when compared to the lowest quartile.

However, further studies including clinical and randomized controlled trials are needed to clearly state the importance of magnesium and to recommend magnesium rich food stating its protective effect for cardiovascular outcomes.

9 REFERENCES

Adamopoulos C, Pitt B, Ahmed A. Low serum magnesium and cardiovascular mortality in chronic heart failure: a propensity-matched study. International Journal of Cardiology 2009;136(3):270-277.

Al-Delaimy WK, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Magnesium intake and risk of coronary heart disease among men. J Am Coll Nutr 2004 Feb;23(1):63-70.

Altura BT, Altura BM. Measurement of ionized magnesium in whole blood, plasma and serum with a new ion-selective electrode in healthy and diseased human subjects. Magnes Trace Elem 1991 -1992;10(2-4):90-98.

Ascherio A, Rimm EB, Hernan MA, Giovannucci EL, Kawachi I, Stampfer MJ, *et al*. Intake of potassium, magnesium, calcium, and fiber and risk of stroke among US men. Circulation 1998 Sep 22;98(12):1198-1204.

Assaadi F. Hypomagnesia:an evidence based approach to clinical cases. Iranian Journal of Kidney disease 2010; 4(1):13-19.

Cardiovascular diseases: Fact sheet. World Health Organisation. 2011 http://www.who.int/mediacentre/factsheets/fs317/en/index.html accessed on 20th may 2012)

Causes of Death in 2012. Statistic Finland. Accessed on 3rd Jan 2014. Available at http://www.stat.fi/til/ksyyt/2012/ksyyt_2012_2013-12-30_kat_001_en.html

Chakraborti S, Chakraborti T, Mandal M, Mandal A, Das A, Ghosh S. Protective role of magnesium in cardiovascular disease: a review. Molecular and Cellular Biochemistry 2002;238:163-179.

Chiuve SE, Korngold EC, Januzzi JL,Jr, Gantzer ML, Albert CM. Plasma and dietary magnesium and risk of sudden cardiac death in women. Am J Clin Nutr 2011 Feb;93(2):253-260.

Chiuve SE, Sun Q, Curhan GC, Taylor EN, Spiegelman D, Willett WC, *et al*. Dietary and plasma magnesium and risk of coronary heart disease among women. J Am Heart Assoc 2013 Mar 18;2(2):e000114.

Corsonello A, Ientile R, Buemi M, Cucinotta D, Mauro VN ,Macasone S, Corica F. Serum ionized magnesium levels in type 2 diabetic patients with microalbuminuria or clinical proteinuria. American journal of Nephrology 2000;20(3):187-192.

Cunnane SC, Soma M, McAdoo KR, Horrobin DF. Magnesium deficiency in the rat increases tissue levels of docosahexaenoic acid. J Nutr 1985 Nov;115(11):1498-1503.

Curiel-Garcia JA, Rodriguez-Moran M, Guerrero-Romero F. Hypomagnesemia and mortality in patients with type 2 diabetes. Magnes Res 2008 Sep;21(3):163-166.

Dai LJ,Ritchie G, Kerstan D, Kang HS, Cole DEC, Quamme GA.Magnesium Transport in the Renal Distal Convoluted Tubule.Physiological reviews 2001;81(1):51-84.

Dasgupta A, Sarma D, Saikia UK. Hypomagnesemia in type 2 diabetes mellitus. Indian J Endocrinol Metab 2012 Nov;16(6):1000-1003.

de Baaij JHF, Hoenderop JGJ, Bindels RJM. Regulation of magnesium balance:lessons learned from human genetic disease. Clinical Kidney Journey 2012;5(1):i15-i24.

De Francisco ALM, Rodriguez M. Magnesium - its role in CKD.Nefrologia 2013;33(3):389-399.

Eilat-Adar S,Sinai T, Yosefy C, Henkin Y. Nutritional Recommendations for Cardiovascular Disease Prevention. Nutrients 2013;5:3646-3683

Elin RJ. Assessment of magnesium status. Clinical Chemistry1987;33(11):1965-70.

Fawcett WJ, Haxby EJ, Male DA. Magnesium: physiology and pharmacology. Br J Anaesth 1999 Aug;83(2):302-320.

Fine KD, Santa Ana CA, Porter JL, Fordtran JS. Intestinal absorption of Magnesium from Food and supplements. The Journal of Clinical Investigation 1991;88(2):396-402.

Ford ES. Serum magnesium and ischaemic heart disease: findings from a national sample of US adults. Int J Epidemiol 1999 Aug;28(4):645-651.

Gallagher CM, Black LJ, Oddy WH. Micronutrient intakes from food and supplements in Australian adolescents. Nutrients 2014 Jan 14;6(1):342-354.

Guasch-Ferre M, Bullo M, Estruch R, Corella D, Martinez-Gonzalez MA, Ros E, *et al*. Dietary magnesium intake is inversely associated with mortality in adults at high cardiovascular disease risk. J Nutr 2014 Jan;144(1):55-60.

Gyamlani G, Parikh C, Kulkarni AG. Benefits of magnesium in acute myocardial infarction: timing is crucial. Am Heart J 2000 Apr;139(4):703.

Hruby A, O'Donnell CJ, Jacques PF, Meigs JB, Hoffmann U, McKeown NM. Magnesium intake is inversely associated with coronary artery calcification: the Framingham Heart Study. JACC Cardiovasc Imaging 2014 Jan;7(1):59-69.

Huijgen HJ, Sanders R, van Olden RW, Klous MG, Gaffar FR, Sanders GT. Intracellular and extracellular blood magnesium fractions in hemodialysis patients; is the ionized fraction a measure of magnesium excess? Clin Chem 1998 Mar;44(3):639-648.

Iso H, Stampfer MJ, Manson JE, Rexrode K, Hennekens CH, Colditz GA, *et al*. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. Stroke 1999 Sep;30(9):1772-1779.

Jahnen-Dechent W, Ketteler M. Magnesium Basics. Clinical Kidney Journal 2012;5:i3-i14.

Joosten MM, Gansevoort RT, Mukamal KJ, van der Harst P, Geleijnse JM, Feskens EJ, *et al*. Urinary and plasma magnesium and risk of ischemic heart disease. Am J Clin Nutr 2013 Jun;97(6):1299-1306.

Kaluza J, Orsini N, Levitan EB, Brzozowska A, Roszkowski W, Wolk A. Dietary calcium and magnesium intake and mortality: a prospective study of men. Am J Epidemiol 2010 Apr 1;171(7):801-807.

Khan AM, Lubitz SA, Sullivan LM, Sun JX, Levy D, Vasan RS, *et al*. Low serum magnesium and the development of atrial fibrillation in the community: the Framingham Heart Study. Circulation 2013 Jan 1;127(1):33-38.

Khan AM, Sullivan L, McCabe E, Levy D, Vasan RS, Wang TJ. Lack of association between serum magnesium and the risks of hypertension and cardiovascular disease. Am Heart J 2010 Oct;160(4):715-720.

Khatami MR, Mirchi E, Khazaeipour Z, Abdollahi A, Jahanmardi A. Association between serum magnesium and risk factors of cardiovascular disease in hemodialysis patients. Iran J Kidney Dis 2013 Jan; 7(1):47-52.

King JL, Miller RJ, Blue JP,Jr, O'Brien WD,Jr, Erdman JW,Jr. Inadequate dietary magnesium intake increases atherosclerotic plaque development in rabbits. Nutr Res 2009 May;29(5):343-349.

Koch SM, Warters RD, Mehlhorn U. The simultaneous measurement of ionized and total calcium and ionized and total magnesium in intensive care unit patients. J Crit Care 2002 Sep;17(3):203-205.

Kousa A, Havulinna AS, Moltchanova E, Taskinen O, Nikkarinen M, Eriksson J, *et al*. Calcium:magnesium ratio in local groundwater and incidence of acute myocardial infarction among males in rural Finland. Environ Health Perspect 2006 May;114(5):730-734.

Kozielec P, Kotkowiak L, Pozniak J, Salacka A, Hornowska I, Brodowski J, *et al*. Assessment of serum ionized magnesium levels in healthy volunteers, in patients with coronary artery disease and/or hypertension and in hypertension alone. Magnes Res 2005 Dec;18(4):241-244.

Krasner BS, Girdwood R, Smith H. The effect of slow releasing oral magnesium chloride on the QTc interval of the electrocardiogram during open heart surgery. Can Anaesth Soc J 1981 Jul;28(4):329-333.

Kurl S, laukkanen JA, Niskanen L, Laaksonen D, Sivenius J, Nyyssönen, Salonen JT. Metabolic Syndromme and the Risk of Stroke in Middle-Aged Men.Stroke 2006; 37:806-811.

Laatikainen T, Critchley J, Vartiainen E, Salomaa V, Ketonen M, Capewell S. Explaining the decline in coronary heart disease mortality in Finland between 1982 and 1997. Am J Epidemiol 2005 Oct 15;162(8):764-773.

Lakka TA, Venalainen JM, Rauramaa R, Salonen R, Tuomilehto J, Salonen JT. Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction. N Engl J Med 1994 Jun 2;330(22):1549-1554.

Larsson SC, Virtamo J, Wolk A. Potassium, calcium, and magnesium intakes and risk of stroke in women. Am J Epidemiol 2011 Jul 1;174(1):35-43.

Larsson SC, Virtanen MJ, Mars M, Mannisto S, Pietinen P, Albanes D, *et al*. Magnesium, calcium, potassium, and sodium intakes and risk of stroke in male smokers. Arch Intern Med 2008 Mar 10;168(5):459-465.

Lecube A, Baena-Fustegueras JA, Fort JM, Pelegri D, Hernandez C, Simo R. Diabetes is the main factor accounting for hypomagnesemia in obese subjects. PLoS One 2012;7(1):e30599.

Leone N, Courbon D, Ducimetiere P, Zureik M. Zinc, copper, and magnesium and risks for all-cause, cancer, and cardiovascular mortality. Epidemiology 2006 May;17(3):308-314.

Leurs LJ, Schouten LJ, Mons MN, Goldbohm RA, van den Brandt PA. Relationship between tap water hardness, magnesium, and calcium concentration and mortality due to ischemic heart disease or stroke in The Netherlands. Environ Health Perspect 2010 Mar;118(3):414-420.

Liang W, Lee AH, Binns CW. Dietary intake of minerals and the risk of ischemic stroke in Guangdong Province, China, 2007-2008. Prev Chronic Dis 2011 Mar;8(2):A38.

Liao F, Folsom AR, Brancati FL. Is low magnesium concentration a risk factor for coronary heart disease? The Atherosclerosis Risk in Communities (ARIC) Study. Am Heart J 1998 Sep;136(3):480-490.

Ma J, Folsom AR, Melnick SL, Eckfeldt JH, Sharrett AR, Nabulsi AA, *et al*. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. J Clin Epidemiol 1995 Jul;48(7):927-940.

Maguire ME, Cowan JA.Magnesium chemistry and biochemistry.Biometals2002;15:203-210
Mahalle N, Kulkarni MV, Naik SS. Is hypomagnesaemia a coronary risk factor among Indians with coronary artery disease? J Cardiovasc Dis Res 2012 Oct;3(4):280-286.

Mirzaeian S, Ghiasvand R, Sadeghian F, Sheikhi M, Khosravi ZS, Askari G, *et al*. Assessing the micronutrient and macronutrient intakes in female students and comparing them with the set standard values. J Educ Health Promot 2013 Jan 31;2:1-9531.106636. eCollection 2013.

Muir KW, Lees KR. A randomized, double-blind, placebo-controlled pilot trial of intravenous magnesium sulfate in acute stroke. Stroke 1995 Jul;26(7):1183-1188.

Nielsen FH. The alteration of magnesium,calcium and phosphorus metabolism by dietary magnesium deprivation in postmenopausal women is not affected by dietary boron deprivation. Magnesium Research 2004;17(3):197-210.

Noronha JL, Matuschak GM. Magnesium in critical illness: metabolism, assessment, and treatment. Intensive Care Med 2002 Jun;28(6):667-679.

O Flaherty M, Flores-Mateo G, Nnoaham K, Lloyd-Williams F, Capewell S. Potential cardiovascular mortality reductions with stricter food policies in the United Kingdom of Great Britain and Northern Ireland. Bull World Health Organ 2012 Jul 1;90(7):522-531.

Ohira T, Peacock JM, Iso H, Chambless LE, Rosamond WD, Folsom AR. Serum and dietary magnesium and risk of ischemic stroke: the Atherosclerosis Risk in Communities Study. Am J Epidemiol 2009 Jun 15;169(12):1437-1444.

Panico S, Mattiello A, Panico C, Chiodini P. Mediterranean dietary pattern and chronic diseases. Cancer Treat Res 2014;159:69-81.

Peacock JM, Ohira T, Post W, Sotoodehnia N, Rosamond W, Folsom AR. Serum magnesium and risk of sudden cardiac death in the Atherosclerosis Risk in Communities (ARIC) Study. Am Heart J 2010 Sep;160(3):464-470.

Ramasamy R, Murugaiyan SB, Gopal N, Shalini R. The prospect of serum magnesium and an electrolyte panel as an adjuvant cardiac biomarker in the management of acute myocardial infarction. J Clin Diagn Res 2013 May;7(5):817-820.

Ravn HB, Kristensen SD, Hjortdal VE, Thygesen K, Husted SE. Early administration of intravenous magnesium inhibits arterial thrombus formation. Arterioscler Thromb Vasc Biol 1997 Dec;17(12):3620-3625.

Reffelmann T, Ittermann T, Dorr M, Volzke H, Reinthaler M, Petersmann A, *et al*. Low serum magnesium concentrations predict cardiovascular and all-cause mortality. Atherosclerosis 2011 Nov;219(1):280-284.

Roffe C, Fletcher S, Woods KL. Investigation of the effects of intravenous magnesium sulphate on cardiac rhythm in acute myocardial infarction. Br Heart J 1994 Feb;71(2):141-145.

Romani AM. Magnesium homeostasis and alcohol consumption. Magnes Res 2008 Dec;21(4):197-204.

Romani AMP. Cellular magnesium Homeostasis. Archives of Biochemistry and Biophysics 2011;512(1):1-23

S P, Pasula S, Sameera K. Trace elements in diabetes mellitus. J Clin Diagn Res 2013 Sep;7(9):1863-1865.

Saaranen M, Suistomaa U, Kantola M, Saarikoski S, Vanha-Perttula T. Lead, magnesium, selenium and zinc in human seminal fluid: comparison with semen parameters and fertility. Hum Reprod 1987 Aug;2(6):475-479.

Sakaguchi Y, Fujii N, Shoji T, Hayashi T, Rakugi H, Isaka Y. Hypomagnesemia is a significant predictor of cardiovascular and non-cardiovascular mortality in patients undergoing hemodialysis. Kidney Int 2014 Jan;85(1):174-181.

Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992 Sep;86(3):803-811.

Salonen JT, Salonen R, Seppanen K, Rauramaa R, Tuomilehto J. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. Circulation 1991 Jul;84(1):129-139.

Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. Clin Chim Acta 2000 Apr;294(1-2):1-26.

Scarborough P, Morgan RD, Webster P, Rayner M. Differences in coronary heart disease, stroke and cancer mortality rates between England, Wales, Scotland and Northern Ireland: the role of diet and nutrition. BMJ Open 2011 Nov 3;1(1):e000263-2011-000263.

Seelig MS. Electrographic patterns of magnesium depletion appearing in alcoholic heart disease. Ann N Y Acad Sci 1969 Aug 15;162(2):906-917.

Shils ME, Shike M, Ross AC, Caballero B,Cousins RJ. Modern Nutrition in Health and Disease. 10th edition. Lippincot williams and wilkins; 2006;p.223-247.

Song Y, Manson JE, Cook NR, Albert CM, Buring JE, Liu S. Dietary magnesium intake and risk of cardiovascular disease among women. Am J Cardiol 2005 Oct 15;96(8):1135-1141.

Spiegel DM. Magnesium in chronic kidney disease: unanswered questions. Blood Purif 2011;31(1-3):172-176.

Stasiukyniene V. Blood plasma potassium, sodium and magnesium levels in chronic alcoholism during alcohol withdrawal. Medicina (Kaunas) 2002;38(9):892-895.

Stevanovic S, Nikolic M, Stankovic A. Dietary magnesium intake and coronary heart disease risk: a study from Serbia. Med Glas (Zenica) 2011 Aug;8(2):203-208.

Swaminathan R. Magnesium metabolism and its disorders. Clin Biochem Rev 2003 May;24(2):47-66.

Takaya J, Higashino H, Kobayashi Y. Intracellular magnesium and insulin resistance. Magnes Res 2004 Jun;17(2):126-136.

Van den Bergh WM, Dijkhuizen RM, Rinkel GJ. Potentials of magnesium treatment in subarachnoid haemorrhage. Magnes Res 2004 Dec;17(4 Nutritional recommendation for cardiovascular disease prevention):301-313.

Virtanen JK, Voutilainen S, Rissanen TH, Mursu J, Tuomainen TP, Korhonen MJ, *et al*. Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in eastern Finland. Arterioscler Thromb Vasc Biol 2005 Jan;25(1):228-233.

Voutilainen S, Rissanen TH, Virtanen J, Lakka TA, Salonen JT, Kuopio Ischemic Heart Disease Risk Factor Study. Low dietary folate intake is associated with an excess incidence of acute coronary events: The Kuopio Ischemic Heart Disease Risk Factor Study. Circulation 2001 Jun 5;103(22):2674-2680.

Weisinger JR, Bellorin-Font E. Magnesium and Phosphorus. The lancet 1998;352(9125):391-396.

Wong GK, Poon WS, Chan MT, Boet R, Gin T, Ng SC, *et al*. Plasma magnesium concentrations and clinical outcomes in aneurysmal subarachnoid hemorrhage patients: post

hoc analysis of intravenous magnesium sulphate for aneurysmal subarachnoid hemorrhage trial. Stroke 2010 Aug;41(8):1841-1844.

Yang CY, Chang CC, Tsai SS, Chiu HF. Calcium and magnesium in drinking water and risk of death from acute myocardial infarction in Taiwan. Environ Res 2006 Jul; 101(3):407-411.

Yang CY. Calcium and magnesium in drinking water and risk of death from cerebrovascular disease. Stroke 1998 Feb; 29(2):411-414.

Zhang W, Iso H, Ohira T, Date C, Tamakoshi A, JACC Study Group. Associations of dietary magnesium intake with mortality from cardiovascular disease: the JACC study. Atherosclerosis 2012 Apr;221(2):587-595.