

11. DRINKING WATER HARDNESS AND CARDIOVASCULAR DISEASES: A REVIEW OF THE EPIDEMIOLOGICAL STUDIES 1979-2004

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I. INTRODUCTION

Cardiovascular diseases (CVDs) are among the main causes of mortality and morbidity in the industrialised countries and their main risk factors are hypertension, dyslipidemia, smoking, alcohol abuse, dietary habits and physical inactivity (1, 2). However, these classic factors do not entirely explain the variability of CVD mortality in different countries. In order to better understand the determinants of CVD, particular attention has been paid to environmental factors, such as weather, air pollution or the mineral content of drinking water (DW). Since the 1950s a causal relation between DW hardness and some CVDs has been hypothesized. The relationship between cardiovascular mortality and the mineral content of DW was first described by Kobayashi (3) in Japan and by Schroeder (4) in the United States. Since then, many epidemiological studies have been conducted worldwide, most of them describing a protective relationship between CVD mortality rates and DW hardness. The first series of studies were performed in the 1960s and 1970s. As noted in the previous chapter by Calderon and Craun, most studies had an ecological design and used mortality data from national registers; geographical areas were defined on the basis of administrative boundaries as units of analysis.

Critical reviews of these early studies emphasized that these studies had a major weakness. Because they considered average values of DW parameters, such as total hardness, calcium (Ca), or magnesium (Mg), exposures were likely subject to non-differential misclassification bias. Moreover, the temporal sequence of exposure and supposed effect (i.e., exposure to the DW risk factor precedes CVD mortality) was not always verified. In fact, the mineral content of DW was often determined at the time of the study and thus, may not represent the quality of the water the subjects had ingested during their lives. However, it should be pointed out that DW hardness is usually quite stable in time and that the health effects related to Ca and Mg concentrations may be both long- and short-term. Moreover, the main risk factors for CVD, which may confound the relationship between DW hardness and CVD mortality, had often not been taken into account in the analysis. More recently, several ecological studies were performed with more attention paid to exposure assessment and confounding factors. Epidemiological studies performed with a cohort design or with a case-control design were also carried out, but these studies are less numerous than the ecological studies even though they offer a greater potential for understanding the relationship between DW hardness and CVD mortality.

The hypothesized beneficial effect of DW hardness on CVD mortality may be due to: (1)

present in hard water (e.g., selenium, lithium, silicon, zinc, vanadium); (3) the reduced adverse effect of toxics such as lead, which may be present at a higher concentration in soft, low pH corrosive water (5-7). In fact, the presence of lead, even at low blood levels, has long been associated with hypertension and also with stroke (8, 9). In this review, we evaluated epidemiological studies on this issue published since 1979.

II. METHODS

In order to evaluate the cardiovascular effects of Ca and Mg, the principal minerals responsible for the hardness of DW, we collected articles published from 1979 through 2003. First we retrieved from the Medline database all articles using the keywords (Mesh terms) “hardness” or “calcium” or “magnesium” or “drinking water” and “human health”. Secondly, we selected articles of interest from the first list by examining the abstracts. Thirdly, we checked the references of the articles retrieved to find other papers of interest. We did not attempt to retrieve unpublished research. An additional paper available on-line in February 2004 (10) was retrieved when preparing the final version of this chapter.

Since studies published before 1979 have already been evaluated (11, 12), we excluded them from our review. Other exclusion criteria were: articles written in languages other than English; experimental studies on animals; studies on dietary intake of Ca or Mg; studies not reporting quantitative measures of associations between water hardness (or Ca/Mg concentration in DW) and human diseases. The articles were divided into the following categories: geographic correlation (ecological), case-control and cohort studies. After a critical review of these studies, we reached conclusions about the possible beneficial effects of water hardness, Ca, and Mg on the basis of the overall findings. We did not, however, perform a formal meta-analysis due to the heterogeneity of measures of effect and of exposure levels (Ca and Mg concentration in DW) in the studies reviewed. Although we did not include physiological, pathophysiological or experimental in-vitro or in-vivo studies, we considered this research in interpreting the results of the epidemiological studies.

III. RESULTS

1. Geographic Correlation Studies

Nineteen correlation studies were reviewed (Table 1). Some of them took into account potential confounders such as socio-economic status, income or climate (13-17), but only two of the 19 studies considered some of the major CVD risk factors in the populations that were compared (18, 19). Significant inverse correlations between water hardness and CVD mortality were found in 10 studies (10, 13, 15, 20-26). Lacey and Shaper (21) reported in males a 7.5% reduction of CVD mortality for 100 mg/L increased water hardness (as CaCO₃), and Yang et al. (15) reported a 10% increase in the risk of ischemic heart disease (IHD) mortality in persons with <75 mg/L hardness compared to persons with >150 mg/L hardness (as CaCO₃). Kousa et al. (10) reported that one unit in German degrees of water hardness decreased the risk of acute myocardial infarction (AMI) by 1%. The remaining eight studies reported a correlation coefficient (r). In the studies where Ca and Mg were evaluated separately, similar associations with CVD mortality were found for each of these minerals.

Although positive correlations were found between Mg concentration and both IHD and stroke mortality rates in the study by Flaten and Bolviken (27) in Norway, virtually all the municipalities investigated have soft water, and the findings may not have relevance to hard water. Six studies found either a very small or no association (14, 16, 17, 28-30). The study by

Zielhuis and Haring (30) considered various data sets; the most recent data were collected in 30 municipalities with >40,000 inhabitants and where there was no change in water hardness over the previous 20-30 years. Although an inverse correlation was found for stroke and IHD mortality rates in 1977 and Ca and Mg in water, the reported correlation coefficient was very small and not statistically significant.

The most informative of the correlation studies were carried out by Nerbrand et al. (18, 19) in Sweden. In the first study Nerbrand et al. found significant inverse associations for both IHD and stroke mortality. However, they further investigated 14,675 randomly selected subjects in the study areas using a postal questionnaire, and they found that water hardness and Ca were significantly associated with non-fatal IHD when the effects of the traditional risk factors were taken with account by multiple logistic regression analysis. Nerbrand et al. (19) conducted an additional investigation comparing a sample of 207 subjects from two municipalities, one with hard DW (Ca=66 mg/L and Mg=4.1 mg/L) and the other with soft DW (Ca=8.8 mg/L and Mg=0.74 mg/L). Although this study found increased mortality for CVD, and IHD in the soft water municipality, the study found no difference between the two populations in Ca and Mg level in the subjects' serum and urine, and no correlation between Ca or Mg concentrations in household water samples and the corresponding serum and urine levels in the subjects investigated. These findings suggest a lack of association between Ca or Mg in DW and CVD and IHD mortality when using individual- rather than population-based data, and their significance will be discussed later.

2. Case-control Studies

Seven case-control studies were included in our review. These studies investigated the association of Ca and Mg concentration with CVD mortality in Sweden, Taiwan, and Finland (Table 2). All found an inverse association between Mg levels in DW and mortality risks for AMI, stroke, or hypertension (HT); only one study found an inverse association with Ca (31-36).

In areas with DW Mg levels greater than 6.8 mg/L, Rubenowitz et al. (32) reported a statistically significant decreased risk of death due to AMI among men. Luoma et al. (31) observed that the AMI mortality risk increased among men as DW Mg levels decreased from greater than 3 mg/L to less than 1.2 mg/L, however, the increased risks were not statistically significant. Yang (33), Yang and Chiu (34) reported a statistically significant decreased risk of death due to stroke among men and women that was associated with Mg levels greater than 7.3 mg/L and a large, but statistically non-significant, decreased risk of death due to hypertension that was associated with increasing Mg levels greater than 3.8 mg/L. Rubenowitz et al. (35) found a statistically significant decreased risk of death due to AMI among women in areas with Mg levels greater than 9.8 mg/L, and in areas with Ca levels greater than 70 mg/L.

In only one of the seven studies did the investigators adjust the estimates of association for the major CVD risk factors measured at the individual level (36). In this study, a relatively large, but statistically non-significant, decreased risk for death due to AMI was found for both men and women aged 50-74 in areas with the highest quartile of Mg in DW. The same study also investigated the risk of AMI among subjects who were still alive (AMI survivors) by collecting individual data on exposure (Ca and Mg concentration in household water) and on major risk factors for the disease. No association was found between Ca or Mg levels and the prevalence of AMI among the AMI survivors.

Rosenlund et al. (37) studied the association between AMI risk and the average daily intake of drinking water constituents in a sub-set from a large Swedish population-based case-control study in the period 1992-1994. After adjustment for the matching variables and confounders, the odds ratio for AMI was 0.88 among those with a Mg intake from their drinking water above 1.86

mg per day. Although this odds ratio suggested a protective effect, analyses using multiple categories of exposure showed no exposure-response relationship. The odds ratios for hardness, sodium and Ca were also slightly less than one, but as was found for Mg, the odds ratios were not statistically significant and there was no exposure-response relationship.

3. Cohort Studies

We identified two cohort studies. One of them, conducted in two rural areas in Finland (38), found that populations residing in the area with lower Mg levels in DW (3.1 mg/L vs. 13.1 mg/L) had a proportionally higher mortality from CVD (14.7% vs. 8.7%). The other study, performed in the Washington County, Maryland, found no association between water hardness and CVD mortality (39). Neither study took into account measures of common CVD risk factors.

IV. DISCUSSION

Many, but not all, of the geographic correlation studies showed an inverse association between water hardness and mortality from CVD. Most case-control studies and one cohort study showed a statistically significant inverse relation between mortality from CVD and water levels of Mg, but not Ca levels. The two elements act differently in human cells and may play different roles in CVD development and evolution. The roles of Ca and Mg in DW as they may affect CVD will, therefore, be discussed separately.

1. Total hardness, calcium concentrations and CVD

Since Ca is the main element responsible for total water hardness, the results of studies that considered water hardness may be interpreted as concerning Ca as well. The majority of epidemiological studies on water hardness and CVD carried out so far have provided somewhat controversial results. Of the 19 geographical studies published since 1979 and included in the present review, 10 found a significant association, but the only two geographical studies which took into account the distribution of the main CVD risk factors (18, 19), both from Sweden, reported no association when individuals were considered. Of the 6 case-control studies that specifically considered Ca, only one (35) found a statistically significant protective effect of high Ca levels (>70 mg/L) in DW, but no exposure-response relationship was observed. An alternative explanation for the inverse association found between water hardness and CVD may be that hard water protects against the toxic effects of lead. Soft water is more aggressive and, therefore, richer in lead which may be present in plumbing materials.

One question is the possible role of Ca in the development and control of primary “essential” hypertension in humans (40). Some animal data and observational studies in humans support the hypothesis that Ca supplementation can reduce blood pressure. However, a recent meta-analysis of 42 randomised controlled trials on the influence of dietary and non-dietary Ca supplementation on blood pressure shows only a small, clinically modest, reduction in both systolic (mean reduction: - 1.44 mm Hg) and diastolic (mean reduction: - 0.84 mm Hg) blood pressure (41). These findings support present recommendations of an adequate dietary intake of Ca. The use of Ca supplements to prevent or treat hypertension is not, however, supported by current evidence.

2. Magnesium concentrations and CVD

Mg participates in many different biological functions, ranging from structural roles by complexing negatively charged groups, i.e. phosphates in nucleic acids, catalytic roles in enzyme activation or inhibition, and regulatory roles by modulating cell proliferation, to cell cycle progression and differentiation (42). Mg deficiency accelerates the development of atherosclerosis

and the induction of thrombocyte aggregation, therefore, it is described as a risk factor for AMI and for cerebrovascular disease (43, 44). Mg is known to be a protective agent against soft tissue calcification (particularly for myocytes), and its role in AMI has been well documented (5, 45).

The results of epidemiological studies on the possible association between Mg in DW and CVD are persuasive, although some discrepancies have been noted. Many, but not all, of the geographical correlation, cohort and case-control studies show that a high Mg water concentration protects against CVD and stroke mortality. Some studies found an exposure-response relationship. However, the only study investigating both CVD incidence and mortality (36) found that a high Mg concentration in DW reduced the risk of dying from, but not of developing, CVD, when taking the main CHD risk factors into account. These findings suggest that Mg may reduce the fatality from CHD among those who develop the disease (secondary prevention) rather than prevent the onset of the disease (primary prevention). Accordingly, a cohort study showed that serum Mg concentration was inversely associated with CHD mortality but not with incidence (46), and clinical data show that Mg is useful in treating patients with myocardial infarction and other acute CVDs (47). Furthermore, oral Mg therapy in CHD patients has been shown to have beneficial effects on the outcome (48, 49).

The role of minerals such as sodium, Ca and Mg in the development of human hypertension has been investigated widely since the first geographical studies showing a lower incidence of the disease in populations with a diet poor in sodium yet rich in Ca and Mg. Physiological and pathophysiological studies support the role of Mg in hypertension development (50). The protective role of Mg in reducing the incidence of hypertension has been reported in many observational studies (50). A recent prospective cohort study found a lower risk of hypertension in subjects with high Mg serum concentration among people free of the diseases at baseline (51). Interestingly, well-designed randomized controlled trials have shown that a diet rich in vegetables, fruit and whole grains, therefore, richer in Mg than common western diets, reduced blood pressure significantly (52, 53). However, taking Ca or Mg supplements did not reduce blood pressure in either normotensive or hypertensive subjects (54-56). In fact, the current recommendations of the US Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (57) are in favour of a regular dietary intake of Ca and Mg, but against taking more than the recommended dose of these mineral supplements.

Indirect evidence of the protective effects of Mg against CVD comes from several dietary preventive trials showing that a diet rich in vegetables, fruit and whole grains ("Oriental" diet) significantly reduces CVD incidence compared to the usual US diet ("American" diet) (58, 59). The Oriental diet is richer in Ca and Mg compared to western diets. However, it is also rich in many other putative factors protecting against CVD, such as vitamins, cofactors, anti-oxidants, polyunsaturated fats and selenium. Despite suggesting an association, these trials provide no conclusive evidence that Ca or Mg intake reduces the risk of CVD.

Various experimental studies carried out on animals support the hypothesis of a cause-effect relationship between Mg content in drinking water and ischemic heart disease. Experimentally-induced Mg deficiency changes the blood lipid composition in a more atherogenic direction (60, 61). It has recently been observed that Mg-fortified water reduced atherogenesis in low-density lipoprotein (LDL) receptor-deficient mice with respect to those receiving distilled water, with and without a high-cholesterol diet (62-64). Likewise, Mg supplementation in DW inhibited atherogenesis in apolipoprotein-E-deficient mice not receiving a high-fat diet; Mg supplementation in DW significantly inhibited atherogenesis in female but not in male mice (65).

Another issue of interest is the relationship between biological measures of Ca and Mg concentration in extracellular and intracellular body compartments and CVD development. Some recent cross-sectional (66, 67), case-control (68) and cohort studies (46, 69) showed that subjects

with low Mg serum levels have an increased risk of CHD compared to people with high levels, when also considering all the major risk factors for the disease as possible confounders.

A controversial point is the relevance of Mg intake by water compared to diet. Although food is usually the main source of Mg, modern western diets often contain very little of this mineral. Marx and Neutra (7) pointed out the apparent paradox that waterborne Mg, which contributes less than dietary Mg to total intake, may have as great an effect (relative risks of 1.5-2.0) on CVD mortality as that observed in some studies of other risk factors. Marx and Neutra criticised the methods of previous studies and noted that the studies are not supported by pharmacokinetic data. They also emphasized that confounding by other waterborne or dietary factors associated with Mg is the most serious concern and argued that further, more valid studies using personal data would be needed to clarify the issue once and for all. Nevertheless, it has been shown that the daily intake of Mg in industrialized countries does not reach the current recommended daily allowance in many subjects and therefore marginal Mg deficiencies are common. An extensive national sample survey among US adults showed that about 23% have Mg serum concentrations <0.80 mmol/L, a level considered as hypomagnesemia (46). Mg intake through DW may be important in these populations because of the higher bioavailability of the mineral in DW than in food. It has been pointed out that 2 litres of water rich in Mg (40 mg/L) will provide 80 mg of Mg, which is about 25% of an adult's total requirement (18).

3. Interpreting the epidemiological evidence

The strengths and limitations of epidemiology for investigating the causes of human diseases are well known. Weak associations, especially those regarding environmental factors, are difficult to interpret and require a number of well-designed investigations, with precise measures of both the factor under study and the possible confounders and effect modifiers at an individual level. There is as yet no conclusive evidence of the relationship between DW hardness and CVD, mainly because the numerous epidemiological studies carried out thus far do not satisfy the criteria for establishing causality. The main drawbacks of these studies are the lack of exposure data at an individual level, with the risk of exposure misclassification, and the lack of control of confounders, including the recognized risk factors for CVD. Although the epidemiological study results may be confounded, it should be remembered that a risk factor for a disease is a confounder if it is associated with exposure under investigation (70). Major CVD risk factors such as serum cholesterol, hypertension, diabetes mellitus, cigarette smoking, obesity and physical exercise may confound the relationship between water hardness and CVD mortality only if they are associated with soft DW. However, there is no reason to believe that major CVD risk factors are usually associated with low Ca or Mg concentration in DW. It, therefore, seems unlikely that confounding alone can explain the associations found in several geographical and case-control studies carried out in different populations and at different times. The role of chance in finding an association should be excluded as well, since numerous studies reported an association, whereas only 5% would expect to find a significant association by chance alone.

A study performed in two areas at substantially different levels of DW hardness provides a good example of how a geographical correlation study can produce invalid results (19). The study investigated two communities, one in the west and the other in the east of Sweden, with substantial differences in both DW hardness concentration and CVD mortality rates: the population with the lower DW hardness (west) had about double the mortality rate of the other population (east). However, the authors also collected individual data from 207 subjects, randomly sampled from the two populations (approximately 100 from each), including the following: Ca and Mg measurement in a sample of tap water from the subject's household; total dietary intake of Ca and Mg, determined by means of a questionnaire; serum and urine Ca concentration, and serum, urine and muscle Mg concentration; serum levels of LDL and HDL cholesterol, triglycerides and other risk factors for

CVD. Total Ca and Mg intakes were found to be higher in the population drinking softer water due to a higher intake of food rich in these minerals. However, there was no difference between the two populations in mean serum or urine levels of Ca and Mg. No correlation was found between Ca and Mg levels in DW and the mean values in serum or urine. Of the investigated risk factors for CVD, the LDL:HDL cholesterol ratio was higher in the west than in the east population, possibly explaining the higher mortality rates for CVD in the former. The research by Nerbrand et al. does not show a direct correlation between Ca and Mg in DW and CVD and IHD mortality at an individual level, in spite of an apparent geographical correlation. These findings are consistent with those from another study showing that a large part of the geographical variation observed in CHD incidence in British towns was reduced after adjustment for the main risk factors (71). At least 80% of major CHD events in middle-aged men can be attributed to the three highest risk factors, i.e. serum total cholesterol, cigarette smoking and blood pressure, as recently estimated (72). It follows that the contribution of other risk factors, including environmental ones, in CHD incidence or mortality is necessarily modest.

Should the association between DW Ca or Mg concentration and CVD be true, why have some studies not found an association. The possible reasons include:

- 1.) the relatively small differences between the areas compared. For instance, only one of the 5 rate-based studies considered in the review by Marx and Neutra (7) compared populations with substantial differences in Mg concentration in DW (Figure 2 in reference 7);
- 2.) the dilution of the effect due to heterogeneity of exposure among individuals in the same population, caused by substantial differences in water and food consumption;
- 3.) the relatively weak strength of the association.

V. CONCLUSIONS

There is little evidence that supports an association between water hardness or Ca concentration in DW and CVD. However, the available information from experimental, clinical and epidemiological studies supports the hypothesis that a lower than recommended intake of Mg is a condition that increases the risk of dying from, and possibly developing, CVD, stroke or hypertension. Similar conclusions about the benefits of Mg in water have been reached by others (43, 44, 73, 74). Although some scientists may argue that additional information is needed to establish causality, the following information strongly supports the conclusions reached :

- In-vitro studies show that exposure of endothelial cells to low Mg causes some of the events involved in the pathogenesis of atherosclerosis;
- Animal studies show that a low-Mg diet causes inflammation and high serum cholesterol levels, particularly LDL cholesterol, thus favouring atherosclerosis development, and conversely that high Mg intake protects against the atherosclerotic effects of oxidative stress and hypercholesterolemia-inducing diets;
- In patients with CHD, Mg in pharmacological doses has been shown to reduce endothelial lesions and is a useful anti-ischemic and anti-arrhythmic agent;
- Most correlation studies show a high mortality for CVD and stroke in populations with low Mg concentration in DW, and vice-versa;
- Some cross-sectional and cohort studies show that people with low serum Mg levels are at a higher risk of hypertension, CHD and stroke;
- Dietary trials show that a diet rich in vegetables and fruit, i.e. rich in Mg, reduces the risk of CVD.

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Table 1. Geographic correlation studies on the relationship between cardiovascular diseases or stroke and hardness and/or calcium/magnesium concentration of drinking water.

Authors, year of publication	Country, area and population	Period	Drinking water parameters	CVD or stroke mortality	Results
Scassellati-Sforzolini et al., 1979 (28)	Italy, Umbria Region, 12 municipalities,	1967-1976	Total hardness	Mortality for: - IHD - Stroke	M & F: r= +0.28 M & F: r= - 0.07
			Ca concentration	Mortality for: -IHD - Stroke	M & F: r= +0.37 M & F: r= - 0.05
			Mg concentration	Mortality for: - IHD - Stroke	M & F: r= - 0.26 M & F: r= - 0.28
Masironi et al., 1979 (20)	Europe, 17 towns, 45-64 years	1974	Total hardness	AMI incidence	M & F: r= - 0.46
Pocock et al., 1980 (13)	Great Britain, 253 municipalities, 35-74 years	1969-1973	Total hardness	Mortality for CVD	M & F: r= - 0.67
Zielhuis and Haring, 1981(30)	The Netherlands, 30 communities	1977	Ca concentration	Mortality for: - IHD - Stroke	M: r= - 0.01 F: r= - 0.11 M: r= - 0.14 F: r= - 0.12
			Mg concentration	Mortality for: - IHD - Stroke	M: r= - 0.19 F: r= - 0.10 M: r= - 0.02 F: r= - 0.07
Leary et al., 1983 (23)	South Africa, 12 districts	1978-1982	Mg concentration	Mortality for IHD	M: r = - 0.68

Lacey and Shaper, 1984 (21)	England and Wales, 14 areas, 45-74 years	1968-1972	Total hardness	Mortality for CVD	Males: 7.5% reduction of mortality for 100 mg/L increase of hardness*
Leoni et al., 1985 (22)	Italy, Abruzzo Region	1969-1978	Total hardness	Mortality for: - CVD - IHD - Stroke	M & F: r= -0.55** M & F: r= -0.59* M & F: r= -0.24
Smith and Crombie, 1987 (29)	Scotland, 56 districts	1979-1983	Total hardness	Mortality for IHD	M: r= -0.17
Rylander et al., 1991 (6)	Sweden, 27 municipalities	1969-1978	Total hardness	Mortality for: - IHD - Stroke	M: r= -0.60 F: r= -0.37 M: r= -0.48 F: r= -0.45
			Ca concentration	Mortality for: - IHD - Stroke	M: r= -0.47 F: r= -0.41 M: r= -0.52 F: r= -0.32
			Mg concentration	Mortality for: - IHD - Stroke	M: r= -0.62 F: r= -0.45 M: r= -0.16 F: r= -0.49
Gyllerup et al., 1991 (14)	Sweden, 259 municipalities, (males only) 40-64 years	1975-1984	Total hardness	Mortality for AMI	Inverse association, with lower relevance after adjusting for cold climate
			Mg concentration	Mortality for AMI	
Flaten and Bolviken, 1991 (27)	Norway, 97 municipalities	1974-1983	Ca concentration	Mortality for: - IHD - Stroke	NR NR
			Mg concentration	Mortality for: - IHD - Stroke	M: r= +0.33*** F: r= +0.23* M: r= +0.22** F: r= +0.35**
	Country, area and	Period	Drinking water	CVD or stroke mortality	Results

Authors, year of publication	population		parameters			
Nerbrand et al., 1992 (19)	Sweden, 76 municipalities, males and females	1969-1983	Total hardness	Mortality for: - IHD	M***	F*
				- Stroke	M***	F***
			Ca concentration	Mortality for: - IHD	M**	F***
			- Stroke	M	F***	
			Mg concentration	Mortality for: - IHD	M	F
				- Stroke	M	F
Yang et al., 1996 (15)	Taiwan, 227 municipalities	1981-1990	Total hardness ^a	Mortality for IHD	RR (95% CI)	
			<75 mg/L		1.096 (1.084-1.108)*	
			75-150 mg/L		1.045 (1.032-1.058)*	
			>150 mg/L		Reference	
Maheswaran et al., 1999 (17)	England, 305 areas, (>45 years)	1990-1992		Mortality for AMI	RR (95% CI) for 4-fold increase of Ca and Mg concentration in drinking water:	
			Ca concentration		Ca: 0.99 (0.94-1.05)	
			Mg concentration		Mg: 1.01 (0.96-1.06)	
Sauvant and Pepin, 2000 (25)	France, Puy de Dôme Department, 52 districts	1988-1992	Total hardness	Mortality for: - IHD	M: r = -0.33	F: r = -0.18
				- Stroke	M: r = -0.32	F: r = -0.34
				-CVD	M: r = -0.34	F: r = -0.37

Marque et al., 2003 (26)	France South-West, 69 areas (>65 years)	1990-1996	Ca concentration	Mortality for:	RR (95% CI) for highest vs lowest tertile:
				- CVD	0.90 (0.84-0.96)**
				- IHD	0.90 (0.84-0.97)**
				- Stroke	0.86 (0.77-0.96)*
			Mg concentration	Mortality for:	RR (95% CI) for highest vs lowest tertile:
				- CVD	0.93 (0.86-1.01)
				- IHD	0.96 (0.87-1.05)
				- Stroke	0.92 (0.80-1.06)
Nerbrand et al., 2003 (18)	Sweden, 2 municipalities in the West and East	1989-1998	West (Ca : 8.8 mg/L, Mg: 0.74 mg/L)	Mortality for [§] :	Mortality rates:
				- IHD	M: 21/1000 F: 5/1000
				- CVD	M: 31/1000 F: 11/1000
			East (Ca : 66 mg/L, Mg: 4.1 mg/L)	Mortality for [§] :	Mortality rates:
				- IHD	M: 10/1000 F: 2/1000
				- CVD	M: 20/1000 F: 6/1000
					RR (West /East) for: IHD=M: 2.03 F: 2.56 CVD=M: 1.56 F: 1.71
Miyake and Iki, 2003 (16)	Japan, 44 municipalities	1995	Total hardness ^a <46.5 mg/L 46.5-51.9 mg/L >51.9 mg/L	Mortality for Stroke	RR (95% CI) Reference 0.97 (0.91-1.03) 0.93 (0.84-1.02)
	Country, area and	Period	Drinking water	CVD or stroke mortality	Results

Authors, year of publication	population		parameters			
Kousa et al., 2004 (10)	Finland, whole country (males, 35-74 years)	1983, 1988 and 1993	Total hardness ^a	Incidence of AMI	Rates per 100,000:	
			<30.6 mg/L			562.1
			30.6-93.08 mg/L			469.5
			>93.08 mg/L		437.6	

^aTotal hardness in mg/L of CaCO₃.

IHD = ischaemic heart diseases; AMI = acute myocardial infarction; CVD = cardiovascular diseases; NR = not reported; RR = relative risk; CI = confidence interval; M = males; F = females; r = correlation coefficient.

* p<0.05; ** p< 0.01; *** p<0.001; if no * shown, p>0.05.

§ Study of 207 inhabitants found positive association for Ca and systolic blood pressure; inverse association for Ca in DW and LDL and total cholesterol; no association for Mg and major CVD risk factors.

Table 2. Case-control studies on the relationship between cardiovascular diseases (CVD) and hardness and/or calcium/magnesium concentrations of drinking water.

Authors, years of publication	Country and area	Population	Age (years)	Drinking water parameters	Odds ratio (OR) (95% CI)			
Luoma et al., 1983 (31)	Finland	58 males with AMI, alive or dead (cases) 58 males (hospital controls) 50 males (population controls)	37-64	Hospital controls	Population controls			
						Ca concentration		
						<16 mg/L	0.73 (0.22-1.99)	0.56 (0.25-1.28)
						16-18 mg/L	0.77 (0.30-1.91)	1.07 (0.48- 2.42)
						19-20 mg/L	0.91 (0.35-2.36)	1.64 (0.73-3.85)
						>20 mg/L	Reference	Reference
						Mg concentration		
						<1.2 mg/L	2.00 (0.69-6.52)	4.67 (1.30-25.32)*
						1.2-1.5 mg/L	1.11 (0.41-3.10)	2.29 (0.88-6.58)
						1.6-3.0 mg/L	1.00 (0.36-3.08)	1.63 (0.62-4.52)
>3.0 mg/L	Reference	Reference						
Rubenowitz et al., 1996 (32)	Southern Sweden, 17 municipalities	854 males dead for AMI (cases) 989 males dead for cancer (controls)	50-69	Ca concentration	OR age-adjusted:			
						<34 mg/L	Reference	
						34-45 mg/L	0.88 (0.65-1.19)	
						46-81 mg/L	0.84 (0.64-1.10)	
						≥82 mg/L	1.06 (0.82-1.38)	
						Mg concentration	OR age-adjusted:	

				<3.6 mg/L	Reference
				3.6-6.8 mg/L	0.88 (0.66-1.16)
				6.9-9.7 mg/L	0.70 (0.53-0.93)*
				≥9.8 mg/L	0.65 (0.50-0.84)*
Yang, 1998 (33)	Taiwan, 252 municipalities	17,133 males and females dead for stroke (cases)	50-69	Ca concentration	OR adjusted for age and sex:
				<24.4 mg/L	Reference
		17,133 males and females dead for other causes, excluding CVD (controls)		24.4-42.3 mg/L	1.5 (0.99-1.11)
				42.4-81.0 mg/L	0.95 (0.88-1.01)
				Mg concentration	OR adjusted for age and sex:
				<7.3 mg/L	Reference
				7.4-13.4 mg/L	0.75 (0.65-0.85)*
				13.5-41.3 mg/L	0.60 (0.52-0.70)*

Authors, years of publication	Country and area	Population	Age (years)	Drinking water parameters	Odds ratio (OR) (95% CI)
Yang and Chiu, 1999 (34)	Taiwan, 252 municipalities	2336 males and females dead for HT (cases)	50-69	Ca concentration	OR adjusted for age, sex, urbanization and Mg:
				4.0-11.3 mg/L	Reference
		2336 males and females dead for other causes, excluding CVD (controls)		11.4-30.0 mg/L	1.23 (0.94-1.62)
				30.1-37.7 mg/L	1.32 (0.98-1.78)
				37.8-53.4 mg/L	1.12 (0.83-1.51)
				53.5-81.0 mg/L	1.26 (0.92-2.02)
		Mg concentration		OR adjusted for age, sex, urbanization and Ca:	
		1.5-3.8 mg/L		Reference	
		3.9-8.2 mg/L		0.73 (0.57-0.93)***	
		8.3-11.1 mg/L		0.66 (0.50-0.87)***	
11.2-16.3 mg/L	0.67 (0.50-0.89)***				
16.4-41.3 mg/L	0.63 (0.47-0.84)***				
Rubenowitz et al., 1999 (35)	Southern Sweden, 16 municipalities	378 females dead for AMI (cases)	50-69	Ca concentration	OR adjusted for age and Mg :
				≤31 mg/L	Reference
		1368 females dead for cancer (controls)		32-45 mg/L	0.61 (0.39-0.94)*
				46-69 mg/L	0.71 (0.49-1.02)
				≥70 mg/L	0.66 (0.47-0.94)*
		Mg concentration		OR adjusted for age and Ca:	
		≤3.4 mg/L		Reference	

				3.5-6.7 mg/L	1.08 (0.78-1.49)
				6.8-9.8 mg/L	0.93 (0.64-1.34)
				≥9.9 mg/L	0.70 (0.50-0.99)*
Rubenowitz et al., 2000 (36)	Southern Sweden, 18 municipalities	263 males and females dead for AMI (cases)	50-74	OR adjusted for age and Mg (highest/lowest quartiles) Ca concentration	M: 1.01 (0.64-1.59) F: 0.68 (0.29-1.59)
		258 males and females dead for other causes (controls)		OR adjusted for age and Ca (highest/lowest quartiles) Mg concentration	M: 0.69 (0.43-1.09) F: 0.51 (0.21-1.22)
		823 males and females surviving after an AMI (cases)	50-74	OR adjusted for age and Mg (highest/lowest quartiles) Ca concentration	M: 0.97 (0.75-1.26) F: 0.90 (0.59-1.38)
		853 males and females without AMI (controls)		OR adjusted for age and Ca (highest/lowest quartiles) Mg concentration	M: 1.19 (0.91-1.54) F: 1.09 (0.70-1.70)
Rosenlund et al., 2002 (37)	Sweden	570 males and females with AMI (cases)	45-70	Mg intake in drinking water	OR (95% CI) 0.88 (0.67-1.15)
		753 males and females without AMI (controls)		>1.86 mg per day	

AMI = acute myocardial infarction; CVD = cardiovascular diseases; HT = hypertension; M = males; F = females.

95% CI = confidence interval.

* p<0.05; *** p< 0.001; all others not statistically significant.