

# First report on the association of drinking water hardness and endothelial function in children and adolescents

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## Abstract

**Introduction:** This study aims to investigate the relationship of water hardness and its calcium and magnesium content with endothelial function in a population-based sample of healthy children and adolescents.

**Material and methods:** This case-control study was conducted in 2012 among 90 individuals living in two areas with moderate and high water hardness in Isfahan County, Iran. The flow-mediated dilatation (FMD) of the brachial artery and the serum levels of soluble adhesion molecules (sICAM-1, sVCAM-1) were measured as surrogate markers of endothelial function, and high-sensitivity C-reactive protein (hs-CRP), as a marker of inflammation.

**Results:** Data of 89 participants (51% boys, mean age 14.75 (2.9) years) were complete. Those participants living in the area with high water hardness had higher FMD, hs-CRP, and soluble adhesion molecules (sICAM-1, sVCAM-1) than their counterparts living in the area with moderate water hardness. Multiple linear regression analysis showed that after adjustment for confounding factors of age, gender, body mass index, healthy eating index and physical activity level, total water hardness, as well as water content of calcium and magnesium, had a significant positive relationship with FMD. The corresponding associations were inverse and significant with soluble adhesion molecules ( $p < 0.05$ ).

**Conclusions:** This study, which to the best of our knowledge is the first of its kind in the pediatric age group, suggests that water hardness, as well as its calcium and magnesium content, may have a protective role against early stages of atherosclerosis in children and adolescents.

**Key words:** water hardness, endothelial function, calcium, magnesium, children and adolescents.

## Introduction

Since the 1950s, the hypothesis of an association of drinking water hardness and some cardiovascular diseases (CVD) has been proposed. This hypothesis was first presented by Kobayashi [1] in Japan and some years later by Schroeder [2] in the United States. The association of water hardness with atherosclerotic CVDs has been widely investigated and has been considered as a concern for regional differences in CVD inci-

dence and mortality rates. Several epidemiological studies have proposed an inverse association between drinking water hardness and CVD mortality. Decades ago, some ecological studies [3–5] suggested that high levels of drinking water hardness could be protective against CVDs, mainly against atherosclerotic diseases [6, 7].

Hard water is considered as water containing high concentrations of calcium (Ca) and magnesium (Mg) ions. However, hardness is also caused by other dissolved metals, divalent or multivalent cations, such as aluminum, barium, strontium, iron, zinc and manganese. Monovalent ions, such as sodium and potassium, do not cause hardness [8].

Results of studies on the association of water hardness with CVD and its mortality are conflicting. Some studies showed an inverse correlation between the incidence of mortality due to atherosclerotic CVDs and Mg concentration in drinking water [9, 10].

Several ecological studies comparing different countries revealed an inverse relationship between hardness and CVD mortality. However, a review of these ecological studies showed that the results are inconsistent between studies. A meta-analysis on case-control studies demonstrated a negative association between water Mg concentrations and CVD mortality [11]. Likewise, another review concluded that some case-control studies and one cohort study reported a protective role for water Mg against CVD mortality, but the analytical studies showed little evidence about the association of water Ca and Mg with CVD risk [12].

Water hardness may also be associated with CVD risk factors; for instance it is documented that water Mg and Ca have positive correlations with blood pressure (BP) [13].

Although it is well established that the process of atherosclerotic CVDs originates in early life [14, 15], limited experience exists about the association of water hardness with the process of atherosclerosis from childhood.

Endothelial dysfunction can be assessed as a surrogate marker of atherosclerosis; it can be determined by the calculation of percentage flow-mediated dilatation (FMD) [16], as well as by laboratory tests, such as measurement of soluble cell adhesion molecules (sCAMs) [17].

The aim of this study is to investigate the relationship of water hardness and its Ca and Mg content with endothelial function in a population-based sample of healthy children and adolescents.

## Material and methods

This case-control study was conducted in 2012 among children and adolescents living in two areas with moderate and high water hardness in Isfahan County, Iran. This study was conducted in

accordance with the principles of the Helsinki Declaration. It was approved by the Ethics Committee of Isfahan University of Medical Sciences. Oral assent and informed written consent were obtained from participants and their parents, respectively.

## Study area

Isfahan county is located in the center of the Iranian plateau, with a latitude of 32°37'N, longitude of 051°40'E, and an average altitude of 1500 m above sea level, bounded by a NW-SE mountain range of 3000 m. The two areas under study were 90 km far from each other. The information on water quality (total hardness, Ca and Mg) was obtained from the monitoring data of the Provincial Health Office, affiliated to the Isfahan University of Medical Sciences. Water hardness does not exhibit significant changes over the years, and is quantified twice a year by instrumental analysis [18].

## Participants

The study participants consisted of a population-based sample of 10–18-year-old adolescents, who were selected by cluster random sampling from their households. By considering an  $\alpha$  error of 0.05 and  $\beta$  error of 20%, and by using the variance of FMD according to oral magnesium intake in a previous study among adults [19], the sample size was calculated as 80 (40 in each group); because of possible missing data, we increased the sample size to 90. Participants were selected by random sampling from two areas of Isfahan County: Dehaghan city with high water hardness and Isfahan city with a moderate level of water hardness.

The eligibility criteria were: being aged 10 to 18 years, living for at least 1 year in the above-mentioned areas with high or moderate water hardness, and using the local drinking water for drinking and food preparation. Those individuals with a history of using mineral waters, any chronic disease, long-term medication use, active or passive smoking, and/or acute infectious diseases were not included in the study.

For assessment of dietary habits, the Healthy Eating Index (HEI) was computed as described before [20]. Physical activity level was assessed by a questionnaire validated for Iranian children and adolescents [21].

## Physical examination

A trained team of physicians and nurses conducted the study under standard protocols and by using calibrated instruments. Weight and height were measured with light clothing and without shoes by using a calibrated scale and stadiometer (Seca, Japan). Body mass index (BMI) was com-

puted as weight (kg) divided by height squared ( $m^2$ ). Systolic and diastolic BP (SBP, DBP) were measured on the right arm, with the individual in a sitting position and at rest for at least 5 min, using a standardized mercury sphygmomanometer and appropriate size cuff. Two measurements at 2-minute intervals were recorded and the average was used for the statistical analysis [22].

### Laboratory tests

Participants were instructed to fast for 12 h before screening; compliance with fasting was determined by interview on the morning of examination. While one of the parents accompanied his/her child, venous blood samples were taken from the left antecubital vein. High-sensitive C-reactive protein (hs-CRP) was measured with an autoanalyzer (Hitachi, Tokyo, Japan). Soluble adhesion molecules (sICAM-1, sVCAM-1) were measured by the enzyme-linked immunosorbent assay (ELISA) method by using standard kits (Bender Med Systems, GmbH, Vienna, Austria).

### Study of arterial reactivity

Measurement of the brachial arterial reactivity, which is a non-invasive endothelial function test, was conducted by the same expert cardiologist using the method previously described [23]. It involved measuring the diameter of an artery by non-invasive ultrasound before and after increasing shear stress (provided by reactive hyperemia), with the degree of dilatation reflecting (in large part) arterial endothelial NO release. The diameter of the brachial artery was measured from high-resolution B-mode ultrasound images (Aloka, SSD-2200a). It was conducted in 3 steps: first the basal brachial artery dimension was detected. In the second step, the sphygmomanometer cuff was inflated with 200 mm Hg in the forearm, and in the third stage the cuff was deflated, and after 30–90 s, the brachial artery dimension in response to reactive hyperemia, endothelium-dependent dilation or FMD was determined in the previous site. The percent change of FMD was expressed as:  $(\text{maximum artery diameter after release of the cuff inflated above SBP} - \text{basal brachial dimension}) / \text{basal brachial dimension} \times 100 = \%FMD$  or  $[(\text{max} - \text{basal}) / \text{basal}] \times 100$ .

The experiments were conducted in a quiet environment, and no significant changes in heart rate and BP were observed. The cardiologist conducting the sonographic studies was not aware of the group assignment of participants.

### Statistical analysis

Data analyses were performed using SPSS (version 20.0, SPSS Inc., Chicago, IL) software. The

normality of the distribution of variables was confirmed by the Kolmogorov-Smirnov test. Continuous variables are reported as the mean  $\pm$  SD. The associations of water hardness and its Ca and Mg content with markers of endothelial function and inflammation were assessed by multiple regression analysis after adjustment for age, gender, BMI, HEI, and physical activity level. A *p* value of less than 0.05 was considered as statistically significant.

### Results

Data of 89 participants (51% boys) were complete and included in the analysis. The mean (SD) age of participants was 14.75 (2.9) years, without a significant difference in the two areas studied. Table I presents the characteristics of the variables studied in two areas with moderate and high water hardness. It shows that participants living in the area with high water hardness had significantly higher FMD, as well as lower hs-CRP and soluble adhesion molecules (sICAM-1, sVCAM-1) than their counterparts living in the area with moderate water hardness.

Multiple linear regression analysis showed that after adjustment for confounding factors of age, gender, BMI, HEI, and physical activity level, total water hardness, as well as water content of Ca and Mg, had a significant relationship with FMD. The corresponding associations were inverse and significant with soluble adhesion molecules (sICAM-1, sVCAM-1). The association of hs-CRP with total water hardness was inverse and significant (Table II).

### Discussion

Our study, which to the best of our knowledge is the first of its kind in the pediatric age group, suggests favorable effects of water hardness, mainly water Mg content, on surrogate markers of endothelial function in children and adolescents.

Water hardness and content have important health effects [24, 25]. Controversial results exist on the association of water hardness with CVDs and their risk factors among the adult population. Our findings may serve as evidence for the protective role of water hardness against early stages of atherosclerosis [26, 27].

The health effects of hard water are mainly considered to be because of its dissolved salts, primarily Ca and Mg. Hard water, mainly very hard water, may be a good source for Ca and Mg intake. Some studies have documented that softer water is associated with higher CVD death rates; this protective role is suggested to be because of the water Ca content [28, 29].

Several other studies have highlighted the role of Mg in atherosclerosis, endothelial function

**Table I.** Characteristics<sup>a</sup> of variables studied according to living area

Variables	Area with moderate water hardness	Area with high water hardness	Total
Water quality:			
Total hardness [mg/l]	150.60 (24.11)*	591.48 (46.10)*	329.7 (32.4)
Calcium [mg/l]	42.10 (11.0)*	106.62 (17.11)*	106.66 (21.11)
Magnesium [mg/l]	13.50 (1.24)*	64.40 (10.71)*	14.11 (1.24)
Participants' physical examination:			
N	44	45	89
Age [years]	14.70 (1.20)	15.21 (1.15)	14.64 (1.21)
BMI [kg/m <sup>2</sup> ]	20.10 (1.10)	21.25 (1.21)	20.31 (1.42)
SBP [mm Hg]	106.33 (10.74)	100.71 (10.48)	102.08 (10.11)
DBP [mm Hg]	66.18 (1.81)	66.60 (1.78)	66.31 (1.29)
Vascular reactivity:			
FMD of brachial artery (%)	2.80 (0.41)*	3.75 (0.82)*	3.20 (0.71)
Laboratory tests:			
Hs-CRP [mg/dl]	4.63 (0.71)*	2.14 (0.17)*	3.49 (0.28)
s-ICAM-1 [ng/ml]	896.17 (74.21)*	230.61 (41.80)*	535.19 (81.70)
s-VCAM-1 [ng/ml]	1000.01 (100.20)*	501.70 (69.11)*	737.88 (78.24)

<sup>a</sup>Data are presented as mean (standard deviation) apart than the number of participants and FMD which is presented as percent; \* $p < 0.05$  between inhabitants of the two areas under study. BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, FMD – flow-mediated dilatation, hs-CRP – high sensitivity C-reactive protein

**Table II.** Regression coefficients<sup>a</sup> for the relation of water quality with markers of endothelial function and inflammation

	FMD Beta (SE)	S-ICAM-1 Beta (SE)	S-VCAM-1 Beta (SE)	Hs-CRP Beta (SE)
<b>Total hardness</b>	0.41 (0.002)*	-0.59 (0.001)*	-0.51 (0.008)*	-0.37 (0.002)*
<b>Magnesium</b>	0.59 (0.006)*	-0.51 (0.001)*	-0.61 (0.001)*	-0.21 (0.001)
<b>Calcium</b>	0.34 (0.001)*	-0.05 (0.001)*	-0.47 (0.007)*	-0.24 (0.002)

<sup>a</sup>Standardized coefficient (standard error) adjusted for age, gender, anthropometric measures, dietary and physical activity habits; \* $p < 0.05$ . SE – standard error, FMD – flow-mediated dilatation, hs-CRP – high-sensitivity C-reactive protein

and CVD mortality [12, 13, 29]. A growing body of evidence supports the beneficial roles of Mg for endothelial function. Magnesium reduces susceptibility to oxygen-derived free radicals, improves endothelial function and inhibits platelet function. Many studies have documented the association of serum Mg concentrations and endothelial function [30, 31]. Low Mg in drinking water is found to be associated with cardiometabolic risk factors [32].

Degeneration of the sub-endothelium is one of the first effects of Mg deficiency, which is associated with intimal thickening, weakening and break-up of the elastic membrane and calcification. The

mechanisms of the pathological effects of Mg deficiency may be mediated by lipid peroxidation [33].

A review study revealed that a low-Mg diet may cause inflammation and consequently it may have a role in the development of atherosclerosis [34]. Low Mg intake may increase the adverse effects of oxidative stress and high fat diets [35].

An experimental study demonstrated that low serum Mg worsens endothelial dysfunction. This may be mediated by producing a pro-inflammatory, pro-thrombotic and pro-atherogenic environment by low Mg concentration [30].

Magnesium deficiency results in up-regulation of adhesion molecules such as s-ICAM and s-VCAM-1.

It has a direct role in promoting endothelial dysfunction by generating a pro-inflammatory, pro-thrombotic and pro-atherogenic environment.

Moreover, low Mg levels may increase the markers of inflammation including CRP [36]. Dietary Mg supplementation is considered as a preventive element in atherosclerosis and ischemic CVDs [37]. A clinical trial in diabetic elderly showed that oral Mg improves endothelial function [19].

Such beneficial effects of oral Mg are also reported for improvement of endothelial function in symptomatic heart failure [38] and hemodialysis patients [39].

Atherosclerosis origins from early life [40, 41], and the role of environmental factors on the process of atherosclerotic diseases, are well documented [20, 42]. Most previous studies on the association of environmental factors with markers of endothelial function have focused on the adverse effects of exposure to secondhand smoke [43] and air pollutants [44, 45], but no previous study has evaluated the association of the hardness of drinking water with endothelial function in children and adolescents. The current findings on the association of water hardness and its Ca and Mg content with surrogate markers of endothelial function can be useful for interventions aiming for health promotion and disease prevention.

Given the importance of preventing CVDs and the role of environmental factors in this regard [46, 47], special attention should be focused to the water hardness of each region.

The main limitation of the current study is its cross-sectional nature, thus the associations documented in this study should be considered with caution. We should also acknowledge that in addition to water content of Ca and Mg, some minor elements might also be associated with the markers of endothelial function and inflammation. However, similar to previous studies in adult populations we only considered total water hardness and its Ca and Mg content; we suggest that other elements should also be considered in future studies. The strengths of this study are its novelty in the pediatric age group, and considering various confounding factors, such as lifestyle habits, to determine the independent association of water hardness on markers of endothelial function and inflammation.

In conclusion, the hardness as well as the Ca and Mg content of drinking water may have a protective role against early stages of atherosclerosis in children and adolescents. Further experimental studies are necessary to determine the underlying mechanisms, and longitudinal studies are required to determine the clinical impacts of the current findings.

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