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# Association Between Drinking Water Mineral Composition and Bone Mineral Density Among Children Aged 9–13 Years: A Comparative Cross-Sectional Study

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## Abstract

# Background

Childhood and adolescence are critical periods for skeletal growth and bone mineral acquisition. Nutritional status, physical activity, body size and environmental exposures influence bone mineralization. Drinking water may contribute calcium and magnesium, whereas reverse osmosis (RO) treatment can substantially reduce mineral content. The relationship between drinking-water composition, fluoride exposure and bone mineral density (BMD) in children remains unclear.

# Objective

To compare BMD among children aged 9–13 years consuming RO-treated, non-RO and high-fluoride drinking water and assess associations between drinking-water minerals and BMD.

# Methods

A comparative cross-sectional study included 180 schoolchildren aged 9–13 years. Participants were equally categorized into RO, non-RO and high-fluoride water groups. Children consuming the same water type for at least 3 years were included. Sociodemographic, dietary and lifestyle information was collected using structured questionnaires and a food frequency questionnaire. Drinking-water samples were analyzed for calcium, magnesium, phosphorus, fluoride and total dissolved solids. BMD was assessed using pediatric dual-energy X-ray absorptiometry.

# Results

Mean age was 11.18 years. The high-fluoride group had higher concentrations of calcium, magnesium, fluoride and total dissolved solids, whereas the RO group had the lowest mineral concentrations. Total-body, lumbar spine and femoral BMD differed significantly among groups, with the highest values generally observed in the high-fluoride group. In multivariable analysis, age, BMI and vitamin D remained significant predictors. Age, sex, physical activity and BMI were significantly associated with lumbar spine BMD.

# Conclusion

Drinking-water mineral composition was associated with BMD in children. Longitudinal studies are needed to clarify these relationships and determine their clinical significance.

## Introduction

Bone health during childhood and adolescence is an important determinant of skeletal health throughout the life course. Approximately 40%–60% of adult bone mass is acquired during adolescence, and approximately 90% of peak bone mass is accumulated by 18 years of age.[1, 2] Consequently, childhood and adolescence represent critical periods during which adequate skeletal mineralization may contribute to the attainment of optimal peak bone mass.

Bone mass is influenced by a combination of genetic and environmental factors. Genetic factors account for a substantial proportion of the interindividual variation in bone mass, while potentially modifiable factors include calcium and vitamin D intake, protein and mineral nutrition, physical activity, body weight and composition, hormonal status and lifestyle.[1, 2] Calcium and phosphorus constitute the principal mineral components of hydroxyapatite, while magnesium also participates in bone mineral metabolism and skeletal structure.[2, 3]

Although dietary intake is an important determinant of skeletal mineralization, drinking water may also contribute to mineral intake. The mineral composition of drinking water varies considerably according to its geological source and treatment process. Reverse osmosis is an effective water-treatment process that removes a broad range of dissolved substances and may substantially reduce concentrations of calcium, magnesium and other minerals.[4] Consumption of very low-mineral water has previously been associated with lower calcium intake, reduced bone mineral content and changes in biochemical markers of bone formation and resorption in children.[5]

Fluoride is another component of drinking water that is relevant to skeletal health. At appropriate concentrations, fluoride has recognized dental health benefits; however, chronic exposure to higher concentrations is associated with an increased risk of dental fluorosis and, at sufficiently high exposure, skeletal fluorosis. The World Health Organization guideline value for fluoride in drinking water is 1.5 mg/L.[6] The relationship between fluoride exposure and BMD is complex, and studies in children have produced inconsistent findings.[7, 8] Therefore, the presence of higher fluoride concentrations should not automatically be interpreted as beneficial for bone health.

Body size is also an important determinant of BMD in growing children. A large population-based study involving 6,143 adolescents aged 8–19 years demonstrated a positive association between BMI and total BMD, although the association was nonlinear and showed a saturation effect.[9] Interpretation of pediatric DXA measurements must therefore consider age, sex, body size and skeletal maturation.[10]

The assessment of BMD in children is particularly challenging because bone mineral accrual occurs concurrently with changes in body size and pubertal development. Dual-energy X-ray absorptiometry (DXA) is widely used for pediatric assessment of bone mineral content and areal BMD. However, pediatric DXA results should be interpreted using appropriate age- and sex-specific reference data, and size adjustment should be considered where appropriate.[10, 11] Importantly, pediatric osteoporosis should not be diagnosed solely on the basis of a low DXA measurement.[10]

Despite the importance of childhood skeletal development, relatively little is known about whether differences in drinking-water mineral composition are associated with BMD during the pubertal transition. The present study therefore compared BMD among children aged 9–13 years consuming RO-treated, non-RO and high-fluoride drinking water and examined the association between drinking-water mineral composition and BMD.

We hypothesized that children consuming RO-treated water would have lower BMD than children consuming non-RO or higher-fluoride drinking water.

## Methods

# Study design and setting

A comparative cross-sectional study was conducted among schoolchildren aged 9–13 years in and around Mysuru district, Karnataka, India, between March 2022 and October 2023.

The study was designed and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations for cross-sectional studies.[12, 16]

## Study population and participant selection

Schools were selected according to the requirements of the study, and eligible children were recruited from the selected schools.

Participants were categorized into three groups according to their habitual drinking-water source:

1. **RO group**: children consuming reverse osmosis-treated drinking water.
2. **Non-RO group**: children consuming non-RO drinking water.
3. **High-fluoride group**: children consuming drinking water with fluoride concentration > 1.5 mg/L.

Each group consisted of 60 participants, resulting in a total sample of 180 children.

The type of drinking water was determined by interviewing the child and parent or legal guardian. Children were included only if they had consumed the same type of drinking water continuously for at least 3 years.

## Inclusion criteria

Children were eligible if they:

- were aged 9–13 years;
- had consumed the same type of drinking water continuously for at least 3 years;

- provided assent to participate; and
- had written informed consent from their parent or legal guardian.

## Exclusion criteria

Children were excluded if they had:

- malnutrition, defined as weight-for-height below the fifth percentile;
- chronic systemic disease, including tuberculosis, diabetes mellitus or endocrine disorders;
- chronic inflammatory or intestinal disease;
- a history of conditions known to substantially affect skeletal metabolism;
- use of nutritional supplements in medicinal form; or
- systemic corticosteroid use.

## Ethical considerations

The study received approval from the Institutional Ethics Committee of JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysuru, India (Research Protocol No. 11/2020, dated 30 April 2020). Written informed consent was obtained from parents or legal guardians, and assent was obtained from the participating children. The study was conducted in accordance with the principles of the Declaration of Helsinki.

# Sociodemographic and dietary assessment

A structured questionnaire was administered to collect information on sociodemographic characteristics, medical history, sunlight exposure, physical activity, lifestyle factors and nutritional supplement use.

Dietary intake was assessed using a previously published and validated food frequency questionnaire (FFQ) developed for the Indian population,[17] administered in the presence of the child's parent or guardian. The FFQ assessed consumption during the preceding month and was used to estimate intake of calcium, magnesium, phosphorus, vitamin D and fluoride.

Dietary nutrient estimation was performed using the Nutrition Support Resource (NSR Nutrical 5.1) database, incorporating the Indian Food Composition Tables 2017 and USDA food-composition data where applicable.

Body weight and height were measured using standardized procedures, and BMI was calculated as:

**BMI = weight (kg) / height² (m²).**

## Drinking-water sample collection and analysis

Participants were asked to provide samples of the drinking water consumed at home. The samples were analyzed for calcium, magnesium, phosphorus, fluoride and total dissolved solids.

Water-quality measurements were performed according to standardized procedures described in *Standard Methods for the Examination of Water and Wastewater* published by the American Public Health Association and collaborating organizations.[13]

The fluoride concentration was used to classify participants into the high-fluoride group. The WHO guideline value for fluoride in drinking water is 1.5 mg/L.[6]

## Bone mineral density assessment

Bone mineral density was assessed using dual-energy X-ray absorptiometry (DXA). Total-body DXA scans were performed using a DPX-IQ 240 pencil-beam densitometer (Lunar Corp., Madison, WI, USA) using the pediatric acquisition protocol and manufacturer's recommendations.

The scans were analyzed for total-body and regional bone outcomes. Measurements included bone mineral content (BMC) and areal BMD for the total body, lumbar spine (L1–L4), and right and left femur.

Quality-assurance procedures were performed daily using the manufacturer's phantom and standardized protocol. Interobserver variation was assessed to maintain measurement quality, and coefficients of variation for the reported outcomes were < 0.4%.

Because the study population consisted of children, DXA measurements were interpreted in accordance with pediatric principles. Pediatric DXA assessment is affected by age, sex, body size and maturation, and size adjustment should be considered where appropriate.[10, 11]

# Sample-size calculation

The sample size was calculated using a previous study and assuming a 95% confidence level and 80% statistical power. A mean difference of 0.22 kg in bone mineral content between normal-water and very-low-mineral-water groups and an effect size of 0.56 were used for the calculation. The estimated sample size was 50 children per group.

To compensate for potential exclusions or loss of participants, the sample size was increased to 60 children per group, giving a total sample size of 180 participants. The calculation was performed using NMaster sample-size calculation software using a hypothesis test for two means with equal variance.

# Statistical analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 23.0.

Categorical variables were summarized using frequencies and percentages. Continuous variables were summarized using means and standard deviations for normally distributed data and medians with interquartile ranges where appropriate.

For normally distributed continuous variables, independent-samples *t* tests and one-way analysis of variance (ANOVA) were used. For non-normally distributed data, the Kruskal–Wallis test and Mann–Whitney U test were applied as appropriate.

Pearson's correlation coefficient was used for normally distributed continuous variables, whereas Spearman's rank correlation coefficient was used for non-normally distributed variables.

Simple linear regression was performed to examine unadjusted associations between relevant nutritional, biochemical and drinking-water variables and BMD. Multivariable linear regression was subsequently performed to assess independent associations after adjustment for relevant covariates, including age, sex, BMI, physical activity and nutritional variables, as appropriate.

A two-sided *P* value < 0.05 was considered statistically significant.

## Results

# Participant characteristics

A total of 180 children aged 9–13 years were included, with 60 participants in each drinking-water group. The overall mean age was 11.18 years. Mean age was 10.98 years in the RO group, 11.20 years in the non-RO group, and 11.35 years in the high-fluoride group, with no significant difference among groups (P = 0.351).

The sex distribution did not differ significantly between groups (P = 0.243). Boys represented 51.7% of the RO group, 46.7% of the non-RO group, and 36.7% of the high-fluoride group.

A marked difference in socioeconomic status was observed among the groups (P < 0.001). Most children in the RO group were classified as above the poverty line, whereas most children in the non-RO and high-fluoride groups were classified as below the poverty line.

Mean height and weight did not differ significantly between groups. However, BMI differed significantly (P = 0.005), with mean BMI values of 16.47 kg/m², 14.87 kg/m², and 16.39 kg/m² in the RO, non-RO, and high-fluoride groups, respectively.

Detailed participant characteristics are presented in Table 1.

**Table 1. Sociodemographic and anthropometric characteristics of study participants**

| variables | RO water | Non RO | High fluoride water | p-value |
|---|---|---|---|---|
| Age | 10.98(1.39) | 11.20(1.34) | 11.35(1.44) | 0.351 |
| Gender | n (%) | n (%) | n (%) | |
| Boys | 31(51.7) | 28(46.7) | 22(36.7) | 0.243 |
| Girls | 29(48.3) | 32(53.3) | 38(63.3) | |
| Socioeconomic status | n (%) | n (%) | n (%) | |
| APL* | | | | |
| BPL** | 49(81.7) | 4(6.7) | 16(26.7) | 0.001 |
| | 11(18.3) | 56(93.3) | 44(73.3) | |
| Height | 138.31(11.02) | 137.80(10.81) | 136.18(9.2) | 0.505 |
| Weight | 31.87(8.16) | 28.78(9.83) | 30.80 ( 8.12) | 0.149 |
| Body Mass Index | 16.47(2.75) | 14.87(3.06) | 16.39(3,03) | 0.005 |

Age (in years),height ( in metres), weight (in kilograms), Body Mass Index ( in kilogram/metre $^2$) were compared using analysis of variance test. Gender and socio economic status values are presented in number and percentages and were compared using chi square test . p value represents the comparison between the sociodemographic variables of RO, non-RO and high fluoride drinking water children.

*APL-Above poverty line, **BPL- Below poverty line,

# Drinking-water mineral composition and dietary characteristics

Substantial differences in drinking-water composition were observed across the three exposure groups.

Mean calcium concentration was 5.24 mg/L in RO water, 35.86 mg/L in non-RO water, and 65.49 mg/L in high-fluoride water ($P < 0.001$). Corresponding magnesium concentrations were 1.72, 17.71, and 80.91 mg/L, respectively ($P < 0.001$).

Fluoride concentrations were 0.14 ppm in the RO group, 0.56 ppm in the non-RO group, and 1.58 ppm in the high-fluoride group ($P < 0.001$). TDS also differed substantially, with mean concentrations of 13.06 mg/L, 361.63 mg/L, and 553.02 mg/L in the RO, non-RO, and high-fluoride groups, respectively ($P < 0.001$).

In contrast, estimated dietary calcium, magnesium, phosphorus, and vitamin D intake did not differ significantly among groups.

These findings indicate that the drinking-water groups represented markedly different mineral-exposure environments, while measured dietary nutrient intake was broadly comparable between groups.(Table 2)

Table 2
Mineral composition of drinking water and estimated dietary nutrient intake

| variables | RO water | Non RO | High fluoride water | p-value |
|---|---|---|---|---|
| Calcium in drinking water (mg/l) | 5.24(3.59) | 35.86(12.76) | 65.49(18.32) | 0.001 |
| Magnesium in drinking water(mg/l) | 1.72(1.18) | 17.71(15.91) | 80.91(28.60) | 0.001 |
| Fluoride in drinking water(ppm) | 0.14(0.13) | 0.56(0.30) | 1.58(0.15) | 0.001 |
| Total dissolved solids in drinking water(mg/l) | 13.06(8.15) | 361.63(284.26) | 553.02(130.72) | 0.001 |
| Calcium assessed through food frequency questionnaire(mg) | 531.98(138.24) | 538.34(130.03) | 560.97(63.36) | 0.381 |
| Magnesium assessed through food frequency questionnaire(mg) | 727.05(160.12) | 667.56(186.66) | 705.25(142.10) | 0.257 |
| phosphate assessed through food frequency questionnaire(mg) | 1781.90(381.28) | 1625.28(473.83) | 1731.29(347.96) | 0.205 |
| Vitamin D assessed through food frequency questionnaire (mcg) | 23.02(10.49) | 22.45(6.65) | 23.81(6.08) | 0.725 |

# Bone mineral density according to drinking-water group

Significant differences in BMD were observed across all measured skeletal sites.

Mean total-body BMD was highest among children consuming high-fluoride water (0.8328 ± 0.0789), followed by non-RO water (0.7807 ± 0.0839) and RO water (0.7689 ± 0.0791; $P < 0.001$).

For the lumbar spine (L1–L4), mean BMD was 0.7566 ± 0.1233 in the high-fluoride group, 0.6916 ± 0.1065 in the non-RO group, and 0.6826 ± 0.1050 in the RO group ($P < 0.001$).

Similarly, right femoral BMD was 0.8038 ± 0.1031, 0.7391 ± 0.0992, and 0.7217 ± 0.0985, respectively ($P < 0.001$), while left femoral BMD was 0.7933 ± 0.1003, 0.7352 ± 0.0996, and 0.7173 ± 0.0940, respectively ($P < 0.001$).

Post-hoc comparisons indicated that the high-fluoride group differed significantly from both the non-RO and RO groups. (Table 3)

Table 3
Comparison of BMD across skeletal sites according to drinking-water group

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | |
| Total BMD | HF | 60 | .8328 | .07894 | .01019 | .8124 | .8532 | 0.001 |
| | NonRO | 60 | .7807 | .08394 | .01084 | .7591 | .8024 | |
| | RO | 60 | .7689 | .07909 | .01021 | .7485 | .7893 | |
| | Total | 180 | .7941 | .08493 | .00633 | .7817 | .8066 | |
| L1 to L4 | HF | 60 | .7566 | .12334 | .01592 | .7247 | .7885 | 0.001 |
| | NonRO | 60 | .6916 | .10651 | .01375 | .6641 | .7191 | |
| | RO | 60 | .6826 | .10496 | .01355 | .6555 | .7097 | |
| | Total | 180 | .7103 | .11609 | .00865 | .6932 | .7274 | |
| Right Femur | HF | 60 | .8038 | .10312 | .01331 | .7771 | .8304 | 0.001 |
| | NonRO | 60 | .7391 | .09923 | .01281 | .7134 | .7647 | |
| | RO | 60 | .7217 | .09851 | .01272 | .6963 | .7472 | |
| | Total | 180 | .7549 | .10584 | .00789 | .7393 | .7704 | |
| Left Femur | HF | 60 | .7933 | .10034 | .01295 | .7674 | .8192 | 0.001 |
| | NonRO | 60 | .7352 | .09958 | .01286 | .7095 | .7610 | |
| | RO | 60 | .7173 | .09395 | .01213 | .6931 | .7416 | |
| | Total | 180 | .7486 | .10273 | .00766 | .7335 | .7637 | |

# Determinants of total-body BMD

In unadjusted linear regression, total-body BMD was positively associated with calcium concentration in drinking water ($R^2$=0.057; P = 0.001), magnesium concentration in drinking water ($R^2$=0.064; P = 0.001), TDS ($R^2$=0.044; P = 0.005), age ($R^2$=0.216; P = 0.001), and BMI ($R^2$=0.274; P = 0.001).

No significant unadjusted associations were observed for sex, socioeconomic status, physical activity, sunlight exposure, or the variables identified as serum calcium and vitamin D in the supplied regression table.

In the multivariable model, age (β = 0.022; 95% CI, 0.015–0.030; P < 0.001), BMI (β = 0.013; 95% CI, 0.009–0.016; P < 0.001), and vitamin D (β = 0.001; 95% CI, 0.000–0.003; P = 0.040) remained significantly

associated with total-body BMD. Associations with drinking-water calcium, magnesium, and TDS were attenuated and were no longer statistically significant after adjustment.

These findings indicate that the apparent unadjusted relationships between water mineral content and total-body BMD were substantially explained by other characteristics of the participants, particularly age and body size.(Table 4 &5)

Table 4
Univariable linear regression analysis of predictors of total-body BMD

| **Total BMD- Unadjusted** | | | | | |
|---|---|---|---|---|---|
| **Parameter** | **$R^2$** | **Constant** | **B** | **Confidence interval** | **p-value** |
| Serum calcium | 0.007 | 0.918 | -0.013 | -0.036-0.010 | 0.273 |
| Vitamin D | 0.005 | 0.777 | 0.001 | 0.001–0.002 | 0.349 |
| Physical activity | 0.003 | 0.802 | 0.000 | -0.001-0.001 | 0.517 |
| Sunlight exposure | 0.002 | 0.788 | 0.000 | 0.000-0.001 | 0.595 |
| **Calcium in water** | **0.057** | **0.768** | **0.001** | **0.000-0.001** | **0.001** |
| **Magnesium in water** | **0.064** | **0.776** | **0.001** | **0.000-0.001** | **0.001** |
| **TDS** | **0.044** | **0.775** | **6.245E-5** | **0.000–0.000** | **0.005** |
| **Age** | **0.216** | **0.477** | **0.028** | **0.020–0.036** | **0.001** |
| gender | 0.009 | 0.819 | -0.016 | -0.041-0.009 | 0.209 |
| Socioeconomic status | 0.001 | 0.787 | 0.004 | -0.021-0.030 | 0.731 |
| **BMI** | **0.274** | **0.015** | **0.560** | **0.011–0.018** | **0.001** |

Table 5
Multivariable linear regression analysis of predictors of total-body BMD

| Model | | Unstandardized Coefficients | | Standardized Coefficients | T | Sig. | 95.0% Confidence Interval for B | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Lower Bound | Upper Bound |
| 1 | (Constant) | .435 | .123 | | 3.527 | .001 | .191 | .679 |
| | Ca | − .016 | .011 | − .104 | -1.448 | .150 | − .038 | .006 |
| | **vitaminD** | **.001** | **.001** | **.131** | **2.068** | **.040** | **.000** | **.003** |
| | Calcium water | .000 | .000 | .089 | .578 | .564 | − .001 | .001 |
| | Magnesium water | .000 | .000 | .066 | .493 | .623 | .000 | .001 |
| | TDS | 2.417E-5 | .000 | .083 | .929 | .354 | .000 | .000 |
| | **Age** | **.022** | **.004** | **.373** | **5.796** | **.000** | **.015** | **.030** |
| | Sex | .005 | .011 | .031 | .474 | .636 | − .016 | .027 |
| | SES | .011 | .013 | .062 | .840 | .402 | − .014 | .035 |
| | Physical activity | .000 | .000 | − .069 | -1.111 | .269 | − .001 | .000 |
| | Sun light exposure | 2.610E-5 | .000 | .009 | .138 | .891 | .000 | .000 |
| | **BMI** | **.013** | **.002** | **.446** | **6.729** | **.000** | **.009** | **.016** |

a. Dependent Variable: total BMD

# Determinants of lumbar spine BMD

In unadjusted analyses, lumbar spine BMD was positively associated with calcium in drinking water ($R^2$=0.036; P = 0.012), magnesium in drinking water ($R^2$=0.040; P = 0.008), TDS ($R^2$=0.026; P = 0.035), age ($R^2$=0.156; P = 0.001), and BMI ($R^2$=0.226; P = 0.001).

In the multivariable model, age (β = 0.027; 95% CI, 0.016–0.038; P < 0.001), sex (β = 0.057; 95% CI, 0.026–0.089; P < 0.001), physical activity (β=-0.001; 95% CI, -0.002 to 0.000; P = 0.036), and BMI (β = 0.018; 95% CI, 0.013–0.023; P < 0.001) were statistically significant.

Neither calcium nor magnesium concentration in drinking water remained significantly associated with lumbar spine BMD after multivariable adjustment.(Tables 6 & 7)

Table 6
Univariable linear regression analysis of predictors of lumbar spine BMD

| **Lumbar spine L1 TO L4- Unadjusted** | | | | | |
|---|---|---|---|---|---|
| **Parameter** | **$R^2$** | **Constant** | **B** | **Confidence interval** | **p-value** |
| Serum calcium | 0.005 | 0.857 | -0.015 | -0.047-0.017 | 0.352 |
| Vitamin D | 0.002 | 0.726 | -0.001 | -0.003-0.002 | 0.515 |
| Physical activity | 0.013 | 0.738 | -0.001 | -0.002-0.000 | 0.141 |
| Sunlight exposure | 0.000 | 0.713 | -5.138E-5 | -0.001-0.001 | 0.870 |
| **Calcium in water** | **0.036** | **0.681** | **0.001** | **0.000-0.001** | **0.012** |
| **Magnesium in water** | **0.040** | **0.689** | **0.001** | **0.000-0.001** | **0.008** |
| **TDS** | **0.026** | **0.690** | **6.561E-5** | **0.000–0.000** | **0.035** |
| **Age** | **0.156** | **0.336** | **0.034** | **0.022–0.045** | **0.001** |
| gender | 0.019 | 0.660 | 0.032 | -0.003-0.067 | 0.071 |
| Socioeconomic status | 0.000 | 0.707 | 0.002 | -0.034-0.039 | 0.901 |
| **BMI** | **0.226** | **0.419** | **0.018** | **0.013–0.023** | **0.001** |

Table 7
Multivariable linear regression analysis of predictors of lumbar spine BMD

| Coefficients[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Model** | | **Unstandardized Coefficients** | | **Standardized Coefficients** | **t** | **Sig.** | **95.0% Confidence Interval for B** | |
| | | **B** | **Std. Error** | **Beta** | | | **Lower Bound** | **Upper Bound** |
| 1 | (Constant) | .267 | .194 | | 1.375 | .171 | − .117 | .650 |
| | Ca | − .029 | .017 | − .142 | -1.695 | .092 | − .064 | .005 |
| | Vitamin D | .000 | .001 | .032 | .471 | .638 | − .002 | .002 |
| | **age** | **.027** | **.006** | **.328** | **4.769** | **.000** | **.016** | **.038** |
| | **sex** | **.057** | **.016** | **.252** | **3.637** | **.000** | **.026** | **.089** |
| | SES | .008 | .019 | .035 | .428 | .669 | − .030 | .046 |
| | Calcium water | -7.410E-5 | .001 | − .018 | − .108 | .914 | − .001 | .001 |
| | Magnesium water | .000 | .000 | .150 | .961 | .338 | .000 | .001 |
| | TDS | 2.624E-5 | .000 | .067 | .691 | .490 | .000 | .000 |
| | **Physical activity** | **− .001** | **.001** | **− .141** | **-2.112** | **.036** | **− .002** | **.000** |
| | Sunlight exposure | -8.547E-5 | .000 | − .022 | − .309 | .758 | − .001 | .000 |
| | **BMI** | **.018** | **.003** | **.473** | **6.649** | **.000** | **.013** | **.023** |
| | Mg | − .011 | .026 | − .030 | − .438 | .662 | − .063 | .040 |
| | phosphorous | .020 | .011 | .156 | 1.793 | .075 | − .002 | .043 |

a. Dependent Variable: L1TOL4

# Discussion

The present study evaluated the association between drinking-water mineral composition and BMD among 180 children aged 9–13 years. The principal findings were that (1) the mineral composition of drinking water differed substantially among RO, non-RO and high-fluoride groups; (2) children consuming high-fluoride water had higher BMD at the measured skeletal sites than children consuming RO and non-RO water; (3) BMD increased with age; and (4) calcium, magnesium and TDS in drinking water, together with age and BMI, were associated with total-body BMD in unadjusted analyses, while age, BMI and vitamin D remained significant in multivariable analysis.

These findings are biologically plausible but should be interpreted cautiously because skeletal mineralization is influenced by numerous interacting factors. In addition, the cross-sectional design prevents determination of whether drinking-water characteristics preceded changes in BMD.

Age was strongly associated with BMD in the present study. BMD increased progressively from 9 to 13 years of age across all three drinking-water groups. This observation is consistent with the established pattern of rapid bone mineral acquisition during childhood and adolescence. Approximately 40%–60% of adult bone mass is acquired during adolescence, with substantial mineral accrual occurring around the period of peak height velocity.[1, 2]

Nakavachara et al. reported age-related increases in total-body and lumbar spine BMD among 381 healthy children and adolescents aged 5–18 years, supporting the age-related increase observed in the present study.[14]

The RO group had the lowest mineral concentrations and TDS in drinking water. Children consuming RO water also demonstrated comparatively lower BMD values than those consuming high-fluoride water and, to a lesser extent, non-RO water.

One possible explanation is reduced mineral intake from drinking water. Reverse osmosis can substantially reduce dissolved mineral concentrations, including calcium and magnesium.[4] Because drinking water may contribute to total daily mineral intake, particularly in populations with relatively low dietary mineral intake, long-term consumption of very low-mineral water could potentially contribute to differences in mineral availability.

Huang et al. conducted a retrospective cohort study among children aged 10–13 years who had consumed either normal-mineral or very-low-mineral water for four years. Children consuming very-low-mineral water had lower calcium intake from drinking water, lower BMC and lower serum bone alkaline phosphatase, together with higher serum CTX, suggesting reduced bone formation and increased bone resorption.[5]

These findings provide biological support for the possibility that drinking-water mineral content may contribute to skeletal mineral acquisition. Nevertheless, the present study cannot establish that the lower BMD observed in RO consumers resulted directly from reduced mineral intake from water.

Calcium concentration in drinking water was positively associated with total-body BMD in the present study. Calcium is a major component of the mineral phase of bone and is essential for normal skeletal development. Adequate calcium intake is particularly important during adolescence, when bone mineral accrual is accelerated.[2]

The association observed in the present study is consistent with findings from Huang et al., who reported positive associations between calcium intake from drinking water and BMC in children consuming very-low-mineral water.[5]

However, total dietary calcium intake is likely to be substantially more important than water-derived calcium alone in most populations. The relative contribution of drinking water will depend on the mineral concentration of the water, daily water consumption and dietary patterns.

Consequently, the present finding should be interpreted as evidence that drinking-water calcium may contribute to the overall mineral exposure of children rather than as evidence that water calcium independently determines BMD.

Magnesium was also positively associated with total-body BMD in the present study. Magnesium is an important component of the skeletal system and participates in bone mineral metabolism. Approximately 60% of body magnesium is stored in bone, and magnesium contributes to hydroxyapatite formation and bone structure.[15]

Magnesium deficiency has been associated with alterations in osteoblast and osteoclast activity and with disturbances in calcium and vitamin D metabolism.[15] In a large cross-sectional study of children younger than 3 years, Wu et al. reported positive associations between serum magnesium concentrations and BMD-related measurements after adjustment for potential confounders.[15]

Although the age range of that study differed substantially from that of the present study, the biological mechanisms provide support for a possible relationship between magnesium status and skeletal mineralization.

The present findings may therefore partly reflect the contribution of magnesium from drinking water. Nevertheless, dietary magnesium intake, rather than drinking-water magnesium alone, is likely to be a major determinant of overall magnesium status.

The highest BMD values in the present study were observed among children consuming high-fluoride water. However, this finding requires careful interpretation.

Fluoride has a complex relationship with skeletal tissues. The WHO guideline value for fluoride in drinking water is 1.5 mg/L, based on the increasing risk of dental fluorosis above this concentration and the potential for skeletal effects at higher exposure levels.[6]

Grobler et al. studied children exposed to drinking-water fluoride concentrations of approximately 0.19 and 3.0 mg/L and reported higher BMD values in children from the high-fluoride area, although the relationship between fluoride exposure and BMD was not straightforward.[7] Other studies have produced inconsistent results,[8] and therefore a higher BMD in a population with higher fluoride exposure should not be interpreted as evidence that higher fluoride exposure is beneficial.

In the present study, the high-fluoride group consumed water containing approximately 1.3–1.55 mg/L fluoride according to the study measurements. These concentrations are close to or slightly above the WHO guideline value of 1.5 mg/L.[6]

The higher BMD observed in this group may therefore reflect the combined influence of fluoride, calcium, magnesium, phosphorus, TDS, dietary factors, age, BMI, physical activity and other environmental characteristics rather than an isolated effect of fluoride.

Importantly, fluoride exposure at concentrations exceeding recommended limits may increase the risk of fluorosis. Thus, the present findings should not be interpreted as supporting intentional consumption of high-fluoride drinking water.

BMI showed a significant positive association with total-body, lumbar spine and femoral BMD in the present study.

This finding is consistent with the population-based study by Ouyang et al., which included 6,143 adolescents aged 8–19 years and demonstrated a positive association between BMI and total BMD. The relationship was nonlinear and demonstrated a saturation effect, with different BMI thresholds according to age.[9]

Several mechanisms may explain the relationship between body size and BMD. Greater body mass increases mechanical loading on the skeleton and may stimulate bone adaptation. Lean mass also contributes to mechanical loading and is positively associated with bone mineral accrual. In addition, adipose tissue participates in sex-steroid metabolism.

However, BMI does not distinguish between fat mass and lean mass and therefore should not be interpreted as a direct measure of skeletal loading. The positive association observed in the present study does not imply that increasing adiposity is an appropriate strategy for improving bone health.

Sex was significantly associated with lumbar spine BMD in the multivariable model. This is biologically plausible because skeletal development differs between boys and girls, particularly during puberty.

In the present study, girls were more numerous overall, although the distribution differed between drinking-water groups. Because sex distribution and socioeconomic status were not identical across the three groups, these variables may have acted as confounders.

Future analyses should therefore provide sex-stratified estimates and, where sample size permits, investigate interactions between sex, pubertal development and drinking-water exposure.

Physical activity was significantly associated with lumbar spine BMD in multivariable analysis. This is consistent with the established role of mechanical loading in skeletal development. Weight-bearing activities such as running, jumping and other impact-based activities can stimulate bone formation during childhood and adolescence.[2]

Because physical activity was assessed using questionnaire-based information, measurement error cannot be excluded. Objective measures such as accelerometry would provide more precise estimates in future studies.

A statistically significant difference in socioeconomic status was observed among the three drinking-water groups. Most children in the high-fluoride and non-RO groups belonged to families below the poverty line, whereas most children in the RO group belonged to families above the poverty line.

Socioeconomic status may influence diet, physical activity, access to healthcare, type of drinking water, sunlight exposure and other determinants of bone health. Therefore, socioeconomic status is a potential confounder in the relationship between drinking-water type and BMD.

This observation is particularly important because the water groups were not randomized. Families who use RO systems may differ from those using non-RO or naturally high-fluoride water in multiple social and behavioral characteristics. Consequently, the observed differences in BMD cannot be attributed solely to drinking-water composition.

The findings suggest that drinking-water mineral composition may be one component of the environmental determinants of skeletal mineralization during childhood. In particular, the low calcium and magnesium concentrations observed in RO-treated water warrant consideration in populations where drinking water represents a substantial source of dietary minerals.

However, these findings should not be interpreted as a recommendation to consume high-fluoride water. Fluoride concentrations above recommended levels may increase the risk of fluorosis, and the WHO guideline value is 1.5 mg/L.[6]

The practical implication is instead that children should obtain adequate calcium, magnesium, phosphorus and vitamin D through an overall balanced diet, while drinking water should meet established safety standards.

The study has several strengths. First, it included an equal number of participants in three distinct drinking-water exposure groups. Second, drinking-water samples were directly analyzed rather than relying solely on participant recall. Third, the study assessed multiple minerals in drinking water and examined BMD at several skeletal sites. Fourth, DXA was used to assess bone mineral parameters, and quality-assurance procedures were performed.

Several limitations should be considered.

First, the cross-sectional design prevents determination of temporal sequence and causality. Second, the three drinking-water groups differed in socioeconomic characteristics, creating the possibility of residual confounding. Third, chronological age was used as the principal indicator of maturation, while pubertal stage was not included in the available analysis. Pubertal development is an important determinant of pediatric BMD.

Fourth, DXA provides areal rather than volumetric BMD, and measurements in children are influenced by body size. Pediatric interpretation should therefore include appropriate age-, sex- and size-adjusted

reference data.[10, 11] Fifth, dietary intake was estimated using an FFQ and is therefore subject to recall and reporting bias.

# Conclusion

In this comparative cross-sectional study of children aged 9–13 years, significant differences in BMD were observed among children consuming RO-treated, non-RO and high-fluoride drinking water. Children consuming high-fluoride water demonstrated the highest BMD values, whereas children consuming RO-treated water demonstrated comparatively lower BMD values.

Calcium, magnesium and TDS concentrations in drinking water were positively associated with total-body BMD in unadjusted analyses. Age and BMI were also important determinants of BMD, while age, BMI and vitamin D remained significantly associated with total-body BMD in multivariable analysis. For lumbar spine BMD, age, sex, physical activity and BMI were significant independent correlates.

These findings indicate that drinking-water mineral composition may contribute to skeletal mineralization during childhood. However, the results should be interpreted as associations rather than causal effects. In particular, the higher BMD observed in children consuming high-fluoride water should not be interpreted as evidence that high fluoride intake is beneficial or recommended.

Longitudinal studies incorporating repeated assessment of drinking-water exposure, dietary mineral intake, pubertal stage, physical activity, body composition and pediatric DXA Z-scores are required to clarify the independent contribution of drinking-water mineral composition to bone development.

# Declarations

### Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available because they contain information relating to minor participants but may be made available from the corresponding author on reasonable request and subject to appropriate ethical and institutional restrictions.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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# Author Contribution

1. Dr Thippeswamy HM -Project conception, development of overall research plan, and study oversight.2. Dr Nanditha Kumar M -primary responsibility for final content Wrote paper.3. Dr. Raghavendra Shanbhog - study oversight and data collection4. Dr Prashanth S N-hands-on primary responsible for the final content 5. Dr Smitha P -hands-on conduct of the experiments and data collection6. Dr Debasmita Hajra - overall analysis

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