

## Calcium Bioavailability from Mineral Waters with Different Mineralization in Comparison to Milk and a Supplement

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### ABSTRACT

**Objective:** The aim of the present study was to compare the bioavailability of calcium from 3 mineral waters with different concentrations of minerals with that of milk and a calcium supplement.

**Methods:** A single-center, randomized controlled trial with a crossover design with 21 healthy men and women was conducted at the Institute of Food Science and Human Nutrition, Leibniz University Hannover. The participants consumed the 5 test products providing 300 mg of calcium each on 5 examination days with 1-week wash-out phases in between. Primary outcome variables were the area under the curve of serum calcium levels for 10-hour ( $AUC_{0-10h}$ ) and 24-hour urinary calcium excretion.

**Results:** In all groups, no significant differences in the  $AUC_{0-10h}$  of serum calcium levels as well as in the 24-hour urinary calcium excretion were observed. Likewise, mean changes in serum phosphate and urinary phosphate, as well as serum parathormone, showed no differences between the groups.

**Conclusion:** Given an equivalent bioavailability of calcium in all test products, neither a high concentration of  $SO_4^{2-}$  or of  $HCO_3^-$  influenced the bioavailability of calcium. Accordingly, the use of mineral water with high concentrations of calcium constitutes a calorie-free calcium source that can improve calcium supply.

**Abbreviations:** AUC, area under the curve; BMI, body mass index; Ca, calcium;  $CaCO_3$ , calcium carbonate; DRV, dietary reference value; MW, mineral water;  $n$ , number of subjects; NHANES, National Health and Nutrition Examination Survey; RDA, recommended dietary allowance; RNI, reference nutrient intake; SD, standard deviation.

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### Introduction

Adequate calcium (Ca) intake is essential for normal growth and development of the skeleton and teeth as well as adequate bone mineralization. Furthermore, small but essential quantities of Ca are required for nerve conductivity, muscle contraction, hormone and enzyme secretion, and blood clotting [1]. Low dietary intake has been linked to age-related bone loss, increased risk of osteoporosis, bone fractures, hypertension, cardiovascular disease, and colon cancer [2,3]. The current recommendations of Ca for adults vary between 1,300 mg/d for the United States (recommended dietary allowance), 1,000 mg/d for Germany (dietary reference value), 800 mg/d for the European Union (dietary reference value), and 700 mg/d for the U.K. (reference nutrient intake) [4–7]. When usual Ca consumption patterns were analyzed, most population groups consumed less than the recommended dietary allowance. Data from the National Health and Nutrition Examination Survey indicate that 68% of U.S. adults do not meet the current dietary recommendation for Ca [8]. For females in the U.K., 13%–18% of 13- to 34-year-olds and 8%–15% of those over 65 years do not reach the reference nutrient intake [9].

Dairy products are a rich source of dietary Ca. Nevertheless, the consumption of these foods is relatively low due to lactose

intolerance and/or taste aversions [6]. Furthermore, in view of the rising prevalence of obesity, low-calorie Ca sources should be preferred [10]. Mineral water is a promising candidate, because it is calorie free, contains no potential allergens, and ensures hydration. Furthermore, the Ca content of mineral waters can reach more than 500 mg per liter, although the content varies widely depending on its origin [11].

According to the literature, the bioavailability of Ca from mineral water is comparable or even better than that from milk [12]. However, the bioavailability may be influenced by the concentration of other minerals in the mineral water, such as magnesium, as well as the presence of anions, such as phosphates and sulfates [13].

Therefore, the aim of the present study was to compare the Ca bioavailability of 3 mineral waters with different types of mineralization with that of milk and a Ca supplement.

### Material and methods

#### Study design

A single-center, randomized controlled trial with a crossover design was conducted by trained professionals according to standardized methods at the Institute of Food Science and

Human Nutrition, Leibniz University Hannover, Germany. The study consisted of a screening phase, a 4-week depletion phase, and 5 examinations with 1-week wash out phases in between.

Ethical approval was provided by the Ethic Commission at the Medical Chamber of Lower Saxony (Hannover, Germany). Written informed consent was obtained from all subjects prior to participating in the study according to the guidelines of the Declaration of Helsinki. The study is registered in the German Clinical Trial Register (DRKS00010411).

## Subjects

Healthy participants ( $n = 21$ ) were recruited from the general population in Hannover, Germany, by advertisements. They were selected according to inclusion and exclusion criteria that were assessed via questionnaires. The main inclusion criteria were age between 18 and 50 years and a body mass index between 18.5 and 29.9 kg/m<sup>2</sup>. Exclusion criteria were defined as an allergy to any of the test products or foods, the intake of Ca supplements, the regular intake of laxatives, and chronic gastrointestinal disorders as well as prior gastrointestinal surgical procedures.

## Test products and procedure

Three mineral waters (MW 1, MW 2, and MW 3) with different concentrations of minerals, milk, and a supplement containing calcium carbonate (CaCO<sub>3</sub>) were investigated in the bioavailability study. The mineral concentrations of the 5 test products are shown in Table 1. Test products were adjusted to provide an amount of 300 mg of Ca, and the volume was, if necessary, filled with demineralized water to ensure consumption of equal amounts of fluids in all groups. All participants received each test product in a randomized order.

The participants consumed 25 µg of vitamin D (Doppelherz Vitamin D 1000 I.E. EXTRA, Queisser Pharma GmbH & Co. KG, Flensburg, Germany) daily 4 weeks before and during the study to ensure adequate vitamin D status. Additionally, the participants were instructed to minimize their Ca intake 2 days before each examination and to avoid excessive exercise on the day prior to the examinations. A list of restricted foods was given to the participants before the intervention.

On examination days, each participant consumed one of the test products in a randomized order after an overnight fast. Participants were instructed to drink Ca-poor water (12 mg Ca per liter) at defined time points during the 12 hours prior to the first draw of fasting blood. Test products had to be

**Table 1.** Mineral composition of the 5 test products.

Mineral value	MW 1 (mg/L)	MW 2 (mg/L)	MW 3 (mg/L)	Milk <sup>a</sup> (mg/100 ml)	Supplement <sup>b</sup> (mg/pill)
Ca <sup>2+</sup>	348	528	290	125	300
Mg <sup>2+</sup>	108	124	137	n.a.	10
Na <sup>+</sup>	118	28.8	100	n.a.	—
Cl <sup>-</sup>	40	28.9	181	n.a.	—
SO <sub>4</sub> <sup>2-</sup>	38	1,463	8.8	n.a.	—
HCO <sub>3</sub> <sup>-</sup>	1,816	403	1,519	n.a.	—

MW = mineral water, n.a. = not analyzed.

<sup>a</sup>Lactose-free ultra-high-temperature milk.

<sup>b</sup>Calcium supplement (CaCO<sub>3</sub>).

consumed within 30 minutes and were given with a standardized breakfast (6.4 mg Ca/portion). Blood samples were drawn initially as well as 1, 2, 3, 4, 5, 6, 8, and 10 hours after the intake of the test product. Urine samples were collected predose and at defined intervals up to 24 hours after dosing (0–2, 2–4, 4–6, 6–8, 8–12, and 12–24 hours). During the experimental period (24 hours), participants consumed standardized Ca-poor meals and water. After the 10-hour blood draw the participants were allowed to drink water at any time, not exceeding 1 L.

The primary outcome variables were the area under the curve (AUC) of serum Ca levels for 10 hours (AUC<sub>0-10h</sub>) and the 24-hour urinary Ca excretion [14–16]. Serum concentrations of Ca, phosphate, and parathormone as well as the urine excretion of Ca and phosphate were examined as secondary outcome variables. The quantity of 25-hydroxyvitamin D was determined only for controls in the baseline blood samples. The blood and urine samples were prepared and analyzed by the Hannover Medical Care Center of the LADR (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V., Geesthacht, Germany) network.

## Data analysis and statistical methods

Data are presented as means ± SD. All serum levels were corrected to their respective baseline levels. The AUC<sub>0-10h</sub> of Ca serum levels was calculated geometrically using the trapezoidal rule, ignoring the area below the baseline. Missing values were caused by faulty of incomplete analysis.

The data were not found to be normally distributed using the Kolmogorov-Smirnov test; therefore, log transformation was applied and nonparametric tests were used.

Differences between baseline levels, AUC<sub>0-10h</sub> of Ca serum levels, and urinary Ca excretion were analyzed by analysis of variance for repeated measurements. Mauchly's test was used to determine sphericity. When sphericity could not be assumed, the Greenhouse-Geisser correction was applied.

Values of  $p \leq 0.05$  were considered statistically significant. All statistical analyses were performed using SPSS software (Version 23.0; SPSS Inc., Chicago, IL).

## Results

### Study population

Twenty-one healthy males and females (men:  $n = 5$ , women:  $n = 16$ ) participated in the study. The mean age was 24.1 ± 2.6 years and the mean body mass index was 23.1 ± 3.3 kg/m<sup>2</sup>. Due to the crossover design, baseline levels (including 25-hydroxyvitamin D) did not significantly differ between groups. The mean fasting 25-hydroxyvitamin D concentrations were above 75 nmol/L.

### Serum and urine concentrations of calcium, phosphate, and parathormone

Statistical group comparisons based on the AUC<sub>0-10h</sub> of serum Ca levels revealed no significant differences between the treatment groups. Likewise, no significant differences in the 24-hour urinary Ca excretion were observed (Table 2). In all

**Table 2.** Area under the Curve (AUC<sub>0-10h</sub>) Serum Calcium Concentrations Corrected to Baseline Levels and Urinary Calcium Excretion over 24 Hours after Consumption of the Test Products.

	AUC <sub>0-10h</sub> Serum Ca		24-Hour Urinary Ca Excretion (mmol)	
MW 1	0.76 ± 0.59	n = 19	4.02 ± 0.40	n = 14
MW 2	0.57 ± 0.49	n = 21	3.69 ± 0.99	n = 17
MW 3	0.77 ± 0.73	n = 21	3.84 ± 1.18	n = 16
Supplement <sup>a</sup>	0.53 ± 0.63	n = 21	3.40 ± 1.11	n = 19
Milk <sup>b</sup>	0.68 ± 0.56	n = 20	3.30 ± 1.08	n = 20
p	0.4261 <sup>c</sup>		0.6192 <sup>d</sup>	

AUC = area under the curve, Ca = calcium, MW = mineral water.

<sup>a</sup>Calcium supplement (CaCO<sub>3</sub>).

<sup>b</sup>Lactose-free ultra-high-temperature milk.

<sup>c</sup>Greenhouse-Geisser correction.

<sup>d</sup>Sphericity given.

groups, after an initial increase, serum Ca levels decreased after 2 hours to approximately half of the maximum concentration and remained nearly consistent. Urinary Ca excretion was initially high and decreased constantly over the following 2 hours in all groups (Fig. 1).

Figure 2 shows the time-dependent courses of the mean changes in serum phosphate and urinary phosphate. Serum phosphate levels decreased initially. Then, the levels increased to approximately one third of the maximum concentration in all groups and remained relatively constant afterwards. In all groups, after an initial increase, the urinary phosphate levels decreased after 2 hours to nearly zero (Fig. 2).

Serum parathormone levels showed an initial increase. Over the following 2 hours, the levels slightly decreased in all groups (Fig. 3).

## Discussion

In this randomized crossover study with healthy volunteers, substantial increases in Ca serum levels were observed for all 5 test products (3 mineral waters with different types of mineralization, milk, and a Ca supplement) after intake of 300 mg Ca. No significant differences regarding the AUC<sub>0-10h</sub> of serum Ca were found between the test products. The general findings are consistent with previous studies showing equivalent Ca absorption from mineral water, milk or other dairy products, and dietary supplements over a maximum time of 24 hours [17-21].

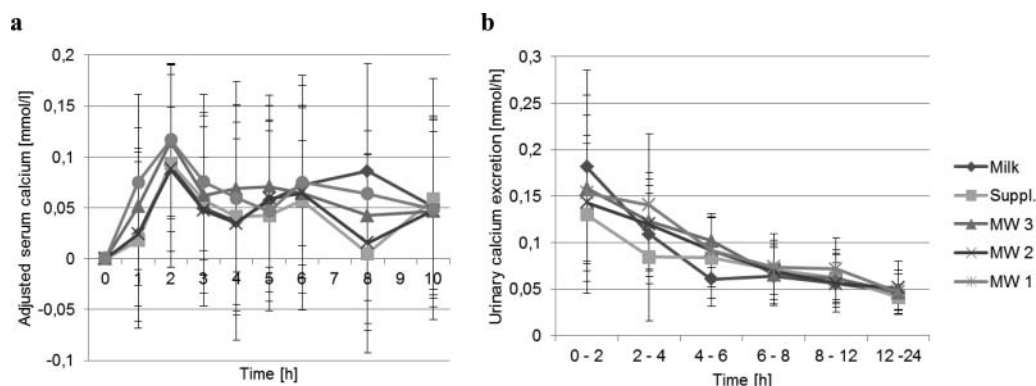
Even in studies with treatments over 3 to 5 days, no significant differences in Ca absorption between mineral water and milk as well as milk and dietary supplements were observed [11,16].

Likewise, the 24-hour urinary Ca excretion was not significantly different between the test products. This corresponds to findings from previous studies showing equivalent 24-hour urinary Ca excretion after intake of mineral water, milk or other dairy products, and dietary supplements [11,16,18,22,23].

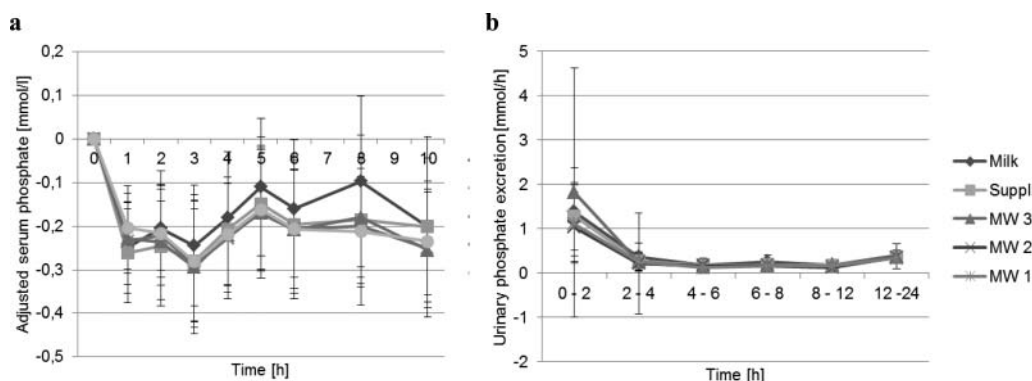
Regarding the mean serum phosphate concentrations, it is notable that the waveform was nearly inverse that of the mean serum Ca concentrations. This is due to the solubility product of Ca and phosphate, which must be avoided to prevent the precipitation of calcium phosphate [24]. Therefore, the 24-hour urinary phosphate excretion increased in all groups and exhibited no significant differences. Similarly, Mortensen and Charles [16] found no significant difference in the 24-hour urinary phosphate excretion over 3 days between a dietary supplement and milk.

All test products induced a decrease in serum parathormone. The oral intake of a Ca load increases serum Ca and lowers parathormone concentrations, which induces a decrease in bone turnover [24]. Similar studies that compared Ca bioavailability from mineral waters with different Ca concentrations with those of Ca from milk demonstrated the same serum parathormone decreases after the consumption of mineral water as after the ingestion of Ca from milk [22,25]. The oral intake of mineral water containing Ca in similar amounts (344 mg Ca) acutely inhibits parathormone secretion and bone resorption [25]. Furthermore, the use of high Ca mineral water (408 mg/L of calcium) over 1 year significantly maintained bone mineral density and resulted in significantly reduced serum osteocalcin levels compared to low-Ca mineral water (80 mg/L of calcium) in postmenopausal women [26].

Vitamin D promotes Ca absorption in the gut by inducing the synthesis of calbindin and maintaining adequate serum Ca concentrations to enable normal mineralization of bone. A poor vitamin D status induces insufficient Ca absorption [27]. In this study, participants consumed 25 µg of vitamin D daily for 4 weeks before and during the study to standardize vitamin D status. The fasting 25-hydroxyvitamin D concentrations of the participants were above 75 nmol/L, which ensured optimal Ca absorption. These findings are supported by studies that compared the absorption of a Ca supplement with that of milk and with a vitamin D supplement plus Ca. The bioavailability



**Figure 1.** (a) Mean serum calcium concentrations corrected to baseline (MW 1: n = 19, MW 2: n = 21, MW 3: n = 21, milk: n = 20, supplement: n = 21) and (b) mean urinary calcium excretion (MW 1: n = 14, MW 2: n = 17, MW 3: n = 16, milk: n = 20, supplement: n = 19) after consumption of the test products.



**Figure 2.** (a) Mean serum phosphate concentrations corrected to baseline (MW 1:  $n = 19$ , MW 2:  $n = 21$ , MW 3:  $n = 21$ , milk:  $n = 20$ , supplement:  $n = 21$ ) and (b) mean urinary phosphate excretion (MW 1:  $n = 14$ , MW 2:  $n = 17$ , MW 3:  $n = 16$ , milk:  $n = 20$ , supplement:  $n = 19$ ) after consumption of the test products.

of Ca was better than or as good as that from milk but could be increased by additional vitamin D [16,23].

The main finding of this study concerns the effect of different types of mineralization of mineral waters on the bioavailability of Ca. Notably, MW 2 was rich in  $\text{SO}_4^{2-}$  (1,463 mg/L), which is suggested to potentially enhance calciuria and therefore decrease the bioavailability of Ca. To the best of our knowledge, only 2 studies have investigated the potential influence of  $\text{SO}_4^{2-}$  on the bioavailability of Ca. A study by Couzy et al. [11] showed a nonsignificant increase of 17% in calciuria with Ca and  $\text{SO}_4^{2-}$ -rich mineral water compared to milk in 9 subjects. Likewise, a longer intervention over 3 weeks showed a significantly higher urinary volume (ml/day) and a higher urinary Ca excretion with an  $\text{SO}_4^{2-}$ -rich mineral water compared to milk [22]. However, this effect was not observed in the present study. Urinary volumes over 24 hours were comparable in all groups ( $p = 0.526$ ). Additionally, MW 2 was low in  $\text{HCO}_3^-$  (403 mg/L) compared to MW 1 (1,816 mg/L) and MW 3 (1,519 mg/L). To the best of our knowledge, no study has determined the effect of  $\text{HCO}_3^-$  on the bioavailability of Ca. The bioavailability of Ca was comparable and not significantly different in products tested in this study. Therefore, the content of  $\text{HCO}_3^-$  does not influence the bioavailability of Ca. Likewise, the potential effect of the magnesium [13] content can be ignored because the magnesium concentration was comparable in all tested mineral waters.

There are some limitations to the present study. In this study, participants received only a single dose of mineral water. However, the bioavailability of Ca can increase when mineral water consumption is evenly distributed throughout the day and additionally when it is consumed together with other food [12]. These effects should be investigated in more depth and taken into consideration in future trials. Furthermore, the potential influence of magnesium on Ca bioavailability should be examined in studies comparing Ca-rich mineral water with magnesium-rich mineral water.

## Conclusion

The results of serum and urine analysis indicated that the bioavailability of Ca from mineral water with different mineralization levels, milk, and dietary supplements is comparable. Specifically, the Ca bioavailability was not influenced by the presence of  $\text{SO}_4^{2-}$  or  $\text{HCO}_3^-$ . Thus, the use of mineral water with higher concentrations of Ca constitutes a calorie-free Ca source that contributes the optimal Ca supply. Future studies should be conducted regarding usual consumption patterns of mineral water, because multiple portions consumed throughout the day may increase the bioavailability of Ca. Additionally, the results should be validated in forthcoming intervention studies, which should determine the effect of high-Ca mineral water on bone mineral density and postmenopausal bone loss.

## Acknowledgment

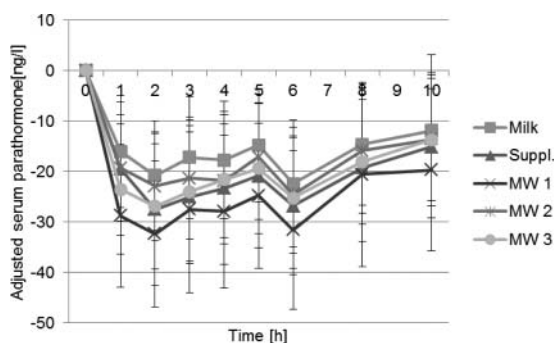
The authors thank all of the subjects who took part in this study.

## Declaration of interest

This study was funded by the Association of German Mineral Water Bottlers (VDM). Study realization, data analysis, and reporting were undertaken independently from the sponsor. The vitamin D was kindly provided by Queisser Pharma GmbH & Co. KG, Flensburg, Germany, and the supplements were provided by Dr. Paul Lohmann GmbH KG, Emmerthal, Germany. The authors declare no potential conflicts of interest.

## Human and animal rights and informed consent

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or



**Figure 3.** Mean serum parathormone concentrations corrected to baseline (MW 1:  $n = 19$ , MW 2:  $n = 21$ , MW 3:  $n = 21$ , milk:  $n = 20$ , supplement:  $n = 21$ ) after consumption of the test products.



national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval was given by the Ethic Commission at the Medical Chamber of Lower Saxony (Hannover, Germany). Informed consent was obtained from all individual participants included in the study.

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