

# Effect of nutritional factors on urinary calcium excretion in a sample of Moroccan children and adolescents

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**Abstract.** In addition to age, gender, body weight, hormonal status as well as certain physiological and pathological states, other factors exert considerable effects on calcium (Ca) retention and therefore on its urinary excretion. Among these other factors, many dietary nutrients have all been discussed as factors affecting urinary Ca through different studies. Thus, our study aims to evaluate the impact of various nutritional factors on urinary Ca excretion among a sample of Moroccan children and adolescents aged from 6 to 18 years old. A total of 133 Moroccan children and adolescents aged between 6 and 18 years were involved in this study. Participants were required from public and private schools in Rabat-Salé-Kenitra region. For each participant, anthropometry data, food record and 24-h urine samples were collected. Urinary Ca was estimated using ICP-mass spectrometry. The urinary Ca average was  $73.58 \pm 37.13$  mg/day. Urinary Ca excretion had no significant association ( $p > 0.05$ ) with different studied nutrients ( $r = -0.082$ ,  $r = 0.118$ ,  $r = -0.025$ ,  $r = 0.142$ ,  $r = 0.084$ ,  $r = 0.119$  for Ca, proteins, vitamin D, sodium, phosphorus and magnesium intakes respectively). The current impact evaluation of various nutritional factors on urinary Ca excretion suggests that Ca requirement may be not influenced by nutrients intake but it is highly recommended for researchers to carry out more studies and define all factors that cause Ca deficiency in Moroccan children and adolescents.

**Key words:** Children and adolescents, Dietary intake, Morocco, Nutritional factors, Urinary Ca.

## Introduction

Childhood and adolescence present the important periods of the bone growth that are conditioned by an adequate intake of Calcium (Ca). This nutritional element is considered as the most important nutrient that plays essential roles in the human health. Moreover, its bioavailability, reflecting its urinary excretion, is largely influenced by different factors [1, 2, 3]. Indeed, it can vary depending on several parameters such as sun exposure, that depend on age changes (growth, senescence), transient conditions such as pregnancy and lactation, nutritional and hormonal status, medical conditions [2, 4], geographical location, physical activity, environmental conditions [5], probably some genetic and physiological factors and also dietary habits. In the context of dietary habits factors, different nutrients play a significant role on affecting the Ca retention in the human body as it was shown by different several studies [1]. Thus, the relation of nutrition to the Ca urine liberation affects the overall state of Ca balance acting on its retention and its losses [6].

In general, nutritional habits and their influence on the urine Ca excretion are related with a considerable relation that must be evaluated to demonstrate their contribution to the onset of any Ca deficiency. According to FAO and WHO reports, three major nutrients are taken into consideration [7] for their remarkable influence on the Ca retention into the body. First, the vitamin D: because of its major role in the homeostasis and the absorption of Ca that it is also recognized experimentally to be a key promoter involved in the reduction of the renal excretion of this mineral element [8]. It is mostly found that this element, under his blood circulating form (1,25(OH)<sub>2</sub> vitamin D<sub>3</sub>) is positively associated with urinary Ca excretion [9] in addition to its role as an hormonal Ca regulator in the human body [10].

The other two important nutritional factors are sodium and animal proteins that shown as mostly implicated elements in promoting the increase of urinary Ca [11]. Indeed, sodium competes glomerularly with Ca, which leads to an increased urinary Ca excretion [12]. For proteins and especially animal proteins, the mechanism by which they influence the excretion of Ca is not clearly demonstrated. An increase in the glomerular

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filtration rate in response to the protein intake has been suggested, but does not seem to be able to explain the principle [7]. However, previous studies in this context have reported that elevated urinary Ca loss is associated to increasing levels of proteins in human diets and especially purified proteins [13].

In this context, other nutrients intake have also a considerable impact on stimulating the total Ca absorption, essentially in intestines, while others inhibits its absorption with an increase urinary excretion. The phosphorus, for example, presents a hypocalciuriant compound acting directly on the renal reabsorption of Ca by promoting this processes in the distal part of the nephron [14]. However, the magnesium has a stimulating effect on urinary Ca excretion by a probable mechanism of competition at the level of Henle's loop between its reabsorption and that of Ca [15].

Thus and through recent studies on the Ca status in the same category of population, it has been shown that Moroccan children and adolescents have a Ca deficiency evaluated both by intake and urinary excretion analysis [16, 17]. On this basis, the study of the different factors that can cause Ca deficiency in this critical age group is of a major necessity in order to act in response to this situation and to respond to the need of studies in around the world [7]. Therefore, the purpose of this study was to evaluate the impact of various nutritional factors on urinary Ca excretion among a sample of Moroccan children and adolescents aged from 6 to 18 years.

## Methods

### 1. Subjects

The present cross-sectional study was performed on a sample of 133 children and adolescents aged from 6 to 18 years old and recruited from public and private schools in Rabat and its regions. All objectives, measurements and details of this study were explained to the participants and their parents or guardians. Apparently, healthy subjects with the signed written consent by their parents participated and those presenting diseases or using Ca supplementation were excluded from the study.

Information about age, sex, medical history, medication and supplementation were requested for each participant and completed by their parents. Data of anthropometric parameters, 24 hours dietary recall and 24 hours urine were collected.

### 2. Anthropometry

Participant's weight and height were measured the day of the interview at schools according to the WHO recommendations [18]. Body weight was taken by a Seca scale at the nearest of 0.1 kg and body height was measured using a portable stadiometer (SECA® 208) with a precision of 0.1 cm. During the

measurement, the participants were asked to stay in standing position, with light clothing and barefoot.

The Body Mass Index (BMI) was calculated according to the relation:  $\text{weight}/(\text{height})^2$  (Kg/m<sup>2</sup>). The nutritional status classification of children and adolescents, developed by the WHO [19], was evaluated by different Z-score calculating by the WHO Anthro Plus software [20].

### 3. Dietary intake assessment

Dietary survey was conducted following the 24h recalls protocol. Indeed, parents or guardians were asked to collect all information about foods and drinks consumed by their children in the day corresponding to the urine collect [21]. To avoid any changes in eating habits and preferences, visits were made unexpectedly. Children, with the help of their mothers, were challenged to memorize and list all the foods and drinks they had consumed the day before. Also, in order to limit quantization errors, two food notebooks were used:

- A photo manual (SU.VI.MAX) for estimating portions of the food and the drink consumed [22];
- A food book prepared in Belgium by professionals in nutrition especially describing the Moroccan diet (description of foods and their average weight and nutritional composition of the preparations) [23].

The quantities consumed were converted to "mg" using the food quantification tables presented in the books used. These quantities were then entered on the Nutrilog food information software (SAS, version: 2.31) which is used to translate each food in nutrients and vitamins through the Moroccan food table, inserted in the software, to have their values in mg/day. In parallel, all details of any vitamin and mineral supplements consumed in the survey period were noted. The mean of the Ca intake was reported.

### 4. Urine collection and analysis

Before collection, each child and his parent received oral and written instructions on the procedure of the collect of the 24-h urine sample. Indeed, the participant was asked to empty its bladder at a specific time and the urine of this first ejection must be discarded. From that moment, 24-hour urine samples must be collected in a container of sufficient capacity.

Urine samples were collected in chemically clean bottles and were brought to the laboratory, where they were treated and then analyzed. Indeed, after collection and volume determination, the pH was measured for each sample of urine collected. Then, the aliquot samples were acidified with 1ml of 6mol HCl/l for 100 ml of urine. This acidification is

necessary to avoid the mineral precipitation that could bias the results. After acidification, samples were thoroughly centrifuged and finally stored at 4°C until analyzed [24].

In the prepared samples, the Ca amount was, then, analyzed by the Inductively Coupled Plasma Mass Spectrometry method (ICP-MS method), which presents a very sensitive analytical technique for measuring the content of almost all the elements of the periodic table present in aqueous, organic or solid solutions. Measurement of creatinine was also realized to avoid possible errors of incomplete urine collection [25].

### 5. Statistical analysis

Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences) software; version 13.0. Variables were presented as medians (inter-quartile) ~~or as mean±standard deviation (SD)~~. The normality of the distribution was tested by the Kolmogorov–Smirnov test. Significance between means was determined using ANOVA or t-student test. To examine the correlation between urinary Ca excretion and different influencing

nutritional intakes, Pearson correlation test was used.

The Mann–Whitney U test was applied to compare medians between two independent samples. The statistical significance level was set at  $p$ -value<0.05.

### 6. Ethical considerations

The study approval was taken from the Ethics Committee for Biomedical Research, Faculty of Medicine and Pharmacy of Rabat, Morocco, and from each parent of the recruited child, a written informed consent was obtained.

### Results

Table 1 summarized results of descriptive statistics of anthropometric parameters and age by sex. The total population was aged  $10.14 \pm 2.54$  years and the studied parameters (Age, Height, Weight and BMI) were statistically the same between boys and girls with the absence of any statistical significance ( $p > 0.05$ ).

**Table 1:** General characteristics of the study population by sex

	Total (n=133)	Girls (n=65)	Boys (n=68)	p-value*
Age. yrs	9.7 (8.35-11.19)	9.9 (8.9-11.42)	9.43 (8.10-11.01)	0.130
Height. cm	137.0 (130.0-144.6)	136.0 (130.0-148.0)	137.90 (126.75-144.0)	0.530
Weight. kg	30.4 (25.0-38.4)	31.60 (25.90-37.90)	29.45 (24.75-39.35)	0.314
BMI. kg/m <sup>2</sup>	16.5 (14.65-18.65)	16.84 (14.83-18.86)	16.08 (14.54-18.25)	0.853

Results are presented as medians (inter-quartile).

\*p-values were calculated by one-way ANOVA for means.  $p < 0.05$  is taken as the level of significance;

BMI: body mass index; p values were calculated by one-way ANOVA for means.

The results of the analysis of the biological urinary parameters according to sex are presented in table 2. The average Ca excretion varies between 76.33 mg/d in boys and 70.66 mg/d in girls, with a total average of 73.58 mg/day and no significant difference was observed between the two sexes ( $p=0.387$ ).

**Table 2:** General biological parameters by sex

	Total (n=133)	Boys (n=68)	Girls (n=65)	p-value*
UF (l/d)	0.89±0.40	0.89±0.43	0.91±0.37	0.388
UCa (mg/d)	73.58±37.13	76.33±44.04	70.66±28.05	0.387
UCr (mg/d)	852.79±325.71	853.42±324.45	852.10±329.68	0.982

Results are presented as mean ± standard deviation.

\*p-values were calculated by one-way ANOVA for means.  $p < 0.05$  is taken as the level of significance;

UF: Urine Flow; UCa: Urinary Calcium; UCr: Urinary Creatinine; d: day

**Table 3:** Dietary intake of nutritional factors on urinary Ca excretion by sex

	Total	Boys	Girls	<i>p</i> -value*
<b>Nutritional intake</b>				
<b>Proteins (g/d)</b>	63.1±29.10	72.82±30.37	63.90±29.10	0.093
<b>Vitamin D (µg/d)</b>	3.73±4.93	4.08±5.75	3.34±3.83	0.394
<b>Magnesium (mg/d)</b>	235.42±109.34	245.41±112.64	224.29±105.35	0.271
<b>Phosphorus (mg/d)</b>	971.49±427.11	1028.06±422.29	908.53±427.00	0.11
<b>Sodium (mg/d)</b>	2078.42±948.93	2118.8±1080.94	2033.47±782.69	0.609
<b>Ca (mg/d)</b>	506.45±238.42	521.13±247.28	490.60±229.39	0.46

Results are presented as mean ± standard deviation. d=day

\* *p*-values were calculated by one-way ANOVA for means. *p* < 0.05 is taken as the level of significance;

The food intake in different nutritional factors on urinary excretion is presented in Table 3. The total daily intake averages are 63.1±29.10 g/d, 3.73±4.93 µg/d, 235.42±109.34 mg/d, 971.49±427.11mg/d, 2078.42±948.93 mg/d and 506.45mg/d±238.42 mg/d for proteins, vitamin D, magnesium, phosphorus, sodium and Ca respectively. According to sex, no statistical significant difference was shown between boys and girls for the different parameters.

Table 4 reported results of correlation test between urinary Ca and different influencing nutritional factors. The results of this test (Pearson coefficient) showed the absence of a significant association between the 24-hour urinary excretion of Ca with the intake of Ca, protein, vitamin D, phosphorus, magnesium and sodium.

**Table 4:** Correlation of urinary Ca with influencing nutritional factors

	Urinary Ca (mg/d)	
	<i>r</i> of Pearson	<i>p</i> -value
<b>Ca intake (mg/d)</b>	-0.082	0,351
<b>Proteins intake (g/d)</b>	0.118	0,179
<b>Vitamin D intake (µg/d)</b>	-0.025	0,775
<b>Sodium intake (mg/d)</b>	0.142	0,105
<b>Phosphorus intake (mg/d)</b>	0.084	0,342
<b>Magnesium intake (mg/d)</b>	0.119	0,175

## Discussion

This is the first study in Morocco to provide information on the impact of a number of nutritional factors on urinary excretion of Ca in a sample of schoolchildren and adolescents aged from 6 to 18 years. Among this the same population, a deficiency on urinary Ca excretion had already shown with the same average reported in this work (73.58±37.13 mg/d) in 73.3 % of studied children and adolescents [7]. Hence, the current study was conducted to evaluate the interrelation between some nutritional elements considered as one of the major important factors which present a considerable impact on urinary Ca excretion.

On this basis, results from this study, based on the correlation between urinary Ca excretion and the intake of Ca, proteins, vitamin D, sodium, phosphorus and magnesium, respectively, showed no significant association for these various nutritional factors with the Ca excretion in the urine.

Generally, a large part of our results differ with findings reported in several previous studies while the other part expresses a similarity. This heterogeneity of results can be explained by various physiological factors such as Ca homeostasis, the complex interaction of these nutrients, as well as the characteristics of the study population such as age and nutritional status, which can be considerably involved. Indeed, in a study on the variance of urinary Ca excretion in relation to the Ca intake,

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Nordin et al., 1988 showed a positive correlation of Ca intake with the urinary Ca excretion in adults [27], results that also found through some animal experiences [28]. Recently, Rasheed et al have been reported that an important calcium intake is responsible of a significant increase in urinary calcium excretion [29]. However, in our population of rapidly growing participants, the data suggest a lack of correlation between the dietary intake and the urinary Ca excretion. These results corroborate those of Matkovic and his collaborators who concluded that the absence of such relationship could be linked to the Ca homeostasis that characterizes subjects in the rapid growth phase. In addition, another study on the Ca balance in a group of adolescent girls also confirmed that the excretion of Ca in the urine is not linked to its intake [30, 31], as well as for another study which demonstrated the absence of correlation between the Ca intake and its urinary excretion ( $r=0.08$ ) in young girls aged between 4.6 and 16.7 years [32]. These findings consistent with the old confirmations of a group of experts, from FAO and WHO, who concluded that the urinary Ca excretion is on average less influenced by the daily Ca intake [6].

The main explanation for these results is that a larger fraction of the Ca absorbed, in the growing population, goes into the skeletal compartment rather than the urine [31]. It is, in fact, the physiological system Ca homeostasis [33].

In the same context, the effect of proteins on the urinary excretion of Ca has been revealed by various studies which have proved a positive association between hypocalciuria and the protein intake [34, 35]. In addition, Kerstetter et al. found that high protein intakes actually improved the absorption of Ca [36]. Through another study carried out by Linkweiler, it has been shown that the urinary Ca excretion is mostly influenced by dietary proteins [37]. Also, using purified proteins or protein hydrolysates, other research scientific works have consistently shown a representative 1mg increase in urinary Ca excretion for every 1gram of protein ingested [38]. However, studies taking proteins from their entire food matrix, through ingestion of meat and/or dairy products, have reported no increase in urinary Ca [39, 40], result that consistent with the findings of our study.

Regarding vitamin D effect, a negative association between the urinary Ca and the vitamin D intake has been widely shown. Vitamin D is the major element involved in the absorption of the Ca, thereby reducing its elimination in the urine [9]. The absence of a significant correlation in this study can be explained by the inadequate intake of Ca and also that of vitamin D which acts by reducing the release of Ca in the urine and by promoting its bone mobilization [41], this result consistent with that of a very ancient study that reported no relation

between vitamin D intake and the Ca excretion [42]. On the contrary, Vitamin D supplementation studies to prevent deficiency have shown that participants in the high-dose vitamin D group were 3.6 times more likely to develop hypercalciuria than those in the lower-dose group [43].

Computerized research into phosphate and its effect on the urinary Ca excretion has identified a close relationship between these two biological variables. Indeed, higher phosphate intakes are associated with a decrease in Ca in the urine and with an increased retention of this mineral [44]. It is most critically implicated on Ca retention in the human body altering the Ca economy operation [45] and whatever the Ca intake [44]. Thus, the results obtained from a study on the factors influencing the urinary excretion of Ca and including children and adolescents, showed that a high Ca/phosphorus ratio generates a considerable increase in the urinary Ca level, often above normal maximum limits [1]. Similarly, many older studies have demonstrated that urinary Ca decreases with the intake of phosphorus supplements [46, 47]. According to Henkin, in his studies about Calcium and Magnesium Levels Change in Relationship to Variations in Usual Dietary Nutrient Intake, it has been shown negligible variations in urinary calcium excretion with the high phosphorus intake [48].

Concerning magnesium, several researchers, through extensive studies, have well documented the positive association of dietary magnesium intake and Ca urinary excretion. For explanatory purposes, in the intestine, magnesium can compete directly or indirectly with Ca absorption. In addition to this competition principle of absorption, a previous study had shown that magnesium supplementation increased urinary Ca excretion at a Ca intake < 800 mg/day [49], suggesting that magnesium can inhibit the reabsorption of Ca when the Ca/Mg consumption ratio is very low. Thus, in older studies, it has been shown that magnesium prevents Ca from entering the cell when the Ca is present in excess [50, 3].

And for urinary excretion of Ca depending on the sodium intake, several studies have reported a positive association. As an example, the study by Jacqueline et al., 1985 who have well explored this interaction, and having shown that the excretion of Ca in the urine increased considerably ( $p < 0.05$ ) after the increase in the rate of sodium in the diet [26], similar conclusion have been reported by Ferraro et al [12]. In another study done among young female, it was found that sodium presents an important determinant that impact Ca excretion in the urine with a positive association [25].

Summary, the effect of these nutritional factors remains even less well understood, knowing that this study presents great disparities with the results presented in the literature. It is possible that other factors, such as physiological adaptation, bone

retention capacities, intestine absorption, etc. influence the release of Ca in the urine. Importantly, the actual effects of nutrients on the Ca economy can be managed differently, either by increasing the intake of the nutrient concerned, or by increasing it [51]. Also, the quantification of these effects provides valuable data to public health professionals, clinicians and nutritionists to advise their patients to achieve body Ca satisfaction. Actually, the most reliable approach to achieve this goal is to increase the consumption of foods naturally rich in Ca, vitamin D, protein and phosphorus and to minimize foods inhibiting Ca absorption by increasing its urinary excretion (Sodium for example).

## Conclusion

The present study presents an important starting point for researchers in our country to carry out studies on the various factors that cause Ca deficiency in children and adolescents. Overall, all of the findings from this study showed the absence of association between urinary Ca excretion and different nutritional influencing factors for both girls and boys. Hence, additional studies are needed to assess the influence of other factors such as bone retention rate, intestinal absorption rate and all physiological factors in order to define their impact on the Ca balance.

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