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ORIGINAL ARTICLE

Calcium status in Moroccan children and adolescents assessed by 24-hour urinary excretion

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Abstract

Background: Worldwide, calcium (Ca) deficiency represents one of the most important deficiencies of all micronutrients. When associated with vitamin D deficiency, it constitutes a major health issue, responsible for many functional diseases especially osteoporosis and fracture risk at the later life. In the Moroccan population, data on Ca deficiency risks are really limited. The urinary losses present a significant determinant of Ca urinary excretion being a crucial determinant of its retention in the human body. **Aims:** This study was conducted to determine the level of urinary Ca excretion in a sample of Moroccan children and adolescents, and to assess the prevalence of Ca deficiency. **Subjects and Methods:** 131 children and adolescents, aged between 6 and 18 years, were recruited from public schools at Rabat-Salé-Kénitra region in the framework of a descriptive cross-sectional study. Socio-economic status, morbidity, and anthropometric parameters were assessed for each participant. Ca assessment was estimated through 24-hour urine measurements by ICP-mass spectrometry. **Results:** The total mean of urinary Ca was 72.48 mg/day and about 73% of participants presented a urinary Ca deficiency. No significant differences were observed according to sex and nutritional status. **Conclusions:** Moroccan children and adolescents might be at risk of Ca deficiency complications. Based on this finding, an appropriate intervention strategy should be adopted to control this deficiency through an overall assessment of Ca deficiency in general population.

Keywords: Moroccan children and adolescents, urinary Ca, Ca deficiency.

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1 Introduction

Nowadays, young people's lifestyle predisposes them to Ca deficiency that can lead to several detrimental consequences, especially bone health, including osteomalacia (osteopenia) [1], rickets [2,3], and osteoporosis [4,5]. The Ca is stored during years of childhood and adolescence stages, and the more important reserves of Ca are consumed during adulthood period. In this context, the quality of diet in childhood is crucial. Almost 99 percent of total body calcium is found in bones and teeth playing a key role during growth, development, and maintenance of bone throughout the life stage. Indeed, during fetal development, a significant amount of calcium is required. Although increased intestinal absorption of calcium satisfies a part of the need, adequate calcium intake by the mother presents a great condition [6]. Calcium continues strengthening our bones till the age of 20-25 when density becomes the highest. Over that age, bone density decreases. Although this reduction, that is considered as a natural aging process, Ca is still maintaining bones strength and integrity. The rate of Ca deposition in bone is proportional to the rate of growth. Moreover, during childhood, adolescence and around the age of 30, Ca accumulates in the skeleton with a rate of about 150 mg per day [7]. During maturity, the body can absorb more or less Ca and around the age of 50 in men and menopause in women, the Ca balance becomes negative and the bone loss becomes significant [8]. The reason for what, it is mandatory that Ca balance should be maintained for target individuals concerned by growth, which provides a sufficient quantity for the skeletal consolidation, especially during adulthood and

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old age [9]. Thus, an adequate daily intake of Ca is highly recommended. Several recommendations were established concerning dietary calcium needs, depending on age and gender and varying with some genetic and environmental factors [10]. According to the Institute of Medicine, Food and Agriculture Organization (FAO) and Food and Nutrition Board, about 1000 mg/d of Ca is recommended for children aged from 6 to 8 and about 1300 mg/d for those aged from 9 to 13 and adolescents [11].

The Ca is accounting about two percent of body weight [12] representing approximately 1200 g [13]. The overall concentration of Ca is under the control of the body's hormonal system namely parathyroid hormone, vitamin D, and calcitonin [14]. Furthermore, calcium availability is also regulated by other nutritional factors that affect its requirement, including phosphorus, phytic acid, oxalates, etc. [15,16] especially sodium and animal proteins [17,18].

It is well established that ingested Ca comes from our diet such as dairy products (milk, yogurt, and cheese) providing the major source (~1150 mg Ca/L). Some vegetables, cereals, beans, eggs, fish consumed with their bones, and mineral drinking water can represent particular sources of this nutrient [19]. Moreover, in some situations, calcium could be provided by supplementation.

Concerning calcium status, several studies have been undertaken in different countries based on 24h dietary recalls or urinary examination [20-22]. Therefore, calcium deficiency has been universally reported, especially in children and adolescents who represent the optimal period of peak bone involvement [9,20,22].

Referring to the latest published data from a national survey, Moroccan diet is unbalanced and based on low consumption of dairy products [23]. This result was supported by a further recent study on dietary calcium intake that confirmed a deficiency [24]. However, complementary biological data conducted in this direction are limited.

In this context and to achieve the World Health Organization (WHO) and the FAO's objectives, this study was implemented to target specific vulnerable populations like the Moroccan schoolchildren and adolescents. The investigation is essentially frame worked on urinary and dietary analysis of calcium. The effectiveness of urinary Ca analysis was clearly demonstrated; indeed, the urinary Ca excretion presents a key determinant of its retention in the body [16]. Aiming to assess the national nutrition strategy developed and conducted by the Moroccan Ministry of Health, the present study was designed to study the urinary Ca in Moroccan schoolchildren and adolescents aged from 6 to 18 years.

2 Subjects and Methods

2.1 Subjects of the study

This was a descriptive cross-sectional study that enrolled a sample of schoolchildren and adolescents aged from 6 to 18 years, randomly selected from different schools in Rabat and surrounding areas. After clinical examination, only healthy participants were included, while those with symptoms of diseases related to Ca deficiency were excluded knowing that some diseases and the consumption of some drugs are associated with a decrease in urinary Ca [12].

At the start of the study, 280 children were invited to participate. 131 subjects were selected accordingly to inclusion criteria to undertake urinary and nutritional assessment.

2.2 Anthropometry

Anthropometric parameters were measured according to the WHO references [25,26]. At the morning of survey day, measurements of height and weight were recorded using standard equipment specific for each measure. A calibrated portable balance (Seca gmbh & Co. kg. Hamburg, Germany) was utilized. Body weight was measured to the nearest of 0.1 kg; and height was obtained to the nearest of 0.1 cm using Shorr Board portable (formerly Shorr Productions, LLC; USA). BMI (Body Mass Index) was calculated as weight in kilogram divided by the square of height in meter (Kg/m^2) following the WHO recommendations. BMI was measured and classified, as indicated in the reference 5-19 years and by referring to the z-score limit values [26]: obese (z-score > +25D, equivalent to BMI >30kg/m^2 at 19 years), overweight (z-score > +1SD, equivalent to BMI > 25 kg/m^2 at 19 years) and normal weight (-2SD ≤ z-score ≤ +1SD, equivalent to 18 ≤ 25kg/m^2 < BMI ≤ 25 kg/m^2 at 19 years).

2.3 Questionnaires

Data on sex, date of birth, school level and socioeconomic variables of the participants, were obtained using a questionnaire including data about parents' level of education, general expenses, and household size.

Health status and presence of any eventual condition that requires the exclusion of participants were examined by a health practitioner using a questionnaire adapted to this study. Indeed, Participants with any of the following conditions were excluded from the study:

- Consumption of dietary supplements of Ca or vitamin D;

- Allergy to milk proteins and lactose intolerance;
- And any disease that may make urine collection difficult (ex: kidney disease) or some medications or supplements that directly affect urinary excretion [12].

2.4 Urine collection

For each participant, parents received oral and written instructions on how to collect the 24-h urine sample. Moreover, the subject is asked to discard the first ejection and to collect all urine from that moment during the twenty-four-hour period. As recommended, the duration of the collection was limited in 20-28 hours, and urinary Ca and creatinine (Cr) volumes were normalized to 24 hours [27]. Collected urine samples in sterile recipients, were then analyzed at the level of the laboratory.

2.5 Urine analysis

After collection and volume determination, the pH was measured for each collected sample. Then, the aliquot samples were acidified with 1 ml of 6 mol HCl/L for 100 ml urine to prevent mineral precipitation that may affect the accuracy of the results, then thoroughly centrifuged and finally stored at 4°C until analyzed [28]. Ca concentration was assessed using the Inductively Coupled Plasma Mass Spectrometry method (ICP-MS method: ICP-MS; Thermo Scientific X-SERIES 2), which presents a highly sensitive analytical technique for measuring the content of almost all the elements of the periodic table present in aqueous, organic or solid solutions.

2.6 Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) Statistics version 21.0 and Microsoft Office Excel version 2007. Description of each variable was performed based on descriptive statistics. Means calculation was performed and results were compared in order to show relationships between body weight, height, BMI and urinary/dietary calcium. Sex comparison of variables was analyzed using Student's t-test and ANOVA for independent samples. Normality assumption was checked by the Kolmogorov– Smirnov test.

Results are expressed as means ± SD (Standard Deviation) for quantitative variables and as percent for qualitative variables. Statistical significance, was set at $p<0.05$.

3 Results

The descriptive statistics of anthropometric parameters are summarized in Table 1. The study participants were divided into 68 boys and 63 girls with a sex ratio of 1.1/1.

For all variables, there was no statistically significant difference according to gender. Values of BMI ranged from 17 to 18 with an average z-score of -0.13 indicating a normal body weight and height in the study population [29].

Table 1: General characteristics of the studied population

	Girls (n=63)	Boys (n=68)	Total (n=131)	p-value
Age (years)	10.49±2.63	9.82±2.41	10.14±2.54	0.1
Weight (Kg)	34.10±12.43	32.73±11.33	33.39±11.84	0.4
Height (cm)	138.56±13.84	137.01±14.42	137.76±14.11	0.53
BMI	17.3±3.47	17.05±3.79	17.17±3.63	0.7
BMI z-score	-0.15±1.38	-0.10±1.83	-0.13±1.63	0.8

BMI: body mass index; p values were calculated by one-way ANOVA for means; *p<0.05 is taken as the level of significance; Results are presented as mean ± SD; BMI and BAZ were determined according to [26].

Table 2: Biological parameters by sex

	Total	Boys (n=68)	Girls (n=63)	p-value*
Urinary output (L/day)	0.88±0.40	0.86±0.43	0.91±0.37	0.388
Urinary Ca (mg/day)	73.58±37.13	76.33±44.04	70.66±28.05	0.387
Ca/Cr	0.09±0.05	0.09±0.05	0.09±0.05	0.459

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; Ca: calcium; Cr. Urinary Creatinine

The biological parameters are presented in Table 2. Results showed no statistical differences between genders.

Overall Ca excretion was 73.6±37.13 mg/day (mean ± SD), 76.3±44 mg/day for boys and 70.6±28 mg/day for girls (p=0.387). The calcium/urinary creatinine (Ca/Cr) ratio was about 0.094 for all participants with no gender difference (p=0.459). The urinary Ca profile of participants is shown in Table 3 and figure 1. We noted that about 73 % of the study population had a value of urinary Ca lower than 100 mg/day and only 27 % that had normal values situated between 100 and 250 mg/ day [17].

Table 3: Urinary Ca deficiency

		Urinary Ca groups				
		Deficiency (UC<100 mg/d)	Normal (UC>=100 and UC<=250 mg/d)	Excess (UC>250 mg/d)	Total	p-value *
Boys	n	50	18	0	68	
	%	73.5	26.5	0	100	

Girls	n	46	17	0	63	0.552
	%	73	27	0	100	
Total	n	96	35	0	131	
	%	73.3	26.7	0	100	

* p values were calculated by one-way ANOVA for means. Results are presented as sample size and percentages. UC=Urinary Ca

Table 4: Urinary Ca deficiency according to sex and to nutritional status

		Urinary Ca		
	Nutritional status	Ca deficiency	Normal Ca	Total
Boys (n=68)	Thinness	2	0	2
	Normal	38	14	52
	Overweight	5	3	8
	Obesity	5	1	6
	Total	50	18	68
Girls (n=63)	Thinness	7	2	9
	Normal	30	11	41
	Overweight	8	2	10
	Obesity	2	1	3
	Total	47	16	63
Total	Thinness	9	2	11
	Normal	68	25	93
	Overweight	13	5	18
	Obesity	7	2	9
	Total	97	34	131

Data, summarized in Table 4, on urinary Ca deficiency with sex and nutritional status, were treated according to the value of the BMI z-score which allowed classifying our population into 4 distinct groups: malnutrition state, normal state, overweight state, and obesity state. The results showed that the majority of participants with urinary Ca deficiency had normal nutritional state: n=68 (38 of boys and 30 of girls). For those with normal urinary Ca excretion, 25 participants showed normal nutritional profile and for the rest of the study population with urinary Ca deficiency, only 9 subjects (2 boys and 7 girls) suffered from thinness, 13 overweight (5 boys and 8 girls) and 7 obese (5 boys and 2 girls).

According to age groups defined based on repartition recommended by The Institute of Medicine [16], results of Ca deficiency are presented in Table 5. We observed that participants aged from 9 to 13 years (n=57) presented the major prevalence of deficiency (28 of boys and 29 of girls), while participants aged from 6 to 8 years old n=29, were represented by 18 boys and 11 girls. The age group: 14-18 years, n= 11 was divided into 7 Boys and 4 girls).

Table 5: Urinary Ca deficiency according to age groups

		Urinary Ca		
	Age groups (Years)	Ca deficiency	Normal Ca	Total
Boys (n=68)	6 à 8	18	11	29
	9 à 13	28	5	33
	14 à 18	4	2	6
	Total	50	18	68
Girls (n=63)	6 à 8	11	5	16
	9 à 13	29	9	38
	14 à 18	7	2	9
	Total	47	16	63
Total	6 à 8	29	16	45
	9 à 13	57	14	71
	14 à 18	11	4	15
	Total	97	34	63

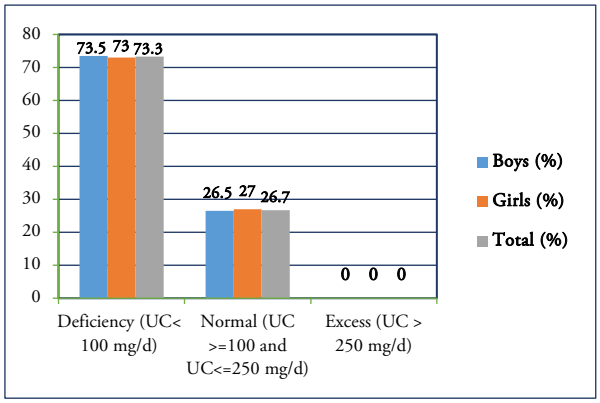

Figure 1: Urinary Ca Profile

4 Discussion

This study is in accordance with the goals set by the Ministry of Health as an important part of the National Nutrition Strategy to tackle micronutrient and macronutrient deficiencies. To the best of our knowledge, the current study is the first to investigate calcium status among Moroccan population-based in 24h urinary excretion and that complete results obtained by the last study treating the 24 hours Ca intake in the same sample of participants (131 schoolchildren aged from 6 to 18 years) [24]. Thus, the purpose of this study point in this direction. Obtained results will constitute a key element for the department of health. They will permit determination of the prevalence of Ca deficiency in Moroccan population, particularly in children and

adolescents. This vulnerable category of individuals presents the most age group concerned with adequate Ca intake to boost good bone development and prevent Ca-related diseases.

Urinary excretion of Ca is among the most important biological processes involved in the regulation of this mineral in the human body. It is well known to decrease with all age groups mostly in early childhood [30] and after age of 10 years [20] because of an increase in the minerals retention rate due to the rapid growth [31]. Hence, it will be crucial to maintain normal Ca concentration in our body, in each group age, particularly among children and adolescents that constitute critical periods of growth [32].

Our results showed that approximately 73% (72.476 mg/day) of subjects had a value of Ca inferior to 100 mg/day. This is considered as the minimum value of the normal range [17] in case of adequate Ca intake which ranged from 800 to 1300 mg/day in children and adolescents (6 to 18 years), as recommended by the Institute of Medicine and the World Health Organization [4,17]. However, 27% of our Moroccan schoolchildren and adolescents sample presented a normal Ca urinary excretion. Identical results were obtained by a previous study focusing on the same population showing that 85.5% had an insufficient intake of Ca [24]. Depending on urinary Ca excretion according to sex, no significant difference was noticed between girls and boys. In this context, different studies were carried out on samples of children and that showed higher values for the means of urinary Ca excretion [28]. These findings can also be confirmed by the Ca/Cr ratio that provides a reliable indicator of urinary Ca status and that the reference value for urine Ca/Cr is 0.2 [12].

In term of calcium urinary excretion, the average amount found in the current studied population remains low compared to the average found in children and adolescents from different populations around the world with values exceeding 200 mg/day [33].

The mean Ca/Cr ratio in our study was 0.09, indicating a too low urinary Ca excretion. Compared with two Iranian studies [34,35] on children that reported significant Ca/Cr ratio means of 0.53 and 0.24 respectively. Another ratio of 0.20 among American children aged 7 to 14 years was found [36], and about 0.1 in Korean [37], Argentinian [38], and Thai children [39]. For Lebanese, Turkish and, German children, mean Ca/Cr ratio was found low with averages of 0.084, 0.092 and 0.096 respectively [40]. Similarly, to other studies, Ca/Cr ratio was not significantly different between genders ($p>0.05$) [21].

Results from our investigation and specifically the large inter-individual variation of urinary Ca excretion, compared with other findings [27,40] can result from several factors such as: dietary culture [41], physical activity, mineral content of water, climate, genetics, and race [21]. Generally, nutritional habits constitute the principal factor known to influence the renal loss of Ca [42], especially diets poor in calcium-rich products such as dairy products that provide a significant contribution [42]. Three major nutrients are taken into account [17]; Vitamin D and animal protein, then urinary sodium that are the major nutrients involved. Indeed, Vitamin D increases renal Ca retention due to its role in Ca homeostasis and Ca absorption [12]. Whereas, animal proteins increase urinary Ca excretion [43]. While, sodium was found to compete Ca for reabsorption at the level of renal tubules and therefore increase its excretion. It should be noticed that a positive association between urinary Ca and urinary sodium was reported in numerous studies carried out in different countries with participants presenting similar age range [28,44,45]. For animal proteins, it has been proven a positive effect of dietary proteins on Ca release in urine. Hight protein intake could lead to increase urinary Ca loss [43]. Further secondary factors influence Ca excretion rate must be taken into consideration, such as: phosphate ion, phytates, oxalates, etc. [17,26].

The current study highlighted a considerable number of individuals with Ca deficiency among Moroccan population regardless of age and gender. This could expose them to a high risk of several related diseases as osteoporosis, primarily in women and other bone issues in men [46-48].

Further research is required to examine, on one hand, the association between the urinary Ca and Ca intake and on the other hand, to establish a relationship between urinary Ca and other important minerals such as phosphate and sodium.

5 Conclusion

It should be underlined that studies on Ca status in Moroccan population are rare. To the best of our knowledge, this is the first study in our country targeting the status of Ca using urinary analysis in schoolchildren and adolescents. This group category is exposed to serious health issues in future, if inadequate amount of calcium is not correctly covered. Our findings, that showed a large percentage of subjects presenting Ca deficiency, are paving the way to develop strategies and action plans to fight against Ca deficiency and prevent relevant diseases.

List of abbreviations

BAZ: BMI z-score of body mass index for age, BMI: Body Mass Index, Ca: Calcium, cm: centimeter, Cr: Creatinine, FAO: Food and Agriculture Organization, ICP-MS: Inductively Coupled Plasma Mass Spectrometry, kg: kilogram, l: liter, mg: milligram, n: Sample size, SD: Standard Deviation, UC: Urinary Ca, WHO: World Health Organization.

Ethical approval

The protocol of the current study was approved by the Ethics Committee for Biomedical Research of the Faculty of Medicine and Pharmacy of Rabat. It is in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Thus, all subjects were informed in a simplified and clear way of the purpose's work and received a summary of the study with a detailed protocol description. They only took part in the study after having signed informed consent by one of their parents or guardians. Personal information of each participant was kept confidential and each one was free to leave the study at any time if he/she wished.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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