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Calcium Biochemical Indicators in a Group of Schoolchildren with Low Socioeconomic Status from Barranquilla, Colombia

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***Abstract*—**Calcium is an essential element for good growth and development of the organism, and its requirement is increased at school age. Low socio-economic populations of developing countries such as Colombia may have food deficiency of this mineral in schoolchildren that could be reflected in calcium biochemical indicators, bone alterations and anthropometric indicators. The objective of this investigation was to evaluate some calcium biochemical indicators in a group of schoolchildren of low socioeconomic level from Barranquilla city and to correlate with body mass index. 60 schoolchildren aged 7 to 15 years were selected from Jesus's Heart Educational Institution in Barranquilla-Atlántico, apparently healthy, without suffering from infectious or gastrointestinal diseases, without habits of drinking alcohol or smoking another hallucinogenic substance and without taking supplementation with calcium in the last six months or another substance that compromises bone metabolism. The research was approved by the ethics committee at Universidad del Atlántico. The selected children were invited to donate a blood and urine sample in a fasting time of 12 hours, the serum was separated by centrifugation and frozen at -20 ℃ until analyzed and the same was done with the urine sample. On the day of the biological collections, the weight and height of the students were measured to determine the nutritional status by BMI using the WHO tables. Calcium concentrations in serum and urine (SCa, UCa), alkaline phosphatase activity total and of bone origin (SAPT, SBAP) and urinary creatinine (UCr) were determined by spectrophotometric methods using commercial kits. Osteocalcin and Cross-linked N-telopeptides of type I collagen (NTx-1) in serum were measured with an enzyme-linked inmunosorbent assay. For statistical analysis the Statgraphics software Centurium XVII was used. 63% (n = 38) and 37% (n = 22) of the participants were male and female, respectively. 78% (n = 47), 5% (n = 3) and 17% (n = 10) had a normal, malnutrition and high nutritional status, respectively. The averages of evaluated indicators levels were (mean ± SD): 9.50 ± 1.06 mg/dL for SCa; 181.3 ± 64.3 U/L for SAPT, 143.8 ± 73.9 U/L for SBAP; 9.0 ± 3.48 ng/mL for osteocalcin and 101.3 ± 12.8 ng/mL for NTx-1. UCa level was 12.8 ± 7.7 mg/dL that adjusted with creatinine ranged from 0.005 to 0.395 mg/mg. Considering serum calcium values, approximately 7% of school children were hypocalcemic, 16% hypercalcemic and 77% normocalcemic. The indicators evaluated did not correlate with the BMI. Low values were observed in calcium urinary excretion and high in NTx-1, suggesting that mechanisms such as increase in renal retention of calcium and in bone remodeling may be contributing to calcium homeostasis.

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I. Introduction

CALCIUM is an essential element for improvement of many metabolic pathways of the body and mainly in bone development, whose dietary recommendations should be appropriate from school age to have a healthy bone metabolism in the future life [1]. The process of bone remodeling occurs throughout life, through mechanisms of bone resorption and formation. [2]. Resorption is a mechanism mediated by osteoclasts with loss of old bone and production of new bone performed by osteoblasts. The process bone formation is high during growth stage, especially in school age children, whose calcium requirement is higher; therefore, an optimal diet intake of this mineral must be guaranteed, achieving maximum bone development at early ages. Studies show that individuals who have consumed a recommended intake during its early stages are less susceptible to osteoporosis in adulthood [3], [4].

Malnutrition and deficiency of macronutrients and micronutrients such as calcium is marked in Latin America and Caribbean countries, such as Argentina, Bolivia, Colombia, Ecuador, Mexico and Peru, as indicated by surveys of eating habits [4], [5]. In Colombia, it was observed that 39% of the population aged 5 to 64 years regardless of the socioeconomic status did not consume dairy products [6] suggesting that a large children population may be deficient in calcium dietary requirements. Even that could be reflected in alterations, either bone metabolism, anthropometric indicators, as well as in blood calcium biochemical indicators.

Within the biochemical indicators of calcium are the biomarkers of bone formation, which are the total alkaline phosphatase activity (SAPT), the bone-specific alkaline phosphatase activity (SBAP) and osteocalcin; and bone resorption biomarkers, which are amino-terminal crosslinked telopeptide of type 1 collagen (NTX-1) [7] These indicators behave according to organism homeostatic mechanism which involves hormones such as paratohormone, calcitonin and 1,25 dihydroxyvitamin, all of them keep serum calcium level (SCa) in normal ranges, possibly by means of three main mechanisms: increasing calcium intestinal absorption; stimulating renal calcium reabsorption; and, mobilizing calcium into blood from the bone [8], [9].

In this work, behavior of calcium biochemical indicators level was evaluated in a group of schoolchildren of low

socioeconomic status from Barranquilla-Colombia. In serum were evaluated: total calcium levels, activity of total alkaline phosphatase, activity of bone-specific alkaline phosphatase, osteocalcin and Cross-linked N-telepeptides of type I collagen (NTx-1). In urine were evaluated total calcium excretion and its ratio with creatinine. Correlations between calcium biochemical indicators with schoolchildren body mass index were also evaluated.

II. Materials and Methods

A. Subjects and Collection of Blood Samples

The population in this investigation was all students with ages of 7 to 15 years enrolled at the Jesus's Heart educational public institution located in the center region from Barranquilla–Colombia, with a population of low socioeconomic level. The sample consisted of 60 students who voluntarily agreed to participate in this research, signing a consent and consent from the parents or guardians and who met inclusion criteria: Schoolchildren aged 7 to 15 years, apparently healthy, non-smokers, non-consumers of alcohol or other hallucinogenic substances and who did not take calcium supplements in the last six months. The research work was approved by the ethics committee of the Universidad del Atlántico from Barranquilla-Colombia.

The participating group of students was invited to donate fasting blood and urine samples in an approximate volume of 5 ml. Blood samples was collected through a disposable syringe and placed in suitable tubes to separate the respective serum. The urine samples were acidified with hydrochloric acid, and both serum and urine samples were aliquoted in small volumes and stored at -30 ℃ until analyzed.

Body mass index (kg/m^2) was calculated for students as weight in kilograms divided by height in meter, measured by a digital balance and meter tape, respectively. Nutritional status was determined taking into account tables of the World Health Organization stipulated in the Resolution of the Ministry of Social Protection from Colombia (2465/16) [10].

B. Biochemical Analysis

The total calcium levels in serum and urine were determined by spectrophotometric method by reaction of the sample with methylthymol blue. The activity of total serum alkaline phosphatase was determined by enzymatic spectrophotometric method and for determination of activity of bone-specific alkaline phosphatase was necessary to inactivate it using a thermal treatment to serum at 56 ℃ for 15 min, in a drying oven and calculating the activity by difference between the activity of the total serum phosphatase and activity of phosphatase resistant to heat treatment [9]. Serum osteocalcin and cross-linked *N*-telopeptides of type I collagen (NTx-1) were measured by enzyme-linked immunosorbent assay. Urine creatinine was determined by colorimetric methods on the Jaffe reaction to express urinary calcium values as ratios of total calcium and creatinine.

C. Statistical Analysis

The statistical analyzes included descriptive analyzes of each variable. The values were expressed as mean ± standard deviation. Comparisons between school groups according to age and BMI were made by One–way analysis of variance (ANOVA). Pairwise significant differences were assessed by using Tukey's range test. Analyzes considered significant at p less than 0.05. Associations or correlations between the anthropometric and biochemical parameters were determined with the Pearson correlation coefficient. The criterion that was taken into account in this analysis was that value of the correlation coefficient (r) should be between 1 and -1 and be closest to these values; in addition to that, p values below 0.05 indicate correlations significantly different from zero, with a confidence level of 95%. The statistical analyzes were carried out with help of Statgraphics plus 5.1 computer program; SPSS version 17, Statgraphics Centurión XV.II.

III. Result and Discussion

A. Characteristic of the Study Sample

The population of students between 7 to 15 years old enrolled in the Jesus's Heart Educational Public Institution was 300. From these 150 were interested in participating in the study. But only 90 signed the informed consent. 22 did not meet the inclusion criteria. On the day of the appointment, for the collection of biological samples, blood and urine, 8 schoolchildren did not arrive fasting. Therefore, 60 schoolchildren participated in the study. From these 60 schoolchildren, 63% (n = 38) were male and 37% (n = 22) were female. 69% were from socioeconomic stratum 1, 17% stratum 2 and 14% stratum 3.

The mean age of participants was 9.0 ± 1.9 years. The classification of schoolchildren by nutritional status (Resolution 2465/16 Ministry of Social Protection from Colombia) [10], showed that 78% (n = 47) presented normal nutritional status, 5% (n = 3) low, 7% (n = 4) overweight and 10% (n = 6) were obese, respectively. It is observed that 17% of the schoolchildren presented an increase in body weight, a situation that has been of concern in many countries of Latin America independent of the socioeconomic stratum, where there had been an increase in overweight and obesity in children, attributed to changes in dietary habits, double-load overfeeding, increased sugary drinks, sedentary lifestyle among others [6], [11], [12].

B. Values of Biochemical Indicators Evaluated

Table I shows the values of biochemical indicators evaluated; serum calcium had average of 9.5 ± 1.06 mg/dL, values similar to those obtained by [13], but different from those found by [4] in schoolchildren from a developing country. However, 77% of students participating in this study had normal serum calcium levels, 16% hypercalcemia and only 7% had hypocalcemia, compared to normal values (8.6 to 10.0 mg/dL) [14]. Result different from what was found in Venezuelan schoolchildren [11], but similar with non-variation in serum calcium levels with sex and anthropometric measurements. Although schoolchildren were of low socioeconomic status, the nutritional status in calcium was

favorable, possibly to a dynamic homeostatic mechanism [15]. On the other hand, serum calcium values did not present a significant correlation with age of the students. This observation is contrary to that observed in [16], in children from India. In that research, serum calcium levels in 6-year-old children showed higher values compared to children of higher age.

In this work, there was no correlation of serum calcium values with the body mass index of schoolchildren.

TABLE I
LEVELS OF CALCIUM BIOCHEMICAL INDICATORS EVALUATED IN THE GROUP OF SCHOOLCHILDREN (N=60)

Compartment	SERUM					URINE	
Level	Calcium total (mg/dL)	SAPT (U/L)	SBAP (U/L)	Osteocalcin(ng/mL)	NTX-1 (ng/mL)	Calcium total (mg/dL)	Ca/Creatinine (mg/mg)
Mean ± SD	9.5 ± 1,06	181.3 ± 64.3	143.8 ± 73.9	9.0 ± 3.4	101.33 ± 12.8	12.8 ± 7,7	0.104 ± 0.07
Minimum	6.21	60.81	24.0	3.3	76.14	0.62	0.005
Maximum	12.84	290.2	269.5	15.6	120.0	30.8	0.395
Range	6.63	229.41	245.5	12.3	43.86	20.16	0.39

Total serum alkaline phosphate activity was lower than that observed by [16] in Indian children and adolescents, but similar range to those reported by [14] in Brazilian children, and by [17] in Italian children, but without difference by sex and age, differently to that was found by [17]. The activity of bone-specific alkaline phosphatase was observed quite high compared to activity of total phosphatase, corresponding approximately 80%, possibly to an active and normal state of bone remodeling in these children, observation that may be associated with the positive correlations found between serum levels of total calcium and total alkaline phosphatase activity ($r = 0.5$ $p < 0.05$) and with SBAP ($r = 0.6$ $p < 0.05$). Correlations between total alkaline phosphatase and SBAP with BMI did not present.

Osteocalcin, a protein synthesized by osteoblasts that plays an important role in bone mineralization and calcium homeostasis, was presented on average in the participant group of 9.0 ± 3.4 ng/mL, with variations of 3.3 to 15.6 ng/mL. Few studies report this protein in serum samples of humans at school age. However, the values found in this study were lower compared to those reported by [13], in Iranian schoolchildren with an average of 50.7 ng/mL, this difference may be due to use of sampling methods, collection and storage, among others [16]. It is necessary to carry out more studies on the assessment of this indicator in children.

Amino-terminal Crosslinked Telopeptide of type 1 collagen (NTX-1), an indicator of bone degradation or resorption was determined in serum of participating children group, observing a mean ± SD of 101.33 ± 12.8 nL/mL, with variations of 76.14 a 120.00 ng/mL, values that could not be compared with other studies in children. Few studies report levels of this indicator. They were only found in adults and in urine sample and different physiological conditions [18].

Calcium concentrations in fasting urine samples were on average 12.8 ± 7.7 mg/dL, and expressed as creatinine ratios were 0.1039 ± 0.068 mg/mg, values that were comparable to those reported by [19], in schoolchildren from Turkic countries and by [20], in school Lebanese children. However, values of urinary calcium/creatinine ratios above 0.21 mg/mg are considered by pediatricians as hypercalciuria in children older than 6 years and may be associated with several metabolic disorders such as enuresis, abdominal pain, hematuria, urolithiasis among others. But factors such as dietary habits, geographical area, cultural habits, can influence excretion and homeostasis of calcium in the body [18]-[20]. None of the evaluated indicators presented correlation with the BMI; levels appear to be independent of nutritional status of the participating infants. In conclusion, low values were observed in calcium urinary excretion and high in NTx-1, suggesting that mechanisms such as increase in renal retention of calcium and in bone remodeling may be contributing to calcium homeostasis.

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