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# **Original**

# Prevalence of vitamin D deficiency in Costa Rican children

(Prevalencia de deficiencia de vitamina D en niños de Costa Rica)

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## **Abstract**

**Objective:** To determine the prevalence of vitamin D deficiency in children in the South-Central Region of Costa Rica.

**Methods:** A cross-sectional study was performed with Costa Rican children aged 1-7 years from the South-Central Region of the country belonging to the Nutrition and Education Centers and Children's Nutrition and Comprehensive Care Centers. Serum samples were collected from all participants between August 2014 and May 2016 and stored at -80 ° C. Vitamin D status was assessed by immunoassay on an ARCHITECT Plus i1000 instrument. Descriptive statistics were performed with the SPSS statistical software package (V20, IBM Corp). A value of p<0.05 was considered significant. Spearman and Pearson correlation were also performed to study the association between vitamin D status, anthropometric and hematological variables.

**Results:** Atotal of 428 samples were analyzed. According to the cut-off points established by the Endocrine Society, 4.9% of the children tested presented deficiency, 50.2% had insufficiency and 44.9% had vitamin D sufficiency. The mean concentration of 25-hydroxyvitamin D in the studied population was 29.7 ng/mL (SD 6.5) in boys and 29.8 ng/mL (SD 7.0) in girls. A high prevalence of hypovitaminosis D (55.1%) was found, but only 7.9% of the children presented 25-hydroxyvitamin D  $\leq$  20 ng/mL. No correlation was found between vitamin D status and any of the evaluated anthropometric or hematological variables.

**Conclusions:** More than half of the young population presented hypovitaminosis-D. Therefore, in order to overcome this situation, the recommendation is to supplement the population with vitamin D and improve its fortification in widespread accessible food in Costa Rica.

**Keywords:** children, vitamin D, vitamin D deficiency, prevalence, Costa Rica.

#### Resumen

**Objetivo:** Determinar la prevalencia de la deficiencia de vitamina D en niños de la Región Central Sur de Costa Rica.

**Métodos:** Se realizó un estudio transversal con niños de 1-7 años de la Región Central Sur de Costa Rica pertenecientes a los Centros de Educación y Nutrición y Centros de Atención Integral y Nutrición Infantil. Se recolectaron muestras de suero de todos los participantes entre agosto de 2014 y mayo de 2016 y se almacenaron a -80 ° C hasta su

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List of abbreviations: CEN-CINAI, Nutrition and Education Centers and Children's Nutrition and Comprehensive Care Centers; API, Child Care and Protection. DAF, Families Food Distribution. 25(OH)D, 25-hydroxyvitamin D or Vitamin D; ES, Endocrine Society; IPS, Italian Pediatric Society; AAP, American Academy of Pediatrics; BMI, Body Mass Index; SD, Standard deviations; IU, international unit.

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uso. Se evaluó las concentraciones de vitamina D mediante un inmunoensayo con el instrumento ARCHITECT Plus i1000. Los análisis estadísticos descriptivos se realizaron con el paquete de software estadístico SPSS (V20, IBM Corp). Se consideró significativo un valor de p <0,05. También se realizó la correlación de Spearman y Pearson para estudiar la asociación entre el estatus de vitamina D con variables antropométricas y hematológicas.

**Resultados:** Se analizaron un total de 428 muestras. Un 4,9% de los niños presentaban deficiencia, 50,2% insuficiencia y el 44,9% suficiencia de vitamina D, según los puntos de corte establecidos por Endocrine Society. La concentración media de 25-hidroxi vitamina D en la población estudiada fue de 29,7 ng/mL (DE 6,5) en niños y de 29,8 ng/mL (DE 7,0) en niñas. Se encontró una alta prevalencia de hipovitaminosis D (55,1%), pero solo el 7,9% de los niños presentó 25-hidroxi vitamina D  $\leq$  20 ng/mL. No se encontró correlación del estatus de vitamina D con ninguna de las variables antropométricas o hematológicas evaluadas.

**Conclusiones:** Más de la mitad de la población infantil evaluada presentó hipovitaminosis D. Por lo tanto, se recomienda suplementar y mejorar la fortificación con vitamina D en alimentos ampliamente accesibles en la población costarricense.

Palabras claves: niños, vitamina D, deficiencia vitamina D, prevalencia, Costa Rica.

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Vitamin D plays a pivotal role in bone health by influencing calcium and phosphorus homeostasis, regulating pathways involved in bone mineralization and bone mass acquisition.<sup>1,2</sup> Although nutritional rickets in infants and children could develop secondary to severe calcium deficiency, vitamin D deficiency is the main cause for this condition.<sup>3</sup>

In addition to its central role in bone development, vitamin D is involved in a broad spectrum of possible pleiotropic and extra skeletal implications. Vitamin D deficiency has studied in recent decades, including its effects on the cardiovascular, nervous and immune systems, as well as its possible roles in the development of infectious and autoimmune diseases. 4,5,6,7 Optimal levels of vitamin D have been associated with lower incidences of type I diabetes mellitus,6-8 reduced complications of asthma, 9,10 and prevention of cancer, 11-13 among others. Nevertheless, most of these studies have not been exhaustive and have failed to establish a definitive consensus on what levels of 25-hydroxyvitamin D [25(OH)D] are required to accomplish the desired effect.

Serum 25(OH)D measurement is usually the analysis of choice to assess vitamin D status in patients, due to its stability (half-life is two to three

weeks in circulation), its concentration in the blood and its resistance to being affected by parathyroid hormone concentration. Despite disagreements in the scientific community regarding the establishment of a minimal desired 25(OH)D concentration, vitamin D hypovitaminosis remains a global health issue for people with low sun exposure, pregnant women, people with increased skin pigmentation, obese individuals and especially, children. 1,5,14

Although there is limited data available regarding the vitamin D status of children, vitamin D hypovitaminosis is considered common among this age group worldwide. This has been attributed primarily to a decrease in sunlight exposure – the primary source for vitamin D – and other factors such as a decrease in milk intake, reduced consumption of food that naturally contains vitamin D, and an increased incidence of obesity. 11,14 Furthermore, the association of skin cancer with ultraviolet rays has contributed to sun exposure avoidance and use of sun protection strategies (sunscreen, clothing). 7,15

There is limited information regarding the vitamin D status of children in Central American countries and very few studies in Costa Rica. Montero-Arias *et al* (2013)<sup>16</sup> have reported that 28% of asthmatic children in Costa Rica presented vitamin D insufficiency, yet no vitamin D status

assessment was made in otherwise healthy individuals. Nevertheless, a study carried out in Puerto Rico – a country located on a geographical latitude similar to Costa Rica (18° 15' North) – demonstrated a high prevalence of vitamin D insufficiency in children.<sup>17</sup>

Since an inadequate vitamin D status is associated with considerable public health repercussions –and children are particularly susceptible due to their high bone growth requirements<sup>11</sup> –we aimed to determine the prevalence of hypovitaminosis D among children in the South-Central Region of Costa Rica and its association to anthropometric or hematological variables.

#### Materials and methods

## Study design and population

The study population consisted of children aged 1-7 years who live in the South Central Region of Costa Rica and who attend the Nutrition and Education Center and the Children's Nutrition and Comprehensive Care Center (CEN-CINAI by its acronym in Spanish), a dependence of the Costa Rican Health Ministry. These children were beneficiaries of one of two programs: 1) Child Care and Protection (API, an intramural program in which children attend the centers daily to receive education and nutrition) or 2) Family Food Distribution (DAF, an extramural program allocating a monthly food package to each participating family, in part due to the poor nutritional status of the children.) Once a month, children in this program are weighed and measured at these centers. A total of 13 CEN-CINAI were randomly selected to make a census: CINAI Aserri, CINAI Paso Ancho, CEN Río Azul, CINAI Uruca, CINAI La Facio, CEN Pavas, CEN San Miguel, CINAI Gravilias, CINAI Alajuelita, CEN Salitrillos, CINAI Santiago de Puriscal, CEN San Rafael, and CEN San Juan de Tibás. Serum samples were collected between August 2014 and May 2016 and stored frozen at -80° C. For this study, a sub-sample of 50% of the frozen samples corresponding to 428 samples was randomly selected, without affecting the representativeness of the study population.

All stored samples were associated with participants whose parents consented to the storage of these samples, to their transfer to other studies

and to the use of this data in future research, all in accordance with the Scientific Ethics Committee of the University of Costa Rica (VI-2884-2014).

#### Vitamin D measurement

Vitamin D status was assessed through the measurement of 25(OH)D. Serum 25(OH)D concentrations were analyzed according to the manufacturer's commercial kit ARCHITECT 25-OH Vitamin D Abbott, an electrochemiluminescence immunoassay performed using an ARCHITECT Plus i1000 instrument. Vitamin D status was categorized according to recommendations of the Endocrine Society (ES): deficiency was defined as a 25(OH)D level below 20 ng/mL, insufficiency as a 25(OH)D level between 20-29 ng/mL, and sufficiency as a 25(OH)D level between 30–100 ng/ mL.18 Vitamin D deficiency or insufficiency was classified as hypovitaminosis. Results were also compared with the guidelines from 1) the Italian Pediatric Society (IPS), which defines deficiency as 25(OH)D below 20 ng/mL, insufficiency between 21-29 ng/mL, sufficiency between 30-50 ng/mL, and optimum above 50 ng/mL,2 and with the American Academy of Pediatrics (AAP), whose guidelines sets severe deficiency as an 25(OH)D value below 5 ng/mL, deficiency between 5-15 ng/ mL, insufficiency between 16-20 ng/mL, sufficiency between 21-100 ng/mL, excess between 101-149 ng/mL, and intoxication above 150 ng/mL.19 For this study, hypovitaminosis was defined as 25(OH) D values below 30 ng/mL. All anthropometric measurements and hematological values were obtained from a previous study conducted on this population.

#### Statistical analysis

The serum 25(OH)D levels were described using mean, standard deviations (SDs), and ranges. Frequencies and percentages (%) were reported for categorical variables. Tests to determine differences in mean serum 25(OH)D levels and vitamin D status by sex, age, assistance program (API-DAF) and center (CEN-CINAI) were performed using unpaired Student's t-test, Analysis of Variance (ANOVA) or Chi square, as appropriate. The statistical significance level was set at p <0.05. A possible correlation between discrete variables of the vitamin D status classification and the categories of weight/height, height/age, weight/age, body mass index (BMI) and anemia were also evaluated using a Spearman

correlation. The nutritional and anemia categories were constructed according to the Training Course on Child Growth Assessment: Module C Interpreting Indicators cut-off values and to Nutritional Anaemias: Tools for Effective Prevention cut-off values, both from the World Health Organization (WHO).<sup>20,21</sup> The correlation of continuous variables of vitamin D levels with anthropometric variable values (weight, height, BMI, z-scores for weight/ height, height/age, weight/age) and hematological values (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, leukocytes, lymphocytes, monocytes, or neutrophils counts) were assessed using Pearson's correlation. Analyses were performed using the SPSS statistical software package (V20, IBM Corp).

#### Results

A total of 428 samples were analyzed. Table 1 shows the status of vitamin D (prevalence of vitamin D deficiency, insufficiency, and sufficiency), using the cut-off values for serum vitamin D concentrations, as established by different organizations. According to the ES and IPS guidelines, 4.9% of the children presented vitamin D deficiency, 50.2% had insufficiency and 44.9% presented sufficiency. Conversely, when considering the cut-off values of the AAP, 92.0% of the children had vitamin D sufficiency.

The data was analyzed using the ES cut-off points. The mean concentration of 25(OH)D in the study population was 29.7 ng/mL, with levels ranging from 13.7 to 63.9 ng/mL. A high prevalence of hypovitaminosis D (55.1 %) was found. Only 34 (7.9 %) children presented  $25(OH)D \le 20 \text{ ng/mL}$ . The averages of the 25(OH)D concentration as well as the prevalence of hypovitaminosis D were stratified according to sex, age, and the program the children were assigned to. No statistically significant differences were observed in the averages or in the prevalence of hypovitaminosis D in any of these categories. Nevertheless, a trend towards an increase in the prevalence of hypovitaminosis D was observed as the children's ages increased and depending on which program they were assigned (higher in the DAF extramural program than in the API intramural program) (Table 2). The prevalence

of hypovitaminosis D in children at or above 5 years ( $\geq$  60 months) was significantly higher than that in children younger than 5 years (52.1% and 47.1% respectively; p = 0.015).

Differences were observed in the average concentration of vitamin D according to the particular CEN-CINAI the children attended (p <0.001), with Aserrí and Paso Ancho being the centers with the lowest average concentration of vitamin D (26.1 ng/mL and 27.1 ng/mL, respectively) and the highest percentage of children with hypovitaminosis D (80% and 65%, respectively), in contrast to San Rafael and San Juan, the centers with the highest average concentrations of serum 25(OH)D (32.5 ng/mL and 33.2 ng/mL respectively) and the lowest percentage of children with hypovitaminosis D (35% and 29%, respectively) in this study (Table 3).

No correlation was found between 25(OH)D vitamin D status (deficiency, insufficiency, sufficiency) and categorized nutritional variables (weight/height, height/age, weight/age, BMI) or anemia (correlation coefficient <0.6 in all cases, data not shown). This means there was no association between vitamin D status and height or weight categories (severe thinness, underweight, normal weight, overweight risk, overweight or obesity) in this study.

There was no correlation found between 25(OH)D concentration and any of the nutritional values (weight, height, BMI, z-scores for weight/height, height/age, weight/age) or hematological

Table 1. Vitamin D status in children from CEN-CINAI from the South-Central Region of Costa Rica, 2014-2016, according to the classification of three reference organizations, n = 428

Category	Endocrine Society	Italian Pediatric Society	American Academy of Pediatrics
Severe deficiency	-	0 (0.0)	0 (0.0)
Mild to moderate deficiency	21 (4.9)	21 (4.9)	4 (0.9)
Insufficiency	215 (50.2)	215 (50.2)	30 (7.0)
Sufficiency	192 (44.9)	192 (44.9)	394 (92.0)
Excess	-	-	0 (0.0)
Intoxication			0 (0.0)

**Abbreviations:** CEN-CINAI: Nutrition and Education Centers -Children's Nutrition and Comprehensive Care Centers.

Table 2. Serum 25(OH)D levels and hypovitaminosis D prevalence in children in the South-Central Region of Costa Rica, aged 1-7 years who attended CEN-CINAI programs, 2014-2016, stratified by sex, age and assistance program, n=428

Variables         n (%)         Mean ± SD         p           Sex         ————————————————————————————————————	n (%) 120 (56.1) 116 (54.2)	<b>p</b> 0.697
Male 214 (50.0) 29.7 (6.5) 0.281		0.697
		0.697
Female 214 (50.0) 29.8 (7.0)	116 (54.2)	
` '		
Age (years)		
1 2 (0.5) 23.8 (10.9) 0.117	1 (50.0)	0.408
2 17 (4.0) 29.8 (8.4)	8 (47.1)	
38 (8.9) 29.0 (5.5)	20 (52.6)	
4 110 (25.7) 30.5 (7.2)	56 (50.9)	
5 137 (32.0) 30.4 (6.7)	73 (53.3)	
6 110 (25.7) 28.5 (6.4)	67 (60.9)	
7 14 (3.3) 25.2 (1.6)	11 (78.6)	
Program		
API 278 (65.0) 29.8 (6.8) 0.618	149 (53.6)	0.681
DAF 121 (28.3) 29.3 (6.9)	70 (57.9)	
API+DAF 29 (6.8) 30.4 (5.6)	17 (58.6)	

**Abbreviations:** CEN-CINAI: Nutrition and Education Centers - Children's Nutrition and Comprehensive Care Centers; API: Child Care and Protection (intramural program); DAF: Families Food Distribution (extramural program), API+DAF: Children who belong to API and DAF programs simultaneously, SD: standard deviation, 25(OH)D: 25-hydroxyvitamin D or Vitamin D. \*Children with vitamin D deficiency or insufficiency were considered hypovitaminosis D (i.e., 25(OH)D levels <30 ng / mL).

variables evaluated (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, leukocytes, lymphocytes, monocytes, or neutrophils counts) in this study (correlation coefficient <0.6 in all cases, data not shown).

#### Discussion

Despite the increasingly recognized clinical relevance of vitamin D deficiency, there are still discrepancies between the cut-off values that different organizations use to classify vitamin D status in individuals. Consequently, the threshold (cut-off value) at which hypovitaminosis D is

defined will alter the percentage of people with this condition.<sup>15</sup> In the present study, the prevalence of hypovitaminosis D obtained using the AAP cut-off values was inverted when compared with that obtained with both ES and IPS cut-off values. This controversial topic, previously discussed by others, <sup>2,5,5,12,22</sup> highlights the imperative need to reach an unambiguous consensus, because the classification criteria will affect each country's prevention and treatment strategies, including oral vitamin D supplementation, increased vitamin D consumption through diet, and natural or artificial ultraviolet radiation exposure.<sup>15</sup>

For the present study, we decided to employ the ES cut-off values for vitamin D, one of the most widely used criteria, because it categorizes vitamin

Table 3. Percentage of children with hypovitaminosis D and average concentration of serum 25(OH)D prevalence in children in the South-Central Region of Costa Rica, aged 1-7 years who attended CEN-CINAI programs, 2014-2016, n = 428

Center	Percentage of children with hypovitaminosis D* (%)	Average concentration of vitamin D (ng/mL) Mean ±SD	Minimum concentration of vitamin D (ng/mL)	Maximum concentration of vitamin D (ng/mL)
CINAI Aserrí (n=40)	80	26.1 ± 5.5	14.8	38.0
CINAI Paso Ancho (n=23)	65	27.1 ± 6.1	13.7	40.0
CEN Río Azul (n=46)	63	29.8 ± 5.2	22.8	43.8
CINAI Uruca (n=52)	58	28.4 ± 5.6	16.0	42.9
CINAI La Facio (n=33)	58	$27.5 \pm 5.7$	18.2	38.2
CEN Pavas (n=21)	57	31.2 ± 8.1	21.1	54.8
CEN San Miguel (n=42)	55	$31.0 \pm 8.4$	18.3	63.9
CINAI Gravilias (n=45)	51	31.4 ± 8.3	14.4	57.1
CINAI Alajuelita (n=32)	50	29.6 ± 5.3	19.1	39.9
CEN Salitrillos (n=24)	50	30.6 ± 7.2	18.9	45.8
CINAI Puriscal (n=36)	39	31.7 ± 6.1	16.3	43.7
CEN San Rafael (n=20)	35	$32.5 \pm 6.0$	18.1	42.1
CEN San Juan (n=14)	29	33.2 ± 6.8	23.1	47.3

**Abbreviations:** CEN-CINAI: Nutrition and Education Centers - Children's Nutrition and Comprehensive Care Centers \* Children with vitamin D deficiency or insufficiency are were classified in the category of hypovitaminosis D (i.e., 25(OH) D levels <30 ng / mL)

level as "sufficient" starting from levels of 25(OH) D > 30 ng /mL. It has been shown that at levels of 25(OH)D below 30 ng/mL, an inverse relationship occurs between parathyroid hormone and vitamin D. Consequently, optimal musculoskeletal health is achieved only at levels of 25(OH)D above 30 ng/mL.<sup>14</sup>

Our study showed a high prevalence of hypovitaminosis D (levels of 25(OH)D < 30 ng/mL) in children (55.1%), in concordance with what has been reported worldwide.<sup>1,2,8</sup> The possible causes and consequences of hypovitaminosis D were previously mentioned in the introduction of this article. Despite the high prevalence of hypovitaminosis D observed in the present study, only 7.9% of children presented  $25(OH)D \le 20 \text{ ng/mL}$ ; this is considered a critical cut-off value for optimal bone health.<sup>23,24</sup>

Regarding the differences of vitamin D levels according to sex, some studies have pointed towards an increased prevalence of hypovitaminosis D among

females,¹⁵ but our study did not find a statistical difference by this variable. Regarding the age, a statistically higher prevalence of hypovitaminosis D was observed in children older than 5 years (≥60 months) compared to younger children, in line with what has been previously reported by others elsewhere. Some authors state that this higher prevalence of hypovitaminosis D with increasing age may be explained by different factors such as an absence of vitamin D supplementation in older children, supplementation with an inadequate dosage, changes in diets or lifestyles, decreased sun exposure and less time spent outdoors, imbalance between intake and requirements during growth, or even a reduction in the body surface area to volume ratio.¹,2,15

Although no statistically significant differences were found according to the children's assistance program, there are higher percentages of hypovitaminosis D in children from extramural programs (DAF). This can be partially explained

because children of the intramural program drink fortified milk [400 international units (IU) of vitamin D/100 g milk] in the educational establishments and receive milk for home consumption, while children in the extramural program receive fortified milk only for home consumption, so the consumption by the child is less reliable. CEN-CINAI's fortified milk can provide a high percentage of the recommended daily intake (600 IU/day), 18 so education strategies should emphasize the importance of the consumption of milk by the child and not by other family members.

In addition, the correlation between low vitamin D levels and overweight or obesity is well documented both in children and in adults.<sup>25-27</sup> The literature indicates that the increase in adipose tissue provokes a greater distribution of the fat soluble vitamin D in this tissue, thereby reducing the blood circulating 25(OH)D levels; nevertheless, this does not necessarily implicate a deficiency, as obese individuals tend to have a high mineral bone density.<sup>28,29</sup> In our study, we found no significant association between the children's anthropomorphic metrics and the 25(OH)D concentration, probably because the vitamin D reduction is more prominent in obesity rather than in overweight individuals, and in the present study only 2 (0.5%) of children were obese and 39 (9.1%) were overweight.

We observed a statistically significant difference in the prevalence of hypovitaminosis D among the children's adscription centers (CEN-CINAI). However, the study's limitations do not allow us to elucidate the causes of these differences. To achieve this, the diet of the children in each center and the amount of sun exposure must be investigated, as there are differences in the availability of courtyards and time spent by caregivers playing outdoors with the children, among other factors. Also, based on the observations made during the study, there are no apparent differences in ethnic background, skin pigmentation or clothing among the children in the different centers that would explain these results.

For the present study, we faced some limitations regarding the lack of access to information regarding the conditions of the study population that could affect vitamin D levels, such as possible treatment with vitamin D in the study population (although it is infrequent for infants in Costa Rica), amount of sun exposure,

use of sunscreen, measurement of skin color, specific diet or eating habits, possible vitamin D supplementation in food, and information on how long the children have benefited from the CENCINAI programs. Moreover, this study was limited to children attending the CEN-CINAI in the South-Central Region of Costa Rica.

Despite the high prevalence of hypovitaminosis D, due to its high cost, a generalized screening for vitamin D status at population level is usually not recommended. Some authors have pointed out that the promotion of food fortification programs, the implementation of oral vitamin D supplementation to the affected population, and moderate sun exposure could be more cost-effective strategie. 14,30

Several studies have demonstrated that a lack of uniformity, quality and access to fortified food products contributes to an ineffective strategy against hypovitaminosis D in the population. Some food products brands in Costa Rica do fortify their products with vitamin D; nevertheless, to achieve an impact on the general population, adequate fortification of food products that are accessible to the whole population and that are consumed on a regular basis must be promoted.

Oral vitamin D supplementation used to be considered imperative for infants with risk factors that may lead to hypovitaminosis D. Therefore, supplementation was mainly oriented towards children with little sun exposure and dark skin pigmentation, nutritional deficiencies, obesity, and others. Nevertheless, today, several international organizations recommend supplementation regardless of the presence of these factors. <sup>2,7,11,18</sup> Currently, a range of 600-1000 IU per day is recommended for infants from the first through the eighteenth year of life. <sup>11,18</sup>

The guidelines of the ES may be employed as a guide to implement a possible supplementation plan at a national level.<sup>18</sup> Furthermore, it is worth emphasizing that the risk for vitamin D intoxication associated with supplementation is negligible in children of this age; for instance, the upper limit of recommended vitamin D intake for children between 4 and 8 years is 3000 IU per day.<sup>11,18</sup> Considering all the potential benefits related to vitamin D – from the intestinal absorption of calcium to its possible immune functions – the fear of toxicity through supplementation must not compromise the goal of an optimal vitamin D status.

Because this study highlights the high prevalence of hypovitaminosis D in the child population, it is important to work on strategies to supplement and improve the fortification with vitamin D in widely accessible food in Costa Rica. However, to achieve this, it will be necessary to establish a regulatory framework that requires specific food products to be properly fortified and periodically verified to ensure compliance to labeling specifications.

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