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Sugar Sweetened Beverages Consumption in Preadolescent Children: 25-Hydroxy Vitamin D and Bone Mineral Density Affection

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Authors' contributions

Author MFN had primary responsibility for the idea and the protocol development, patient screening, enrollment, outcome assessment, preliminary data analysis and writing the manuscript. Author EKE shared the protocol development, supervised patient screening and enrollment. He helped in data interpretation and manuscript revision. Author RHS supervised the design and execution of the study and participated in the data analysis and preparation of the manuscript. Author DAF supervised the laboratory work of the study and contributed to the editing of this section in the manuscript. Author AGZ was responsible for patient screening, enrollment and performance of the DXA scan. Author MSA was responsible for patient screening, enrollment and obtaining the blood samples. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Objective: To study the effect of heavy consumption of sugar sweetened beverages on serum 25-hydroxy vitamin D (25OHD) level and bone mineral density (BMD) in preadolescents.
Study Design: Case-control.
Place and Duration of Study: Outpatient clinic in Children`s hospital, Ain Shams University, between September, 2009 and July, 2010.
Methods: A questionnaire to define the frequency of beverage consumption was filled by one thousand children within the age range of 8-12 years. Fifty children were selected from the heavy sugar sweetened beverages consumers and were compared to 50 clinically healthy age and sex matched children who gave no history of more than

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average sugar sweetened beverages intake. Each enrolled case filled a questionnaire that assesses duration of beverage intake, frequency and type as well as milk consumption. Questions concerning symptoms of pain, limitation of movement and fractures were also included. Anthropometric measurements were taken. Serum calcium and phosphorus were measured as well as the circulating 25OHD and dual-emission x-ray absorptiometry scan assessed their BMD Z-score.

Results: Children who heavily consumed the sugar sweetened beverages had significantly lower 25 (OH) vitamin D, and BMD Z-score as compared to the controls (t= 4.05, *P*<0.001 and t= 3.73, *P*<0.001, respectively). There was significant negative correlations between duration of beverage intake and both BMD Z-score and 25OHD among heavy sugar sweetened beverage consumers(r=0.38, *P*=0.01 and r=0.4, *P*<0.001, respectively). A significant negative correlation was detected between duration of beverage intake and serum calcium among heavy sugar sweetened beverage consumers (r = -0.46 and *P*<0.001) and significant positive correlation between BMD Z-score and 25OHD among them (r = 0.69 and *P*<0.001).

Conclusion: Sugar sweetened beverages intake is associated with a decrease in 25OHD which contributes to low bone mineralization in preadolescents and the duration of intake is the most determinant factor for this association. Consumption of carbonated beverages had more hazardous effect on 25OHD and BMD than packed fruit juice while milk intake showed an opposite effect. We recommend more awareness programs for preadolescents and their parents whether at school or as a part of national campaigns to boost healthy trends in beverage consumption.

Keywords: Calcium; Sugar Sweetened beverages; Preadolescents; Bone mineral density; Milk; Serum vitamin D.

1. INTRODUCTION

Dairy products, particularly fluid milk, are recognized as the primary source of dietary calcium and vitamin D and an important source of protein, phosphorous, riboflavin, potassium, magnesium, vitamin B_{12} and vitamin B_6 in young children [1]. During last few decades, soft drinks consumption has steadily increased while milk intake has decreased. Excess consumption of soft drinks and low milk intake may pose risks of several diseases such as dental caries, obesity, and osteoporosis [2]. Goulding [3] reported that poor nutrition (including an inadequate intake of dietary calcium, milk avoidance and excessive consumption of carbonated beverages) may influence fracture risks in the general pediatric population. When dietary calcium intake is sufficient, it interacts with vitamin D metabolism at both the renal and bone tissue levels to direct an anabolic action on bone through a bone autocrine or paracrine system [4]. Fisher et al. [5] provided new longitudinal evidence that calcium intake predicts bone mineral status during middle childhood and reflects beverage choice patterns in girls. Evidence also supports an association between soft drink consumption and decreased bone mineral density [6,7]. These observations further demonstrate the interaction between diet, nutrients and bone health which could thus be in stake in preadolescents consuming excess sugar sweetened beverages.

The aim of this work was to study the effect of heavy consumption of sugar sweetened beverages on both 25-hydroxy vitamin D (25 OHD) level and bone mineral density (BMD) in preadolescents.

2. METHODS

2.1 Study Population

After approval of the local ethical committee this study was performed in 2 phases. Phase I was a cross - sectional study which included one thousand preadolescent recruited from the outpatient clinic of Pediatric hospital, Ain- Shams University. They were randomly recruited amongst the relatives of patients coming for vaccination or routine follow up. Children who were on medications like steroids, anticonvulsants, calcium supplement, vitamin D and those who were receiving chemotherapy or had systemic diseases, especially bone and kidney diseases were excluded. After obtaining an informed consent from their parents, the selected children were given a questionnaire in order to define the frequency of beverage consumption among them. Out of the 1000 children only 632 (63.2%) were considered heavy sugar sweetened beverage consumers (consume more than 12 ounces or 354.84 ml/day[8]).

Phase II was a case – control study in which 50 children were selected from the heavy sugar sweetened beverage consumers of phase I by systematic randomized sampling.They were contacted and summoned back to participate in the phase II. A group of 50 clinically healthy age and sex matched children were chosen from those who gave no history of more than average sugar sweetened beverages intake (that does not exceed 0-8 ounces or 0-236.56 ml daily [9]) and enrolled as the control group.

Each preadolescent was asked to fill a questionnaire that assesses their socio-demographic data, daily intake of beverages with special emphasis on the frequency, duration and type of beverage intake, as well as milk consumption. Questions concerning symptoms of pain, limitation of movement and fractures were also included. Kuppuswamy's socioeconomic scoring classification (2005) was used to determine their socioeconomic standard.

General examination of each child was done and Tanner assessment of pubertal development was performed [10]. Anthropometric measurements were taken and this included body weight (kg) and height (cm) and weight % and height % from mean for age. Body Mass Index (BMI) was calculated by dividing the weight with square the height (kg/m^2).

2.2 Laboratory Investigations

Venous blood samples were taken under complete aseptic conditions using sterile dry disposable syringes and left to clot in clean closed glass tube then centrifugation was performed at 3000g for 15 minutes at room temperature.

Serum calcium and phosphorus were assessed by photometric assay using Synchron CX9, Bechman, USA. Measurement of circulating 25OHD level in serum was done using ELISA by commercially available kit supplied by Immundiagnostik, Bensheim and Biomedica, Wien Children who showed levels less than 20 ng/ml were considered vitamin D deficient as agreed by most researchers [11].

2.3 Radiological Assessment

Dual-emission x-ray absorptiometry (DXA) scan (Manufacturer Hologic, Inc. 35 Crosby Drive, Bedford, MA 01730-1450 USA) with pediatric software was applied to lumbar spine of

cases and controls upon which they were said to have low bone mineral density (BMD) if their BMD Z-score was less than-2 [12].

2.4 Statistical Methods

The IBM SPSS statistics (V. 19.0, IBM Corp., USA, 2010) was used for data analysis. Kolmogorov-Smirnov test was used to test for normality of data. Data were expressed as mean ± Standard Deviation (SD) for quantitative measures in addition to both number and percentage for categorized data. Comparison between two independent mean groups was performed using Student t and Wilcoxon Rank Sum tests for parametric and non-parametric data respectively. Pearson correlation test was used to study the possible association between two variables for parametric data. Chi-square test was performed to compare categorized data. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 it was considered as highly significant.

3. RESULTS AND DISCUSSION

3.1 Results

The study included fifty heavy sugar sweetened beverage consumers with mean age of 10.26 $\pm$ 1.44 years and male to female ratio of 1.27:1.00. A group of 50 clinically healthy age and sex matched children (mean age 10.64+1.25 years) who gave history of no more than average sugar sweetened beverages intake were enrolled as the control group. Both groups of preadolescents were from low middle social class and their socio-economic data and anthropometric measurements showed no statistical differences.

Table (1) shows that cases had significantly higher number and duration of beverage intake as compared to the controls. Additionally, this table demonstrates that cases drank significantly more carbonated beverages and less packed fruit juices and milk as compared to the controls.

Regarding symptoms suggestive of low BMD; cases significantly reported bony pains as compared to controls (χ^2 = 6.83 and p = 0.01). Other complains like limitation of movements and repeated fractures did not show statistical significance.

Regarding the laboratory parameters, 25OHD was significantly lower in cases as compared to the controls (Table 2). Additionally, serum calcium and phosphorus were significantly lower in cases with t (*P*) values of 4.29 (<.001) and 3.65 (<.001), respectively. The results of the current study also revealed that the duration of beverage consumption was significantly higher in the 18 cases with low 25OHD as compared to the 32 cases with normal 25OHD (t = 3.74 and *P*<.001).

Table (2) also shows that cases had significantly lower BMD Z-score as compared to the controls. The current study showed that out of the 50 children with heavy sweetened beverages intake, twenty were found to be of low BMD Z-score according the DXA scan results. When we compared those to the preadolescents with normal BMD Z-scores, the present study revealed that the age, sex and anthropometric measurements were not statistically different. Among the heavy sugar sweetened beverage consumers, preadolescents with low BMD had significantly longer duration of beverages intake (4.25+1.21 years) as compared to those with normal BMD (2.67+1.15 years) (t = 4.62 and

P<.001). They drank significantly more carbonated beverages (χ^2 = 9.78 and *P*<.001) and significantly less milk (χ^2 = 17.01 and *P*<.001). Additionally, preadolescents with low BMD complained of pain, limitation of movement and repeated fractures but only the first two reached statistical significance (χ^2 = 8.68, 14.49 and 4.68 and *P*<.001, <.001 and >.05 respectively).

As regard to the laboratory parameters, Table (3) demonstrates that serum 25OHD, calcium and phosphorus were significantly lower in preadolescents with low as compared to those with normal BMD Z-score.

Among the heavy sugar sweetened beverage consumers; BMD Z-score and serum 25OHD level were significantly lower in the 32 predominant carbonated beverages consumers as compared to the 18 packed fruit juice users (Table 4). Additionally, among the heavy sugar sweetened beverage consumers; BMD Z-score and serum 25OHD were significantly lower in the 20 children who did not drink milk as compared to the 30 who did [t = 4.97 (*P*<.001) and 4.39 (*P*<.001) respectively].

Table 1. Comparison between heavy sugar sweetened beverage consumers and the controls as regard beverage intake

Variable	Cases number=50	Controls number=50	t/χ^2*	*P*
Number of sugar sweetened beverages (/day)	3.16±0.37	1.08±0.64	-15.03	<.001
Duration of sugar sweetened beverages` intake (years)	3.30±1.40	1.86±0.73	-5.68	<.001
Predominant type of sugar sweetened beverages	Packed fruit juice=36.0% Carbonated drinks =64.0%	Packed fruit juice=48.0% Carbonated drinks =36.0%	10.99*	<.001
Milk intake	Yes=40.0% No=60.0%	Yes=72.0% No=28.0%	4.01*	.03

- P>0.05 = insignificant
- P<0.05 = significant
- P<0.01 = highly significant

Table 2. Comparison between heavy sugar sweetened beverages consumers and the controls as regard 25OHD level and BMD Z-score

Variable	Cases number=50 mean±SD	Controls number=50 mean±SD	t	*P*
25OHD (ng/ml)	45.6±16.22	57.2±8.43	4.05	<.001
BMD Z-score	-0.62±1	0.03±0.52	3.73	<.001

- P<0.01 = highly significant

Table 3. Comparison between low and normal bone mineral density cases as regard to laboratory data

Variable	Low BMD number=20 mean±SD	Normal BMD number=30 mean±SD	t	*P*
Serum 25OHD (ng/ml)	29.9±11.10	56.11±8.95	8.81	<.001
Serum Ca (mg/dl)	8.88±0.62	9.75±0.47	5.37	<.001
Serum P (mg/dl)	3.99±0.65	5.28±0.49	7.56	<.001

- P< 0.01 = highly significant

Table 4. Comparison between predominant carbonated and packed fruit juice consumers as regards serum 25OHD and BMD Z-score

Variable	Carbonated beverages number=32 mean±SD	Packed fruit juice number=18 mean±SD	t	*P*
25OHD(ng/ml)	40.76±16.59	54.28±11.5	3.38	<.001
BMD Z-score	-0.94 ±0.95	0.03±0.77	3.92	<.001

- *P*<0.01 = highly significant

The correlation studies revealed significant negative correlations between duration of sugar sweetened beverage intake and both BMD Z-score and 25OHD among heavy consumers (r=-0.34, *P*=.01 and r=-0.4 *P*<.001 respectively). Additionally, a significant negative correlation was detected between duration of sugar sweetened beverage intake and serum calcium among cases (r=-0.46 and *P*<.001) and a significant positive correlation between BMD Z-score and 25OHD among heavy consumers (r =0.69 and *P*<.001).

In a trial to calculate the relative risk factors for low BMD and for deficient 25OHD status, the current study revealed that the sugar sweetened beverage intake showed a positive significant risk while milk showed a negative significant risk for both conditions.

Fig. (1) demonstrates the ROC curve analysis for the diagnostic performance of duration of beverage intake to discriminate patients with low BMD from those without and at cut off value of 0.773 year the curve showed 90% sensitivity, 53.3% specificity and 68% efficacy. In addition, Fig. (2) shows the ROC curve analysis for the diagnostic performance of duration of beverage intake to discriminate patients with low serum 25OHDfrom those without and at cut off value of 0.662 year the curve showed 77.8% sensitivity, 68.8% specificity and 70% efficacy.

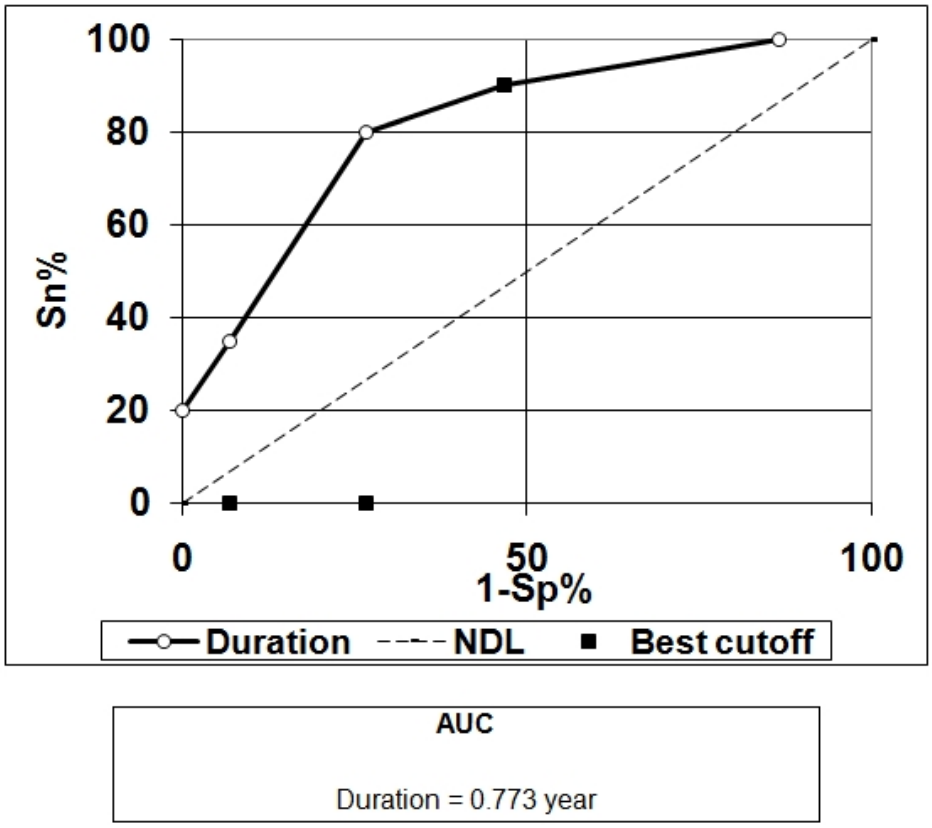

Fig. 1. ROC curve analysis showing the diagnostic performance of duration of drinking for discriminating patients with low BMD from those without and at cut off value of 0.773 year (the curve showed 90% sensitivity, 53.3% specificity and 68% efficacy)

3.2 DISCUSSION

The prevalence of heavy sugar sweetened beverage consumption among the studied cases in the first phase was 63.2% where males represented 56% and the rest were females. In 2008, a Canadian report mentioned that 53% of boys and 35% of girls between 14 – 18 years of age were sweetened beverage consumers [13].This was reinforced by Guthrie and Morton [14] who revealed that sweetened drinks constitute the primary source of added sugar in the daily diet of children. Additionally, Harrington [15] stated that soft drinks consumption increased by 300% in the last 20 years.

In the present study, we couldn`t trace any significant difference in BMI between heavy sweetened beverage consumers and the controls. This is in accordance with Rajeshwari et al. [16] who reported that there is no significant correlation between soft drink consumption and BMI. In 2006, Striegel-Moore et al. [17] reported that the observational studies in children and adolescents include several limitations that preclude a definitive conclusion that sugar sweetened beverages cause excessive weight gain. The latter authors even concluded that the increased caloric requirement associated with increased BMI may in itself

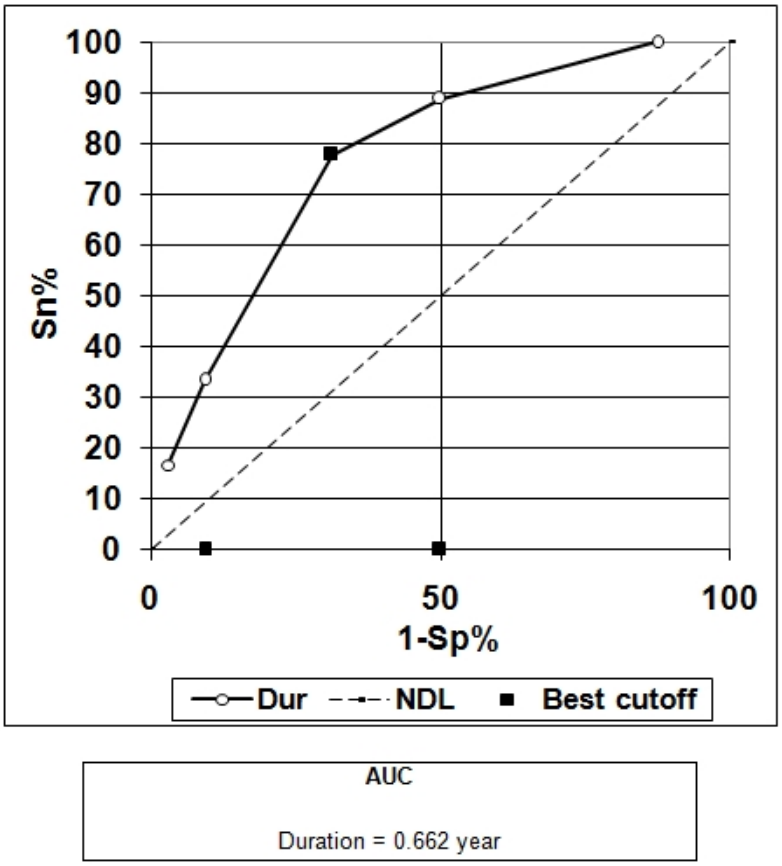

Fig. 2. ROC curve analysis showing the diagnostic performance of duration of intake for discriminating patients with low 25OHDfrom those without, and at cut off value of 0.662 year (the curve showed 77.8% sensitivity, 68.8% specificity and 70% efficacy)

account for the greater intake of calories and sugar sweetened beverages. On the other hand, Kosova et al. [18] reported that sugar-sweetened beverage intake was independently associated with alterations in lipid profiles, increased markers of inflammation, and increased waist circumference in children.

The present study revealed that significantly less number of heavy sweetened beverages consumers drank milk as compared to the controls. This is consistent with Blum et al. [19] who reported in their longitudinal cohort study significant decreases in milk consumption in children who consume soft drinks. Similarly, Marshal*l* et al. [1] found that milk intakes for 1–5 years were inversely associated with intakes of juice drinks, carbonated drinks and added sugar beverages. Additionally, Libuda et al. [20] reported that the change in consumption of all soft drinks was negatively associated with a concurrent change in milk consumption and calcium intake. In 2009, Rangan et al. [21] explained that children who consume large amounts of sugary drinks may also have poor nutritional intake due to the displacement of milk from the diet.

The current study reported that pain, limitation of movement and fractures were more common in cases as compared to the controls. This could be explained by the fact that increase cola consumption is associated with decrease calcium intake. This is supported by *Holick* [22] who reported that the cause of pain in diminished mineralization of bone is thought to be hydration of the demineralized gelatin matrix beneath the periosteum; the hydrated matrix pushes outward on the periosteum, causing throbbing, aching pain. Additionally, *Wyshak* [7] showed an association between carbonated beverage consumption and bone fractures among teenage girls.

In the current study, serum 25OHD was significantly lower in heavy sweetened carbonated beverage consumers versus the controls. *Gordon* et al. [23] reported an inverse correlation between consumption of juices and soft drinks and serum 25OHD levels. Previous studies also highlighted that the intake of sweetened beverages was negatively associated with intake of not only milk but also calcium as well as vitamin D [24,25].

In the present study, mean serum calcium was significantly lower in cases as compared to the controls. Though serum calcium is not a good marker of calcium intake Mazariegos-Ramos et al. [26] previously linked soft drink consumption with hypocalcaemia in children. Additionally, *Striegel*-Moore et al. [17] reported that soda consumption was associated with a statistically significant decrease in calcium intake. Because milk provides an important source of calcium in the diets of children and adolescents, the authors emphasized that the decline in milk consumption at a time of bone mineral deposition may predispose to eventual osteoporosis which is a major concern.

The DXA scan results showed that 40% of the children were having low BMD and BMD Z-score value was significantly lower in heavy sugar sweetened beverage consumers as compared to the controls. The preadolescents with low BMD drank significantly more carbonated beverages and less milk. This is in accordance with Ma and Jones, [6] who reported an association between soft drink consumption (the predominant sugar sweetened beverages taken by the studied cases) and fractures in children. Additionally, McGartland et al. [27] reported that carbonated soft drink consumption seems to be inversely related to BMD effect at the dominant heel in girls. The latter authors added that it is possible that the apparent association results from the displacement of more nutritious beverages from the diet. In a trial to explain the cause for this BMD affection Ogur et al. [28] suggested that the high phosphate and caffeine content of these drinks, the very low pH causing acid load in the body and the decreased consumption of milk and drinks containing milk when cola drinks are consumed in large amounts are to blame. Additionally, caffeine is a main ingredient in most colas and it has been shown that caffeine induces calcium loss [29].

The current study reported that duration of beverages consumption was significantly higher in preadolescents with low BMD as compared to those with normal BMD. This is in accordance with *Guesry* [30] who reported that chronic ingestion of soft drinks (the predominant sugar sweetened beverages taken by the studied cases) is thought to cause slow dissolution of bone minerals due to phosphoric acid effect.

Among the studied heavy sugar sweetened beverage consumers, the serum 25OHD was significantly lower in preadolescents with low BMD as compared to those with normal BMD. Ryan et al. [4] recently explained that vitamin D deficiency contributes to the etiology of osteomalacia and osteoporosis. The latter authors added that the key element of osteomalacia, or rickets in children, is a delay in mineralization and a large body of clinical

data indicates that an adequate serum 25OHD level improves BMD protecting against osteoporosis and reducing fracture risk.

The current study reported that serum 25OHD was significantly higher in cases who consumed milk. This is in accordance with Gordon et al. [23] who reported that milk consumption was among the significant independent predictors of hypovitaminosis D, with consumption having a protective effect against this problem

The present study reported that 25OHD shows significant positive correlation with BMD Z-score. This is in accordance with Lehtonen-Veromaa et al. [31] who reported that the primary role of sufficient vitamin D during youth and adolescence is optimization of BMD. Similarly, Bischoff-Ferrari et al. [32] reported that vitamin D levels in adulthood are important for maintaining BMD.

The present study showed that BMD Z-score, serum calcium and 25OHD showed significant negative correlations with duration of beverages consumption. The results revealed a cutoff value of 0.773 year for occurrence of low BMD and 0.662 year for the decrease in vitamin D level in serum of children. This finding shows that vitamin D is affected first then the BMD which further suggests the role of vitamin D in maintaining bone integrity.

3.3 Study Strengths and Limitations

The study strengths are the clinical measures of both nutrients and actual bone mineral density. Nevertheless, the study was limited by the small number of enrolled preadolescents, the lack of some investigations such as parathyroid hormone level measurement and the fact that data concerning bone pain and limitation of movement were subjective. Other limitations are the study timing and duration which didn`t take into consideration the seasonal variation regarding beverage consumption and sun exposure. However, we tried to minimize some of the limitation factors by recommending further larger scale studies, performing a face to face questionnaire and collecting all the blood samples during one and the same season.

4. CONCLUSION

Sugar sweetened beverages intake is associated with decrease in 25OHD which contributes to low bone mineralization in preadolescents and the duration of intake is the most determinant factor for this association. Consumption of carbonated beverages had more hazardous effect on 25OHD and BMD than packed fruit juice while milk intake showed an opposite effect. We recommend further larger scale studies and more awareness programs for preadolescents and their parents whether at school or as a part of national campaigns to boost healthy trends in beverage consumption.

CONSENT

Written informed consents were obtained from the parents or care givers of the enrolled preadolescents.

ETHICAL APPROVAL

Authors obtained the necessary ethical approval from their Institutional Committee. The parents of the enrolled preadolescents were briefed with the results of the tests and their implications.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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