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Efficacy of different modes of vitamin D supplementation strategies in Saudi adolescents

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ABSTRACT

Vitamin D deficiency is rampant in the Middle East, even in children and adolescents. This study was designed to investigate the effects of different vitamin D repletion strategies commonly used on serum vitamin D levels of Saudi adolescents. Study design: A 6-month multi-center, controlled, clinical study, involving 34 schools in the central region of Riyadh, Saudi Arabia. Different strategies of vitamin D supplementation were tested (200 ml fortified milk of different brands or vitamin D tablet (1,000IU). Anthropometrics were taken and fasting blood samples withdrawn at baseline and after intervention for the quantification of serum glucose, lipid profile and 25(OH) vitamin D. A significant increase in 25(OH)D level was observed in subjects supplemented with vitamin D tablet, milk brand 2 and milk brand 4, whereas subjects supplied with fortified milk brands 1 and 3 respectively, exhibited a significant decrease in 25(OH)D levels. Analysis of covariance showed that after adjusting for baseline 25(OH)D, age, gender and BMI, the mean 25(OH)D levels of children who were taking vitamin D tablet (9.1 ± 0.8 nmol/l) and milk brand 4 were significantly higher (7.3 ± 1.1 nmol/l) than children taking milk brand 2 (1.6 ± 1.0 nmol/l). Subjects supplied with milk brands 1 and 2 exhibited a significant increase in total cholesterol level, while it dropped significantly in subjects taking milk brand 3, while no changes were observed in other groups. Different strategies in vitamin D supplementation used in this clinical study elicited varying degrees of improvement in serum 25(OH)D level. The observed outcomes were dependent on the strategy and gender in the Saudi adolescent population, with oral tablet supplementation being favored in boys.

1. Introduction

Vitamin D deficiency is a global public health concern affecting people of all ages and sexes [1–5]. Extensive research has been carried out on the pleiotropic effects of vitamin D on human health in the recent decade due to the pandemic of vitamin D deficiency. Of particular interest is the extra-skeletal effects of vitamin D in the development of chronic, non-communicable (mostly metabolic) diseases in both children and adults [2,6].

Humans acquire vitamin D through two sources: one through endogenous production in the skin via sunlight exposure [7,8] and through diets such as natural vitamin D-fortified food sources (fish oil, liver, sun-exposed mushrooms, etc.) [9,10] and oral supplementations [11,12].

The Kingdom of Saudi Arabia (KSA) is drenched with sufficient sunlight throughout the year so, hypothetically, should not have issues regarding vitamin D deficiency. But this is not the case, as recent and previous studies consistently point out to a very high prevalence of vitamin D deficiency [13–15]. This is mainly attributed to indoor lifestyle, avoidance of sunlight to prevent from scorching heat effects on

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Table 1
Mean differences in Baseline Characteristics among Vitamin D Tablet versus Milk Brands.

Parameters	Vitamin D tablet	Milk Brand 1	Milk Brand 2	Milk Brand 3	Milk Brand 4
N	272	177	154	113	168
F/M	168/104	118/59	86/68	40/73	103/65
Age (years) #	15.0 (4.0)	14.0 (2.0)^{AC}	15.0 (3.0)	11.0 (1.0)^{ABC}	13.0 (2.0)^{ACD}
BMI (m/kg^2)	24.1 ± 6.7	22.2 ± 5.3^{A}	23.7 ± 5.6	18.4 ± 4.4^{ABC}	23.6 ± 5.3^{D}
BMI (Z-Score)	0.22 ± 1.11	−0.11 ± 0.89^{A}	0.14 ± 0.93	−0.74 ± 0.74^{ABC}	0.13 ± 0.88^{D}
WHR	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1^{B}	0.9 ± 0.1^{AB}	0.8 ± 0.1^{D}
Systolic BP (mmHg)	117.8 ± 14.3	114.7 ± 13.6	118.0 ± 11.6	108.1 ± 12.0^{ABC}	116.0 ± 15.7^{D}
Diastolic BP (mmHg)	73.2 ± 11.8	67.3 ± 10.1^{AC}	73.4 ± 16.3	68.9 ± 12.1^{AC}	71.8 ± 10.5^{B}
Total Cholesterol (mmol/l)	4.9 ± 0.9	4.6 ± 0.7^{AD}	4.8 ± 0.8	5.0 ± 0.6	4.7 ± 0.7^{AD}
HDL-Cholesterol (mmol/l)	1.3 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.4 ± 0.5 ^{BC}	1.3 ± 0.4^{D}
Glucose (mmol/l)	5.1 ± 0.6	5.0 ± 0.6	5.2 ± 0.8^{BD}	4.9 ± 0.5	5.2 ± 0.6^{BD}
Triglycerides (mmol/l)	1.2 ± 0.5	1.1 ± 0.4	1.1 ± 0.4	0.9 ± 0.3^{A}	1.2 ± 0.5^{CD}
25(OH)Vitamin D (nmol/l)	37.2 ± 16.8	37.9 ± 17.2	34.7 ± 14.5	44.9 ± 19.7^{ABC}	33.1 ± 14.8^{D}

Note: Data presented as Mean ± SD for continuous variables and frequencies for categorical variables; # denotes non-Gaussian variables presented as Median (IQR); A denotes significance compared to vitamin D tablet; B denotes significance compared to milk brand 1; C denotes significance compared to milk brand 2; D denotes significance compared to milk brand 3.

skin and women covering the entire body with dark veils for cultural and religious reasons [16].

For most countries including KSA, fortified foods is an efficient means to provide the needed vitamin D [17–19]. However, fortified foods in the Middle East, particularly dairy products, have never been tested as to whether their claimed vitamin D content is effective in at least raising vitamin D status of those consuming such products. Hence, the main objective of this interventional study was to evaluate whether intake of locally available vitamin D fortified milk results in the restoration of physiological vitamin D levels in Saudi adolescents.

2. Materials and methods

A total of 889 apparently healthy Saudi adolescents aged 11–17 years were randomly enrolled from 34-different schools in Riyadh city during the months of November-May 2014–2015. Written informed consents from parents as well as assent from children and adolescents were obtained prior to inclusion in the study. Ethical approval was obtained from the Ethics Committee of the College of Science Research Center, King Saud University, Riyadh, KSA.

2.1. Exclusion criteria

Subjects with chronic conditions such as asthma, type 1 diabetes mellitus, hypertension, history of cardiac, kidney or liver disease, use of medications known to affect body weight (such as glucocorticoids), afflicted by psychiatric conditions, and those taking calcium, vitamin D, or multivitamin supplements were excluded from the study.

2.2. Study implementation

A previously approved questionnaire [20] that included demographic information and medical history were provided to all participants and completed with the assistance of their parents. Subjects were randomly allocated into five [5] groups of supplementations. Each day for six months, all the subjects in group 1 were given vitamin D tablet (1000 IU) (VitaD1000®, Synergy Pharma, Dubai, UAE) and the other four groups were given four different brands of locally available fortified milk, 200 ml/tetra pack, respectively.

2.3. Anthropometry and blood collections

Subjects were requested to visit their respective schools after an overnight fast (≥10 h). Physical examination was carried out by the attending physician to determine whether the participants met the inclusion criteria. Weight (kg) and height (cm) were recorded using an international standard scale (Digital Pearson Scale, ADAM Equipment Inc., USA) and body mass index (BMI) was calculated as kg/m^2.

Systolic and diastolic blood pressure (mmHg) was measured using a calibrated, mercurial sphygmomanometer and was measured twice, with a 15-minute interval and the mean of the two readings was recorded. Fasting blood samples were collected and transferred to a non-heparinized tube for centrifugation. All measurements were repeated after the 6-months intervention. Collected serum samples were transferred to pre-labeled plain tubes, placed on ice, and delivered to the Biomarkers Research Program (BRP) laboratory in King Saud University, Riyadh, KSA, for storage at −20 C.

2.4. Sample analyses

Fasting glucose and lipid profile were measured using a chemical analyzer (Konelab, Espoo, Finland). Serum 25(OH)D was measured with a Roche Elecsys modular analytics Cobas e411 using an electrochemiluminescence immunoassay (Roche Diagnostics, GmbH, Mannheim, Germany) and commercially available IDS kits (IDS Ltd, Boldon Colliery, Tyne & Wear, UK). BRP laboratory is participating in the Vitamin D External Quality Assessment Scheme (DEQAS), and Quality Assurance (QA) standards are maintained by ISO 9000 and 17,025. The QA department audits the BRP laboratory at regular intervals.

2.5. Data analyses

Data were analyzed using SPSS (version 16.5 Chicago, IL, USA). Mean and standard deviations were used to represent data for normal variables, while the median and interquartile range was used to report non-normal variables. Normality was assessed using the Kolmogorov-Smirnov test. Analysis of Variance (ANOVA) and paired sample t-test were used to assess mean differences, while GLM univariate analysis was used to control for age, BMI, gender and baseline vitamin D. P-value < 0.05 was considered statistically significant.

3. Results

The median age, number of male and female, BMI, 25(OH)D levels, along with other biochemical parameters of the subjects are illustrated in Table 1. These subjects were randomized into five-groups; one group received vitamin D tablet (1000 IU) (VitaD1000®) (Synergy Pharma, Dubai, UAE) and other four groups received different brands of 200 ml of fortified milk. ANOVA was used to identify the difference in baseline characteristics among treatments. Significant differences were observed in age, BMI, systolic BP, and vitamin D levels. No significant

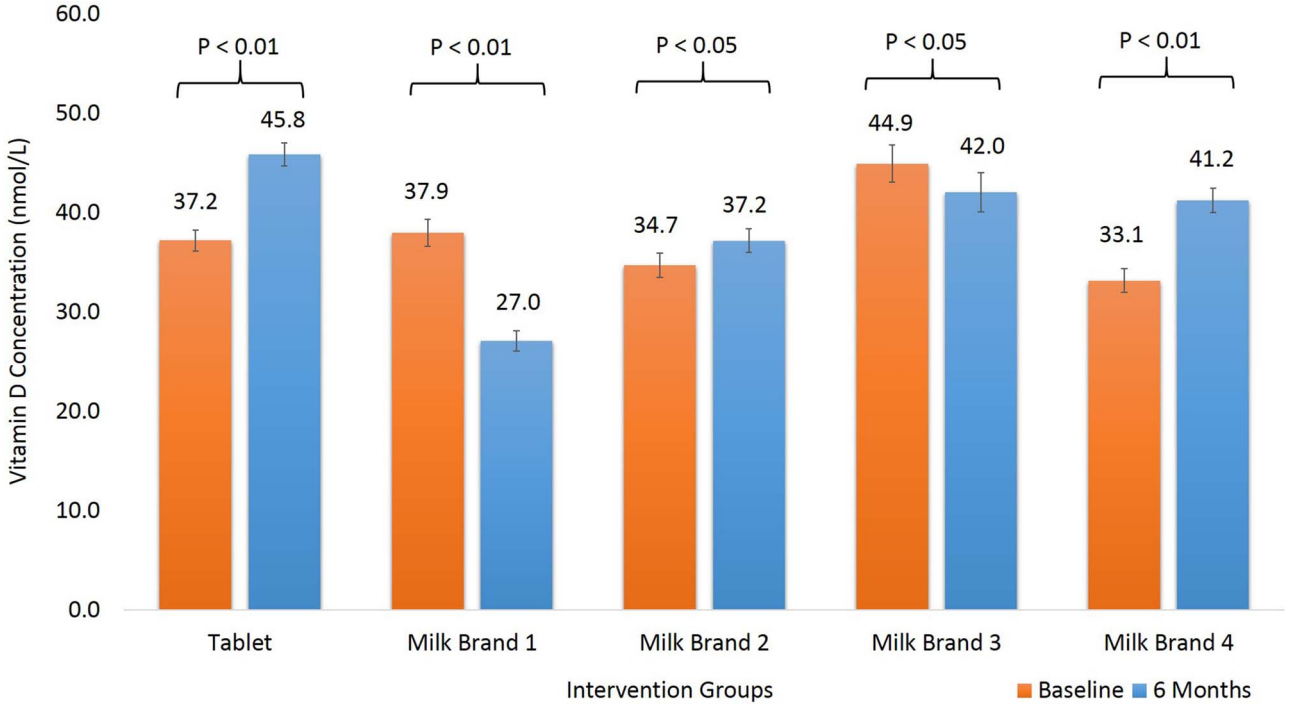

Fig. 1. Differences between Baseline and Follow-up Vitamin D Concentrations among Intervention Groups (Note. ** Significantly different at 0.05 level; * significantly different at 0.01 level).

Table 2
Mean Differences between Baseline and 6 Month follow-up in Vitamin D Tablet versus Milk Brands.

Parameters	Vitamin D tablet		Milk Brand 1		Milk Brand 2		Milk Brand 3		Milk Brand 4	
	Baseline	6 Months	Baseline	6 Months	Baseline	6 Months	Baseline	6 Months	Baseline	6 Months
BMI (m/kg^2)	24.1 ± 6.7	24.4 ± 6.8**	22.2 ± 5.3	22.2 ± 5.5	23.7 ± 5.6	23.8 ± 5.5	18.4 ± 4.4	18.7 ± 4.6**	23.6 ± 5.3	23.5 ± 5.6
BMI (Z-score)	0.2 ± 1.1	0.2 ± 1.1	−0.1 ± 0.9	−0.1 ± 0.9	0.1 ± 0.9	0.1 ± 0.9	−0.7 ± 0.7	−0.7 ± 0.8**	0.1 ± 0.9	0.1 ± 0.9*
WHR	0.8 ± 0.1	0.8 ± 0.1**	0.8 ± 0.1	0.8 ± 0.1*	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1**	0.8 ± 0.1	0.8 ± 0.1**
Systolic BP (mmHg)	118 ± 14	113 ± 13**	115 ± 14	115 ± 14	118 ± 12	116 ± 17	108 ± 12	110 ± 14	116 ± 16	111 ± 15**
Diastolic BP (mmHg)	73.2 ± 11.8	69.7 ± 11.7**	67.3 ± 10.1	67.5 ± 9.5	73.4 ± 16.3	78.6 ± 15.8**	68.9 ± 12.1	71.8 ± 13.4*	71.8 ± 10.5	71.3 ± 12.8
T. Cholesterol (mmol/l)	4.9 ± 0.9	4.9 ± 0.9	4.6 ± 0.7	4.9 ± 0.8**	4.8 ± 0.8	5.0 ± 0.8*	5.0 ± 0.6	4.4 ± 0.6**	4.7 ± 0.7	4.8 ± 0.8
HDL-Chol (mmol/l)	1.3 ± 0.4	1.4 ± 0.4**	1.3 ± 0.3	1.3 ± 0.3**	1.2 ± 0.3	1.3 ± 0.3*	1.4 ± 0.5	1.3 ± 0.2**	1.3 ± 0.4	1.1 ± 0.3**
Glucose (mmol/l)	5.1 ± 0.6	4.9 ± 0.7**	4.9 ± 0.6	4.7 ± 0.6**	5.2 ± 0.8	5.1 ± 0.8*	4.9 ± 0.5	5.1 ± 0.6**	5.2 ± 0.6	5.3 ± 0.8
Triglycerides (mmol/l)	1.2 ± 0.5	1.1 ± 0.6**	1.1 ± 0.4	1.0 ± 0.4*	1.1 ± 0.4	1.1 ± 0.6	0.9 ± 0.3	1.0 ± 0.5	1.2 ± 0.5	1.2 ± 0.5
25(OH)D (nmol/l)	37.2 ± 16.8	45.8 ± 18.6**	37.9 ± 17.2	27 ± 13.1**	34.7 ± 14.5	37.1 ± 14.2*	44.9 ± 19.7	42.0 ± 20.5*	33.1 ± 14.8	41.2 ± 15.1**

Note: Data presented as Mean ± SD; Paired sample t-test is used to identify significant differences; ** denotes significantly different at 0.01; * denotes significantly different at 0.05.

Table 3
Comparison of Mean Change in Study Parameters between Vitamin D Tablet versus Milk Brands.

Parameters	Tablet	Milk Brand 1	Milk Brand 2	Milk Brand 3	Milk Brand 4
Δ BMI (kg/m^2)	0.25 ± 0.06	0.02 ± 0.07	0.09 ± 0.09	0.28 ± 0.09	−0.12 ± 0.10^{AD}
Δ BMI (Z-score)	0.13 ± 1.02	−0.09 ± 0.81	−0.02 ± 1.02	0.16 ± 0.92	−0.22 ± 1.14^{AD}
Δ WHR	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.09 ± 0.01^{ABC}	0.02 ± 0.01^{D}
Δ Systolic BP (mmHg)	−4.65 ± 0.86	0.62 ± 1.04^{A}	−2.36 ± 1.48	1.91 ± 1.57^{A}	−5.02 ± 1.37^{BD}
Δ Diastolic BP (mmHg)	−3.52 ± 0.82	0.15 ± 0.84	5.26 ± 1.74^{AB}	2.82 ± 1.43^{A}	−0.57 ± 1.17^{C}
Δ Total Cholesterol (mmol/l)	0.02 ± 0.05	0.29 ± 0.06^{AD}	0.15 ± 0.07^{D}	−0.56 ± 0.06^{A}	0.06 ± 0.07^{D}
Δ HDL-Cholesterol (mmol/l)	0.04 ± 0.02	0.05 ± 0.01^{D}	0.03 ± 0.01^{D}	−0.14 ± 0.05^{A}	−0.14 ± 0.02^{ABC}
Δ Glucose (mmol/l)	−0.19 ± 0.05	−0.28 ± 0.05	−0.17 ± 0.07	0.21 ± 0.07^{ABC}	0.03 ± 0.06^{B}
Δ Triglycerides (mmol/l)	−0.08 ± 0.03	−0.07 ± 0.03	0.07 ± 0.05^{A}	0.08 ± 0.04	−0.05 ± 0.04
Δ Vitamin D (nmol/l)	8.65 ± 1.08	−10.90 ± 1.16^{A}	2.47 ± 0.90^{ABD}	−2.88 ± 1.34^{AB}	8.08 ± 0.79^{BCD}

Note: Data presented as Mean ± SE; ANOVA is used to identify significant differences; A, B, C, and D denotes significance different from tablet, milk brands 1, 2, and 3 respectively.

Table 4
Mean Change in Vitamin D Concentrations between Vitamin D Tablet and Milk Brands.

Interventions	Mean ± S.E*	P-values				
		Tablet	Milk Brand 1	Milk Brand 2	Milk Brand 3	Milk Brand 4
Tablet	9.1 ± 0.8	–	< 0.001	< 0.001	< 0.001	0.17
Milk Brand 1	−8.4 ± 1.1	< 0.001	–	< 0.001	< 0.001	< 0.001
Milk Brand 2	1.6 ± 1.0	< 0.001	< 0.001	–	0.04	< 0.001
Milk Brand 3	−1.8 ± 1.3	< 0.001	< 0.001	0.04	–	< 0.001
Milk Brand 4	7.3 ± 1.1	0.17	< 0.001	< 0.001	< 0.001	–

Note: Data presented as adjusted Mean ± SE; * denotes that Means were adjusted for baseline Vitamin D, Age, Gender and BMI.

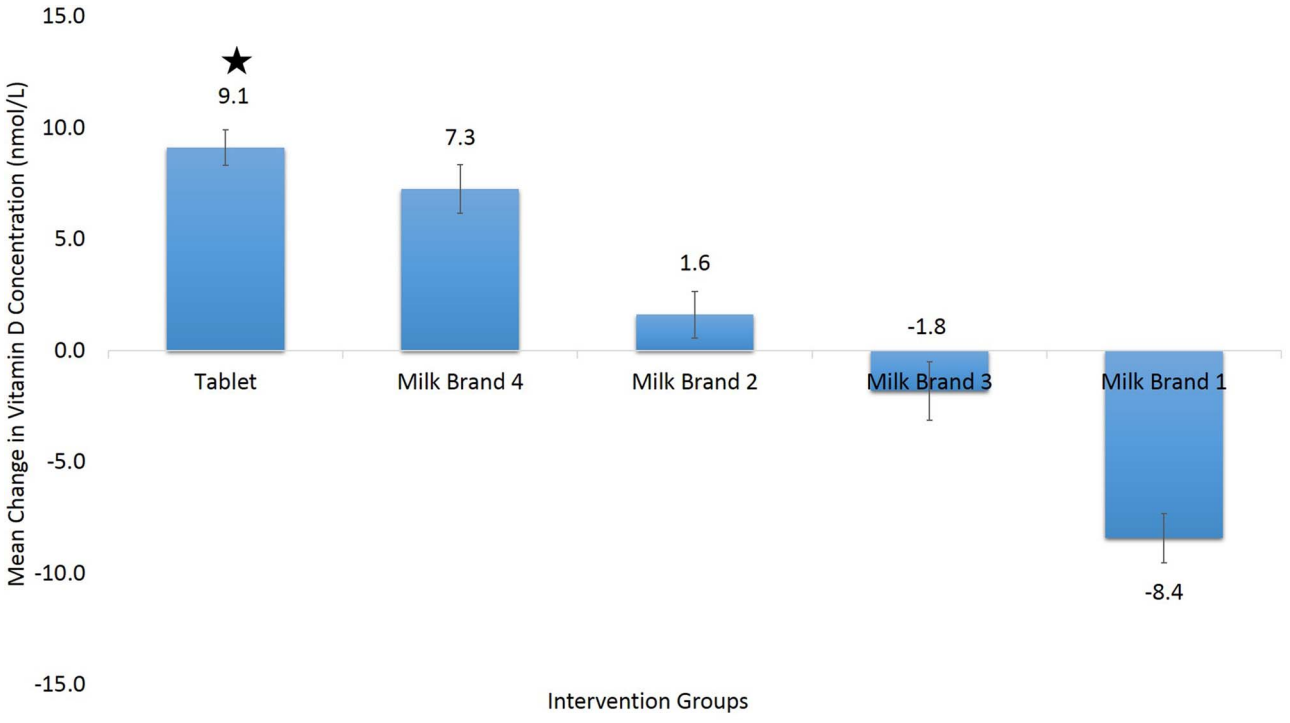

Fig. 2. Change in Vitamin D Concentration from the Baseline among Intervention Groups (Note: Indicates significant Increase in Vitamin D concentration from Baseline as compared to interventions except Milk 4).

associations were noted between vitamin D levels and other biochemical parameters.

3.1. Vitamin D levels at baseline and post intervention

No significant differences were observed in the 25(OH)D levels in the groups supplied with vitamin D tablet and other milk brands at the baseline. The group supplied with milk brand 3 exhibited significantly higher vitamin D levels (44.9 ± 19.7 nmol/l) as compared to vitamin D tablet (37.2 ± 16.8; p-value < .05), milk brand 1 (37.9 ± 17.2; p-value < 0.05) and milk brand 2 (34.7 ± 14.5; p-value < 0.05). After intervention, significantly elevated 25(OH)D concentrations were observed in subjects supplemented with vitamin D tablet (p-value < 0.01), milk brands 2 (p-value < 0.05) and 4 (p-value < 0.01). Whereas subjects provided with milk brands 1 (p-value < 0.01) and 3 (p-value < 0.05) had a significant decrease in vitamin D levels (Fig. 1).

Total cholesterol levels in groups under milk brands 1 and 2 significantly increased after the intervention. In milk brand 3, total cholesterol levels dropped significantly and no difference was observed in the other two groups. No significant differences were observed in other parameters (Table 2).

3.2. Comparison between vitamin D tablet and milk brands

The groups who received milk brands 2, 4 and vitamin D tablets had significantly higher serum 25(OH)D concentrations than those who received milk brands 1 or 3. The greatest improvement was observed in subjects given with vitamin D tablets followed by milk brands 2 and 4 (Table 3).

To adjust for baseline differences in age, BMI, gender and serum 25(OH)D concentrations, GLM univariate analysis was used. The adjusted means are presented in Table 4 and (Fig. 2). The increase in 25 (OH) D concentrations in subjects receiving the vitamin D tablet was significantly greater than those receiving milk brands 1–3. However, no differences were observed in the 25(OH)D concentrations between milk brand 4 and vitamin D tablet groups.

4. Discussion

The present study highlighted variations in improving vitamin D status of Arab children and adolescents based on locally available dairy products that claim to be vitamin D-fortified versus oral vitamin D supplementation. The study is the first of its kind in the Middle-Eastern region where vitamin D deficiency is extremely common in the general population and where policies have been enforced to fortify dairy products with vitamin D. Mean changes in vitamin D status indicate

that one milk brand was at par with oral vitamin D tablets in significantly increasing circulating 25(OH)D levels and was superior to the other milk brands in this regard. One milk brand a showed modest increase in serum 25(OH)D while 2 milk brands surprisingly had a negative effect on vitamin D status. These observations were somehow in alignment with a previous cross-sectional study done in the same cohort, indicating a modest but significant association between dietary milk intake and vitamin D status in children [21]. As vitamin D deficiency in the Gulf Arabian youth, particularly in Saudi Arabia, is extremely common [16,22], there is a need for a good evidence that food fortification strategies done in Saudi Arabia are working, as it did in other countries which opted for mandatory food fortification of commonly used dairy products with vitamin D [23–25].Another highlight in the present study was that 25(OH)D levels dropped in groups fed with milk brand, 1 (37.9 ± 17.2–27.0 ± 13.1 p-value < 0.01) and the milk brand 3 (44.9 ± 19.7–42.0 ± 20.5 p-value < 0.05). These observations echo the results observed on 10–14 year old healthy Indian children who were allocated into 200 mL of unfortified milk (control) or fortified milk with, 600 IU and 1000 IU, respectively, for 3 months [12]. The non-favorable change in other milk brands observed in the present study were similar to those in a study of girls aged 12–18 years from Finland [26] who failed to show any improvement in their 25(OH)D levels (19.3 ng/ml vs 19.2 ng/ml) even after a year of initiation of the food fortification program. The lack of improvement in 25(OH)D levels were probably due to (a) very small amount of vitamin D that was added to milk (0.5 μg/100ml), and (b) girls did not consume milk in adequate quantities [26]. Other possible reasons for failure to achieve improved 25(OH)D levels may include (a) small amount of vitamin D used for fortification and (b) non-consumption of fortified milk on weekends and holidays [27].

A recent evidence in Saudi Arabia pointed to completely absent or deficient vitamin D fortification of dairy and other local products as one of the major reasons for widespread deficient levels of vitamin D than that in other Western countries [28]. The study also found wide variations in the vitamin D content of fortified products. For example: fresh milk (from 5 different manufacturers) had 0 to 400IU/L; powdered milk (from 4 manufacturers) had 65–350IU/100 gm; cheese (5 manufacturers) had 0–350IU/100 gm and yoghurt (6 manufacturers) from 0 to 400IU/L. Sadat Ali et al., also compared these levels with those of corresponding items in US and confirmed completely absent or highly inadequate fortification in Saudi Arabia [28].

The conflicting evidence on the favorable effects of vitamin D supplementation may partially be explained by the genetic make-up and variations involved in vitamin D pathways [29]. Vitamin D receptor (VDR) gene is one factor responsible for regulating vitamin D response. Our previous study showed that VDR polymorphisms influence metabolic response to vitamin D supplementation [30]. Patients with VDR *Fok*-I CC genotype showed the least improvement in serum 25(OH)D levels and as such might need higher doses of vitamin D to achieve sufficient levels [30]. In conclusion, our study highlighted the need for food fortification, particularly milk provided to children in providing effective strategies to combat vitamin D deficiency, including high-quality vitamin D supplements.

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Conflict of interest

The authors declare no conflict of interest.

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