

## Original Article

# Community Interventional Trial (CITFOMIST) of Vitamin D Fortified Versus Non-fortified Milk on Serum Levels of 25(OH) D in the Students of Tehran

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## Abstract

**Background:** As prevention of osteoporosis becomes more imperative with the global ageing of the population, establishing different measures to fight vitamin D deficiency will also become increasingly important. The aim of this study is to help assess the efficacy of vitamin D–fortified milk on circulating concentrations of 25(OH)D (as the primary outcome), a widely accepted indicator of vitamin D status, in Tehran students. Another objective of the protocol is to help assess the compliance with fortified dairy in students of different socioeconomic classes.

**Method:** The cluster-randomized trial (CITFOMIST) is conducted on 15- to 19-year olds guidance and high school students of both genders from different districts of Tehran, in wintertime. The schools enrolled in this study are randomly assigned to receive one of the three groups of milk (whole milk, milk that contained 600 IU Vit D/1000 cc, or milk that contained 1000 IU Vit D /1000 cc) for a 30-day period. In order to study the effect of vitamin D–fortified milk on the circulating concentrations of 25(OH) D, a serum vitamin D levels are checked in a subgroup before and after the intervention.

**Conclusion:** There are few data on the efficacy of incremental doses of vitamin D from fortified foods among adolescents. This is while developing an optimal model to fight vitamin D deficiency needs further research on bone health outcomes and the safety of vitamin D-fortified products. The modified version of this protocol could be applied in different parts of the country to assess the efficacy of a vitamin-D product.

**Keywords:** Fortification, osteoporosis, vitamin D

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## Introduction

As prevention of osteoporosis becomes more imperative with the global ageing trend, establishing different measures to fight vitamin D deficiency as the main risk factor attributing to osteoporosis has become increasingly important.<sup>1,2</sup>

Despite its abundant sunshine, the Middle East has the highest rates of hypovitaminosis D worldwide. Hypovitaminosis D, as a major public health problem is believed to affect all individuals of different age groups.<sup>3</sup> It is reported that 20% – 80% of apparently healthy individuals from several countries in the Middle east

have suboptimal vitamin D levels, depending on the cutoff used for defining hypovitaminosis D, the country, season, age group, and gender.

There have been several reports detailing the high prevalence of vitamin D deficiency across Iran. According to an Iranian national Multi-center Osteoporosis Study (IMOS) in five major cities, 75.1% of women and 72.1% of men aged between 20 and 69 years suffered from different degrees of vitamin D deficiency.<sup>4</sup> The highest prevalence of moderate to severe vitamin D deficiency was observed in the Iranian Capital, Tehran. In a cross-sectional study on 963 students aged 7 – 18 years (424 boys and 539 girls) in Tehran, similarly, 53.6% of the girls and 11.3% of the boys suffered from vitamin D insufficiency. The condition was about five times more common among female than male students.<sup>5</sup> As a result of these studies, fighting against vitamin D deficiency should start from early years of life.

Current recommendations for vitamin D intake established by the Standing Committee on Dietary Reference Intake suggested that, adults under the age of 50 should consume 200 IU of vitamin D per day.<sup>6</sup>

This comes while many studies have estimated that 1,000 to 2,000 IU of vitamin D would be necessary to satisfy the body's needs for most people.<sup>7</sup> Many experts suggest that the recommended daily intake of vitamin D is difficult to achieve without

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supplementation, particularly in higher latitudes and in areas of extreme winter climate.<sup>8,9</sup>

In Iran, similarly, air pollution and sedentary westernized lifestyle have contributed to low serum levels of vitamin D in different age groups, making supplementation a necessity.<sup>10,11</sup> Considering the fact that there are few natural food sources of vitamin D, the need for alternative vitamin D sources becomes more apparent.<sup>12</sup>

These facts highlight the importance of food fortification as an alternative in obtaining adequate vitamin D, which is required for maintaining overall health.<sup>13,14</sup> Food fortification can effectively compensate for low dietary intake of vitamin D and lack of sufficient sun exposure.<sup>15,16</sup> This is while food-fortification practices vary around the world.<sup>17,18</sup> Adding to the variability of vitamin D content in different fortified products, surveys have reported low compliance with the consumption of vitamin D fortified foods in different parts of the world.<sup>19</sup>

While milk and orange juice are the only products fortified with vitamin D in our country, fortification of dairy products is not commonly practiced among Iranian companies.<sup>16,20</sup>

Considering the abovementioned facts, the present study was conducted to assess the effect of vitamin D-fortified foods on circulating concentrations of 25(OH) D (as the primary outcome) in Tehran students. The other objective of the study was to assess the compliance with fortified dairy in students of different socioeconomic classes.

## Methods

### Study Design

The cluster-randomized trial (CITFOMIST) is conducted in collaboration with the Food and Drug Laboratory Research Center of Food and Drug Organization of the Iranian Ministry of Health and Medical Education, Department of Health and Department of Nutrition of the MOHME, the Ministry of Education, and the UNICEF. The CITFOMIST is approved by the Ethical Board Committee of the Endocrinology and Metabolism Research Institute and ClinicalTrials Gov (NCT02035423).

The trial is conducted on 15- to 19-year olds guidance and high school students of both genders from different districts of Tehran, the Iranian capital, in wintertime. A mixed sampling method con-

sisted of stratified random sampling and two-stage cluster sampling is applied in the study.

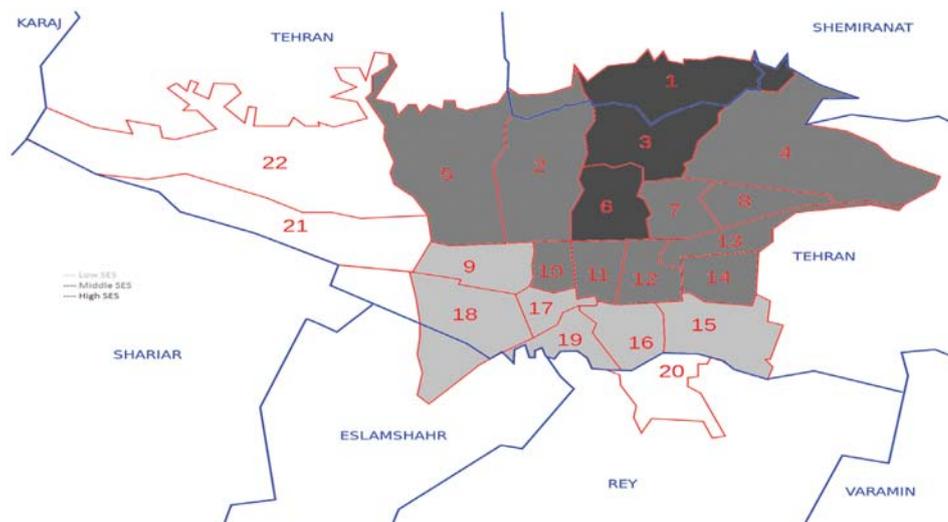
Separate sample sizes were calculated for each of the primary objectives of the study (the prevalence of vitamin D deficiency and the compliance with milk consumption).

First, Tehran districts are broken into three strata (high, middle and low) based on the socioeconomic status (SES) using the Urban Health Equity Assessment and Response Tool Study (Urban HEART) conducted by Tehran municipality (Figure 1).<sup>21</sup> The tool combines total measures of unemployment rate, being illiterate and individualized healthcare cost. Then, the total number of students in each district is retrieved from the Iranian Ministry of Education. Based on this data, a proportional to size allocation method is used to select the districts based on their socioeconomic status. District in this regard, the districts 16 and 17 from the 'low SES,' 10, 11 and 13 from 'middle SES,' and 3 from 'high SES' are selected (Figure 1).

Regarding the sample size, a subgroup of the students is selected to study the efficacy of vitamin D fortification. Same sample size calculation is performed based on a previous study conducted in the Osteoporosis Research Center.<sup>16</sup> Seventy students are needed in each group (Total = 210). The sample size is increased to 390 considering a design effect of 1.85. Additionally, the sample size is increased by 25% to lower the missing rate, and bringing the required number of subjects to 468. In this regard, 45 students are recruited from 12 random schools from each SES group.

As the next step, another sample size is calculated to assess the compliance with milk use. Based on the study conducted by Neyestani, et al. 84 students are needed in each group and the total sample is increased to 155 considering the sampling design effect (1.85). Then, the number is multiplied by 12 ( $155 \times 12 = 1860$ ) considering the effect of the three studied strata (the students' gender (boy/girl), school level (guidance/high school), and socioeconomic status (low, middle, high)). Since, there are intervention arms (Simple Milk/Milk fortified with 120 IU vitamin D/Milk fortified with 200 IU vitamin D) in the study, a total of 5580 students are needed ( $1860 \times 3 = 5580$ ).

In this study each school is considered as a cluster, therefore 12 schools are selected from each strata (socioeconomic status) based on the students' gender, school level and intervention. Based on



**Figure 1.** Tehran districts based on the socioeconomic status (SES), according to the Urban Health Equity Assessment and Response Tool Study (Urban HEART).<sup>21</sup>

the mean number of students from selected schools, 6256 students enrolled in this study. Among them, 468 students are tested for serum vitamin D levels at two intervals to assess the efficacy of the fortified milk.

In summary, using Block Balanced Randomization Method 12 schools from each socioeconomic classes of Tehran are selected and allocated to the three intervention arms (four schools for each intervention). Therefore, 36 schools are enrolled in this study.

Three middle schools and three high schools for boys are randomly selected from the list, provided by the education ministry for each district. A similar strategy is used to select the girl schools. All the students in these schools are recruited.

Each school is randomly assigned to receive one of the three groups of milk—whole milk, milk that contains 600 IU Vit D/1000 cc and 1000 IU Vit D /1000 cc for a 30-day period (starting in the second week of February).

To study the effect of vitamin D–fortified milk on the circulating concentrations of 25(OH) D, a subgroup is selected out of the studied schools using proportional to size sampling method. For this purpose, a cumulative list is prepared for each grade level in the selected schools. The first student is selected based on the random number attributed to each school in the project. Then, 15 consecutive students from each grade are selected. In case there are not sufficient students in each class, the students of another class of the same grade level would be enrolled in the study until adequate number of the students is obtained. All the parents sign an informed consent. If parents do not agree with their child giving blood samples, the next student on the list would be selected through the same procedure.

A team of two skilled technicians, a physician and a fully equipped ambulance would be sent to each school. They examine student's history for possible factors interfering with serum vitamin D levels. Students who have taken vitamin D supplements (different forms of Calcium and Vitamin D supplements or multivitamins containing Calcium and Vitamin D) during the past three months, suffering from underlying conditions (liver, kidney, gastrointestinal, cancer, endocrine, bone and biliary disease) or consuming medication affecting bone metabolism (anti-convulsants, anti-tuberculosis medication, HMG-CoA inhibitors, cimetidine, theophylline, and cholestyramine), as well as those who are following special diets such as vegetarian diet or consuming fortified products regularly, would be excluded.

Blood samples are taken in two phases: before starting the project (February) and within 30 days after consuming milk (March). Approximately 10cc of blood is drawn between 7:00 A. M. and 9:30 A. M. after the student has fasted for at least eight hours. Blood samples are used to determine calcium, phosphorus, parathyroid hormone (PTH), alkaline phosphatase and bone-specific alkaline phosphatase, 25(OH)D, osteocalcin, cross-linked C-telopeptide (CTX), total protein, albumin and creatinine.

Blood samples are taken in a sitting position according to the standard protocol. Participants rest in the seated position for 15 minutes prior to blood collection. This waiting period will allow equilibration of the concentrations of blood components. The vacuum tubes are immediately placed on wet ice and transferred to the EMRI laboratory for centrifugation (at 3000 rpm for 10 min). Blood samples are aliquoted into four vials for freezing at -70 °C until assayed.

Calcium (Man, Arsenazo), phosphorus (Man, Phosphomolybdate, UV), albumin (Pars Azmoon, Bromocresol Green), total

protein (Pars Azmoon, Biuret), creatinine (Man, Jaffe Kinetic), and alkaline phosphatase (Pars Azmoon, DGKC) are determined with inter assay coefficient variation (CV) of 3.9%, 6%, 2.2%, 3%, 4.5% and 2.1% respectively. Intact PTH is measured by Immunoradio Metric Assay (IMMUNOTECH) with CV of 6.6%. Bone specific Alkaline phosphatase (QUIDEL) and 25–Hydroxy Vitamin D, Osteocalcin (N- MID), CTX (Immunodiagnostic systems) are measured by Enzyme Immunoassay with CV% of 9%, 8%, 6% and 10% respectively. All the samples with high concentration of analytes (more than reportable ranges) are diluted according to kit protocol and assessed again.

The students are then asked to fill a questionnaire on their demographic data, possible underlying diseases and bone health-related lifestyle habits. They are also asked to fill a weekly questionnaire on their compliance with milk consumption and possible reasons for not consuming milk regularly. The questionnaire in the subgroup also contains a section for assessing Ca and vitamin D food intake in the past three months.

Supervision and monitoring are conducted at two levels by school representatives and the administration team. The representatives of each school supervise the distribution and consumption of the milk among the students; therefore every student would receive and drink a 200 cc package of milk everyday. The representatives of each school also supervise the process of completing the questionnaires. The administration team supervises the whole process from distributing to consuming milk in each school, completing the questionnaires, and finally performing the lab tests. They also check the milk storeroom in each school, to make sure the packages are kept in a suitable place.

#### Intervention

Each school is randomly assigned to receive one of the three groups of milk (whole milk, milk that contained 600 IU/1000 cc or 1000 IU/1000 cc) for a 30-day period, starting in the second week of February. The schools are allocated to the intervention groups by Block Balanced Method.

The students are asked to drink 200 cc milk on school days. They are also asked not to change their eating habits and diet during the study period. There is no significant difference between the packaging, taste and color of the milk provided in each group. The subjects and supervisors are blind to the group assignment.

Milk is provided by Pegah Co. and distributed in two phases. Fortification is performed under the supervision of the Quality Control Laboratory Research Center of FDA of the Iranian MOHME. Liquid milk is sterilized in all the three groups.

To assess the stability of vitamin D in the fortified milk, the vitamin D content of milk is determined at four stages, the time vitamin D<sub>3</sub> is added to the milk and within 20, 40 and 60 days. Analytical HPLC assay and AOAC method is used in this regard. During this study, the products are stored at 4 °C and the bioavailability of vitamin D during the shelf life is assessed through measuring vitamin D levels in the products in a two-month period.

#### Outcomes

The primary outcome of this protocol is to assess the compliance with fortified milk consumption among guidance and high school students. Secondary outcome is to assess the effect of fortified milk on serum levels of vitamin D and other bone biomarkers.

## Discussion

Most circulating 25(OH) D originate from sunlight exposure, because foods naturally rich in vitamin D are limited. This is while many studies have suggested that due to many factors such as pollution, there is an inefficacy of sunlight exposure.<sup>22</sup> As a result, the use of fortified foods and dietary supplements are necessary in many regions to maintain adequate serum concentrations of this vital vitamin.<sup>23</sup> Fluid milk, margarine and breakfast cereals are the predominant vehicles for vitamin D in many countries such as Finland, the United States and Canada.<sup>13,24</sup> Other studies have suggested fortification of orange juice with vitamin D as an alternative, particularly in those complaining of lactose intolerance.<sup>14</sup> However, many studies have reported low vitamin D intake even in countries where almost all retail milk and margarine are fortified with vitamin D, suggesting that a model for optimal, safe and efficient food fortification practice is needed.<sup>25</sup>

There have been several reports detailing the high prevalence of vitamin D deficiency, particularly among children, across Iran. However, there are difficulties in comparing the outcomes of these studies due to differences in study design, and variability in assays measuring circulating levels of 25-hydroxyvitamin D [25(OH)D]<sub>3</sub>.<sup>26</sup>

This is the reason behind conducting this community intervention trial on a group of school children. Majority of previous studies had been conducted on the high-school, therefore this is the first Iranian study to evaluate the effects of fortified products on vitamin D levels of children who have not yet reached the peak bone mass. Blood samples were obtained in midwinter; therefore these students had been exposed to insufficient sunlight for at least four months and experienced little or no cutaneous synthesis of vitamin D. Moreover, this is the first to assess the efficacy of receiving high doses of vitamin D (1000 IU/L) on serum levels of vitamin D, and to compare it with that of lower doses of the product. If the present study confirms the efficacy of milk fortification as well as an acceptable compliance with using such products among Iranian adolescents, more efforts should be focused on implementing National policies on food fortification or supplementation with vitamin D. Such policies could help improve peak bone mass and subsequently reduce vitamin D deficiency and its complications, the most important of which is osteoporosis.

### Limitations

Considering the severity of vitamin D deficiency in the Iranian population; it is possible that fortified milk should be consumed for a longer period to overcome vitamin D requirements for this group of individuals.

In conclusion, there are few data on the efficacy of incremental doses of vitamin D from fortified foods among adolescents; this is while developing an optimal model to fight vitamin D deficiency needs further research on bone health outcomes and the safety of vitamin D-fortified products. The modified version of this protocol could be applied in different parts of the country to assess the efficacy of a vitamin-D product.

The present protocol is developed to study a group of students from Tehran, the Iranian capital. In order to adopt the protocol for other cities, the demographic characteristics of the region should be taken into account. Performing such studies would help policy-makers implement guidelines for safe and efficient distribution of fortified milk among the students based on their needs.

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**Competing Interest:** None

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