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Short Communication
Bioavailability of Vitamin D_3 from a Fortified Cereal Based Snack: A Pilot Study

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Abstract

Background and Objective: There is a widespread prevalence of vitamin D deficiency among Indian children and adolescents. Fortification of commonly available cereals with vitamin D_3 could be a viable strategy to improve their vitamin D status. This study was undertaken to address the issue of vitamin D deficiency among adolescents by investigating the bioavailability of vitamin D_3 from a cereal legume snack (laddoo) fortified with vitamin D_3 versus the commercially available cholecalciferol supplement. **Materials and Methods:** Two groups of six adolescents aged 16-17 years. with low serum 25-hydroxy vitamin D were randomly divided. Subjects in the test group were given the fortified laddoo (1000 IU vitamin D_3) while those in the reference group were given commercially available vitamin D_3 (1000 IU) supplement. Serum calcium was measured at 0 h and serum 25-(OH)D was measured at 0, 6 and 24 h intervals. **Results:** The serum 25(OH)D levels raised from 12.28±3.86 to 13.78±4.54 and to 14.71±3.66 ng mL^{-1} in the test group and 12.91±4.78 to 13.78±4.54 and to 14.53±4.61 ng mL^{-1} in the reference group at 0, 6 and 24 h intervals respectively. No significant differences ($t = -0.252$, $p>0.05$ at 0 h, $t = 0.084$, $p>0.05$ at 6 h, $t = 0.075$, $p>0.05$ at 24 h) were observed in the increase of serum 25(OH)D concentrations in between both the groups. **Conclusion:** Hence fortified cereal legume snack (laddoo) could be novel tool in improving the vitamin D status of adolescents.

Key words: Adolescents, Indian snacks, serum 25(OH)D, vitamin D status, vitamin D_3 supplement

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Owing to the rising incidence of vitamin D deficiency and insufficiency in India[1-5], the Food Safety Standards Authority of India recommends vitamin D fortification of oil and milk. However only certain brands of oil and milk are fortified with vitamin D and accessibility and affordability are questionable. Also, consumption of milk among lower socio economic class is observed to be lower in children and adolescents. Plus, due to varied Indian cooking methods, the bioavailability of vitamin D from such fortified products remains to be uncertain. Hence researchers have expressed the need for fortification of cereals with vitamin D[1].

Vitamin D is a fat soluble vitamin and plays an important role in skeletal health[2]. Chronic vitamin D deficiency is now observed to be linked with number of extraskeletal conditions such as cancer, diabetes, cardiovascular diseases and acute lower respiratory infections[3,6,7]. Although, vitamin D is sufficiently available from sunlight, around 50-90% individuals in the Indian subcontinent are reported to be vitamin D deficient[8] despite of it being a tropical country. Among children and adolescents, a prevalence of 90% has been observed in North India. When sunlight exposure is insufficient, oral intake of vitamin D either dietary or supplementary becomes essential[3]. Vegetarianism coupled with low availability and low accessibility of vitamin D_3 fortified foods in India adds to the already existing woe. Researchers have thus indicated a nationalized need to fortify foods with vitamin D[1,3,9]. However it is also important to consider the bioavailability of the fortified foods in order to ensure appropriate effect of fortification.

The Indian market commonly has two forms of vitamin D supplements, vitamin D_3 (alfacalcidol, cholecalciferol and calcitriol) and vitamin D_2 (ergocalciferol). Cholecalciferol is proved to be better in potency, elevating and sustaining concentrations of 25(OH)D and maintaining the storage form of vitamin D, as compared to ergocalciferol. Also, in relation to the other forms of vitamin D_3, cholecalciferol is the preferred form for treatment vitamin D deficiency[10].

However, adherence of children and adolescents towards supplements has reported to be low. Hence fortification of commonly available foods is a viable strategy to improve vitamin D status among children. Cereals and legume consumption is found to be higher among adolescents and can be considered as a vehicle for vitamin D fortification. Few authors have fortified cereals with vitamin D and found beneficial results[11,12].

Laddoo is a traditional Indian snack loved by children and adolescents. Ekbote *et al.*[11] used laddoos as a vehicle for administering calcium to children and observed good acceptance among the group. In their experiment, they also fortified laddoos with vitamin D using a commercial cholecalciferol supplement and administered to the children once a month and noted improved serum Vitamin D levels[11].

A premix is generally the preferred choice for long term fortification. However there is limited evidence on the bioavailability of vitamin D premix in cereals. Hence, the current study aimed to evaluate the bioavailability of a cereal legume snack fortified with vitamin D_3 and ensure efficient absorption of vitamin D_3.

MATERIALS AND METHODS

Ethics approval: Ethical approval was obtained from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (IEC/14/DEC/113/26). The study is registered under the Clinical Trial Registry of India (CTRI/2017/04/008408).

Preparation of fortified flour: *Eleusine coracana* L. (finger millet), *Pennisetum typhoideum* (pearl millet) grains and *Vigna mungo* (black gram dhal) were purchased from a local store. Each of these grains and the dhal were ground together in the ratio of 34:17:8% respectively and the flour obtained was fortified with vitamin D_3.

Vitamin D premix containing cold water soluble form of vitamin D_3 was procured from Hexagon Nutrition Pvt Ltd and was used for fortification.

The ground flour was fortified with 0.11 g of the premix such that 100 g of the flour had 8250 IU of vitamin D_3, thus ensuring that the final product had the recommended dosage of 1000 IU per serving. The premix was added into the flour following the inching method wherein a small portion of the flour was first taken in a vessel and divided into four quadrants; a small quantity of premix was then added and mixed into each of the quadrants. This procedure was followed to prepare the entire batch of flour required for the study.

This mixture was then blended together for 20 min. After this process of fortification, laddoos, each weighing 25 g, were prepared by blending the fortified flour with ghee and sugar.

Sample size calculation: Based on the study by Natri *et al.*[12], we anticipated the mean change in serum 25(OH)D to be 6.41 and 7.81 ng mL^{-1} in the test and the experimental group. A sample size of 4 in each arm would be sufficient to detect this difference with 80% power and 5%level of significance based on a one-way ANOVA test.

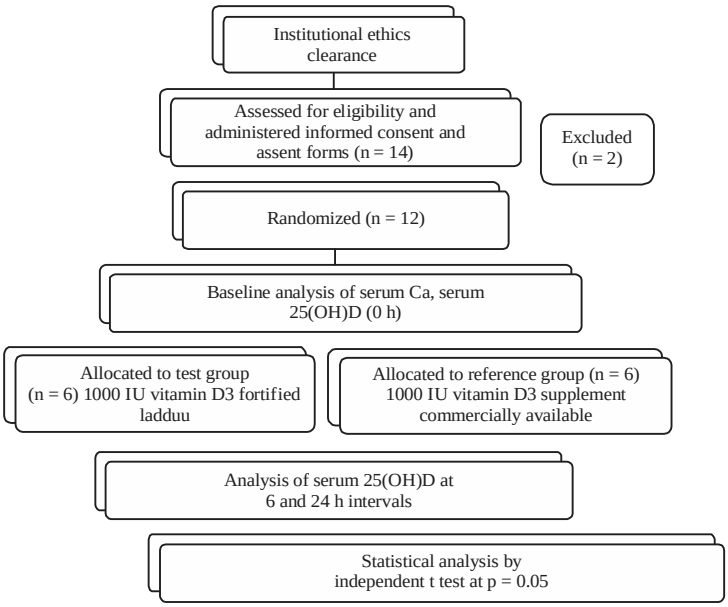

Fig. 1 : Flow of work according to consort guidelines

Subjects: A total of 14 volunteers aged 17 years were enrolled for this bioavailability study phase. Individuals with severe illness, hypercalcemia, intestinal malabsorption, allergies or taking supplements containing vitamin D_3 were excluded from the study. Signed informed consent and informed assent forms were obtained from parents and children respectively.

Design: Volunteers were randomly divided into two groups namely, (1) Test group (fortified laddoos containing 1000 IU vitamin D_3) and (2) Reference group (vitamin D_3 cholecalciferol tablets containing 1000 IU vitamin D_3) using a research randomizer software. Each volunteer was required to consume either the fortified laddoo or the vitamin D_3 tablet depending upon the group to which they were assigned. Blood was collected at 0, 6 and 24 h, to assess serum 25(OH)D levels. Hypercalcemia was ruled out in the subjects by assessing serum calcium at baseline. The flow DF work has been described in Fig. 1.

Analytical methods: The Microparticle Enzyme Immunoassay (MEIA) technique was employed to evaluate serum 25(OH)D and O-cresolphthalein technique was employed to assess serum calcium at the Central Laboratory of Sri Ramachandra Institute of Higher Education and Research, Chennai, India.

Vitamin D_3 levels in the fortified and non-fortified foods were analysed by High performance Liquid Chromatography (HPLC) technique at SGS Laboratories, Chennai, India.

Statistical analysis: Statistical calculations were carried out by using SPSS version 16 software. Independent t test was used to compare the means of vitamin D_3 levels between the treatment groups. All significant differences are reported at $p \leq 0.05$ level.

RESULTS

Fortification of flour and laddoos: On analysis with HPLC, the non fortified flour and laddoos were found to contain below detectable unit of vitamin D_3. The vitamin D_3 content of the fortified flour was 367.7 µg/100 g and that of the fortified laddoos was 119.19 µg/100 g.

Baseline characteristics: Of the total volunteers enrolled for the study, two were excluded as they did not adhere to the protocol. Six in each of the study groups completed the entire protocol and results are presented for these participants.

Age, weight and height of all the volunteers were not statistically significant ($p>0.1$, Table 1). Among the two study groups, baseline values of serum 25(OH)D and serum calcium did not differ significantly from each other. All the participants were found to be deficient in serum 25(OH)D levels, the normal levels being >30 ng mL^{-1}, as per the guidelines of Endocrine Society. The mean baseline serum calcium levels were found to be within the reference range.

The adolescents did not report use of multivitamins or vitamin D supplements.

Table 1: Baseline characteristics of the participants

Characteristics	TEST (n = 6)	Reference (n = 6)	't' value	Significance
Age (years)	17.35±0.27	17.55±0.37	0.305	0.771^{NS}
Weight (kg)	54.08±11.84	55.58±7.82	0.065	0.882^{NS}
Height (cm)	157.00±4.35	160.00±5.61	0.890	0.335^{NS}
Baseline Serum 25(OH) (ng mL^{-1})	12.28±3.86	12.91±4.78	0.439	0.641^{NS}
Baseline Serum Ca (mg dL^{-1})	9.31±0.42	9.43±0.17	0.567	0.561^{NS}
Dietary vitamin D_2 intake (µg d^{-1})	5.15±1.32	5.23±1.67	0.325	0.661^{NS}

^{NS}Indicates not significant

Table 2: Bioavailability of vitamin D_3 from fortified laddoo and the cholecalciferol supplement

	Serum 25(OH)D		
Study groups	0 h	6 h	24 h
Test	12.28±3.86	13.98±3.67	14.71±3.66
Reference	12.91±4.78	13.78±4.54	14.53±4.69
t value	-0.252	0.084	0.075
Significance	0.806^{NS}	0.935^{NS}	0.941^{NS}

^{NS}Indicates not significant

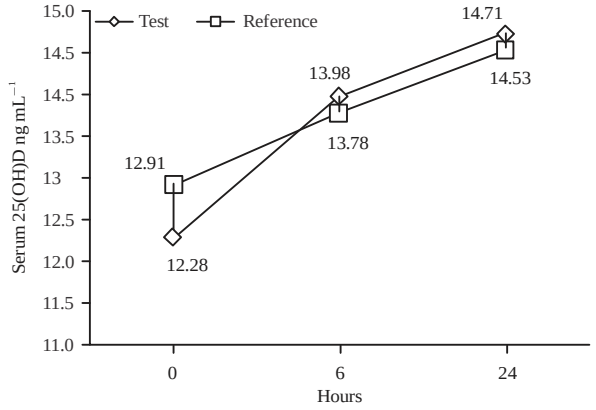

Fig. 2: Bioavailability of vitamin D_3 from fortified laddoo vs the cholecalciferol supplement

Bioavailability of vitamin D_3 from the fortified laddoos: After the administration of the vitamin D fortified laddoo and the cholecalciferol supplement to the test and reference groups respectively, there was a steady rise in the serum 25(OH)D levels of the subjects. The changes in the serum 25(OH)D levels were 13.98±3.67 and 14.71±3.66 ng mL^{-1} in the test group and 13.78±4.54 and 14.53±4.61 ng mL^{-1} in the reference group at 6 and 24 h intervals respectively. The increases in the serum 25(OH)D concentration in the test and the reference groups followed a similar pattern ($p>0.05$, Table 2, Fig. 2).

DISCUSSION

To the best of our knowledge, this is the first study in which bioavailability of vitamin D_3 (using vitamin D_3 premix containing a cold water soluble source of vitamin D_3) from millet laddoos has been reported in India.

Results of this study indicate that vitamin D_3 from the fortified laddoo was absorbed as effectively as that from a synthetic source. This is interesting since some previous studies have reported that vitamin D absorption is inhibited in the presence of high fibre[12]. Knowing that millets and pulses are good sources of fibre, the absorption of vitamin D_3 could have decreased but instead, the S25(OH)D levels showed steady increase, indicating good efficiency of absorption. This particular finding is in accordance with a study wherein rye bread fortified with vitamin D_3 was equally effective in raising vitamin D status, equivalent to that of a synthetic form Natri *et al.*[12].

Vitamin D deficiency is prevalent, irrespective of age or sex, with an estimate of nearly 1 billion people worldwide are vitamin D deficient[7]. In a study among adults in Tirupati, it was observed that 82% males and 62% females in urban areas were deficient in vitamin D, despite living in a tropical area[5]. High incidence of Vitamin D deficiency was also observed among healthy children and adolescents in South Chennai[13]. Although, the present study with its limited sample size cannot be indicative of the vitamin D deficiency status across Chennai, observations with baseline serum 25(OH)D levels in the population group are in accordance with previously reported studies[2-7,13,14].

Srinivasa and Harinarayan[9] reported that an intake of 27 µg day^{-1} of vitamin D is required to raise the serum 25(OH)D levels above 30 ng mL^{-1}. Thus it is not unexpected that in the present study, the adolescents were deficient. Unfortunately, the best bioavailable form of vitamin D does not naturally occur in many foods; hence, fortification of commonly consumed foods with vitamin D_3 may be a significant option to combat the observed deficiency.

Cereals form the staple diet among Indians, thus making it a desirable prospect for fortification. For best results with vitamin D fortification, it is of utmost importance that the chosen vehicle be rich in calcium. Hence, in this study, millets such as, finger millet and pearl millet, were chosen as the vehicle for fortification of vitamin D_3. As per known literature, vitamin D absorption is positively influenced by dietary calcium and negatively influenced by phytates and fibre[4]. In the current study, the flour comprising of finger millet, pearl millet and black gram dhal was fortified with vitamin D_3; analysis of flour and the laddoos revealed good distribution of vitamin D_3. The calcium content of each laddoo was 162 mg and total dietary fibre was 8.9 g.

Since the study involved adolescents, large number of participants could not be recruited. A cross over design was not employed since the parents would not have approved of repeatable measuring of blood samples. Also, further field studies need to be carried out to understand the impact of the snack fortified with vitamin D_3 on vitamin D status and the bone mineral density status of adolescents. Vitamin D_3 fortified laddoos could serve as a beneficial strategy to cope with vitamin D deficiency but, more investigations need to be carried out to understand the stability of vitamin D_3 in the given snack and sustained bioavailability over a period of time.

SIGNIFICANCE STATEMENT

This study discovers the possible effect of a cereal based snack fortified vitamin D_3 in improving the vitamin D status of individuals in comparison with that of a cholecalciferol supplement that can be beneficial in reducing the burden of vitamin D deficiency in the country. This study will help the researchers to uncover the critical area of bioavailability of vitamin D in cereals fortified with vitamin D_3 that many researchers have not explored. Thus, a new theory on cereal based snack fortified with vitamin D may be arrived at.

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