Vitamin D-fortified bread is as effective as supplement in improving vitamin D status: a randomized clinical trial

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Context: Bread can potentially be a suitable vehicle for fortification with vitamin D.

Objective: This study was undertaken to evaluate (1) bioavailability of vitamin D from the fortified Iranian bread, and (2) the possible effects of daily consumption of the fortified bread on certain health aspects.

Design, Setting, and Participants: This was a randomized double blind placebo-controlled trial conducted over 8 weeks in 90 healthy subjects aged 20–60 yr.

Intervention: Subjects were randomly allocated to 1 of 3 groups: (a) fortified bread (FP; 50 g bread fortified with 25μg vitamin D3 plus placebo daily; $n_1 = 30$); (b) supplement (SP; 50 g plain bread plus 25μg vitamin D supplement daily; $n_2 = 30$); and (c) control (CP; 50 g plain bread plus placebo daily; $n_3 = 30$).

Outcome: Measures: Initial and final anthropometric and biochemical assessments were performed.

Results: The within-group changes of serum 25(OH)D concentrations were 39.0±22.6 ($p<0.001$), 28.9±31.2 ($p<0.001$) and -9.2±12.3 nmol/L in the FP, SP and CP groups, respectively. Only in FP and SP groups, serum iPTH concentrations decreased approximately 13.5% and 14.5%, respectively. Visceral fat also showed a significant decrement in FP (-1.05±1.4%; p=<0.001) and SP (-0.96±1.7%; p=0.006). Serum LDL-C concentration showed a within-group reduction in FP (-10.4±11.2 mg/dL; p <0.001) and an insignificant decrement in SP (-6.6±20.2 mg/dL; p=0.083). Serum HDL increased in both vitamin D supplemented groups (FP: 9.7±7.6 vs. SP: 5.7±6.7 mg/dL; $p<0.001$).

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Abbreviations: 25(OH)D: 25-hydroxycalciferol, BMI; Body mass index, CP: Control (plain) bread plus placebo, Cr: Creatinine, DBP: Diastolic blood pressure, FP: Fortified bread plus placebo, HDL-C: High density lipoprotein cholesterol, iPTH: Intact parathyroid hormone, LDL-C: Low density lipoprotein cholesterol, MOH: Ministry of Health, NFNS: National Food and Nutrition Surveillance Program, SP: Vitamin D supplement plus plain bread, SBP: Systolic blood pressure, U: urinary

Conclusion: Vitamin D-fortified bread could be a potentially effective in raising circulating 25(OH)D of the population to nearly adequate levels.

Key words: vitamin D; fortification; fortified bread; lipid profile

Hypovitaminosis D is one of the most important public health concerns occurring in all racial, gender and age subpopulations with almost no geographical boundaries (1, 2). The recent National Food and Nutrition Surveillance Program (NFNS) report demonstrated that even during summer over 70% of the Iranian population aged 12–65 years had hypovitaminosis D (3). Considering the economic burden of vitamin D deficiency especially due to its contribution in many human diseases (4), optimization of vitamin D status of the community is among the top priorities of public health interventions in most, if not all, countries (5). The alarming occurrence rates from different studies have been, and continue to be, a tremendous stimulus for the stakeholders of the Iran Ministry of Health (MOH) to make a decision for a proper action against this epidemic.

Naturally, vitamin D supply depends mostly on cutaneous synthesis upon exposure to solar ultra violet beam (UVB), at wavelengths of 290 to 315 nm. However, environmental, cultural, social as well as individual factors can all influence the amount of UVB reaching the skin and hence the amount of calciferol synthesis. Thus reliance on intake, mostly from fortified foods and supplements, has become almost the sole way to achieve vitamin D adequacy. Though supplementation as a preventive strategy is of interest especially for high risk subpopulations, the population coverage, compliance, costs, regular use and sustainability are all challenging (6). Consequently, much attention is being paid to food fortification.

Many countries have established mandatory fortification program for several staple food items whereas in some countries, fortification with vitamin D is voluntary. In any case, the key question arises as to "what is the appropriate food vehicle for fortification with vitamin D?" The selected food source should be widely available especially to those with moderate to severe food insecurity and its consumption should not remarkably differ by such socio-economic factors as education or income. Though milk and dairies may seem the best choice at the first glance, fortification of dairy products has not been as effective for preventing of vitamin D inadequacy in the general population as expected probably due to the high variations of milk intake across population subgroups (7). The latest nationwide household dietary pattern survey in Iran reported that the per capita consumption of dairy products was about 140 g/d, including only 38 g milk a day (8). At the time of that survey, milk was covered by governmental subsidies. Sporadic reports indicate that milk consumption has dramatically decreased following removal of subsidies (9). In Iran, bread as a staple food which is consumed almost with each meal, has per capita consumption of over 300 g/d (8). This has made bread a good vehicle for fortification with several micronutrients including iron and folic acid, the two fortificants having been mandatory added to flour in Iran since over 8 years ago. Scarcity of experience with flour fortification with vitamin D and, on the other hand, especial features of the Iranian traditional breads both are challenging. Iranian breads are flat and baked by direct heat exposure, thus increasing the possibility of destruction of vitamin D during baking process. Bioavailability of vitamin D from this matrix was another important issue to address.

We have performed several trials on the effects of vitamin D on different aspects of health in diabetic subjects (10–12). In the current study, we aimed to evaluate the effects of daily consumption of vitamin D-fortified flat bread on anthropometric measures as well as blood glucose and lipids of healthy individuals. Our main questions were: (1) is vitamin D bioavailable from a fortified Iranian flat bread enough to improve vitamin D status of a healthy consumer? If the answer is positive (2) how comparable is the bioavailability of vitamin D from fortified bread with that from a supplement pill? (3) is improvement of vitamin D status following consumption of fortified bread accompanied by a decrement in serum parathyroid hormone? if the answer is positive (4) how serum and urinary concentrations of calcium, phosphorous, magnesium, sodium and potassium are affected? And (5) does improvement of vitamin D status affect consumers' general health as judged by body mass index (BMI), percent of body fat, systolic and diastolic blood pressure (BP) and blood glucose and lipid profile? To answer these questions, we first estimated the amount of vitamin D loss in the baked Lavash bread, the most commonly eaten bread in Iran. Then, a randomized clinical trial (RCT) was conducted on apparently healthy subjects.

Subjects and Methods

Preparation of the fortified breads. A premix containing 100′000 IU vitamin D/g (Xiamen Kingdomway Group, Xiamen, China) was used to fortify the flour. To estimate vitamin D loss during baking process, Lavash breads fortified with 400 IU (10 μg) or 1000 IU (25 μg) per 100 g were baked. The amount of vitamin

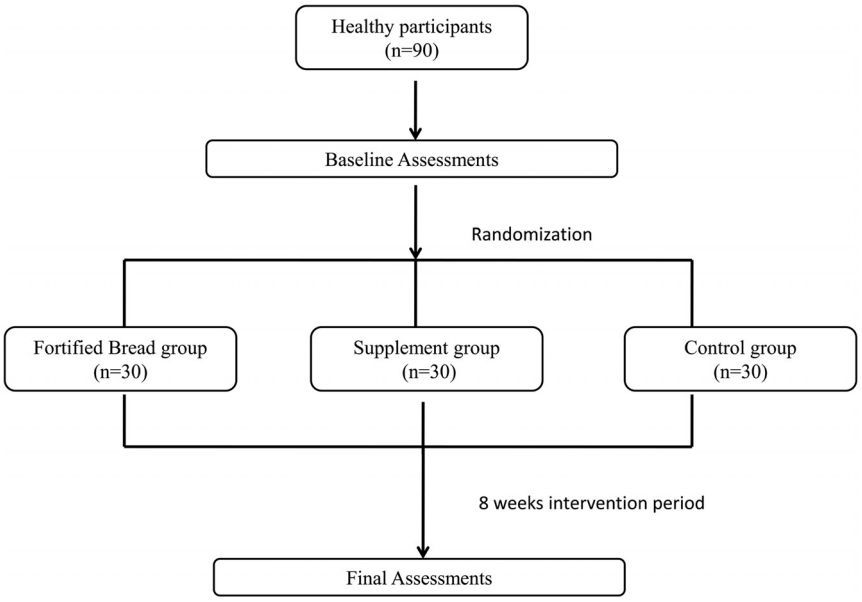

Figure 1. Summary of study design

D in the bread samples were determined using high performance liquid chromatography (HPLC) analysis (13). Then, the concentration of the fortificant was calculated based on the amount of nutrient loss during baking. Using this calculation, sufficient amount of the fortificant was added to the flour so that 1000 IU (25 μg)/50 g of bread was yielded. We used these amounts because: (1) based upon our previous experiences (10, 14), this dosage of vitamin D is considered safe yet potentially efficacious in achieving a serum 25(OH)D concentration to 50 nmol/L and above in most subjects, a concentration considered optimal by many experts (15). Moreover, mandatory fortification of several foods with vitamin D was being planned based on this dosage at MOH at the time of this investigation; (2) this dosage of the fortificant was comparable with 1000 IU vitamin D tablet which was available in the market; and (3) we were almost sure that 50 g bread was eaten up by all participants with every dietary habits. The breads were baked weekly and stored at –20°C. From each batch of the breads, 3 samples were randomly selected for quality control (QC) and evaluation of the stability of vitamin D using HPLC analysis (13). Unfortified wheat flour was used to bake plain breads as controls.

Participants and study design. This study was a prospective, randomized double blind three-arm parallel design trial (RCT) in healthy men and women conducted for 8 weeks during fall and winter 2015 (February through March) when there is minimal sunlight exposure (clinicaltrials.gov registration NCT00789503).

A total of 90 subjects were enrolled in the study. The inclusion criteria were age 20–60 years, no medical conditions or disease that could influence vitamin D metabolism, not taking dietary supplements including calcium, vitamin D or omega-3 in the preceding 3 months; and not planning a holiday during the intervention period.

The participants were randomly allocated by a computer generated randomization list to one of the three groups: (a) fortified bread (FP; 50 g bread fortified with 25 μg vitamin D3 plus placebo daily; $n_1 = 30$); (b) supplement (SP; 50 g plain bread plus 25 μg vitamin D supplement daily; $n_2 = 30$); and (c) control (CP; 50 g plain bread plus placebo daily; $n_3 = 30$). The bread and tablet packages labeled with special codes by main supervisor which were unknown to the investigators, staff and participants.

Participants were instructed to eat the provided breads and pills with one of their meals on a daily basis and also to follow their usual life style, including physical activity and dietary habits. Subjects obtained their packages of breads and tablets weekly from our laboratory and were asked to consume all of them in the way they were instructed and to return any unused breads and tablets on their next attendance at the laboratory. Compliance of the intervention was assessed as the grams of breads consumed divided by expected consumed grams. The subject was excluded if this ratio was less than 0.85 at the end of the study.

The initial and final assessments, including dietary intake, anthropometric and laboratory measures, were performed for all subjects (Figure 1). A written informed consent was obtained from all participants. Ethical approval was obtained from Ethical Committee of the National Nutrition and Food Technology Research Institute (NNFTRI). This trial followed the CONSORT guidelines (16) and is registered at clinicaltrials.gov as NCT00789503.

Dietary assessment. Total dietary intake data were collected using 2-day food recalls. The data were translated to the energy and nutrients intakes using the software Nutritionist IV (version 4.1, 1997; First DataBank, The Hearst Corporation, San Bruno, CA)

Anthropometrics. Weight and height were measured without shoes and with light clothing, weight with a scale (to the precision of 0.1 kg; Seca 808, Hamburg, Germany), and height with a wall-mounted stadiometer to the nearest of 0.1 cm. Body mass index (BMI) was calculated using the equation weight (kg)/height $(m)^2$. Systolic and diastolic BP was measured twice (after 5–10 minutes resting in a sitting position) using a validated digital automated BP monitor (Beurer BC 08; Beurer GmbH, Ulm, Germany). The average of two measurements was used for analysis.

Evaluation of abdominal adiposity. Truncal and visceral fat were estimated using a BIA system (ViScan AB-140, Tanita, Tokyo, Japan) while the subject was in a supine position.

Biochemical analyses. Blood and urine samples were collected between 8:30 and 10:00 am after an overnight fasting at baseline and after the intervention. The samples were processed for storage within 2 hours of collection; blood was allowed to stand for 15 minutes at room temperature (RT) before centrifugation at 800 *g* for 15 minutes at RT to separate serum. The recovered sera were aliquoted, one tube was used for blood glucose and lipid profile tests on the same day of bleeding while the other tubes were immediately transferred to a –80°C freezer for further analyses.

Serum 25(OH)D concentrations were assayed by HPLC

method (17) with intra- and interassay coefficients of variation of 8.1% and 12.6%, respectively. Our laboratory is a part of the Vitamin D External Quality Assessment Scheme (DEQAS, www.DEQAS.org.uk). Based on the concentration of circulating 25(OH)D, vitamin D status was defined as sufficient (≥50 nmol/L), insufficient (≥27.5 to < 50 nmol/L) and deficient (<27.5 nmol/L) (18).

Serum intact parathyroid hormone (iPTH) was determined by enzyme immunoassay (EIA) (DSL, Webster, Texas, USA) with an interassay variations ranging from 6.0 to 6.3%, as claimed by the manufacturer.

Serum total-cholesterol, HDL-C, LDL-C, triglycerides and glucose were determined by enzymatic methods and serum and urinary creatinine, calcium, phosphorous and magnesium were measured by colorimetric methods using commercial kits (all from Pars-Azmoon, Tehran, Iran) and an automatic analyzer (Selecta E; Vitalab, Holliston, Netherlands). Urinary sodium and potassium concentrations were assayed in spot samples by a flame photometer system (Corning 480, Halstead Essex, UK). To avoid interassay variations, all tests (except for blood glucose and lipid profile) of pre and postintervention were carried out in a same run at the end of the study.

Chemical characterization of bread samples. Chemical characteristics of bread samples, including moisture, ash, protein, fat and pH, were all determined by standard methods at Iran Cereals Research Center (ICRC).

Sensory evaluation of breads. Sensory evaluation of bread samples was examined using 37 in-house untrained subjects. Vitamin D-fortified and unfortified bread samples in a same size were cut and put in a disposable plates which were tagged randomly with three-digit codes. The compliance was ranked between 1 (most) to 3 (least) by the evaluators.

Statistical analyses

All analyses were done using Statistical Package for Social Sciences (SPSS, version 21.0) for windows (SPSS Inc, Chicago) with a 2-sided 0.05 level of significance used. Descriptive statistics (mean and standard deviation (SD)) were determined for all variables. Between-group comparison of baseline characteristics was performed by analysis of variance (ANOVA) for continuous variables and chi square tests for categorical variables. A two-way repeated-measures ANOVA was done to explore the impact of time and time × group effects with adjustment by the Tukey's test. Effects of the intervention in each group were examined using the paired *t* test vs the baseline values. Between-group comparisons were examined using ANOVA followed by Tukey's post hoc analysis. Sensory evaluation data were compared using nonparametric Kruskal-Wallis test.

Results

Vitamin D amount in breads and sensory evaluation. Mean amount of vitamin D was 25 ± 2.0 μg per 50g of bread with interbatch variation of less than 9% and no remarkable decline during storage for two weeks. No significant difference in chemical characteristics was observed between fortified and unfortified bread (Table 1). There was no significant difference in sensory evaluation between vitamin D-fortified and plain breads ($P = .248$).

Baseline characteristics of the participant and their dietary intakes. Ninety participants (49 men and 41 women) completed the study with a compliance rate > 90%. Mean age was 37.9 ± 10.9 years in the whole study population. There were no significant differences in baseline characteristics, duration of sun exposure and sunscreen use among the groups (Table 2). None of the subjects reported any significant adverse effects. Dietary intake, including energy, protein, fat, vitamin C, vitamin D, calcium and fiber did not show any intra- and intergroup significant difference (data not shown).

Anthropometric and biochemical measures. Initially, serum 25(OH)D concentrations below 50 nmol/L was detected in 86.4% of the participants that decreased dramatically to 44.6% at the end of intervention. At baseline, there was no significant between-group difference in serum 25(OH)D concentrations ($P = .988$). However, after 8 weeks intervention, the within-group changes of serum 25(OH)D concentrations were 39.0 ± 22.6 ($P < .001$), 28.9 ± 31.2 ($P < .001$) and –9.2 ± 12.3 nmol/L in the FP, SP and CP groups, respectively ($P = .001$). The increments in circulating calcidiol did not differ significantly between FP and SP ($P = .206$) but the changes of 25(OH)D in these two groups were different significantly with that in CP ($P < .001$ for both). Compared with baseline, serum iPTH concentrations decreased approximately 13.5% and 14.5% in FP and SP groups, respectively (80.4 ± 47.3 to 69.6 ± 30.8 pg/mL, $P = .030$; and 71.3 ± 26.4 to 61.1 ± 25.8 pg/mL, $P = .010$; respectively). No significant change in iPTH concentrations was observed in CP group (74.3 ± 30.5 to 81.3 ± 50.5 pg/mL, $P = .275$). There was an inverse association between initial serum 25(OH)D con-

Table 1. Chemical characteristics of vitamin D-fortified and plain (unfortified) breads

Sample/Test	Moisture (%)	Ash (%)	Protein (dried material) (%)	Fat (%)	pH
Plain (unfortified) bread	25.11	1.093	14.52	0.34	6.9
Vitamin D-fortified bread	24.46	1.212	15.41	0.39	6.9

Table 2. Baseline characteristic of the participants in groups

variable		FP (n = 30)	SP (n = 30)	CP (n = 30)	p value
Age		37.2 ± 10.5	37.3 ± 10.9	39.4 ± 11.6	0.699
Sex	**Male n(%)**	16 (53.3)	16 (53.3)	17 (56.6)	0.762
	Female n(%)	14 (46.7)	14 (46.7)	13 (43.4)	
Sun exposure	**> one hr**	26 (86.7)	25 (83.3)	21 (70.0)	0.233
	< one hr	4 (13.3)	5 (16.7)	9 (30.0)	
Time of sun exposure	**10 AM-3 PM**	18 (60.0)	12 (40.0)	17 (56.7)	0.251
	others	12 (40.0)	18 (60.0)	13 (43.3)	
Sunscreen use	**Yes**	9 (31.0)	11 (36.7)	8 (26.7)	0.705
	No	21 (69.0)	19 (63.3)	22 (73.3)	

FP: Vitamin D-fortified bread + placebo pill

SP: Vitamin D supplement + plain bread

CP: Control (plain) bread + placebo pill.

centrations and changes of that after intervention (r= –0.350, P = .001).

The time and group interaction was statistically significant for serum 25(OH)D, BMI, waist circumference, visceral fat, serum LDL-C, HDL-C and iPTH. In vitamin D supplemented groups, BMI had a small, but statistically significant, reduction compared with the control group (Tables 3 and 4). Percent of truncal fat did neither change nor differ between groups but changes of visceral fat showed a significant decrement in FP (-1.05 ± 1.4%; p=<0.001) and SP (-0.96 ± 1.7%; P = .006) and a small insignificant increment in PC (0.61 ± 1.5%; P = .055) (Table 4). Accordingly, serum triglycerides decreased in FP (127.2 ± 50.8 vs. 103.6 ± 46.0 mg/dL, P = .003) and SP (143.2 ± 70.4 vs. 112.8 ± 51.9 mg/dL; P = .003) groups but no significant change occurred in PC (145.1 ±

Table 3. Studied characteristics at baseline and after the intervention[1]

Variable	FP (n = 30)		SP (n = 30)		CP (n = 30)		p^b
	Before	After	Before	After	Before	After	
25(OH)D (nmol/liter)	33.9 ± 21.9	72.9 ± 23.1	35.0 ± 38.7	63.9 ± 31.0	34.7 ± 30.5	25.4 ± 21.8	<0.001
Weight (kg)	74.0 ± 14.0	73.5 ± 13.7	71.8 ± 11.7	71.3 ± 11.5	75.5 ± 15.4	75.7 ± 15.6	0.019
BMI (kg/m^2)	26.5 ± 4.7	26.3 ± 4.6	25.7 ± 3.7	25.5 ± 3.6	27.2 ± 4.0	27.3 ± 4.1	0.015
Waist Circumference (cm)	94.6 ± 10.7	93.6 ± 11.1	93.9 ± 9.7	93.4 ± 9.3	95.2 ± 9.8	96.4 ± 10.2	0.017
Trunk fat (%)	31.8 ± 9.5	31.6 ± 9.0	33.1 ± 8.9	33.0 ± 9.4	32.7 ± 7.0	33.7 ± 6.7	0.151
Visceral fat (%)	10.8 ± 4.4	9.7 ± 4.5	10.7 ± 4.7	9.7 ± 4.6	10.7 ± 4.2	11.3 ± 3.9	<0.001
SBP (mmHg)	123.7 ± 11.6	122.0 ± 9.0	125.5 ± 13.0	122.2 ± 12.8	127.6 ± 20.0	127.2 ± 18.4	0.643
DBP (mmHg)	79.7 ± 9.7	73.4 ± 12.6	78.7 ± 7.3	76.4 ± 8.7	81.7 ± 14.6	80.9 ± 13.9	0.229
Glucose (mg/dL)	80.9 ± 7.5	82.5 ± 6.4	81.8 ± 8.9	82.2 ± 6.6	83.7 ± 11.4	85.7 ± 10.8	0.638
Triglycerides (mg/dL)	127.2 ± 50.8	103.6 ± 46.0	143.2 ± 70.4	112.8 ± 51.9	145.1 ± 84.3	132.0 ± 74.0	0.386
Total cholesterol (mg/dL)	184.1 ± 36.9	175.1 ± 32.2	177.5 ± 38.6	173.6 ± 34.4	178.8 ± 42.8	176.1 ± 40.8	0.372
LDL (mg/dL)	109.9 ± 27.9	99.5 ± 26.5	103.7 ± 36.2	97.1 ± 30.6	104.4 ± 39.5	105.3 ± 37.2	0.018
HDL (mg/dL)	45.0 ± 9.8	54.8 ± 9.6	48.2 ± 14.2	53.9 ± 12.1	42.9 ± 12.7	44.4 ± 14.2	<0.001
iPTH (pg/mL)	80.4 ± 47.3	69.6 ± 30.8	71.3 ± 26.4	61.1 ± 25.8	74.3 ± 30.5	81.3 ± 50.5	0.023
Serum Ca (mg/dL)	10.2 ± 0.5	10.1 ± 0.4	10.0 ± 0.5	10.1 ± 0.4	10.1 ± 0.4	10.1 ± 0.4	0.280
Serum P (mg/dL)	3.5 ± 0.9	3.5 ± 0.6	3.2 ± 0.8	3.5 ± 0.4	3.4 ± 0.7	3.3 ± 0.4	0.202
Serum Mg (mg/dL)	2.0 ± 0.16	2.0 ± 0.13	2.0 ± 0.15	2.0 ± 0.17	2.0 ± 0.20	2.0 ± 0.16	0.194
UNa/Cr (mg/mg creat)	0.78 ± 0.3	0.93 ± 0.5	0.88 ± 0.6	1.09 ± 0.6	1.13 ± 0.6	1.12 ± 0.7	0.914
UK/Cr (mg/mg creat)	0.45 ± 0.2	0.53 ± 0.3	0.48 ± 0.2	0.56 ± 0.2	0.45 ± 0.1	0.45 ± 0.1	0.478
UCa/Cr (mg/mg creat)	0.13 ± 0.04	0.16 ± 0.09	0.13 ± 0.07	0.14 ± 0.07	0.18 ± 0.08	0.17 ± 0.07	0.340
UP/Cr (mg/mg creat)	0.35 ± 0.12	0.4 ± 0.17	0.34 ± 0.14	0.4 ± 0.19	0.42 ± 0.18	0.40 ± 0.14	0.352
UMg/Cr (mg/mg creat)	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.579

[1]All values are means ±SDs, FP: Vitamin D-fortified bread + placebo pill, SP: Vitamin D supplement + plain bread, CP: Control (plain) bread+ placebo pill

25(OH)D, 25-hydroxyvitamin D; SBP, systolic blood pressure, DBP, diastolic blood pressure

p^b Time*group interaction (two-factor ANOVA).

Table 4. The comparison of changes within and between groups after the intervention[1]

Variables	FP (n = 30) changes	p^a	SP (n = 30) changes	p^a	CP (n = 30) changes	p^a	p^b	p^c	p^d
25(OH)D (nmol/liter)	39.0 ± 22.6	<0.001	28.9 ± 31.2	<0.001	−9.2 ± 12.3	0.001	0.206	<0.001	<0.001
Weight (kg)	−0.51 ± 1.3	0.011	−0.52 ± 1.1	0.024	0.21 ± 0.9	0.267	1.0	0.032	0.039
BMI (kg/m^2)	−0.18 ± 0.3	0.008	−0.19 ± 0.4	0.023	0.07 ± 0.3	0.257	0.997	0.028	0.030
Waist Circumference (cm)	−0.95 ± 3.5	0.122	−0.51 ± 2.4	0.255	1.1 ± 2.1	0.013	0.810	0.016	0.087
Trunk fat (%)	−0.25 ± 2.4	0.539	−0.16 ± 2.6	0.740	0.9 ± 2.9	0.091	0.733	0.780	0.364
Visceral fat (%)	−1.05 ± 1.4	<0.001	−0.96 ± 1.7	0.006	0.61 ± 1.5	0.055	0.972	<0.001	0.001
SBP (mmHg)	−1.7 ± 10.7	0.363	−3.36 ± 10.6	0.094	−0.4 ± 14.3	0.884	0.843	0.906	0.618
DBP (mmHg)	−6.2 ± 17.0	0.036	−2.3 ± 7.9	0.120	−0.7 ± 11.6	0.731	0.798	0.434	0.822
Glucose (mg/dL)	1.6 ± 6.9	0.168	0.36 ± 5.2	0.707	2.0 ± 8.4	0.228	0.737	0.980	0.651
Triglycerides (mg/dL)	−23.5 ± 44.3	0.003	−30.4 ± 52.1	0.003	−13.1 ± 44.8	0.139	0.827	0.667	0.355
Total cholesterol (mg/dL)	−9.0 ± 19.0	0.008	−3.8 ± 21.8	0.341	−2.6 ± 16.3	0.411	0.529	0.399	0.968
LDL (mg/dL)	−10.4 ± 11.2	<0.001	−6.6 ± 20.2	0.083	0.8 ± 12.9	0.746	0.580	0.013	0.159
HDL (mg/dL)	9.7 ± 7.6	<0.001	5.7 ± 6.7	<0.001	1.5 ± 6.7	0.239	0.067	<0.001	0.070
iPTH (pg/mL)	−10.4 ± 27.3	0.030	−10.2 ± 20.1	0.010	6.9 ± 32.3	0.275	0.999	0.035	0.048
Serum Ca (mg/dL)	−0.1 ± 0.5	0.259	0.06 ± 0.4	0.428	0.06 ± 0.4	0.487	0.359	0.366	1.00
Serum P (mg/dL)	−0.01 ± 0.9	0.948	0.3 ± 0.8	0.064	−0.1 ± 0.7	0.498	0.341	0.923	0.216
Serum Mg (mg/dL)	−0.03 ± 0.1	0.269	−0.01 ± 0.4	0.824	0.05 ± 0.2	0.228	0.835	0.173	0.452
UNa/Cr (mg/mg)	0.15 ± 0.5	0.121	0.2 ± 0.6	0.091	0.14 ± 0.6	0.275	0.928	1.00	0.927
UK/Cr (mg/mg)	0.08 ± 0.4	0.308	0.07 ± 0.1	0.062	0.01 ± 0.2	0.905	0.994	0.646	0.733
UCa/Cr (mg/mg)	0.02 ± 0.1	0.120	0.01 ± 0.0	0.424	−0.01 ± 0.09	0.765	0.683	0.314	0.815
UP/Cr (mg/mg)	0.04 ± 0.1	0.207	0.06 ± 0.2	0.149	−0.01 ± 0.2	0.665	0.962	0.478	0.367
UMg/Cr (mg/mg)	−0.00 ± 0.01	0.265	−0.00 ± 0.02	0.810	0.001 ± 0.02	0.757	0.801	0.561	0.923

[1]All values are means ±SDs, FP: Vitamin D-fortified bread + placebo pill, SP: Vitamin D supplement + plain bread, CP: Control (plain) bread + placebo pill

25(OH)D, 25-hydroxyvitamin D; SBP, systolic blood pressure, DBP, diastolic blood pressure

p^a Significance of within-group changes (paired-samples t test)

p^b Comparison between the fortified bread (group 1) and supplement (group 2) groups (one-factor ANOVA)

p^c Comparison between the supplement (group 1) and plain (group 3) groups (one-factor ANOVA)

p^d Comparison between the fortified bread (group 2) and plain (group 3) groups (one-factor ANOVA).

84.3 vs. 132.0 ± 74.0 mg/dL; P = .139) (Table 3). Changes of serum LDL-C concentrations differed significantly among the groups (P = .018) which remained significant even after adjustment for changes of BMI (P = .025), waist circumference (P = .024) and visceral fat (P = .015). Interestingly, serum LDL-C concentration showed a within-group reduction in FP (-10.4 ± 11.2 mg/dL; P < .001) and an insignificant decrement in SP (-6.6 ± 20.2 mg/dL; P = .083). Serum HDL increased in both vitamin D supplemented groups with slightly higher increment in FP than in SP (9.7 ± 7.6 vs. 5.7 ± 6.7 mg/dL; P < .001) and no significant change in CP (Table 4). Significant between-group difference of changes of HDL-C was not affected by adjustment for changes of BMI, waist circumference or percent of visceral fat (P < .001 for all).

The changes in circulating 25(OH)D and iPTH were not accompanied by significant changes in serum concentrations of calcium, phosphorous and magnesium. In supplemented groups, an increase in UCa/Cr, UNa/Cr and UK/Cr were observed. However, these changes were all statistically insignificant (Table 3, 4). Systolic and diastolic BP did not show any within- or between-group change throughout the intervention, either.

There were inverse associations between changes of serum 25(OH)D concentrations and changes of weight (r = −0.336, P = .001), BMI (r = −0.341, P = .001), waist circumference (r = −0.245, P = .019) and visceral fat (r = −0.241, P = .017). For all other parameters, no significant main effect of the intervention was detected.

After adjustment for age, gender and baseline BMI, it was found that daily intake of 1000 IU vitamin D either as fortified bread or supplement would result in approximately 40 nmol/L increase in serum 25(OH)D, on average.

Discussion

Daily consumption of 50 g vitamin D-fortified bread resulted in a remarkable improvement of vitamin D status of the healthy subjects which was accompanied by a decline in serum iPTH concentrations as well as a significant improvement of blood lipid components and percent of visceral fat.

The efficacy of vitamin D-fortified bread in raising circulating calcidiol has been already reported (19–23). The dosages used in these studies were 12 μg/100 g bread for

three weeks in 25–45yr healthy women (23), 125 μg/bread bun daily for over 12 months in elderly (19, 21, 22) and 6 μg/100 g bread plus 0.38 μg/100 mL milk in 4–60yr subjects (20). In Denmark experience, the dosage used for fortification of bread and milk could not prevent winter decline in serum 25(OH)D. However, the slope of decrease in supplemented group was much less than in the unsupplemented group (20). It is noteworthy that the initial mean concentration of serum 25(OH)D in Danish subjects was 73.1 nmol/L, which is considered almost adequate (24). Obviously a very high dose vitamin D-fortified food applied in some studies (19, 21, 22) cannot be used for general population. Consequently, this can mostly be applicable for targeted fortification program. Even in that case, pros and cons of fortification against supplementation, which could be more feasible and less expensive, must be carefully evaluated. The other important issue is that baking conditions of flat breads and bread buns and rolls are quite different. Iranian traditional flat breads are baked by direct heat exposure. It has been shown that vitamin D loss during cooking could be dependent upon the foodstuffs and cooking process (25). In bread buns and rolls, destruction of vitamin D during baking is close to zero (23) whereas our results showed some 50% destruction of vitamin D during baking process. We found almost no decline in vitamin D concentration in breads during 2 weeks storage in –20° fridge and even 3 months after conclusion of the study.

The effect of vitamin D on blood lipid profile has been examined in several studies (14, 26–28). Improvement of serum lipid profile and apolipoproteins of the subjects with diabetes following regular consumption of fortified yogurt drink has been already reported (27). The beneficial effect of vitamin D on serum triglycerides which has also been observed by other research groups (29) might be secondary to its association with lipoprotein lipase (30), the key enzyme of plasma triglycerides clearance. On the other hand, weekly consumption o f 50′000 IU vitamin D supplement for eight weeks could not improve lipid profile in adults with high cardiometabolic disease risk (31). In accord with this report, a large retrospective cohort study did not find any significant association between circulating 25(OH)D and lipid profile components (32). In a meta-analysis study of 12 clinical trials including 1346 subjects, no appreciable effect of vitamin D supplementation on serum cholesterol, triglycerides and HDL-C was found and the pooled estimated effect on LDL-C was only 3.23 mg/dL (33). The reasons for inconsistency of the results obtained from different studies could, at least in part, be difference in study population, dosages of vitamin D used, mode of administration (supplements, fortified food or drink) and, more importantly, initial vitamin D status. This latter could especially be a determinant of the outcome as we found an inverse association between baseline values of circulating calcidiol and its increase following supplementation. It is noteworthy that our subjects were mostly normolipidemic and the evaluation of the possible effects of vitamin D in dyslipidemic subjects deserves further specifically designed interventional studies (34).

We found a small, but significant, reduction in percent of visceral fat in both vitamin D supplemented groups. The association of circulating 25(OH)D with body fat has been reported in previous cross-sectional studies (35). The Framingham Heart Study revealed a strong association of vitamin D status with subcutaneous and, more importantly, visceral fat (36). Visceral fat area has been recently suggested as a risk factor for poor vitamin D status in men and premenopausal women (37). However, these cross-sectional studies cannot unveil whether low serum 25(OH)D concentration is a consequence or the cause of increased body fat. Early observations indicated low bioavailability of both dietary and endogenous vitamin D in adiposity due to deposition of 25(OH)D in body fat (38). A few interventional studies, on the other hand, have reported body and visceral fat loss due to vitamin D supplementation (39, 40). Though reduction in visceral fat does not necessarily result in a remarkable decrease in body weight, it can potentially bear a noticeable cardiometabolic benefit. Further studies on the effect of vitamin D on visceral fat are needed.

Some limitations of this study are acknowledged. The results of a short-term intervention cannot necessarily be extended to the long-term consumption of vitamin-D fortified bread. The effectiveness of flour fortification with vitamin D at the population level with inevitable variations in bread consumption must be further evaluated in a pilot study. Apparently fortification of several foods with low concentration of vitamin D would be more preferable and safer strategy than to fortify just a single food with a high dose.

Conclusion

Considering bread as a staple food in Iran, fortification of flour with vitamin D could be a potentially effective strategy to raise circulating 25(OH)D of the population to almost adequate levels. This could be accompanied by several health outcomes including improvement of lipid profile and a reduction of visceral fat. Future effectiveness studies are warranted.

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Authors' contributions: TRN supervised the whole project. BN and TRN designed the study with intellectual assistance of HH, ZA and FS. HPLC analyses were done by MZ. MeM conducted sensory evaluation study. Other laboratory works than HPLC as well as anthropometric measurements and subjects follow up were done by NS, AK, NL, MoM and SJMR. BN also performed the statistical analyses and prepared the preliminary draft of the manuscript, which was revised and finalized by TRN.

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