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ORIGINAL CONTRIBUTION

Vitamin D-fortified cooking oil is an effective way to improve vitamin D status: an institutional efficacy trial

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Abstract

Purpose High prevalence of vitamin D deficiency (VDD) justifies a cost-effective and sustainable strategy to combat VDD in the community. This study was undertaken for the first time to evaluate the efficacy of daily consumption of vitamin D fortified sunflower oil with a meal.

Methods This single-blind trial was conducted in two separate institutions: one as intervention (D-fortified sunflower oil) group (DO, $n_1 = 39$) and the other as control (unfortified sunflower oil) group (SO, $n_2 = 33$). Participants consumed their lunches cooked either with D-fortified or unfortified cooking sunflower oil (500 IU/30 g) for 12 weeks. Dietary, anthropometric and biochemical assessments were done for all participants before and after the intervention.

Results A total of 65 subjects from both sexes aged 32.5 ± 4 years completed the intervention period. Serum 25(OH)D showed a significant increase in DO and a decrease in SO group (8.8 ± 9.3 vs. -7.4 ± 6.4 ng/mL, $p < 0.001$). The rise in serum 25(OH)D in DO group was accompanied by a significant decrease in iPTH (DO: -10.2 ± 29.4 vs. SO: $+9.2 \pm 29.5$ pg/mL; $p = 0.009$). A significant reduction in weight ($p = 0.004$), BMI ($p = 0.029$), waist girth ($p < 0.001$), serum total cholesterol ($p = 0.0290$) and LDL-C ($p = 0.010$) was observed in DO, as compared with SO group.

Conclusions Cooking oil can be considered as an efficacious vehicle for mass fortification program to combat VDD. The improvement of vitamin D status may bring about betterment of certain cardiometabolic risk factors.

Registration number Clinicaltrials.gov: NCT03826654.

Keywords Vitamin D · Fortification · Sunflower oil · Efficacy trial

Introduction

Vitamin D, among all micronutrients, has a unique place in many aspects. It can be synthesized in the body through direct exposure to sun and that is why it is considered a hormone. However, urbanization has fundamentally broken down this natural relation between human and sun. On the other hand, dietary sources of the vitamin are quite limited and usually in quantities not sufficient to meet body needs. Consequently, vitamin D deficiency (VDD) has become prevalent globally including Iran. The National Food and Nutrition Surveillance (NFNS) reported that even during summer over 70% of the Iranian adults and children had vitamin D deficiency/insufficiency, based on serum calcidiol concentrations less than 25 and 50 nmol/L, respectively [1, 2]. Exploration of vitamin D receptor (VDR) in many tissues and unveiling of many other functions of vitamin D than so-called calcemic activities revealed the importance and

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influence of vitamin D on various aspects of human health. As a result, high prevalence of VDD for the health professionals now implies as the increased burden of disease in the affected population with its all consequences including increased economic health care costs. It is, therefore, not surprising that health stakeholders seek for a cost-effective as well as sustainable strategy to combat VDD in the community. Though vitamin D supplementation was instructed as a high priority action to be taken in such subpopulations as pregnant women, school children and elderly in Iran, many other people were left outside the supplementation umbrella. Sustainability of this approach was another challenging issue [3]. Consequently, food fortification as a more sustainable and cost-effective strategy attracted policy-makers' attention. Preliminary experiments with milk, orange juice and yogurt drink (*doogh*) as vehicles for targeted fortification encouraged market-driven fortification of these items with vitamin D [4, 5]. However, for a mass fortification program with wide population coverage, the vehicle must preferably be among the staple foods [6, 7]. From the very beginning, bread and cooking oil were considered for this purpose. Though the results of an efficacy trial on daily consumption of flat bread baked from D-fortified wheat flour were promising [8], there was no similar published report of D-fortified cooking oil. We, therefore, decided to conduct an efficacy study. Notwithstanding, there were several challenges to address initially. Firstly, Iranian cuisines commonly comprise mixed dishes that are prepared with different time and temperature conditions and we had no idea of the percent of destruction of added vitamin D in oil during these diverse conditions. Secondly, unlike bread, milk or orange juice, fortified oil could not be offered to the participants in a given amount as the oil would be used for cooking in a household and hence it was close to impossible to have even a rough estimation of the intake. To address the first issue, a separate study was performed by imitating different cooking conditions and measuring the retained amount of added cholecalciferol in the cooking oils [9]. To overcome the second problem, we decided to conduct the study in two institutions, ~ 40 km away from each other. All of the participants consumed the lunch prepared in their work place, one institution used fortified sunflower oil and the other used unfortified sunflower oil for cooking. The chefs of the institutions commonly know the exact rations of food items (including oil) being used per person. Using this information, we were able to calculate the amount of fortificant needed to make a significant rise in circulating 25(OH)D by consuming only one meal in the institution. To consider cooking oil as a vehicle for mass fortification, some questions have to be answered: (1) How bioavailable is added vitamin D from a mixed food prepared by a D-fortified cooking oil? (2) In case of improvement of vitamin D status due to D-fortified oil consumption, does it affect other health aspects notably some cardiometabolic risk factors including anthropometric measures and blood glucose and lipids? (3) What are the pros and cons of cooking oil versus wheat flour for a vitamin D mass fortification program? This study was undertaken to answer the first two questions and also to provide additional information to reply the third.

Subjects and methods

Study protocol

This single-blind parallel trial was conducted in two separate institutions both belonged to Kurosh incorporations. Employees in the central office in Tehran were allocated to the intervention group and workers in a factory in Eshtehard (~ 40 km away from west of Tehran) were included in control group. To invite participants, a general presentation session was held through which the whole protocol and objectives of the study were clarified for all employees. Those volunteers who met the inclusion criteria were then enrolled. The inclusion criteria were: (a) age 18–65 years; (b) having no clinical disease including diabetes, liver or kidney disorders; (c) not receiving vitamin D, fish oil or omega-3 supplements at least 3 months before intervention; (d) as for women not being pregnant or lactating. The exclusion criteria included: (a) unwillingness to complete the intervention period at any time during the experiment; (b) being affected by any clinical conditions that interfered with vitamin D metabolism notably diabetes, liver or kidney disease as well as malignancies; (c) not having the prepared lunch at the work place for 3 or more consecutive days. As a whole, 73 subjects were enrolled, 39 in D-fortified (DO) and 33 in unfortified sunflower oil (SO). After a 2 week run-in period, anthropometric assessments were done and fasting blood as well as spot urine samples were taken from all participants. In both caterings, two types of meals were prepared and served. Meal one was without D-fortified oil and meal two was prepared either with fortified oil (intervention group, Tehran) or with regular unfortified oil (control group, Eshtehard). Thus, those employees who were unwilling to participate in the study could have meal one while those enrolled in the study would have meal two. No modification was made in the weekly menus of the two institutions or the way of cooking foods. The duration of the experiment was 12 weeks. At the end of intervention period, all assessments together with a dietary intake assessment were done again for those participants completed the study. All participants signed a written informed consent. Ethical approval was obtained from Ethical Committee of the National Nutrition and Food Technology Research Institute (code: IR.SBMU.

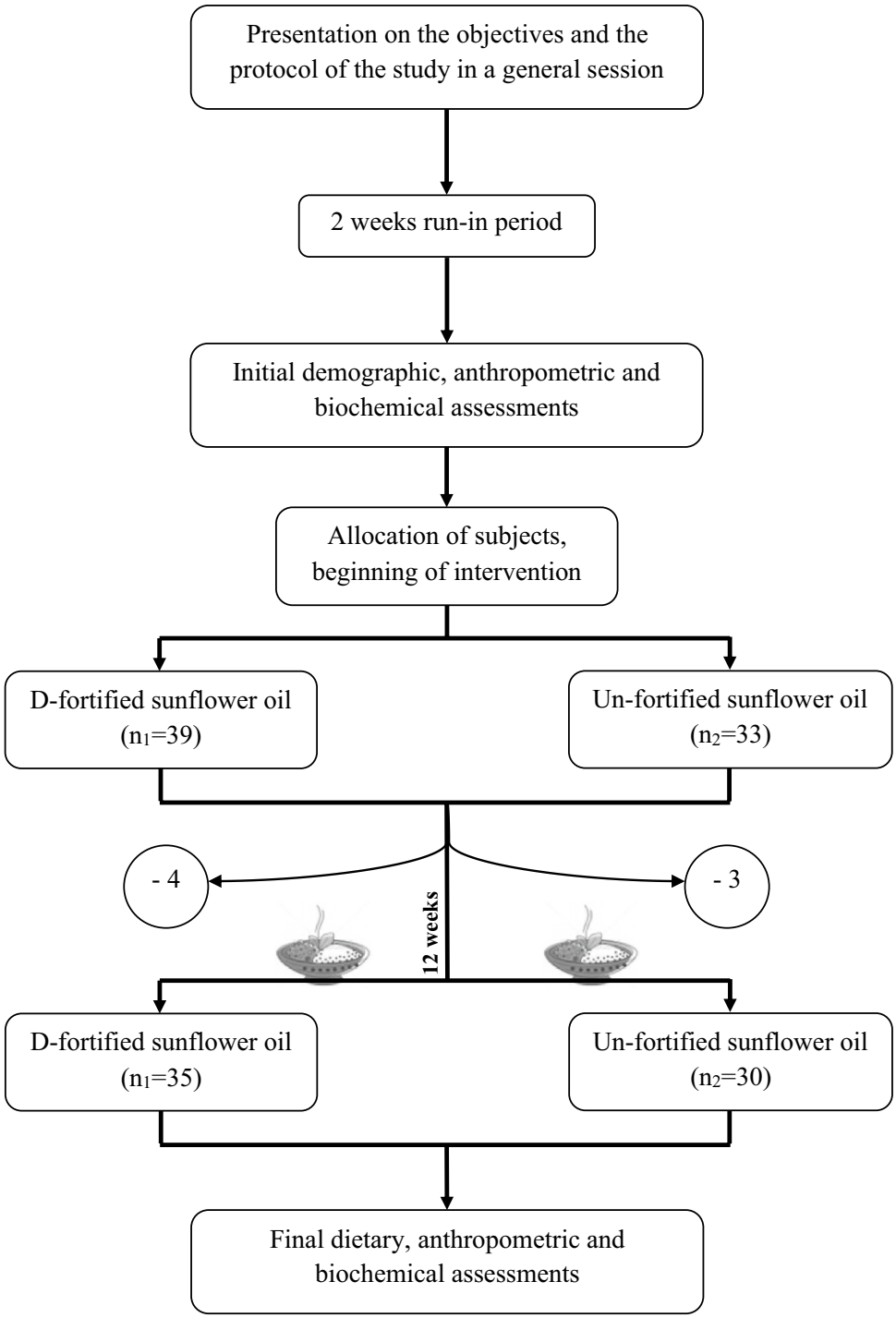

Fig. 1 Protocol of the study at a glance

nnftri.Rec.1396.167). This study is registered at ClinicalTrials.gov (NCT03826654). Figure 1 shows the study protocol in brief.

Fortification of cooking oil

Considering that the about 30% of added vitamin D in canola, corn and sunflower oils may be destroyed during cooking (160 °C for 60 min) [9] and the ration of cooking oil was weighed up 30 g for each person, the cooking oil was fortified by cholecalciferol so that even with 30% destruction, each participant would receive ~ 12.5 µg (= 500 IU) vitamin D by eating up his or her lunch. In this study sunflower oil was fortified as it was the usual cooking oil used in the caterings.

Dietary assessment

To assess the consumption amount of the food prepared by D-fortified sunflower oil, a multiple choice questionnaire was prepared and given to each participant. All subjects were instructed to check the appropriate box in the form denoting the amount of food left in his/her plate as "nothing left, half or 3/4" on a daily basis throughout 12 weeks intervention period. To evaluate the possible within- and between-group changes of dietary intake, a 24 h dietary recall for 2 different days was taken from all participants in both groups just before and after the intervention. Dietary intakes were translated to energy and nutrients using Nutritionist IV software (version 4.1, 1997; First DataBank, The Hearst Corp).

Anthropometric measurements

Weight was measured with light clothing and no shoes using a digital scale to the nearest of 0.1 kg. Height was measured by a stadiometer to the nearest of 0.1 cm. Waist girth was measured using a measuring tape to the nearest of 0.1 cm. Body mass index was calculated by dividing weight (kg) by the square of height (m).

Laboratory tests

Blood sampling and handling

Six milliliters of fasting blood taken from antecubital vein of each subject was collected in a test tube without anticoagulant. Blood samples were kept in a cold box for transportation to the Laboratory of Nutrition Research, NNFTRI. Clot samples were centrifuged at 800*g* at room temperature for 20 min. Sera thus separated were aliquoted in clean microtubes. One of the microtubes was used for serum glucose and lipids measurements on the same day while the other microtubes were kept at − 80 °C until the day of analysis. Table 1 shows performance characteristics of the commercial kits used in this study.

Circulating 25(OH)D and intact parathyroid hormone (iPTH)

Serum 25(OH)D was measured using enzyme immunoassay (EIA) and a commercial kit (Monobind, CA, USA). The accuracy of the test results was checked by high-performance liquid chromatography (HPLC) [10]. To do this, we made several pooled sera from each 7–8 serum samples and analyzed them by HPLC to check the comparability of the mean results. Serum iPTH was determined by an EIA commercial kit (Euroimmun, Leubeck, Germany).

Serum glucose and lipid profile components

Fasting serum glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined using commercial kits (all from Pars-Azmoon, Tehran, Iran) and an auto-analyzer (Selectra E; Vitalab, Holliston, Netherlands).

Urinary analytes

To evaluate the possible effect of vitamin D intake on urinary calcium [11, 12] and hence sodium and potassium excretion [13], we analyzed urine samples of all subjects before and after the intervention. A fasting spot urine sample was analyzed. Sodium and potassium was determined by flame photometry (Corning 480). Calcium and creatinine were measured in an appropriately pre-diluted urine sample using colorimetric method (Pars-Azmoon, Tehran, Iran) and an auto-analyzer (Selectra E; Vitalab, Holliston, Netherlands). We used the ratio of urinary electrolytes to creatinine to eliminate the effect of urine density on the concentrations of the analytes in a spot sample, as reported earlier [8].

Table 1 Performance characteristics of the commercial kits according to their manufacturers

Test	Manufacturer	Sensitivity	Intra-assay CV	Inter-assay CV
25(OH)D	Monobind (USA)	0.67 ng/mL	< 6.36%	< 6.95%
iPTH	Euroimmun (Germany)	0.1 pg/mL	< 9.5%	< 11.0%
Glucose	Pars Azmoon (Iran)	5 mg/dL	< 1.74%	< 2.0%
Triglycerides	Pars Azmoon (Iran)	5 mg/dL	< 1.82%	< 2.0%
Cholesterol	Pars Azmoon (Iran)	5 mg/dL	< 1.62%	< 2.0%
HDL-C	Pars Azmoon (Iran)	1 mg/dL	< 0.82%	< 1.8%
LDL-C	Pars Azmoon (Iran)	2 mg/dL	< 0.67%	< 1.45%
Calcium	Pars Azmoon (Iran)	0.4 mg/dL	< 1.73%	< 2.01%
Creatinine	Pars Azmoon (Iran)	0.2 mg/dL	< 2.38%	< 3.63%

Statistical analyses

Normality of data distribution was assessed by Shapiro–Wilk test. Descriptive statistics were expressed as mean values with standard deviation or as absolute numbers with percentages for categorical variables. Paired samples *t* tests were performed to assess within-group comparisons. Analysis of covariance (ANCOVA) was used to evaluate the differences between groups in continuous outcome variables at the end of intervention with baseline measures of each variable as covariate. Statistical analyses were performed using SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA) and two-sided $p < 0.05$ were considered statistically significant.

Results

A total of 73 subjects from both sexes aged 32.5 ± 4 years were enrolled but 65 completed the intervention period. The main reason of attrition of the subjects was absence at work due to leave or mission for more than 3 consecutive days. There was no significant between-group difference in distribution of age, sex and sun exposure behaviors (Table 2).

Table 2 Baseline characteristic of the participants in groups

Variable	DO ($n=39$)	SO ($n=34$)	p value
Age	32.5 ± 5.1	32.5 ± 3.5	0.930
Sex			
Male *n* (%)	24 (64.1)	27 (79.4)	0.198
Female *n* (%)	14 (35.9)	7 (20.6)	
Sun exposure (%)			
> 1 hr	5 (12.8)	5 (14.7)	1.00
< 1 hr	34 (87.2)	29 (85.3)	
Time of sun exposure (%)			
10 AM–3 PM	15 (38.5)	19 (55.9)	0.163
Others	24 (61.5)	15 (44.1)	
Sunscreen use (%)			
Yes	6 (15.3)	2 (5.9)	0.302
No	33 (84.7)	32 (94.1)	

Between-group comparisons (Independent sample *t* test for quantitative variables and Chi-square test for qualitative variables)

DO vitamin D-fortified oil, *SO* plain oil

Dietary intake

In DO group, 71.4% of the subjects and in SO group 73.1% had consumed at least 90% of their lunch served at their work place. None of the participants reported less than 50% consumption of their meals during the intervention period. There was no significant within- and between-group difference in dietary energy and nutrient intakes (Table 3).

Circulating 25(OH)D and iPTH

Serum 25(OH)D showed a significant increase in DO (from 35.1 ± 9.0 to 44.0 ± 12.4 ng/mL, $p < 0.001$) and a significant decrease in SO group (from 38.2 ± 7.8 to 30.7 ± 7.9 ng/mL, $p < 0.001$) and between group difference was significant (8.8 ± 9.3 vs. − 7.4 ± 6.4 ng/mL, $p < 0.001$). The rise in serum 25(OH)D in DO group was accompanied by a significant decrease in iPTH whereas in SO, serum iPTH

Table 3 Studied selected nutrient intake at baseline and after the intervention

Variable	DO ($n_1=35$)		p^a	SO ($n_2=30$)		p^a	p^b
	Before	After		Before	After		
Energy (kcal/day)	1683.4 ± 534	1765 ± 781	0.600	1923.5 ± 853	1884.2 ± 499	0.819	0.671
Carbohydrate (g/day)	246.1 ± 84.0	233.7 ± 110	0.611	349.5 ± 169.7	321.9 ± 144.3	0.290	0.064
Protein(g/day)	69.4 ± 22.7	76.3 ± 30.4	0.270	88.7 ± 51.4	76.7 ± 23.8	0.190	0.663
Fat (g/day)	48.3 ± 22.8	50.6 ± 30.1	0.733	44.7 ± 28.0	58.2 ± 25.7	0.052	0.281
SFA (g/day)	15.2 ± 6.6	15.6 ± 9.5	0.858	15.0 ± 8.8	16.5 ± 7.6	0.290	0.661
PUFA (mg/day)	8.4 ± 9.0	7.9 ± 7.3	0.773	8.0 ± 7.4	10.4 ± 5.4	0.059	0.108
MUFA (mg/day)	14.6 ± 7.3	16.7 ± 12.3	0.385	12.7 ± 7.9	15.9 ± 9.4	0.192	0.800
Cholesterol (mg/day)	180.2 ± 115	210.9 ± 155.5	0.327	198.4 ± 348	273.8 ± 212	0.357	0.187
Vitamin D (μg/day)	0.4 ± 0.7	0.6 ± 1.0	0.385	0.6 ± 1.0	0.82 ± 1.1	0.395	0.514
Calcium (g/day)	607.7 ± 229.2	563.9 ± 429	0.637	671.3 ± 279	643.7 ± 319	0.724	0.411

All values are means ± SDs

DO vitamin D-fortified sunflower oil, *SO* Plain (unfortified) sunflower oil, *SFA* saturated fatty acid, *PUFA* poly unsaturated fatty acid, *MUFA* mono unsaturated fatty acid

[a]Comparison of within-group changes (paired-samples *t* test)

[b]Comparison of between-group changes (analysis of covariance test with baseline values as covariates)

did not differ significantly by the end of intervention period. Between-group comparison by ANCOVA revealed a significant difference in serum iPTH (− 10.2 ± 29.4 vs. + 9.2 ± 29.5 pg/mL; $p = 0.009$).

Anthropometric measures

After 12 weeks intervention, a significant reduction in weight ($p = 0.004$) and BMI ($p = 0.005$) was observed in DO which was accompanied by a significant reduction in WC ($p < 0.001$). No significant changes in anthropometric measures were observed in SO group. Between-group differences of weight ($p = 0.031$), BMI ($p = 0.029$) and WC ($p < 0.001$) were statistically significant (Table 4).

Serum glucose and lipids and urinary electrolytes

Fasting serum glucose showed a significant decrease in DO group ($p < 0.001$). However, between-group comparison revealed no significant difference ($p = 0.581$). Total cholesterol and LDL-C concentrations both had a significant within-group reduction in DO ($p < 0.001$ for both) as well as a significant between group difference ($p = 0.09$ and $p = 0.010$, respectively). As for HDL-C, there was a small but significant reduction in SO group ($p = 0.004$) and no significant between-group difference ($p = 0.136$). No significant within- or between-group changes were found in serum triglycerides concentrations. The ratios of urinary calcium, sodium and potassium to creatinine did not show any significant changes throughout the intervention period. Table 3 shows the initial and final measures of the studied variables.

Discussion

Consumption of lunch prepared with D-fortified sunflower oil for 12 weeks resulted in almost 8.8 ng/mL increment in circulating 25(OH)D in DO group. Considering our previous experiences and other reports that for each 100 IU (2.5 µg) vitamin D intake through fortified foods there is 1.2–1.76 ng/mL rise in serum 25(OH)D [14, 15], the amount of 25(OH)D increment in this study corresponds to daily intake of 500–733 IU vitamin D. In support of this finding, a recent meta-analysis study reported that with the fortification dosage of 25 µg/day, the efficacy effect is 0.56 ng/mL (1.4 nmol/L) for each 40 IU (1 µg)/day vitamin D consumed [16]. Therefore, with 8.8 ng/mL increase in serum 25(OH)D in DO group, the average intake of vitamin D through fortified sunflower oil is estimated ~ 628 IU/day.

The varying amount of vitamin D intake in this study could be partly due to within- and between-individual variations of portion sizes during the intervention period and also due to varying retention percent of added vitamin D during

Table 4 Studied characteristics at baseline and after the intervention

Variable	DO ($n_1 = 35$)		p^a	SO ($n_2 = 30$)		p^a	p^b
	Before	After		Before	After		
25(OH)D (ng/mL)	35.1 ± 9.0	44.0 ± 12.4	< 0.001	38.2 ± 7.8	30.7 ± 7.9	< 0.001	< 0.001
iPTH (pg/mL)	54.8 ± 26.9	44.5 ± 28.8	0.044	50.6 ± 20.9	59.8 ± 24.3	0.098	0.009
Weight (kg)	78.6 ± 13.6	76.9 ± 12.8	0.004	78.5 ± 12.2	78.3 ± 12.0	0.293	0.031
BMI (kg/m^2)	26.8 ± 3.2	26.2 ± 3.0	0.005	26.8 ± 3.6	26.7 ± 3.7	0.337	0.029
WC (cm)	96.8 ± 10.4	92.2 ± 9.4	< 0.001	93.6 ± 8.4	94.3 ± 8.9	0.412	< 0.001
Glucose (mg/dL)	83.3 ± 7.3	73.5 ± 7.0	< 0.001	74.8 ± 9.4	71.2 ± 10.3	0.059	0.581
TG (mg/dL)	121.6 ± 67.1	123.4 ± 68.1	0.817	137.7 ± 67.9	135.1 ± 69.0	0.786	0.896
TC (mg/dL)	185.0 ± 28.2	166.9 ± 31.5	< 0.001	189.9 ± 30.1	182.8 ± 32.6	0.061	0.029
LDL-C (mg/dL)	109.6 ± 21.4	95.2 ± 26.7	< 0.001	111.7 ± 20.4	109.2 ± 23.7	0.462	0.010
HDL-C (mg/dL)	51.0 ± 9.4	50.3 ± 9.0	0.729	49.3 ± 7.2	46.5 ± 8.6	0.004	0.136
UCa/Cr (mg/mg)	0.06 ± 0.02	0.07 ± 0.05	0.075	0.07 ± 0.04	0.07 ± 0.02	0.881	0.621
UNa/Cr (mg/mg)	0.7 ± 0.3	0.83 ± 0.45	0.059	0.64 ± 0.33	0.8 ± 0.44	0.126	0.918
UK/Cr (mg/mg)	0.48 ± 0.16	0.54 ± 0.21	0.135	0.52 ± 0.34	0.41 ± 0.32	0.212	0.055

All values are means ± SDs

FO vitamin D-fortified sunflower oil, *PO* plain (unfortified)sunflower oil, *25(OH)D* 25-hydroxyvitamin D, *iPTH* intact parathyroid hormone, *BMI* body mass index, *WC* waist circumference, *TG* triglycerides, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *UCa/Cr* urinary calcium to creatinine ratio, *UNa/Cr* urinary sodium to creatinine ratio, *UK/Cr* urinary potassium to creatinine ratio

[a]Comparison of within-group changes (paired-samples *t* test)

[b]Comparison of between-group changes (analysis of covariance test with baseline values as covariates)

various cooking processes (including temperature and time) of Iranian cuisines.

Some studies reported various retention percent of vitamin D in different foods and cooking conditions, from 39 to 45% after heating in oven for 40 min to 82–84% after frying [17]. In a laboratory study, there was a decrease in retention percent of added vitamin D in fortified sunflower oil during cooking in 105 °C by increasing cooking time, from 85.8% after 60 min to 83.1% after 180 min. Similarly, during frying at 160 °C the retention percent of added vitamin D in fortified sunflower oil from 86.5% after 5 min decreased to 68.6% after 60 min [9]. In preparation of a mixed Iranian dish with diverse ingredients, various times and temperatures are applied so the amount of intact added vitamin D in the final dish could actually vary. In the current study, the sunflower oil was fortified with the assumption of a maximum 30% destruction of vitamin D during cooking. Our findings indicate that the destruction percent of added vitamin D is actually much less on average.

A significant decline in serum 25(OH)D concentrations in SO group is noticeable. As the study was performed in winter (from late December to mid March), this decline was expectable. Seasonal variation of vitamin D status in Iranian adults and children has already been reported [18, 19]. Daily consumption of a meal prepared with D-fortified sunflower oil not only prevented winter decline of serum 25(OH)D but remarkably improved vitamin D status, as well.

The changes in circulating 25(OH)D were reciprocally accompanied by significant changes in serum concentrations of iPTH (a significant decline in DO as compared with an insignificant rise in SO). The results of a meta-analysis study revealed that daily intake of 1000 IU vitamin D can bring serum PTH concentrations down [20]. In this study, our subjects were mostly over-weight, as judged by the mean BMI. Notwithstanding, daily consumption of D-fortified sunflower oil could suppress serum iPTH by an average of 10.2 pg/mL. A dose–response study reported that daily intake of 800 IU vitamin D can lift serum 25(OH)D to above 20 ng/mL and suppress PTH in 97.5% of postmenopausal women [21].

Daily consumption of D-fortified sunflower oil interestingly resulted in significant healthy changes of anthropometric measures in DO compared to SO group. The improving effect of daily consumption of yogurt drink and bread fortified with vitamin D on anthropometric measures including waist circumference has already been reported [8, 16, 22]. These findings may be explained by functions of vitamin D in adipose tissue including regulation of adipocyte apoptosis and energy metabolism as well as suppression of inflammation [23]. Animal studies indicate that vitamin D deficiency may enhance lipogenesis and suppress hepatic β-oxidation thereby predispose to weight gain [24]. In support of this, other experimental studies revealed the suppressing effect of vitamin D on lipogenesis and weight gain [25]. The effects of vitamin D on adipose tissue and energy metabolism deserve further studies.

Raised circulating 25(OH)D in DO group was accompanied by a significant reduction in fasting serum glucose concentrations. However, this reduction was not statistically significant compared to the corresponding changes in SO group. The glycemic optimizing effect of vitamin D intake through fortified yogurt drink in the subjects with type 2 diabetes (T2D) has been already reported [5].In the same line of evidence, several meta-analysis studies concluded that vitamin D may be beneficial in improving glycemic and insulinemic status in diabetes [26–28] as well as in polycystic ovarian syndrome [29]. Some experimental studies suggested that vitamin D may selectively inhibit certain signaling pathways within skeletal muscles (like NF-κB) and thereby improve insulin resistance in murine model [30].However, the effect of vitamin D intake on glucose homeostasis is still the subject of debate. In a recent randomized clinical trial, supplementation with a single mega-dose of vitamin D did not result in any improvement in insulinemic response of T2D subjects [31]. Accordingly, a meta-analysis failed to find any optimizing effect of vitamin D supplementation on glucose and insulin metabolism in the subjects with overweight or obesity [32]. Baseline concentrations of 25(OH)D, mode of vitamin D administration (oral vs. injection) and dosages used may, at least in part, explain these discrepancies.

The lowering effect of D-fortified sunflower oil consumption on serum total and LDL-cholesterol is also noteworthy. Observational studies revealed an association between vitamin D status and components of blood lipid profile in Iranian children and adults [19, 33]. Similar studies from other countries supported the link between VDD and increased risk of dyslipidemia [34]. These observations were further endorsed by the findings obtained from RCTs showing the improvement of lipid profile through consumption of D-fortified yogurt drink or bread [5, 8, 35, 36]. Several meta-analyses concluded the improving effect of vitamin D replenishment on some components of lipid profile [37] notably LDL-C [38] with almost no favorable effect on HDL-C. In contrast, a recent randomized clinical trial put quite different results forward. In that study, 200 subjects with hypertension and circulating 25(OH)D concentrations below 30 ng/mL were divided in two groups to receive either 2800 IU vitamin D or placebo. After 8 weeks, total cholesterol and triglycerides significantly increased in supplemented group [39]. The interaction of vitamin D and blood lipids is very complex [40] and needs further elucidations.

On the whole, our findings revealed the efficacy of D-fortified cooking oil and endorsed the previously reported association of vitamin D status and certain cardiometabolic risk factors [19, 33]. However, some limitations of this study

must be acknowledged. The short term effect of consumption of D-fortified sunflower oil may not easily be extended for a longer period of time. Current recommendations by scientific bodies to reduce fat intake may limit the usefulness of cooking oils as suitable vehicles for a mass fortification program.

Conclusions

To the best of our knowledge, this is the first report of the efficacy of D-fortified cooking oil. We concluded that cooking oil can be considered as an efficacious vehicle for vitamin D fortification to combat VDD at the community level. Added vitamin D seems to have more retention in the cooking oil than in flat breads [8, 9]. However, a rather wide range of various types of fats and oils used for cooking in Iran, and perhaps other countries, together with dietary recommendations to reduce fat intake may both limit the effectiveness of cooking oil compared with other staple foods, mainly bakery's flour, as a suitable vehicle for vitamin D fortification. Ideally, both vehicles may be considered for a successful fortification program. Finally, improvement of vitamin D status may bring about betterment of certain cardiometabolic risk factors and thus may reduce the overall burden of disease.

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Author contributions The authors' responsibilities were as follows—TRN and BN: designed and supervised the study. TRN, BN, AK, NS, MZ: were involved in all stages of the research, including all laboratory bench works; AZ, AJ and MK: were involved in field works. TRN, BN and BH: interpreted the data and prepared the final manuscript.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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