Clinical Nutrition 35 (2016) 67–76

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Effects of vitamin D-fortified low fat yogurt on glycemic status, anthropometric indexes, inflammation, and bone turnover in diabetic postmenopausal women: A randomised controlled clinical trial

Tina Jafari [a, 1], Elham Faghihimani [b, 1], Awat Feizi [c], Bijan Iraj [b], Shaghayegh Haghjooy Javanmard [d], Ahmad Esmaillzadeh [a], Aziz A. Fallah [e], Gholamreza Askari [a, *]

[a] Food Security Research Center and Department of Community Nutrition, School of Nutrition & Food Science, Isfahan University of Medical Sciences, Isfahan, Iran
[b] Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[c] Department of Epidemiology and Biostatistics, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran
[d] Department of Physiology, Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[e] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord 34141, Iran

ARTICLE INFO

Article history:
Received 5 August 2014
Accepted 23 February 2015

Keywords:
Type 2 diabetes mellitus
Vitamin D fortification
Low fat yogurt
Glycemic status
Inflammation
Bone turnover

SUMMARY

Background & aims: Low levels of serum 25-hydroxy vitamin D (25(OH)D) are common in type 2 diabetic patients and cause several complications particularly, in postmenopausal women due to their senile and physiological conditions. This study aimed to assess the effects of vitamin D-fortified low fat yogurt on glycemic status, anthropometric indexes, inflammation, and bone turnover in diabetic postmenopausal women.

Methods: In a randomized, placebo-controlled, double-blind parallel-group clinical trial, 59 postmenopausal women with type 2 diabetes received fortified yogurt (FY; 2000 IU vitamin D in 100 g/day) or plain yogurt (PY) for 12 weeks. Glycemic markers, anthropometric indexes, inflammatory, and bone turnover markers were assessed at baseline and after 12 weeks.

Results: After intervention, in FY group (vs PY group), were observed: significant increase in serum 25(OH)D and decrease of PTH (stable values in PY); significant improvement in serum fasting insulin, HOMA-IR, HOMA-B, QUICKI, and no changes in serum fasting glucose and HbA_{1c} (significant worsening of all indexes in PY); significant improvement in WC, WHR, FM, and no change in weight and BMI (stable values in PY); significant increase of omentin (stable in PY) and decrease of sNTX (significant increase in PY). Final values of glycemic markers (except HbA_{1c}), omentin, and bone turnover markers significantly improved in FY group compared to PY group. Regarding final values of serum 25(OH)D in FY group, subjects were classified in insufficient and sufficient categories. Glycemic status improved more significantly in the insufficient rather than sufficient category; whereas the other parameters had more amelioration in the sufficient category.

Conclusions: Daily consumption of 2000 IU vitamin D-fortified yogurt for 12 weeks improved glycemic markers (except HbA_{1c}), anthropometric indexes, inflammation, and bone turnover markers in postmenopausal women with type 2 diabetes.

Trial registration: www.irct.ir (IRCT2013110515294N1).

Abbreviations: 25(OH)D, 25-hydroxy vitamin D; FM, fat mass; FY, fortified yogurt; FSG, fasting plasma glucose; HC, hip circumference; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-B, HOMA of beta cell function; hs-CRP, highly sensitive C-reactive protein; PY, plain yogurt; QUICKI, quantitative insulin sensitivity check index; sBAP, bone alkaline phosphatase; sNTX, N-terminal type-1 collagen; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; METs, metabolic equivalents; WC, waist circumference; WHR, waist to hip ratio.

* Corresponding author. Tel./fax: +98 31 37922776.
E-mail address: askari@mui.ac.ir (G. Askari).

[1] Co-first authors (the authors are contributed to the work equally).

http://dx.doi.org/10.1016/j.clnu.2015.02.014

1. Introduction

International Diabetes Federation has predicted a rise in the number of diabetic patients throughout the world from 387 in 2014 to 592 million in 2035. In Iran, 8.43% (more than 4 million) of adults suffered from type 2 diabetes mellitus (T2DM) [1]. A great deal of therapeutic expenses is dedicated to diabetes and its complications throughout the world [2]. Postmenopausal diabetic women are more vulnerable due to several factors such as aging, probable co-morbidities such as osteoporosis, and socio-economic conditions.

It has been demonstrated that there is an inverse association between serum 25(OH)D levels and the risk of T2DM [3]. Vitamin D plays an important role in insulin resistance. It also has modulatory effects on growth and differentiation of cells involved in immune-response as well as in production of inflammatory and anti-inflammatory cytokines [4]. Therefore, vitamin D deficiency is associated with autoimmune and inflammatory diseases like diabetes and metabolic syndrome. Vitamin D status is defined on the basis of serum concentrations of 25-hydroxy vitamin D (25(OH)D) as deficient i.e.<50 nmol/l (20 ng/ml), insufficient i.e. 50–74 nmol/l (21–29 ng/ml), and sufficient i.e.>75 nmol/l (30 ng/ml) [5].

The major source of vitamin D in human is cutaneous synthesis. Factors such as exposure duration, season, latitude, aging, skin pigmentation, and continuous usage of sunscreens may affect vitamin D synthesis. People also receive vitamin D from foodstuffs such as oily fish, fish liver oil, wild mushrooms, and egg yolk, which are the richest sources of vitamin D but some of these foods are not part of the usual intake in many countries. The average intake of vitamin D varies from one country to another, due to the differences in dietary patterns and food fortification rights. This intake seems to be higher in the countries fortifying foodstuffs [6]. In Iran, fortification of foods with vitamin D is not customary. According to the latest advice from Institute of Medicine, a recommended dietary allowance (RDA) of 600 IU/day of vitamin D is needed for ages 1–70 y to provide at least serum 25(OH)D of 50 nmol/l [7]. The Endocrine Society recommended 600 IU/day based on bone health and muscle function protection; however, it is unknown whether the mentioned dose is enough to supply all the potential non skeletal functions of vitamin D [5]. The Society has also stated that consistent daily intake of at least 1500–2000 IU/day is needed to raise serum 25(OH)D above 75 nmol/l; hence it is necessary to improve vitamin D intakes via supplementation or food fortification.

High prevalence of vitamin D deficiency or insufficiency among Iranian population [8] and lack of accessible vitamin D-fortified foodstuffs warrants conducting scientific-based studies to introduce suitable staple foods for vitamin D fortification. Recently, some clinical trials have been carried out in Iran on the effect of vitamin D fortified foods, like Persian yogurt drink (Doogh; consists in plain yogurt, water, and salt), milk, and orange juice [9,10]. This study aimed to assess the effects of vitamin D-fortified low fat yogurt on glycemic control, anthropometric indexes, inflammation, and bone turnover in diabetic postmenopausal women. Yogurt could be a good choice for vitamin D fortification due to extensive consumption among Iranian people and also an appropriate replacement for milk in subjects who are not able to consume it. Moreover, unlike Doogh (1.6 g/100 g fat and 380 mg/100 g Na) or sugary juices, low-fat yogurt (1.4 g/100 g fat and 65 mg/100 g Na) is safe for those who suffer from diseases like hypertension or diabetes.

2. Methods

2.1. Study design and participants

This single center study was a randomized, placebo-controlled, double-blind parallel-group clinical trial on diabetic postmenopausal women registered at Isfahan Endocrine and Metabolism Research Center. To calculate the sample size, suggested formula for parallel-design randomized controlled trial was used based on $\alpha = 0.05$, 90% power, and a standardized effect size of $\Delta = 1$ in NTX [11] as a key variable. We reached to 22 participants per group.

We studied 148 medical records of diabetic women who did not use insulin. The diagnosis of T2DM was based on WHO criteria [12]. Among the records, postmenopausal women who had not menses for at least 12 months were selected. The cases were enrolled in the study if they met these inclusion criteria: (*i*) not taking vitamin D, calcium, or omega-3 supplements within the past 3 months before the intervention, (*ii*) not taking drugs which have obvious interaction with vitamin D or influence its metabolism i.e. corticosteroids or estrogens, (*iii*) baseline serum 25(OH)D < 125 nmol/l, and (*iv*) not having history of malignancy, renal failure, liver, endocrinologic, or inflammatory disorders. All subjects had to spend a 3 weeks run-in period during which they were instructed by a dietitian to follow a weight-maintenance diet according to American Diabetes Association guidelines [13]. After that period, subjects who had weight changes were excluded and the others were randomly divided to 2 groups. The equivalent amounts of dairy products were replaced by 1 serving (100 g) per day of low-fat yogurt in their diet. During the intervention, the exclusion criteria were: (*i*) any change in type or dosage of oral anti-diabetic drugs or usage of insulin, (*ii*) intake of vitamin D, calcium or omega-3 supplements, and (*iii*) disobedience to the study protocol.

2.2. Study protocol

The study protocol was approved by the Ethical Committee of Research, Isfahan University of Medical Sciences on 19 July 2013 (registration number: 192015). The study protocol and its progress were recorded at www.irct.ir (registration ID: IRCT2013110515294N1). At first, the study protocol and objectives were fully explained to each subject and then written informed consent was obtained from all participants.

2.3. Randomization and blinding

Study's enrolled patients underwent permuted block randomization. Each block had permuted, even-numbered, randomly varying block sizes with 1:1 allocation ratio. The block sizes were concealed till the end of the study. Subjects were randomly allocated to the 'FY' (received vitamin D-fortified low fat yogurt, containing 2000 IU vitamin D in 100 g) or 'PY' (received plain low fat yogurt without additive) treatment groups. The random sequence was generated by an investigator uninvolved in recruiting subjects. Both participants and investigators were blinded to the content of interventions.

2.4. Outcome measurements

The project was launched at late fall (December 2013), going on during the winter, and finished after 12 weeks of intervention in the middle of March, 2014 in order to minimize the cutaneous synthesis of vitamin D. Participants consumed one serving of low

fat yogurt (vitamin D-fortified or plain) every day throughout the intervention period. Blood samples were taken at the beginning and at the end of the intervention, between 7:30 and 8:30 AM, while subjects were fast for more than 12 h. The sera samples were isolated and kept at −80 °C prior to analyses.

2.5. Laboratory measurements

Fasting serum glucose (FSG), total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (TG) were measured using enzymatic methods. Serum highly sensitive C-reactive protein (hs-CRP) was determined by immunoturbidimetric assay. The commercial kits from Pars Azmun Inc. (Tehran, Iran) were used to perform the tests. Serum insulin levels were determined by radioimmunoassay (ADVIA Centaur CP, USA). HbA_{1c} was separated by ion-exchange chromatography and measured by a colorimetric method (DS5 Analyzer, England). Serum parathyroid hormone (PTH) (DiaMetra, Milan, Italy), bone alkaline phosphatase (sBAP), N-terminal type-1 collagen (sNTX), and omentin (Eastbiopharm Company, USA), and serum 25(OH)D (LDS Ltd., USA) were determined by ELISA.

Homeostasis model assessment of insulin resistance (HOMA-IR), Homeostasis model assessment of β cell function (HOMA-B), and quantitative insulin sensitivity check index (QUICKI) were calculated according to the suggested equations. Anthropometric indexes were measured as follows: weight and percentage of body fat mass (FM) were measured by using an electrical body analyzer (Body composition Analyzer, ioi 353, JAWON MEDICAL, Korea) with light clothes and without shoes. Height was measured with a stadiometer to the nearest of 0.1 cm. BMI was calculated as weight in kg/(height in meter)^2. A measuring tape to the nearest of 0.1 cm was used to assess waist circumference (WC) and hip circumference (HC). WC was measured at the midpoint of lower rib and iliac crest when the patients were at the end of breathing out. HC was measured from where the buttocks protrude the most.

2.6. Yogurt manufacture

Preparation and fortification of low fat yogurt were performed in Allas Dairy Products Company (Allas Dairy, Isfahan, Iran). Energy, protein, fat, Na, and Ca contents of 100 g low fat yogurt were 57 kcal, 3 g, 1.4 g, 65 mg, and 150 mg, respectively. Vitamin D used for fortification was a powder "Dry Vitamin D_3 100 SD/S" (contained 100,000 IU Vitamin D_3 per gram, suitable for fortification of water-based foods, product code: 5010950.304; DSM Nutritional Products Ltd., Basel, Switzerland). Concentrations of vitamin D and its stability in yogurt had been checked at the first day of production, after 1, and 2 weeks of refrigerated storage. The results demonstrated that vitamin D was stable during the product shelf-life.

Participants were visited once a week to assess their compliance and receive 7 packets of low-fat yogurt for consumption. They were instructed to tick the consumption calendar, every day. The calendar was planned to make sure that subjects will consume the yogurt regularly. They were also asked to return the empty packets on their next visit.

Demographic data, duration of daily sun exposure, durations of diabetes and menopause, concomitant diseases, drugs, and smoking habits were collected using questionnaires.

2.7. Dietary intake and physical activity assessment

Dietary intake and physical activity of the participants were monitored by an expert dietitian at the beginning, weeks 3, 6, 9, and at the end of the intervention period (including a weekend) using a 24-h recall questionnaire. To derive energy and nutrients, all dietary data were converted to gram and entered to Nutritionist 4 software (based on United States Department of Agriculture (USDA) food composition table and modified for Iranian foods). Then the average of 5 day-dietary recalls was expressed as dietary intake (Table S1). Metabolic equivalent (MET) value for each physical activity [15] was multiplied by the duration of the activity (MET h^{-1} d^{-1}), and the average of 5 day−physical activity recalls was reported (Table 1).

2.8. Statistical analyses

Quantitative data were expressed as mean ± SE, while qualitative data as number and percentage. Normality of studied variables was evaluated using Kolmogorov−Smirnov test and Q−Q plot. Positively skewed data were subjected to logarithmic transformation. Within group analyses were conducted using paired samples t-test based on change from baseline. Between group analyses were conducted using multivariate analysis of covariance (MANCOVA) in different models. In the crude model, the 2 groups were compared based on the final values of the variables studied; in the model 1, adjustment was made for the baseline values, while in the model 2 the adjustment was made, in addition to baseline values, also for age, diabetes duration, menopausal duration, physical activity, energy, and protein. We presented only the results of models 1 and 2. Also, chi-square test was used for comparing the qualitative data between 2 studied groups.

2.9. Subgroup analysis

Subjects who received FY were divided, after the completion of the study, in three subgroups (deficient, insufficient, sufficient) according to the baseline values of serum 25(OH)D concentrations, and in two subgroups (insufficient, sufficient) according to the final values of serum 25(OH)D concentrations. Data of these groups were analyzed, for within-groups differences, with the same methods utilized previously, while between-groups differences were studied with MANCOVA adjusted for baseline values of variables and for age, dietary energy, and protein intake during the study.

3. Results

Ninety three postmenopausal diabetic women were enrolled at first. Among them, 25 patients who did not met the inclusion criteria were kept out. We also excluded 4 women who had high serum 25(OH) D (>125 nmol/l). The remaining 64 participants were randomly divided in to the 2 parallel groups after run-in period. Five women were excluded during the intervention (week 3). All the remaining 59 participants completed the project (Fig. 1). The

Table 1
General characteristics of participants at baseline.

Variables	FY[a] ($n = 30$)	PY[b] ($n = 29$)	P[c]
Age (year)	57.8 ± 5.5	56.8 ± 5.7	0.47
BMI (kg/m^2)	28.00 ± 0.82	29.30 ± 0.72	0.23
FM (%)	36.80 ± 0.70	37.21 ± 0.76	0.68
Diabetes duration (year)	9.3 ± 5.3	8.8 ± 4.8	0.70
Menopausal duration (year)	8.1 ± 6.2	8 ± 4.5	0.94
Physical activity (MET h^{-1} d^{-1})	23.4 ± 1.8	23.3 ± 2.0	0.94
Sun exposure (minute/day)	24.7 ± 11.0	23 ± 11.2	0.84
25-hydroxy vitamin D (nmol/l)	62.23 ± 4.52	62.72 ± 4.27	0.94

All presented values are means ± SEs.
[a] Fortified yogurt group.
[b] Plain yogurt group.
[c] Denote significance of between group changes (t-test).

distribution of age, diabetes and menopausal duration, physical activity, and sun exposure did not differ significantly between 2 groups (Table 1). The percentages of patients taking oral anti-diabetic drugs were as follows: metformin, 66.6% in FY group and 65.5% in PY group; glitazone, 10% in FY group and 10.4% in PY group; oral agent combination, 23.3% in FY group and 24.1% in PY group. The average dietary intakes also did not significantly differ between the 2 groups except for protein (Table S1), which is considered as a confounder in the analyses.

3.1. Vitamin D status and serum PTH

At start, the 79.7% of subjects was vitamin D deficient or insufficient. At the end of the study period, the vitamin D status of the FY group was substantially improved: serum 25(OH)D significantly ($P > 0.001$) increased (Table 3), and no more subject was deficient; the percentage of subjects with a sufficient vitamin D status increased from 20% to 50% (Table 2), and their final serum 25(OH)D was 107.86 ± 5.77 nmol/l, while final serum 25(OH)D in the insufficient group was 65.8 ± 1.32 nmol/l. Otherwise, vitamin D status in PY group got worse: serum 25(OH)D, even if not significantly, decreased (Table 3), and the percentage of sufficient subjects lowered from 20.7% to 3.4% (Table 2). Serum PTH decreased significantly in FY group ($P = 0.01$), while it did not significantly change in PY group ($P = 0.129$; Table 3).

3.2. Glycemic status

Compared to baseline, serum fasting insulin, and the indexes of insulin resistance, secretion and sensitivity improved after intervention in FY group, (but FSG and HbA_{1c} did not significantly change), while all the glycemic control markers (except HOMA-B) worsened in PY group (Table 3). Final values of all glycemic indexes (except HbA_{1c}) were significantly improved in comparison with the PY group (Table 4).

3.3. Lipid profile

Comparing to baseline values, TG decreased significantly in FY group ($P = 0.046$), while cholesterol, LDL and HDL did not change significantly (Table 3). Final values of TG, cholesterol, and HDL did not show significant differences between FY and PY groups both in model 1 and model 2 (Table 4). However, marginally significant differences were observed between the 2 groups in final values of LDL (model 1: $P = 0.056$; model 2: $P = 0.053$).

3.4. Anthropometric indexes

Final values of waist circumference (WC), waist to hip ratio (WHR), and fat mass (FM) significantly decreased in FY group compared to baseline ($P < 0.001$), whereas weight, BMI, and hip circumference (HC) did not change significantly (Table 3). There were significant differences between the 2 groups in final values of WC, WHR, BMI, and FM in model 1 and model 2 (Table 4).

3.5. Blood pressure

Systolic and diastolic blood pressure did not change significantly in FY and PY group compared to baseline (Table 3). There were also no significant differences in final values of blood pressure components between FY and PY groups (Table 4).

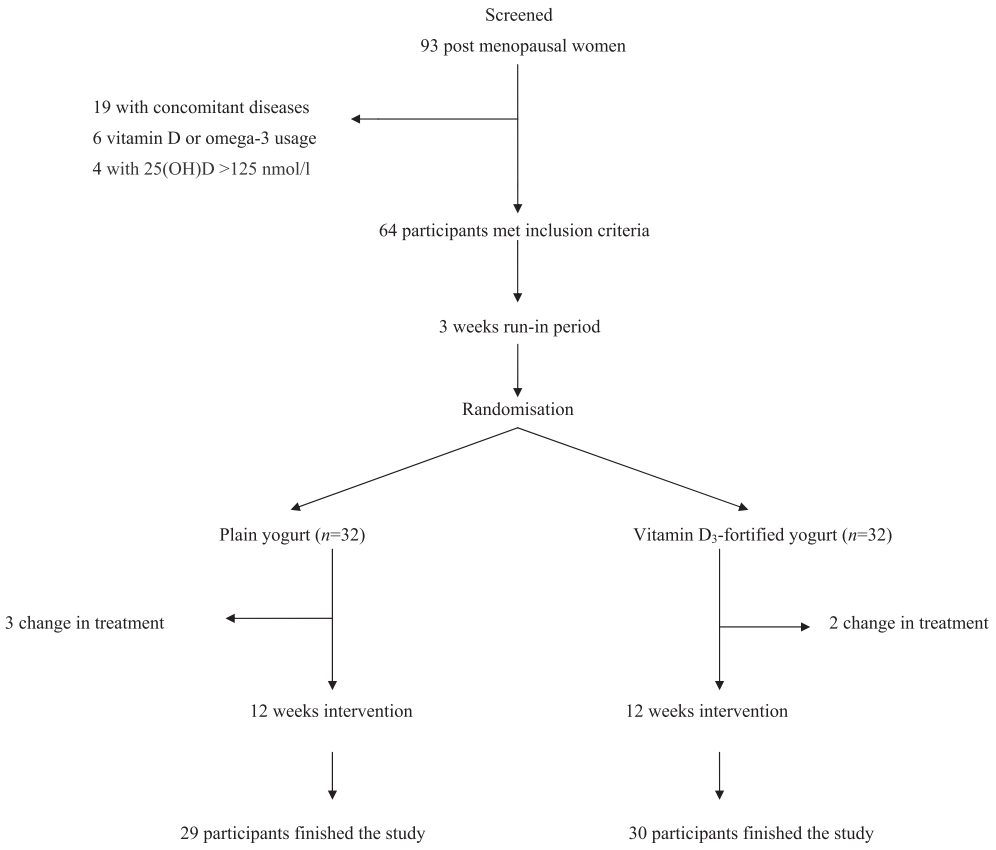

Fig. 1. Flowchart of study design and participants.

Table 2
Distribution of participants based on vitamin D status before and after the intervention.

Group	Before intervention				After intervention			
	Deficient[a]	Insufficient[b]	Sufficient[c]	P^f	Deficient[a]	Insufficient[b]	Sufficient[c]	P^f
PY[d] [*n* (%)]	10(34.5)	13(44.8)	6(20.7)	0.915	12(41.4)	16(55.2)	1(3.4)	<0.001
FY[e] [*n* (%)]	9(30)	15(50)	6(20)		0(0)	15(50)	15(50)	
Total [*n* (%)]	19(32.2)	28(47.5)	12(20.3)		12(20.3)	31(52.5)	16(27.1)	

[a] Participant with serum 25(OH)D < 50 nmol/l.
[b] Participant with serum 25(OH)D between 50 and 75 nmol/l.
[c] Participant with serum 25(OH)D > 75 nmol/l.
[d] Plain yogurt.
[e] Fortified yogurt.
[f] Denotes the significance of differences in vitamin D categories between 2 groups before and after the intervention (chi-square test).

3.6. Inflammation

hs-CRP decreased in FY group compared to baseline but the result was not statistically significant ($P = 0.074$), while omentin increased significantly in this group ($P = 0.001$; Table 3). Table 4 represents that final values of hs-CRP were significantly different between the 2 groups (model 1, $P = 0.037$); however, when other confounders were controlled, final values of hs-CRP did not show statistically significant difference between the groups (model 2, $P = 0.197$). Final values of omentin were significantly different between the groups (model 1: $P = 0.001$; model 2: $P = 0.018$; Table 4).

3.7. Bone turnover

sBAP and sNTX decreased in FY group compared to baseline values, but only the decrement of sNTX was statistically significant (sBAP: $P = 0.210$; sNTX: $P < 0.001$; Table 3). In PY group, final values of sBAP and sNTX increased; however, the increment of sBAP was not statistically significant (sBAP: $P = 0.107$; sNTX: $P = 0.001$; Table 3). Final values of sBAP and sNTX were significantly different between FY and PY groups, both in model 1 and in model 2 (Table 4).

3.8. Multivariate analyses

Multivariate analyses (Wilks Lambda) demonstrated that glycemic status, anthropometric measurements, inflammation, and bone turnover were significantly improved in FY group compared to PY group after adjustment for baseline values (model 1) or even after controlling the other confounders (model 2). Moreover, lipid profile and blood pressure did not significantly improved (Table 4).

Table 3
Comparison of baseline and final values of study variables.

Variable	FY[a] group			PY[b] group		
	Before	After	P^c	Before	After	P^c
25(OH)D (nmol/l)	62.23 ± 4.5	86.83 ± 4.87	<0.001	62.72 ± 4.27	56.13 ± 2.89	0.270
PTH (pg/ml)	54.86 ± 2.7	46.70 ± 2.38	0.010	53.58 ± 3.15	56.75 ± 3.13	0.129
Glycemic markers						
FSG (mg/dl)	168.80 ± 4.48	166.67 ± 5.45	0.177	167.48 ± 2.78	170.62 ± 2.66	0.004
Fasting serum insulin (mU/l)	7.71 ± 1.23	5.17 ± 0.46	0.029	8.78 ± 1.08	11.20 ± 1.04	0.032
HOMA-IR	3.23 ± 0.50	2.13 ± 0.20	0.020	3.58 ± 0.42	4.72 ± 0.44	0.016
HOMA-B	95.28 ± 16.00	64.27 ± 5.98	0.039	109.70 ± 14.53	135.02 ± 13.08	0.074
QUICKI	0.331 ± 0.004	0.348 ± 0.004	0.001	0.327 ± 0.005	0.312 ± 0.005	0.018
HbA_{1c} (%)	7.16 ± 0.23	7.24 ± 0.22	0.744	7.08 ± 0.30	7.58 ± 0.23	0.029
Lipid profile						
Triglycerides (mg/dl)	133.56 ± 10.05	129.10 ± 9.80	0.046	144.96 ± 11.81	149.21 ± 14.92	0.961
Cholesterol (mg/dl)	182.23 ± 8.49	180.77 ± 8.65	0.296	189.96 ± 7.48	191.34 ± 7.15	0.597
LDL (mg/dl)	108.00 ± 6.70	107.33 ± 6.66	0.475	106.20 ± 5.86	120.34 ± 10.31	0.035
HDL (mg/dl)	52.93 ± 1.96	55.93 ± 1.87	0.074	49.03 ± 1.62	50.28 ± 1.77	0.217
Anthropometric indexes						
Weight (kg)	67.10 ± 2.06	66.54 ± 2.13	0.257	69.30 ± 1.87	69.42 ± 2.04	0.799
BMI (kg/m^2)	28.00 ± 0.82	27.84 ± 0.80	0.352	29.30 ± 0.72	29.56 ± 0.76	0.087
WC (cm)	97.60 ± 1.48	96.54 ± 1.46	<0.001	96.45 ± 1.24	96.54 ± 1.23	0.226
HC (cm)	102.20 ± 1.24	102.03 ± 1.24	0.063	102.24 ± 1.40	102.14 ± 1.40	0.319
WHR	0.95 ± 0.006	0.94 ± 0.006	<0.001	0.94 ± 0.004	0.94 ± 0.004	0.290
FM (%)	36.80 ± 0.70	33.91 ± 1.03	<0.001	37.22 ± 0.76	37.90 ± 0.77	0.248
Blood pressure markers						
SBP (mm Hg)	128.31 ± 1.40	128.97 ± 1.28	0.484	128.50 ± 1.50	129.30 ± 1.63	0.473
DBP (mm Hg)	75.12 ± 3.50	75.45 ± 3.51	0.547	81.93 ± 0.94	81.90 ± 1.17	0.958
Inflammatory markers						
hs-CRP (mg/l)	1.32 ± 0.19	0.98 ± 0.04	0.074	1.07 ± 0.05	1.21 ± 0.10	0.201
Omentin (ng/l)	88.69 ± 9.54	122.84 ± 15.12	0.001	99.96 ± 14.73	93.79 ± 14.55	0.289
Bone markers						
sBAP (IU/l)	84.13 ± 12.60	78.46 ± 11.07	0.210	78.05 ± 9.70	87.68 ± 7.69	0.107
sNTX (nmol/l)	57.15 ± 5.95	36.63 ± 5.38	<0.001	52.73 ± 6.67	59.82 ± 6.77	0.001

All presented values are means ± SEs.
[a] Fortified yogurt.
[b] Plain yogurt.
[c] *P* values denote significance of within-group changes (paired t-test).

Table 4
Effects of vitamin D fortified yogurt on glycemic markers, lipid profile, anthropometric indexes, blood pressure, inflammatory, and bone turnover markers.

Variable	Model 1[a]			Model 2[b]		
	FY[c] group	PY[d] group	*P*[e]	FY[c] group	PY[d] group	*P*[e]
PTH (pg/ml)[*]	46.26 ± 1.88	57.21 ± 1.91	<0.001	46.31 ± 2.04	57.15 ± 2.07	0.001
Glycemic markers			**<0.001**[**]			**0.001**[**]
FSG (mg/dl)	166.39 ± 1.06	170.90 ± 1.08	0.005	166.60 ± 1.17	170.68 ± 1.19	0.026
Fasting serum insulin (mu/l)	5.44 ± 0.74	10.92 ± 0.75	<0.001	5.27 ± 0.81	11.01 ± 0.83	<0.001
HOMAIR	2.47 ± 0.31	4.60 ± 0.31	<0.001	2.18 ± 0.34	4.66 ± 0.35	<0.001
HOMA-B	67.37 ± 9.18	131.81 ± 9.35	<0.001	64.10 ± 10.05	134.27 ± 10.25	<0.001
QUICKI	0.346 ± 0.004	0.314 ± 0.004	<0.001	0.35 ± 0.00	0.31 ± 0.005	<0.001
HbA_{1c} (%)	7.17 ± 0.17	7.65 ± 0.18	0.063	7.25 ± 0.17	7.58 ± 0.18	0.227
Lipid profile			**0.127**[**]			**0.115**[**]
Triglycerides (mg/dl)	133.41 ± 6.40	144.74 ± 6.50	0.270	136.50 ± 6.47	141.54 ± 6.60	0.802
Cholesterol (mg/dl)	185.06 ± 2.10	186.90 ± 2.13	0.553	184.60 ± 2.25	187.37 ± 2.30	0.425
LDL (mg/dl)	106.90 ± 5.84	120.80 ± 5.95	0.056	106.27 ± 6.15	121.44 ± 6.27	0.053
HDL (mg/dl)	54.90 ± 1.27	51.35 ± 1.30	0.063	55.08 ± 1.35	51.15 ± 1.38	0.063
Anthropometric indexes			**<0.001**[**]			**0.001**[**]
Weight (kg)	67.50 ± 0.47	68.44 ± 0.48	0.180	67.25 ± 0.50	68.70 ± 0.51	0.069
BMI (kg/m^2)	28.38 ± 0.15	29.00 ± 0.15	0.008	28.32 ± 0.16	29.06 ± 0.17	0.006
WC (cm)	95.99 ± 0.16	97.12 ± 0.17	<0.001	95.95 ± 0.18	97.16 ± 0.18	<0.001
HC (cm)	102.08 ± 009	102.09 ± 0.10	0.901	102.04 ± 0.10	102.13 ± 0.11	0.580
WHR	0.940 ± 0.002	0.951 ± 0.002	<0.001	0.940 ± 0.002	0.951 ± 0.002	<0.001
FM (%)	34.13 ± 0.61	37.68 ± 0.62	<0.001	34.22 ± 0.60	37.59 ± 0.62	0.001
Blood pressure markers			**0.956**[**]			**0.983**[**]
SBP (mm Hg)	129.14 ± 0.97	129.09 ± 0.98	0.969	129.26 ± 1.02	128.97 ± 1.03	0.854
DBP (mm Hg)	78.75 ± 0.60	78.50 ± 0.61	0.767	78.65 ± 0.66	78.59 ± 0.68	0.949
Inflammatory markers			**<0.001**[**]			**0.014**[**]
hs-CRP (mg/l)	0.97 ± 0.08	1.22 ± 0.08	0.037	1.01 ± 0.08	1.18 ± 0.08	0.197
Omentin (ng/l)	129.23 ± 8.07	87.18 ± 8.21	0.001	123.21 ± 8.00	93.41 ± 8.15	0.018
Bone markers			**<0.001**[**]			**<0.001**[**]
sBAP (IU/l)	76.23 ± 4.40	89.99 ± 4.45	0.032	75.54 ± 4.62	90.71 ± 4.70	0.033
sNTX (nmol/l)	34.73 ± 2.62	61.78 ± 2.67	<0.001	34.44 ± 2.85	62.09 ± 2.90	<0.001

Variables are after intervention measurements and represented "estimated marginal means" ± SEs.
[*] Results were obtained from ANCOVA for between group comparisons based on after intervention-values (adjustment was made for baseline values).
[**] *P* values are resulted from MANCOVA, Wilks Lambda tests.
[a] Adjusted for baseline values.
[b] Adjusted for baselines, age, physical activity, diabetes duration, menopausal duration, energy, and protein intake.
[c] Fortified yogurt.
[d] Plain yogurt.
[e] *P* values are resulted from MANCOVA for between group comparisons based on after intervention values.

3.9. Subgroup analyses in "FY" group

3.9.1. Subgroup analyses based on baseline values of serum 25(OH) D

Regarding the baseline value of serum 25(OH)D in participants of FY group, we recognized 3 subgroups as deficient, insufficient, and sufficient; the increment of serum 25(OH)D in the subgroups was 31.33, 21.2, and 23 nmol/l, respectively. Serum PTH decreased in the subgroups, but the result was statistically significant only in deficient subgroup (Table 5). It was also the only marker which was significantly different among the subgroups ($P = 0.019$; Table S2).

Insulin indexes improved in each of the subgroups compared to baseline values, but just participants of the insufficient subgroup showed statistically significant results. However, the result for QUICKI was also significant in deficient subgroup. FSG and HbA_{1c} did not significantly change in each of the subgroups. Final values of lipid and blood pressure markers did not significantly change in the subgroups (Table 5).

Considering the anthropometric indexes, participants in deficient subgroup showed significant decrease in final value of WC compared to baseline, while in the insufficient subgroup, final values of WC, WHR, and FM significantly decreased. In sufficient subgroup, statistically significant reduction was observed just for the final value of FM (Table 5).

The inflammatory markers did not significantly changed compared to baselines in the subgroups except for omentin which increased significantly in the sufficient subgroup (Table 5).

Considering the bone markers, sBAP did not significantly change in the subgroups, but sNTX significantly decreased in the deficient and insufficient subgroups (Table 5).

Results of MANCOVA tests represented that glycemic markers, lipid profile, anthropometric indexes, inflammatory markers, and bone markers did not differ significantly among the subgroups (Table S2).

3.9.2. Subgroup analyses based on final values of serum 25(OH)D

Regarding final values of 25(OH)D in FY group, participants were categorized as insufficient or sufficient subgroups at the end of the intervention. Within subgroup analysis showed that serum PTH significantly decreased in both insufficient and sufficient categories (Table 6) but the result of ANCOVA test represented no statistically significant difference between categories (Table S3).

Fasting serum insulin, HOMA-IR, and QUICKI significantly decreased in the insufficient subgroup. FSG and HOMA-B decreased in this subgroup but the results were not statistically significant. In the sufficient subgroup, glycemic markers did not significantly change compared to baseline (Table 6).

As shown in Table 6, final values of lipid markers in each of the subgroups did not significantly change compared to baseline except for HDL which showed a small but significant increase in the sufficient subgroup. Blood pressure markers also, did not significantly change in the subgroups.

Considering the changes in anthropometric indexes, final values of WC and WHR decreased significantly both in the insufficient and

Table 5
Comparison of initial and final values of study variables based on categorized baseline vitamin D status in fortified yogurt group.

Variable	Deficient[a] ($n = 9$)			Insufficient[b] ($n = 15$)			Sufficient[c] ($n = 6$)		
	Before	After	P^d	Before	After	P^d	Before	After	P^d
25(OH)D (nmol/l)	36.55 ± 3.68	67.88 ± 2.41	<0.001	62.00 ± 1.64	83.20 ± 5.97	0.001	101.33 ± 6.21	124.33 ± 5.34	0.016
PTH (pg/ml)	57.33 ± 6.02	43.77 ± 3.66	0.013	56.60 ± 3.76	52.26 ± 3.38	1.00	46.83 ± 3.46	37.16 ± 3.98	0.142
Glycemic markers									
FSG (mg/dl)	169.84 ± 6.37	169.58 ± 5.46	0.498	167.87 ± 4.13	167.89 ± 3.30	0.095	166.33 ± 6.34	168.75 ± 6.09	0.882
Fasting serum insulin (mU/l)	9.54 ± 1.91	7.53 ± 0.86	0.159	7.73 ± 1.00	4.87 ± 2.08	0.033	5.11 ± 1.02	4.08 ± 1.01	0.247
HOMA-IR	4.01 ± 0.762	3.14 ± 0.372	0.123	3.81 ± 0.401	3.09 ± 0.447	0.024	4.92 ± 0.459	2.05 ± 0.801	0.776
HOMA-B	116.23 ± 24.81	91.90 ± 10.46	0.194	82.88 ± 13.49	51.38 ± 11.14	0.048	65.37 ± 11.25	59.85 ± 12.08	0.546
QUICKI	0.321 ± 0.006	0.342 ± 0.006	0.035	0.322 ± 0.005	0.332 ± 0.005	0.015	0.332 ± 0.006	0.333 ± 0.010	0.065
HbA_{1c} (%)	6.82 ± 0.36	7.16 ± 0.249	0.739	7.58 ± 0.264	7.71 ± 0.234	0.923	6.50 ± 0.328	7.07 ± 0.732	0.763
Lipid profile									
Triglycerides (mg/dl)	115.00 ± 13.35	114.78 ± 13.34	0.799	147.33 ± 14.51	139.13 ± 14.29	0.059	127.00 ± 28.62	125.50 ± 28.50	0.394
Cholesterol (mg/dl)	168.77 ± 13.09	166.55 ± 13.55	0.190	188.00 ± 12.75	187.64 ± 13.47	0.828	188.00 ± 21.46	185.33 ± 18.90	0.375
LDL (mg/dl)	98.11 ± 10.34	96.00 ± 10.33	0.167	114.80 ± 11.13	115.26 ± 10.92	0.751	105.83 ± 11.08	104.50 ± 11.10	0.408
HDL (mg/dl)	48.55 ± 2.55	53.66 ± 2.65	0.108	56.73 ± 3.22	57.53 ± 3.13	0.756	50.00 ± 2.81	55.33 ± 3.66	0.104
Anthropometric indexes									
Weight (kg)	68.12 ± 4.26	67.12 ± 4.57	0.278	68.22 ± 2.94	66.84 ± 2.96	0.078	64.26 ± 4.15	64.43 ± 4.22	0.104
BMI (kg/m^2)	28.45 ± 1.62	28.83 ± 1.65	0.303	28.32 ± 1.19	27.90 ± 1.10	0.096	26.54 ± 1.60	26.21 ± 1.62	0.218
WC(cm)	100.24 ± 3.58	98.74 ± 3.70	0.027	96.98 ± 1.41	96.33 ± 1.40	0.01	95.18 ± 3.87	93.78 ± 3.53	0.074
HC (cm)	103.38 ± 2.90	103.06 ± 2.89	0.175	102.28 ± 1.44	102.20 ± 1.42	0.469	100.23 ± 3.01	100.05 ± 3.09	0.324
WHR	0.968 ± 0.011	0.958 ± 0.014	0.052	0.948 ± 0.006	0.942 ± 0.006	0.002	0.948 ± 0.006	0.937 ± 0.018	0.093
FM (%)	36.66 ± 1.52	34.44 ± 2.50	0.147	36.90 ± 0.797	34.44 ± 1.15	0.024	36.66 ± 2.09	32.00 ± 2.39	0.016
Blood pressure markers									
SBP (mm Hg)	128.72 ± 2.82	130.00 ± 2.32	0.582	128.26 ± 1.64	126.93 ± 1.56	0.063	127.83 ± 4.36	132.50 ± 3.59	0.070
DBP (mm Hg)	63.42 ± 10.83	62.49 ± 10.82	0.602	80.53 ± 1.09	80.33 ± 1.14	0.802	79.16 ± 2.00	80.50 ± 2.92	0.318
Inflammatory markers									
hs-CRP	1.62 ± 0.441	1.11 ± 1.01	0.309	1.01 ± 0.030	0.890 ± 0.056	0.106	1.66 ± 0.692	1.00 ± 0.094	0.333
Omentin (ng/l)	102.42 ± 25.35	117.36 ± 30.08	0.210	78.59 ± 8.65	117.51 ± 19.86	0.042	91.84 ± 21.36	144.39 ± 39.72	0.118
Bone markers									
sBAP (IU/l)	77.54 ± 21.01	70.96 ± 23.33	0.455	81.27 ± 17.86	77.06 ± 13.18	0.566	101.19 ± 35.15	93.02 ± 31.22	0.255
sNTX (nmol/l)	60.24 ± 10.63	31.46 ± 7.67	0.001	56.48 ± 9.02	38.71 ± 8.95	<0.001	54.21 ± 13.51	39.17 ± 11.18	0.267

All presented values are means ± SEs.
[a] Serum 25(OH)D < 50 nmol/l.
[b] Serum 25(OH)D between 50 and 75 nmol/l.
[c] Serum 25(OH)D > 75 nmol/l.
[d] *P* values denote significance of within-group changes (paired t-test).

sufficient subgroups. The decrement of FM was statistically significant just in the sufficient subgroup (Table 6). It was also the only index which was significantly different between the subgroups ($P = 0.009$; Table S3).

Omentin significantly increased in the insufficient and sufficient subgroups compared to baseline values. The decrement of hs-CRP in both subgroups was not statistically significant (Table 6).

In the insufficient subgroup, sBAP did not significantly change compared to baseline, but it decreased significantly in the sufficient subgroup. sNTX decreased significantly in the subgroups compared to baselines (Table 6).

According to the MANCOVA test (Wilks Lambda), glycemic status, lipid profile, anthropometric measurements, inflammation, and bone turnover did not significantly differ between insufficient and sufficient subgroup (Table S3).

4. Discussion

There is no consensus on the threshold for optimal level of serum 25(OH)D. Most experts believe that for maximizing healthy functions of vitamin D, a serum concentration of 25(OH)D > 75 nmol/l is needed. To obtain the mentioned value, regular intake of 1500–2000 IU/day vitamin D is recommended [5]. In this study, approximately 80% of the participants in each group were vitamin D deficient or insufficient. Daily consumption of 2000 IU/d vitamin D fortified low-fat yogurt for 12 weeks significantly increased the serum 25(OH)D concentration in the group treated, and raised the percentage of subjects considered sufficient as vitamin D status (serum 25(OH)D > 75 nmol/l) from 20% to 50%. The use of such high dosage in our study is reasonable due to the fact that some previous studies with lower dosages reported inconclusive results [14]. The mentioned dose is regarded safe due to the current knowledge [5].

The content of fat and Na in low-fat yogurt used in this study was 1.4 g/100 g and 65 mg/100 g, respectively. Regarding the age and cardiovascular risks of the patients participated in this study, it seems that fortification of low-fat yogurt is reasonable compared to Doogh which contain higher levels of fat (1.6 g/100 g) and Na (380 mg/100 g).

No differences in baseline values of serum 25(OH)D and duration of sun exposure were observed among groups; therefore, improvement in vitamin D status of the subjects is due to intervention.

Observational studies revealed inverse association between serum 25(OH)D and incidence of T2DM [3]. A meta-analysis of prospective observational studies demonstrated a decrease of 58% in risk of T2DM in the highest quintile of serum 25(OH)D compared to the lowest [16]. Potential mechanisms for effects of vitamin D on T2DM are currently recognized. Vitamin D is known to have anti-inflammatory effects through the regulation of inflammatory and anti-inflammatory markers. Systemic inflammation is also identified as one of the basic components of T2DM. Furthermore, pancreatic beta cells have specific receptors for $1,25(OH)_2D$, the active form of vitamin D, whose regulatory effects on insulin secretion have been reported [17]. Vitamin D also has some beneficial effects on insulin resistance; it may stimulate the expression of insulin receptors and therefore enhance insulin response to glucose. It also provides an adequate intracellular cytosolic calcium

Table 6
Comparison of initial and final values of study variables based on categorized final vitamin D status in fortified yogurt group.

Variable	Insufficient[a] ($n = 15$)			Sufficient[b] ($n = 15$)		
	Before	After	P^c	Before	After	P^c
25(OH)D (nmol/l)	47.86 ± 3.86	65.80 ± 1.32	<0.001	76.60 ± 6.36	107.86 ± 5.77	<0.001
PTH (pg/ml)	61.93 ± 4.06	52.00 ± 2.99	0.009	47.80 ± 2.63	41.40 ± 3.24	0.038
Glycemic markers						
FSG (mg/dl)	177.53 ± 9.40	172.46 ± 7.55	0.073	160.06 ± 4.90	160.86 ± 4.60	0.559
Fasting serum insulin (mU/l)	9.80 ± 2.32	5.36 ± 0.66	0.047	5.62 ± 0.48	4.96 ± 0.65	0.337
HOMA-IR	4.26 ± 0.91	2.27 ± 0.28	0.027	2.19 ± 0.16	1.98 ± 0.26	0.423
HOMA-B	116.84 ± 30.66	65.07 ± 8.60	0.073	73.71 ± 7.42	63.46 ± 8.61	0.266
QUICKI	0.321 ± 0.007	0.344 ± 0.006	0.002	0.342 ± 0.004	0.351 ± 0.006	0.154
HbA_{1c} (%)	7.40 ± 0.34	7.53 ± 0.35	0.758	6.92 ± 0.32	6.95 ± 0.25	0.910
Lipid profile						
Triglycerides (mg/dl)	140.46 ± 14.50	136.93 ± 14.52	0.220	126.66 ± 14.19	121.26 ± 13.35	0.123
Cholesterol (mg/dl)	182.60 ± 14.74	180.33 ± 15.43	0.125	181.86 ± 8.99	181.20 ± 8.46	0.787
LDL (mg/dl)	115.33 ± 12.46	114.33 ± 12.30	0.250	100.66 ± 4.78	100.33 ± 5.04	0.837
HDL (mg/dl)	51.33 ± 2.85	52.53 ± 2.10	0.672	54.53 ± 2.71	59.33 ± 2.90	0.011
Anthropometric indexes						
Weight (kg)	67.39 ± 3.54	66.56 ± 3.56	0.306	66.81 ± 2.70	66.53 ± 2.47	0.635
BMI (kg/m^2)	28.60 ± 1.43	28.47 ± 1.36	0.624	27.41 ± 0.81	27.21 ± 0.87	0.423
WC(cm)	97.77 ± 2.33	96.73 ± 2.35	0.011	97.42 ± 1.90	96.36 ± 1.83	0.002
HC (cm)	102.17 ± 1.93	102.00 ± 1.90	0.308	102.23 ± 1.65	102.05 ± 1.67	0.070
WHR	0.956 ± 0.007	0.947 ± 0.008	0.017	0.951 ± 0.09	0.942 ± 0.10	0.005
FM (%)	36.19 ± 1.06	35.21 ± 1.45	0.235	37.40 ± 0.93	32.62 ± 1.43	<0.001
Blood pressure markers						
SBP (mm Hg)	130.56 ± 1.87	130.26 ± 1.57	0.835	126.06 ± 1.99	127.66 ± 2.03	0.190
DBP (mm Hg)	72.25 ± 6.91	71.71 ± 6.84	0.533	78.00 ± 1.17	79.20 ± 1.24	0.076
Inflammatory markers						
hs-CRP	1.11 ± 0.05	1.02 ± 0.08	0.380	1.54 ± 0.37	0.94 ± 0.04	0.109
Omentin (ng/l)	90.08 ± 15.45	127.87 ± 23.90	0.055	87.30 ± 11.76	117.81 ± 19.28	0.026
Bone markers						
sBAP (IU/l)	72.29 ± 18.41	73.53 ± 16.85	0.866	95.97 ± 17.29	83.39 ± 14.86	0.019
sNTX (nmol/l)	51.11 ± 8.94	31.76 ± 7.78	<0.001	63.20 ± 7.85	41.50 ± 7.48	0.001

All presented values are means ± SEs.
[a] Serum 25(OH)D between 50 and 75 nmol/l.
[b] Serum 25(OH)D > 75 nmol/l.
[c] *P* values denote significance of within-group changes (paired t-test).

pool necessary for insulin secretion through the regulation of cell membrane calcium flux [18].

Results of randomized controlled trials about the effects of vitamin D on glycemic markers are inconsistent. Sugden et al. reported no significant changes in glycemic markers after administration of 100000 IU single dose of ergocalciferol (vitamin D_2) in diabetic patients, but they found significant improvement in insulin sensitivity in subjects who had a 25(OH)D increase of 11 nmol/l or more after conducting a post-hoc analysis [4]. Shab-Bidar et al. showed that glycemic status improved in diabetic patients after 12 weeks consumption of 1000 IU vitamin D-fortified Doogh [9]. In our study, insulin functions improved in FY group compared to PY group. In agreement to our study, results of a previous meta-analysis demonstrated that vitamin D supplementation did not reduce HbA_{1c} values [14]. It has to be pointed that some previous studies [4,19] even with high dosages and longer interventional period performed on vitamin D deficient patients, did not found significant improvement in glycemic control. Subgroup analyses based on final values of serum 25(OH)D represented more improvement in glycemic status in the insufficient rather than sufficient category. More studies are needed to clarify the optimal levels of 25(OH)D for a good glycemic status.

Results of clinical trials about the effects of vitamin D supplementation on lipid profile are contradicting. Heikkinen et al. reported detrimental effects of 3 y treatment with 300 IU/day vitamin D on lipid profile in postmenopausal women [20]. Data from Women's Health Initiative (WHI) study documented that 5 y calcium (1000 mg/day) and vitamin D (400 IU/day) co-supplementation did not change the lipid profile [21]. Shab-Bidar et al. reported that 1000 IU vitamin D-fortified Doogh improved lipid profile in patients with T2DM [9]. In this study, TG was the only lipid marker demonstrating small significant improvement in FY group. Subgroup analyses revealed no differences on the lipid markers between subject in the insufficient and sufficient categories except for HDL, which improved significantly in the subjects whose serum 25(OH)D levels exceeded 75 nmmol/l.

Previous cross-sectional studies showed that serum 25(OH)D is inversely associated with obesity and body fat [22]. Regarding the positive relationship between serum concentration of PTH and obesity and the role of PTH in increasing lipogenesis and decreasing lipolysis [23], suppressing effect of vitamin D on serum PTH justifies its influence on anthropometric indexes. Vitamin D also decreases the expression and activity of peroxisome proliferator-activated receptor-gamma in adipocytes and therefore inhibits adipogenesis [24]. We found that 2000 IU vitamin D-fortified yogurt improved WC, FM, and BMI specifically when the levels of serum 25(OH)D reached>75 nmol/l. Nikooyeh et al. also reported similar results in their study, in which T2DM patients consumed 1000 IU vitamin D-fortified Doogh [25]. Weight and HC did not significantly change in our study but Nikooyeh et al. reported significant weight reduction in their trial. In the WHI study, a negligible weight reduction (0.13 kg during 7 y) was observed in postmenopausal women received 400 IU vitamin D and 1000 mg calcium per day [21].

Results of studies about the effects of vitamin D on systolic and diastolic blood pressure are inconsistent [4,19,25]. The suppressing effects of vitamin D on renin production and PTH secretion may control blood pressure [4]. Based on the results of a meta-analysis, vitamin D may control blood pressure in hypertensive subjects [26]. In our study, neither systolic nor diastolic blood pressure were

affected by vitamin D due to the fact that most of the patients were not hypertensive.

Anti-inflammatory effects of vitamin D have now been recognized. It is believed that $1,25(OH)_2D$ regulates production of inflammatory cytokines and modulates the function of granulocyte and macrophage [27]. Observational studies reported higher levels of serum hs-CRP in subjects with hypovitaminosis D [28]. In a recent systematic review of clinical trials, no effect of vitamin D supplementation on hs-CRP was reported [29]. Neyestati et al. reported amelioration of hs-CRP and plasma fibrinogen concentrations after 12 weeks consumption of 1000 IU fortified Doogh in diabetic patients [10]. In our study, final values of hs-CRP revealed no remarkable difference between the 2 groups after adjustment for confounders. It was assumed that the anti-inflammatory effects of vitamin D are more prominent in severe inflammatory conditions [30].

Omentin, a novel adipocytokine derived from visceral adipose tissue, was found by Yang et al., in 2003. The omentin gene is located in the 1q22–q23 chromosomal region, which is known to have link with T2DM. Body of evidence around the inverse association between omentin and obesity, insulin resistance, or impaired glucose tolerance is increasing [31,32]. Furthermore, it has been stated that serum omentin levels are lower in postmenopausal women compared to premenopausal women and also there is an inverse relationship between serum omentin and bone turnover markers (sBAP and sNTX) [33]. We demonstrated for the first time the improving effect of vitamin D fortified low fat yogurt on serum omentin in postmenopausal women with T2DM.

An inverse association between serum 25(OH)D levels and bone turnover markers were reported in several studies. Secondary hyperparathyroidism may occur in vitamin D deficiency which increases bone turnover markers. Furthermore, postmenopausal women have higher serum concentration of these markers [34]. In this study, sNTX and sBAP, as bone turnover markers, decreased in FY group after the intervention. The decrement of serum PTH levels in the FY group after the intervention justifies the decreases in bone turnover markers, whilst the markers finally increased in the PY group concurrently with the increment in PTH values. Due to the higher turnover of bone markers in winter months [34], the markers increased in PY group, whereas vitamin D-fortified yogurt overcame this phenomenon and improved the condition.

We could not show significant differences in measured final values of markers and indexes among the subgroups based on baseline or final levels of serum 25 (OH)D. However, based on baseline serum 25(OH)D, the within subgroup changes were more favorable in subjects categorized as insufficient. Considering the final levels of serum 25(OH)D, glycemic markers were improved in the insufficient subgroup, whereas the other improved markers and indexes were in the sufficient subgroup. It can be concluded that the serum 25(OH)D might be raised to a certain level to have beneficial effects. We think that further studies with more participants and longer interventional periods are needed to compare the differences among the subgroups more accurately.

Our different results compared to the results of previous studies could be due to the following reasons:

- *Usage of a fortified food instead of a pharmacological supplement*: It seems that studies which used fortified foods obtained more favorable results than the studies which used pharmacological supplements. It is possible that fortified food by itself may influence the metabolic responses.
- *The difference in the baseline serum 25(OH)D of participants:* In this study, about 32% of the participants were vitamin D deficient, whereas in some previous studies approximately most of the subjects were vitamin D deficient. It is possible that the baseline levels of serum 25(OH)D may affect the metabolic responses of the patients.

Limitations of our study are as follows: (i) 12 weeks intervention might be inadequate to affect long-term markers like HbA_{1c}. (ii) Seasonal effects may influence the results of our study specifically bone markers. As a result, further long intervention clinical trials with different doses of vitamin D fortification are recommended.

5. Conclusions

Our study demonstrated that 2000 IU vitamin D-fortified low fat yogurt could increase the levels of serum 25(OH)D concentration satisfactorily. Glycemic status, anthropometric indexes, inflammatory, and bone turnover markers improved after 12 weeks intervention in postmenopausal women with T2DM. The measured markers improved when serum 25(OH)D concentrations exceeded 75 nmol/l with the exception of glycemic markers which showed more improvement in the level of serum 25(OH)D concentrations between 50 and 75 nmol/l. The desirable compliance of the product by the subjects suggests that low-fat yogurt could be a good choice for vitamin D fortification in Iran.

Funding source

This study was funded by Vice Chancellor for Research, Isfahan University of Medical Sciences, Isfahan, Iran.

Contribution statement

TJ contributed to conception, design, statistical analyses, data interpretation, manuscript drafting, and writing for this study. EF, BI, SH, AE, and AAF contributed to drafting. AF contributed to statistical analyses. TJ, GA, and EF are the guarantors of this study. All authors approved the final manuscript for submission.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was extracted from PhD dissertation which was approved by School of Nutrition & Food Sciences, Isfahan University of Medical Sciences (code 192015). Special thanks go to Mr. Nasrabadi, the director of Allas Dairy Company for providing the yogurt, the staff of Isfahan Endocrine and Metabolism Research Center for their cooperation, and Mr. Aman A. Jafari for editing the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2015.02.014.

References

[1] International Diabetes Federation. Diabetes: facts and figures. IDF; 2014 [accessed 8.7.14], at, www.idf.org/worlddiabetesday/toolkit/gp/facts-figures.
[2] Jafari T, Fallah AA, Azadbakht L. Role of dietary n-3 polyunsaturated fatty acids in type 2 diabetes: a review of epidemiological and clinical studies. Maturitas 2013;74:303–8.
[3] González-Molero I, Rojo-Martínez G, Morcillo S, Gutiérrez-Repiso C, Rubio-Martín E, Almaraz MC, et al. Vitamin D and incidence of diabetes: a prospective cohort study. Clin Nutr 2012;31:571–3.

[4] Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with type 2 diabetes mellitus and low vitamin D levels. Diabet Med 2008;25:320–5.
[5] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
[6] O'Mahony L, Stepien M, Gibney MJ, Nugent AP, Brennan L. The potential role of vitamin D enhanced foods in improving vitamin D status. Nutrients 2011;3:1023–41.
[7] IOM (Institute of Medicine). Dietary reference intakes for calcium and vitamin D. Washington DC: The National Academies Press; 2011.
[8] Heshmat R, Mohammad K, Majdzadeh SR, Forouzanfar MH, Bahrami A, Omrani GR, et al. Vitamin D deficiency in Iran: a multi-center study among different urban areas. Iran J Publ Health 2008;37:72–8.
[9] Shab-Bidar S, Neyestani TR, Djazayery A, Eshraghia MR, Houshiarrad A, Gharavi AA, et al. Regular consumption of vitamin D-fortified yogurt drink (Doogh) improved endothelial biomarkers in subjects with type 2 diabetes: a randomized double-blind clinical trial. BMC Med 2011;9:125–35.
[10] Neyestani TR, Hajifaraji M, Omidvar N, Nikooyeh B, Eshraghian MR, Shariatzadeh N, et al. Calcium-vitamin D-fortified milk is as effective on circulating bone biomarkers as fortified juice and supplement but has less acceptance: a randomised controlled school-based trial. J Hum Nutr Diet 2014;27:606–16.
[11] Lamb JJ, Holick MF, Lerman RH, Konda VR, Minich DM, Desai A, et al. Nutritional supplementation of hop rho iso-alpha acids, berberine, vitamin D_3, and vitamin K_1 produces a favorable bone biomarker profile supporting healthy bone metabolism in postmenopausal women with metabolic syndrome. Nutr Res 2011;31:347–55.
[12] World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva: World Health Organization; 2006.
[13] Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, et al. Nutrition principles and recommendations in diabetes. Diabetes Care 2004;27:S36–46.
[14] George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabet Med 2012;29:e142–50.
[15] Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, et al. 2011 compendium of physical activities: a second update of codes and MET values. Med Sci Sports Exerc 2011;43:1575–81.
[16] Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care 2013;36:1422–8.
[17] Danescu LG, Levy S, Levy J. Vitamin D and diabetes mellitus. Endocrinol 2009;35:11–7.
[18] Ojuka EO. Role of calcium and AMP kinase in the regulation of mitochondrial biogenesis and GLUT4 levels in muscle. Proc Nutr Soc 2004;63:275–8.
[19] Alkharfy KM, Al-Daghri NM, Sabico SB, Al-Othman A, Moharram O, Alokail MS, et al. Vitamin D supplementation in patients with diabetes mellitus type 2 on different therapeutic regimens: a one-year prospective study. Cardiovasc Diabetol 2013;12:113.
[20] Heikkinen AM, Tuppurainen MT, Niskanen L, Komulainen M, Penttila I, Saarikoski S. Long-term vitamin D3 supplementation may have adverse effects on serum lipids during postmenopausal hormone replacement therapy. Eur J Endocrinol 1997;137:495–502.
[21] Caan B, Neuhouser M, Aragaki A, Lewis CB, Jackson R, LeBoff MS, et al. Calcium plus vitamin D supplementation and the risk of postmenopausal weight gain. Arch Intern Med 2007;167:893–902.
[22] LeBlanc ES, Rizzo JH, Pedula KL, Ensrud KE, Cauley J, Hochberg M, et al. Associations between 25-Hydroxyvitamin D and weight gain in elderly women. J Womens Health 2012;21:1066–73.
[23] McCarty MF, Thomas CA. PTH excess may promote weight gain by impeding catecholamine-induced lipolysis implications for the impact of calcium, vitamin D, and alcohol on body weight. Med Hypotheses 2003;61:535–42.
[24] Wood RJ. Vitamin D and adipogenesis: new molecular insights. Nutr Rev 2008;66:40–6.
[25] Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, Kalayi A, et al. Daily consumption of vitamin D- or vitaminD-calcium-fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. Am J Clin Nutr 2011;93:764–71.
[26] Witham MD, Nadir MA, Struthers AD. Effect of vitamin D on blood pressure: a systematic review and meta-analysis. J Hypertens 2009;27:1948–54.
[27] Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev 2005;26:662–87.
[28] Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Intern Emerg Med 2013;8:33–40.
[29] Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol 2014;2:76–89.
[30] Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab 2003;88:4623–32.
[31] Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, et al. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol-Endocrinol Metab 2006;290:E1253–61.
[32] Pan HY, Guo L, Li Q. Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. Diabetes Res Clin Pract 2010;88:29–33.
[33] Wang D, Jiang TJ, Liao L, Huang J. Relationships between serum omentin-1 concentration and bone mineral density, and bone biochemical markers in Chinese women. Clin Chim Acta 2013;426:64–7.
[34] Brown JP, Albert C, Nassar BA, Adachi JD, Cole D, Davison KS, et al. Bone turnover markers in the management of postmenopausal osteoporosis. Clin Biochem 2009;42:929–42.