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

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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 HbA1c (significant worsening of all indexes inPY); 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 HbA1c), omentin, and bone turnover markers were assessed at baseline and after 12 weeks.

Conclusions: Daily consumption of 2000 IU vitamin D-fortified yogurt for 12 weeks improved glycemic markers (except HbA1c), anthropometric indexes, inflammation, and bone turnover markers in postmenopausal women with type 2 diabetes.

Trial registration: www.irct.ir (IRCT2013110515294N1).
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 foodstuffs 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 post-menopausal 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 post-menopausal 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 α = 0.05, 90% power, and a standardized effect size of Δ = 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.iroct 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 immuno-turbidimetric 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). HbA1c was separated by ion-exchange chromatography and measured by a colorimetric method (DSS 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 meter². 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 D₃ 100 SD/S” (contained 100,000 IU Vitamin D₃ 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 yoghurt 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 in the crude model, 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 meet 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
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 HbA1c 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 HbA1c) 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).

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**Fig. 1.** Flowchart of study design and participants.
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 \((P = 0.001; \text{ Table 3})\). Table 3 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: \(P = 0.018\; \text{ Table 4}\)).

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: \(P < 0.001\; \text{ Table 3}\)). In PY group, final values of sBAP and sNTX increased; however, the increment of sBAP was not statistically significant (sBAP: \(P = 0.107\); sNTX: \(P = 0.001\; \text{ Table 3}\)). 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 2

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Before intervention</th>
<th>After intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient(^a)</td>
<td>Insufficient(^b)</td>
</tr>
<tr>
<td>PY</td>
<td>10(34.5)</td>
<td>13(44.8)</td>
</tr>
<tr>
<td>FY</td>
<td>9(30)</td>
<td>15(50)</td>
</tr>
<tr>
<td>Total</td>
<td>19(32.2)</td>
<td>28(47.5)</td>
</tr>
</tbody>
</table>

\(^a\) 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.

### Table 3

Comparison of baseline and final values of study variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>FY(^a) group</th>
<th>PY(^b) group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td>62.23 ± 4.5</td>
<td>85.83 ± 4.87</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>54.86 ± 2.7</td>
<td>46.70 ± 2.38</td>
</tr>
<tr>
<td>Glycemic markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSG (mg/dl)</td>
<td>168.80 ± 4.48</td>
<td>166.67 ± 5.45</td>
</tr>
<tr>
<td>Fasting serum insulin (mU/l)</td>
<td>7.71 ± 1.23</td>
<td>5.17 ± 0.46</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.23 ± 0.50</td>
<td>2.13 ± 0.20</td>
</tr>
<tr>
<td>HOMA-B</td>
<td>95.28 ± 16.00</td>
<td>64.27 ± 5.98</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.331 ± 0.004</td>
<td>0.348 ± 0.004</td>
</tr>
<tr>
<td>HbA(_1c) (%)</td>
<td>7.16 ± 0.23</td>
<td>7.24 ± 0.22</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>133.56 ± 10.05</td>
<td>129.10 ± 9.80</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>182.23 ± 8.49</td>
<td>180.77 ± 8.65</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>108.00 ± 6.70</td>
<td>107.33 ± 6.66</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>52.93 ± 1.96</td>
<td>55.93 ± 1.87</td>
</tr>
<tr>
<td>Anthropometric indexes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.10 ± 2.06</td>
<td>65.54 ± 2.13</td>
</tr>
<tr>
<td>BMt (kg/m(^2))</td>
<td>28.00 ± 0.82</td>
<td>27.84 ± 0.80</td>
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<tr>
<td>WC (cm)</td>
<td>97.60 ± 1.48</td>
<td>96.54 ± 1.46</td>
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<tr>
<td>HC (cm)</td>
<td>102.20 ± 1.24</td>
<td>102.03 ± 1.24</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 ± 0.006</td>
<td>0.94 ± 0.006</td>
</tr>
<tr>
<td>FM (%)</td>
<td>36.80 ± 0.70</td>
<td>33.91 ± 1.03</td>
</tr>
<tr>
<td>Blood pressure markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>128.31 ± 1.40</td>
<td>128.97 ± 1.28</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>75.12 ± 3.50</td>
<td>75.45 ± 3.51</td>
</tr>
<tr>
<td>Inflammatory markers</td>
<td></td>
<td></td>
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<tr>
<td>hs-CRP (mg/l)</td>
<td>1.32 ± 0.19</td>
<td>0.98 ± 0.04</td>
</tr>
<tr>
<td>Omentin (ng/l)</td>
<td>88.69 ± 9.54</td>
<td>122.84 ± 15.12</td>
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<tr>
<td>Bone markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sBAP (IU/l)</td>
<td>84.13 ± 12.60</td>
<td>78.46 ± 11.07</td>
</tr>
<tr>
<td>sNTX (nmol/l)</td>
<td>57.15 ± 5.95</td>
<td>36.63 ± 5.38</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1 a</th>
<th>Model 2 b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FY group</td>
<td>FY group</td>
</tr>
<tr>
<td></td>
<td>57.21 ± 1.91</td>
<td>57.15 ± 2.07</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Glycemic markers</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting serum insulin (mu/l)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMEIR</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Omentin (ng/l)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Blood pressure markers</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>129.26 ± 1.02</td>
<td>128.97 ± 1.03</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>78.65 ± 0.66</td>
<td>78.59 ± 0.68</td>
</tr>
<tr>
<td>Inflammatory markers</td>
<td>&lt;0.001</td>
<td>0.014</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>0.97 ± 0.08</td>
<td>1.18 ± 0.08</td>
</tr>
<tr>
<td>Omentin (ng/l)</td>
<td>123.21 ± 8.00</td>
<td>93.41 ± 8.15</td>
</tr>
<tr>
<td>Bone markers</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>sBAP (IU/l)</td>
<td>75.54 ± 4.62</td>
<td>90.71 ± 4.70</td>
</tr>
<tr>
<td>sNTX (nmol/l)</td>
<td>34.44 ± 2.85</td>
<td>62.09 ± 2.90</td>
</tr>
</tbody>
</table>

Variables are after intervention measurements and represented as "estimated marginal means" ± S.Es.

** 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 0.019; Table S2).

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 HOME-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
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 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 baseline (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 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%.
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 D2) 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 Hba1c values [14]. It has to be pointed that some previous studies [4,9] 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 nmol/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 reported 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 [27]. Observational studies reported higher levels 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].

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 HbA1c. (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.

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