Daily consumption of vitamin D– or vitamin D + calcium–fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial1–3


ABSTRACT

Objective: This study aimed to compare the effects of daily intake of vitamin D– or vitamin D3 + calcium–fortified yogurt drink on glycemic status in subjects with type 2 diabetes (T2D).

Design: Ninety diabetic subjects were randomly allocated to 3 groups to consume plain yogurt drink (PY; containing no vitamin D and 150 mg Ca/250 mL), vitamin D–fortified yogurt drink (DY; containing 500 IU vitamin D3 and 150 mg Ca/250 mL), or vitamin D + calcium–fortified yogurt drink (DCY; containing 500 IU vitamin D3 and 250 mg Ca/250 mL) twice per day for 12 wk. Fasting serum glucose (FSG), glycated hemoglobin (Hb A1c), homeostasis model assessment of insulin resistance (HOMA-IR), serum lipid profile, and percentage fat mass (FM) were assessed before (baseline) and after the intervention.

Results: In both the DY and DCY groups, mean serum 25(OH)D3 improved (+32.8 ± 28.4 and +28.8 ± 16.1 nmol/L, respectively; P < 0.001 for both), but FSG (−12.9 ± 33.7 mg/dL, P = 0.015) and Hb A1c (−0.4 ± 1.2% (P < 0.001) and −0.4 ± 1.9% (P < 0.001)), HOMA-IR (−0.6 ± 1.4 (P = 0.001) and −0.6 ± 3.2 (P < 0.001)], waist circumference (−3.6 ± 2.7 and −2.9 ± 3.3; P < 0.001 for both), and body mass index [in kg/m2]: −0.9 ± 0.6 (P < 0.001) and −0.4 ± 0.7 (P = 0.005)] decreased significantly more than in the PY group. An inverse correlation was observed between changes in serum 25(OH) D3 and FSG (r = −0.208, P = 0.049), FM (r = −0.219, P = 0.038), and HOMA-IR (r = −0.219, P = 0.005).

Conclusion: Daily intake of a vitamin D–fortified yogurt drink, either with or without added calcium, improved glycemic status in T2D patients. This trial was registered at clinicaltrials.gov as NCT01229891. Am J Clin Nutr 2011;93:764–71.

INTRODUCTION

Vitamin D deficiency is still a global health care concern. A growing number of studies have reported widespread vitamin D deficiency and insufficiency in both apparent healthy populations and patients with various pathologies (1). It has been estimated that 1 billion people worldwide are affected by various degrees of vitamin D deficiency (2). Diabetes is the most prevalent endocrinologic disorder in the world. The total number of people with diabetes worldwide is estimated to increase from 171 million in 2000 to >366 million by 2030 (3). Type 2 diabetes (T2D) is associated with increased morbidity and mortality and hence with accelerated health care costs. It can result in many long-term micro- and macrovascular complications clinically presented as blindness, kidney disease, heart disease, and neuropathy, among others. Persons with diabetes are 4 times as likely to have heart disease as are those without diabetes (4).

Since the first report on the influence of vitamin D on insulin secretion (5), which was further supported by animal studies (6, 7), evidence has proposed a role for vitamin D in both the occurrence (8, 9) and treatment (10, 11) of T2D. There is now convincing evidence that vitamin D has some role in both pancreatic insulin secretion (11, 12) and insulin sensitivity (10) and thereby affects the pathogenesis of the disease.

The role of calcium in the development of T2D has been indirectly suggested by cross-sectional studies in which a high calcium intake has been found to be inversely associated with body weight and adiposity (13). Note that adequate calcium intake could indirectly lead to an improvement in vitamin D status because there would be less need for conversion of 25-hydroxyvitamin D [25(OH)D] to 1,25-dihydroxyvitamin D (14). Accordingly, cohort studies have documented associations of dietary calcium and vitamin D intake with a reduced risk of subsequent diabetes (15). These observations have led to the idea that amelioration of glycemic status could be achieved by an
improvement in vitamin D and calcium status in patients with T2D. The high prevalence of vitamin D deficiency (16–18), the metabolic syndrome (18), and T2D (19) in Iran warrants an immediate population-based intervention. Some studies in Iran have shown a high occurrence of vitamin D deficiency in both patients with diabetes and nonpatients with diabetes (20) and in overweight and obese women with and without the metabolic syndrome (TR Neyestani et al, unpublished observations, 2010); no significant difference in serum 25(OH)D3 concentrations was observed between the patients and the healthy control subjects.

The available human data are limited because most, if not all, observational studies have been cross-sectional, whereas prospective studies have not measured circulating vitamin D concentration and there is a paucity of randomized controlled trials with vitamin D and/or calcium supplementation specifically designed for outcomes related to T2D (21). Therefore, a clinical trial was conducted in subjects with T2D to evaluate the possible metabolic effects of regular intake of calcium and/or vitamin D–fortified dairy product. In this study, a native popular yogurt drink (Persian name: doogh) was used for fortification with calcium and/or vitamin D.

SUBJECTS AND METHODS

Study design

The project was conducted during the fall and winter of the year 2009–2010 and started in late October and concluded in early March, during which cutaneous vitamin D3 synthesis is minimal (22).

Diabetic subjects registered at the Iranian Diabetes Society were recruited via phone call. Using data from a previous study on the vitamin D status of Iranian diabetic patients (20), we calculated that a sample of 30 subjects in each group would have 90% power to detect a change in means of 25(OH)D of 1 SD (assuming an effect size of 1).

The inclusion criteria were age 30–60 y and a fasting blood glucose concentration ≥126 mg/dL at the first visit. The exclusion criteria included 1) an inability or unwillingness to participate 2); intake of vitamin D, calcium, or omega-3 (n-3) supplements within the past 3 mo 3); use of medications that could potentially influence vitamin D metabolism (notably estrogens and calcitriol) within the past 3 mo 4); any other concomitant clinical disease that could influence vitamin D metabolism (eg, renal, hepatic, other endocrinologic disorders, and malignancies); and 5) use of insulin or any change in the type or dosage of current hypoglycemic medications during the intervention period.

After a 2-wk run-in period, the subjects were randomly assigned to 1 of 3 groups: 1) plain yogurt drink (PY; containing 150 mg Ca/250 mL and no detectable vitamin D3); 2) vitamin D–fortified yogurt drink (DY; containing 500 IU vitamin D3 and 250 mg Ca/250 mL); or 3) vitamin D + calcium–fortified yogurt drink (DCY; containing 500 IU vitamin D3 and 250 mg Ca/250 mL). The concentrations of calcium and vitamin D3 in the yogurt drinks were determined by a specialized laboratory accredited by the Deputy of Food and Drug of the Iranian Ministry of Health. Patients were instructed to consume one serving of the yogurt drink with both lunch and dinner (ie, 2 servings/d) for 12 wk.

On the first visit, the objectives and the protocol of the study were fully described to the subjects before they signed an informed written consent form. Then, a general questionnaire on demographic data, history of illness, and medication use was completed. In addition, information on duration of sun exposure was also gathered. Participants were asked to recall the number of minutes/hour that they have spent in daylight (23). The subjects were then asked not to change their lifestyle during the run-in period or during the study. All of the subjects were visited at approximately biweekly intervals to both assess their compliance and to deliver the yogurt drinks for the next 2 wk.

All of the participants were also given a consumption instruction manual including a “yogurt drink consumption table” that consisted of 28 empty boxes for each week. The patients were instructed to mount the table where it was readily observed (eg, on the fridge) and to tick each box after consumption of a yogurt drink with each meal. They were also asked to keep the empty bottles and to bring them back on their next visit. Compliance was assessed by checking the consumption tables, counting the empty bottles, and making weekly phone calls. Dietary (24-h recall), anthropometric, and laboratory assessments were done for all of the participants before (baseline) and after the intervention period (Figure 1). The study was approved by the Ethical Committee of the National Nutrition and Food Technology Research Institute.

Diet

The participants were instructed to follow a weight-maintenance diabetic diet for 2 wk (run-in period) according to American Diabetes Association (ADA) guidelines (24), after which
equivalent amounts of dairy products in the diet were replaced by 2 servings of the yogurt drink by a dietitian. Dietary intake was assessed at the beginning and at the end of the intervention period by using a validated 24-h recall questionnaire (25) for 2 d (including a weekend).

**Anthropometric measures and blood pressure**

Weight was measured by using a digital scale to the nearest of 0.1 kg (Seca 808; Seca, Hamburg, Germany) while the participants were wearing light clothing and no shoes. Height was determined with a stadiometer to the nearest of 0.1 cm (Seca). BMI was calculated as weight (kg)/height2 (m). WC was measured at the midpoint between the lower rib and iliac crest at the end of expiration by using a measuring tape to the nearest of 0.1 cm. Percentage body fat mass (FM) was estimated by using a bioelectrical impedance analysis (BIA) system (Quadscan 4000; BodyStat, Douglas, United Kingdom). Blood pressure was measured with a digital system (BC 08; Beurer, Ulm, Germany), whereas the subject was seated for ≥10 min. The average of duplicate measurements was considered for blood pressure. All measurements were done between 0800 and 1000, whereas the subjects were fasting and not allowed to smoke.

**Laboratory investigations**

After 12–14 h of fasting, venous blood samples were collected and kept at room temperature for 30–60 min in the dark. The samples were then centrifuged at 2000 g at room temperature. Sera recovered and transferred to fresh microtubes in aliquots at room temperature for 30–60 min in the dark. The end of expiration by using a measuring tape to the nearest of 0.1 cm. Percentage body fat mass (FM) was estimated by using a bioelectrical impedance analysis (BIA) system (Quadscan 4000; BodyStat, Douglas, United Kingdom). Blood pressure was measured with a digital system (BC 08; Beurer, Ulm, Germany), whereas the subject was seated for ≥10 min. The average of duplicate measurements was considered for blood pressure. All measurements were done between 0800 and 1000, whereas the subjects were fasting and not allowed to smoke.

**Statistical analyses**

Data are expressed as means ± SDs. The normality of data distribution was assessed by using the Kolmogorov-Smirnov goodness-of-fit test. Two-factor repeated-measures analysis of variance (ANOVA) was used to test time × group interactions, with time and treatment as factors. In case of a significant time × group interaction, a between group comparison of changes at 12 mo was done by using ANOVA followed by Tukey’s post hoc analysis. When the time effect was significant, the within-group comparison of values was performed by paired-samples t test with Bonferroni correction. Differences in proportions were evaluated by using a chi-square test. Correlations between variables were evaluated by using either Pearson (r) (for data with normal distribution) or Spearman (rs) (for data with nonnormal distribution) correlations. All statistical analyses were done by using the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc, Chicago, IL). *P < 0.05* was considered significant.

**RESULTS**

The subjects included 55 women and 35 men aged 50.7 ± 6.1 y. Their mean (±SD) ages (PY: 50.8 ± 6.6 y; DY: 51.4 ± 5.4 y; and DCY: 49.9 ± 6.2 y), durations of disease (PY: 9.5 ± 7.1 y; DY: 8.9 ± 5.9 y; and DCY: 8.8 ± 5.4 y), and sex ratios were not significantly different between the groups. All participants completed the study, and overall compliance by the subjects was estimated as being 100%. Categories of vitamin D status improved overall in the 2 groups (Table 1), which was accompanied by a significant increase in serum 25(OH)D3 concentrations in both the DY and DCY groups (Table 2). In contrast, serum 25(OH)D3 in the PY group did not change significantly (Table 2).

Suboptimal vitamin D status observed in 70% of the subjects at baseline in both the DY and DCY groups decreased dramatically to only 10% and 20% (overall 15%) of the subjects, respectively, at the end of the intervention. In the PY group, however, the percentage of the suboptimal vitamin D status did not change.

**Table 1** Comparison of vitamin D status based on serum concentrations of 25-hydroxyvitamin D3 between the 3 groups

<table>
<thead>
<tr>
<th></th>
<th>Before intervention</th>
<th>After intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficiency (n (%))</td>
<td>Insufficiency (n (%))</td>
</tr>
<tr>
<td>PY</td>
<td>14 (46.7)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td>DY</td>
<td>6 (20)</td>
<td>15 (50)</td>
</tr>
<tr>
<td>DCY</td>
<td>15 (50)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>35 (38.9)</td>
<td>31 (34.4)</td>
</tr>
</tbody>
</table>

1 PY, plain yogurt; DY, vitamin D–fortified yogurt drink; DCY, calcium + vitamin D–fortified yogurt drink. Deficiency is defined as <27.5 nmol/L, insufficiency as ≥27.5 to <50 nmol/L, and sufficiency as ≥50 nmol/L.

2 Denotes the significance of differences in the distribution of vitamin D categories between the 3 groups (chi-square test).

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Plain Before</th>
<th>Plain After</th>
<th>Vitamin D–fortified Before</th>
<th>Vitamin D–fortified After</th>
<th>Vitamin D + calcium–fortified Before</th>
<th>Vitamin D + calcium–fortified After</th>
<th>Between-group P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D₃ (nmol/L)</td>
<td>41.6 ± 44.5</td>
<td>37.2 ± 44</td>
<td>44.4 ± 28.7</td>
<td>77.7 ± 28.6</td>
<td>44.5 ± 43.7</td>
<td>74.6 ± 39.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.4 ± 16.7</td>
<td>77.3 ± 16.9</td>
<td>75.0 ± 14.1</td>
<td>72.9 ± 14.3</td>
<td>75.7 ± 11.9</td>
<td>74.6 ± 12.0</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.9 ± 4.7</td>
<td>30.0 ± 4.7</td>
<td>29.2 ± 4.4</td>
<td>28.3 ± 4.4</td>
<td>29.1 ± 5.5</td>
<td>28.6 ± 5.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>97.9 ± 11.0</td>
<td>96.8 ± 11.3</td>
<td>95.6 ± 11.4</td>
<td>92.0 ± 11.7</td>
<td>98.0 ± 10.7</td>
<td>95.1 ± 11.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>102.2 ± 10.5</td>
<td>102.1 ± 9.4</td>
<td>100.5 ± 9.6</td>
<td>99.7 ± 8.5</td>
<td>103.2 ± 9.8</td>
<td>103.3 ± 9.7</td>
<td>0.874</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 ± 0.06</td>
<td>0.94 ± 0.05</td>
<td>0.95 ± 0.07</td>
<td>0.92 ± 0.06</td>
<td>0.95 ± 0.06</td>
<td>0.92 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FM (%)</td>
<td>35.7 ± 7.8</td>
<td>36.9 ± 7.2</td>
<td>33.01 ± 9.5</td>
<td>30.9 ± 9.0</td>
<td>35.1 ± 9.8</td>
<td>34.1 ± 9.8</td>
<td>0.06</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>127.3 ± 14.8</td>
<td>127.7 ± 16.5</td>
<td>131.5 ± 22.6</td>
<td>128.1 ± 16.3</td>
<td>128.3 ± 17.8</td>
<td>131.0 ± 15.5</td>
<td>0.186</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>77.5 ± 10.6</td>
<td>78.0 ± 12.8</td>
<td>77.5 ± 20.0</td>
<td>77.8 ± 8.7</td>
<td>76.9 ± 11.8</td>
<td>78.4 ± 8.7</td>
<td>0.309</td>
</tr>
<tr>
<td>FSG (mg/dL)</td>
<td>187.0 ± 57.1</td>
<td>203.3 ± 64.4</td>
<td>184.1 ± 63.8</td>
<td>171.2 ± 59.2</td>
<td>184.0 ± 57.3</td>
<td>174.3 ± 50.0</td>
<td>0.269</td>
</tr>
<tr>
<td>Fasting serum insulin (mU/L)</td>
<td>7.7 ± 3.2</td>
<td>10.8 ± 5.5</td>
<td>7.7 ± 4.6</td>
<td>6.5 ± 3.3</td>
<td>7.6 ± 4.6</td>
<td>6.4 ± 3.1</td>
<td>0.147</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.4 ± 1.5</td>
<td>5.5 ± 3.7</td>
<td>3.3 ± 2.7</td>
<td>2.7 ± 1.5</td>
<td>3.7 ± 3.3</td>
<td>3.0 ± 1.5</td>
<td>0.282</td>
</tr>
<tr>
<td>Hb A₁c (%)</td>
<td>7.5 ± 1.5</td>
<td>8.7 ± 1.4</td>
<td>7.4 ± 1.8</td>
<td>7.0 ± 2.3</td>
<td>7.8 ± 1.9</td>
<td>7.3 ± 1.3</td>
<td>0.180</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>187.5 ± 43.3</td>
<td>197.3 ± 48.1</td>
<td>177.5 ± 41.9</td>
<td>182.8 ± 43.0</td>
<td>187.7 ± 46.6</td>
<td>178.5 ± 42.4</td>
<td>0.213</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>160.5 ± 100.7</td>
<td>181.3 ± 91.8</td>
<td>155.4 ± 65.9</td>
<td>155.2 ± 55.1</td>
<td>176.6 ± 91.0</td>
<td>161.1 ± 64.3</td>
<td>0.258</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>88.6 ± 25.9</td>
<td>93.5 ± 24.9</td>
<td>87.2 ± 25.1</td>
<td>91.1 ± 19.8</td>
<td>90.2 ± 27.9</td>
<td>96.3 ± 27.8</td>
<td>0.072</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>50.8 ± 8.2</td>
<td>51.8 ± 9.6</td>
<td>46.0 ± 9.6</td>
<td>46.1 ± 10.0</td>
<td>47.2 ± 9.4</td>
<td>48.0 ± 6.8</td>
<td>0.450</td>
</tr>
</tbody>
</table>

All values are means ± SDs. DBP, diastolic blood pressure; FM, fat mass; 25(OH)D₃, 25-hydroxyvitamin D₃; SBP, systolic blood pressure; WHR, waist-to-hip ratio; HOMA-IR, homeostasis model assessment of insulin resistance; Hb A₁c, glycated hemoglobin; FSG, fasting serum glucose.

Denotes the significance within-group changes (paired-samples t-test).

Denotes the significance between the plain and vitamin D–fortified groups (one-factor ANOVA).

Denotes the significance between the vitamin D + calcium–fortified and plain groups (one-factor ANOVA).

Denotes the significance between the vitamin D + calcium– and vitamin D–fortified groups (one-factor ANOVA).

Denotes the time × group interaction (2-factor ANOVA).
Table 3
Comparison of nutrient intakes within and between groups before and after the intervention.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Plan</th>
<th>After Plan</th>
<th>Before Group 1</th>
<th>After Group 1</th>
<th>Before Group 2</th>
<th>After Group 2</th>
<th>Before Group 3</th>
<th>After Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1639.0</td>
<td>1701.9</td>
<td>1745.7</td>
<td>1871.9</td>
<td>1671.9</td>
<td>1824.5</td>
<td>1781.9</td>
<td>1953.7</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>229.2</td>
<td>106.0</td>
<td>212.8</td>
<td>73.5</td>
<td>182.8</td>
<td>249.9</td>
<td>221.2</td>
<td>283.9</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>52.4</td>
<td>26.3</td>
<td>58.4</td>
<td>41.2</td>
<td>58.5</td>
<td>14.6</td>
<td>24.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>18.3</td>
<td>12.7</td>
<td>20.4</td>
<td>8.2</td>
<td>19.2</td>
<td>9.1</td>
<td>16.3</td>
<td>21.7</td>
</tr>
<tr>
<td>Calcium (g/d)</td>
<td>668.9</td>
<td>277.3</td>
<td>693.5</td>
<td>304.8</td>
<td>306.7</td>
<td>30.4</td>
<td>67.7</td>
<td>68.7</td>
</tr>
<tr>
<td>Vitamin D (IU/d)</td>
<td>20.4</td>
<td>8.2</td>
<td>50.2</td>
<td>105.4</td>
<td>12.5</td>
<td>9.1</td>
<td>21.7</td>
<td>28.5</td>
</tr>
</tbody>
</table>

All values are means ± SDs. The extra calcium only in Group 2 and vitamin D in Groups 2 and 3 intakes provided by the fortified yogurt drink are not shown.

DISCUSSION

The present investigation showed that Improvement in the vitamin D status of T2D patients is beneficial for glycemic optimization and weight control. Fortification of a yogurt drink with calcium did not confer further improvement. We found a high occurrence of subclinical vitamin D deficiency (73.3%) and a considerable proportion of severe deficiency (38.9%) in our diabetic subjects. To date, there is no consensus on the optimal concentration of serum 25(OH)D3. However, a wide range between 50 and 100 nmol/L has been proposed as optimal (2). The daily intake of cholecalciferol needed to reach this concentration is likely to be much higher than the recommended 400–600 IU/d for adults (29). In this study, 1000 IU/d in 2 divided doses was used, which is, on the basis of current knowledge, believed to be safe (30). This dose was effective at achieving a serum 25(OH)D3 concentration safely (30). This dose was effective at achieving a serum 25(OH)D3 concentration safely (30). This dose was effective at achieving a serum 25(OH)D3 concentration safely (30).
differences in calcium and vitamin D intakes or in the duration of sun exposure were observed between the 3 groups; therefore, the improvement in vitamin D status was due to the intervention.

**Anthropometric measures**

Our finding on the effect of the daily intake of a vitamin D–fortified yogurt drink (with or without added calcium) on body weight, FM, and WC is noteworthy. Few previous studies have reported the effect of vitamin D supplementation on weight, and the results are contradictory (33, 34). However, several clinical studies have reported the preventive effect of increasing calcium intake against the risk of overweight (35). On the other hand, vitamin D supplementation had a small effect on the prevention of weight gain (36). The weight-lowering effect of vitamin D could be indirectly due to its suppressing effect on parathyroid hormone, which is known to promote fat accumulation by increasing intracellular calcium concentrations (36). Previous cross-sectional studies have shown that 25(OH)D concentrations are associated with body-composition variables, especially body fat (37). In agreement with this, our findings showed a strong inverse association between serum 25(OH)D concentrations and FM or WC. The excess fat, by sequestering vitamin D, makes it less available for use by the body (38). On the other hand, vitamin D supplementation had a small effect on the prevention of weight gain (39). The possible effects of vitamin D on adipocyte and weight gain remain to be clarified.

**Lipid profile**

Despite a relatively healthy lipid profile in our subjects, a slight nonsignificant improvement in serum concentrations of cholesterol and a significant reduction in serum triglycerides were seen at 12 wk in the DCY group compared with the PY group. It is hypothesized that an increase in calcium intake can reduce serum triglycerides by decreasing hepatic triglyceride formation and/or secretion via an effect on hepatocellular calcium (40). The effect of vitamin D and calcium intake on lipid profile in dyslipidemic subjects needs to be investigated further.

**Glycemic status**

The rationale for vitamin D treatment in patients with T2D is based on studies that suggest that vitamin D may have some role in insulin secretion and/or sensitivity (41). Data from several studies have shown that hypovitaminosis D might play an important role in the pathogenesis of T2D (42). In cross-sectional studies, lower serum 25(OH)D concentrations have been associated with an increased risk of developing T2D (9, 15, 43), and serum 25(OH)D3 concentrations have been correlated negatively with glucose and insulin concentrations and also with insulin resistance (9, 10). Interventional studies investigating vitamin D supplementation alone or in combination with calcium for the prevention or treatment of T2D have shown minimal effects, and the results of these studies in humans have been inconsistent (11, 21, 44, 45).

A large 20-y observational study reported a decreased incidence of T2D in women taking vitamin D and calcium supplements (15). However, a randomized placebo-controlled trial did not show a reduction in the development of T2D with vitamin D and calcium supplementation (43). However, despite a similar calcium dosage in both studies, the dosage of vitamin D in the clinical trial (400 IU) was much lower than that in the observational study (800 IU).

The results of the longest and largest vitamin D treatment studies were reported by Pittas et al (44). A post hoc analysis of a 3-y trial designed to assess the effects of vitamin D plus calcium in 314 elderly persons without diabetes found that the increase in fasting blood glucose concentrations during the study was lower with vitamin D therapy than with placebo, but only in the group that had impaired fasting glucose at baseline. Insulin resistance, as assessed by HOMA-IR, increased in the placebo group, but not in the vitamin D group (44). It is not possible to determine whether the observed effect was related to vitamin D therapy, calcium therapy, or both, because only combination therapy was given.

Previous studies had many possible limitations, such as a small sample size (46), inclusion of subjects with or without diabetes (47), the lack of a control group (48), a short intervention period (46, 47), the use of a low dose of vitamin D (47), and low bioavailability from supplements (46, 47). To eliminate the aforementioned limitations, we enrolled a sufficient number of subjects to detect changes with adequate power in a double-blind randomized clinical trial. Our finding of beneficial effects of vitamin D supplementation on glucose homeostasis in T2D are supported by evidence that calcium and vitamin D improve factors contributing to the development of T2D (pancreatic β cell function, insulin sensitivity, and inflammation) (21). For instance, glucose-stimulated insulin secretion is impaired in islets from vitamin D–deficient rats (49). Administration of small doses of 1,25-dihydroxyvitamin D3 to vitamin D–deficient rats led to restoration of insulin secretion (41). The effect of vitamin D on insulin secretion (21, 50) and action (21, 51, 52) may be mediated either directly or indirectly.

Our finding of decreased insulin resistance in the DY and DCY groups indicates an improvement in the glucose-stimulated insulin response (21, 41, 49). Consistent with our findings, a recent study reported that vitamin D supplementation can improve insulin resistance and decrease fasting insulin (53).

Evidence of an association between vitamin D deficiency and insulin resistance is limited, but the reports of the effect of vitamin D supplementation on insulin resistance from short-term, nonrandomized, uncontrolled human trials are conflicting (11, 12, 54). Extrapolation from the observations in the current study suggests that increasing 25(OH)D3 from 45 to 76 nmol/L can improve insulin sensitivity by 13.3%, which is comparable with the effect of metformin (55). Of particular interest, after adjustment for FM and weight, the correlation between changes in serum 25(OH)D and glycemic markers disappeared. However, in an analysis of covariance, differences in changes in FSG (P = 0.015, P = 0.049 respectively), insulin (both P < 0.001), and HOMA-IR (P < 0.001, P = 0.002, respectively) between the DY or DCY and PY groups after adjustment for FM remained significant. These findings indicate both the direct and indirect glycemic optimizing effects of vitamin D in our subjects.

To the best of our knowledge, this is the first report of a controlled clinical trial in diabetic patients to evaluate the effects of daily vitamin D, with or without calcium supplementation, in the form of a fortified yogurt drink, on anthropometric, metabolic, and glycemic status. In conclusion, daily intake of a yogurt drink fortified with cholecalciferol, either with
or without added calcium, for 12 wk improved the anthropometric and glycemic status in the subjects with T2D. Our findings indicated both the direct (on insulin secretion and insulin sensitivity) and indirect (by decreasing weight and FM) glycemic optimizing effects of vitamin D. Therefore, replenishment of vitamin D might be taken into consideration as a preventive nutritional strategy against development and as an adjunct treatment of diabetes.

However, this study had some limitations. The changes observed after the 12-wk intervention period do not necessarily reflect the long-term outcomes of the daily consumption of a fortified yogurt drink. Moreover, no practical approach was used to control intake of the yogurt drinks; intakes were reported by the subjects. A strength of this study was that the expected increases in serum 25(OH)D indicated satisfactory compliance with supplementation by the study subjects.

Our findings have potentially important public health implications, because the modest effect of vitamin D intake on anthropometric and glycemic status in individual persons translates to a dramatic effect in the population as a whole because the high prevalence of hypovitaminosis D carries an attributable risk of T2D and the metabolic syndrome.

We acknowledge the Iranian Diabetes Society for their cooperation, with special thanks to its head, Asadollah Rajab. We also appreciate all of the subjects who participated in this study. All of the laboratory bench work was conducted at the Laboratory of Nutrition Research. The authors' responsibilities were as follows—TRN and MF: designed and supervised the study. TRN, MF, and BN: were involved in all stages of the research, including all laboratory bench work; HA-M: supervised the estimation of the sample size and the statistical analyses; AK and NS: were involved in the anthropometric measures and laboratory assays; AG: performed the HPLC analyses; AH: supervised the dietary assessments; and SH, NT, SS, and MZ: recruited the subjects, arranged the visits, instructed the patients, and conducted the follow-up. None of the authors declared a conflict of interest.

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