Improvement of Vitamin D Status via Daily Intake of Fortified Yogurt Drink Either with or without Extra Calcium Ameliorates Systemic Inflammatory Biomarkers, including Adipokines, in the Subjects with Type 2 Diabetes


Laboratory of Nutrition Research, National Nutrition and Food Technology Research Institute and Faculty of Nutrition and Food Technology (T.R.N., B.N., N.S., A.K., N.T., S.H., S.S., M.Z.), and Department of Biostatistics (H.A.-M.), Shahid Beheshti University of Medical Sciences, 1981619573 Tehran, Iran

Context: Systemic inflammation is thought to have a central role in diabetic long-term complications.

Objective: The aim of this study was to investigate the effects of vitamin D either with or without extra calcium on certain inflammatory biomarkers in the subjects with type 2 diabetes (T2D).

Design, Setting, and Participants: This was a double-blind, randomized, controlled trial conducted over 12 wk in 90 T2D subjects aged 30–60 yr from both sexes.

Intervention: Subjects were randomly allocated to one of three groups to receive two 250-ml bottles a day of plain Persian yogurt drink or doogh (PD, containing 150 mg calcium and no detectable vitamin D₃/250 ml), vitamin D-fortified doogh (DD, containing 500 IU vitamin D₃ and 150 mg calcium/250 ml), or calcium + vitamin D₃-fortified doogh (CDD, containing 500 IU vitamin D₃ and 250 mg calcium/250 ml).

Outcome Measures: The changes in inflammatory markers were evaluated.

Results: Compared to the baseline values, highly sensitive C-reactive protein, IL-1β, IL-6, fibrinogen, and retinol binding protein-4 concentrations significantly decreased in both the DD and CDD groups. Although the decrement in highly sensitive C-reactive protein and fibrinogen was more in CDD compared to DD (−4.0 ± 8.5 vs. −1.3 ± 2.8 mg/liter, and −0.40 ± 0.74 and −0.20 ± 0.52 mg/liter, respectively), the differences were not significant. There was a significant increase in serum adiponectin in both the DD and CDD groups (51.3 ± 65.3 vs. 57.1 ± 33.8 μg/liter; *P* < 0.05). Mean adiponectin changes in CDD were significantly higher than in PD (*P* = 0.021).

Conclusions: Daily intake of vitamin D-fortified doogh improved inflammatory markers in T2D subjects, and extra calcium conferred additional benefit only for the antiinflammatory adipokine, i.e. adiponectin. (*J Clin Endocrinol Metab* 97: 0000–0000, 2012)

The possible roles of low-grade systemic inflammation and activated innate immunity in the pathogenesis of type 2 diabetes (T2D) have increasingly been the focus of interest for both scientists and clinicians during the last decade. Several studies have shown that inflammatory markers, including cytokines and acute-phase reactants, are strong predictors of T2D development (1). Adipokines and their associations with obesity and the related comor-
bidities including systemic inflammation, insulin resistance, and diabetes, are rather a new area of concern. Several adipokines including adiponectin, retinol binding protein (RBP)-4, and lipocalin (LCN)-2 have been demonstrated to affect both insulin activity and inflammatory reactions (2). Osteopontin (OPN), an extracellular matrix proinflammatory cytokine that induces chemotaxis, has been suggested as a link between bone and the immune system and a mediator of macrophage infiltration in adipose tissue and consequent insulin resistance in the murine model (3). These inflammatory cytokines may elicit atherogenic immune reactions with autoreactive T cells and antibodies, mostly of IgG isotype (4).

Vitamin D plays an important role in calcium homeostasis and is essential for bone growth and preservation. Recently, noncalcemic functions of vitamin D, especially antiinflammatory and immunomodulatory effects, have attracted much attention. In animal studies, beneficial effects of vitamin D and its analogs in various autoimmune disorders were demonstrated (5). We recently reported the beneficial effect of vitamin D intake on circulating inflammatory endothelial biomarkers in a separate population of diabetic subjects (6).

Notwithstanding, evidence for the antiinflammatory effect of vitamin D from clinical studies in humans is still scarce. More importantly, there is still no convincing evidence for the possible ameliorating effect of vitamin D in specific human disorders with inflammatory nature. The effect of calcium intake on glycemic status and related systemic inflammation is even more controversial. Although some studies have suggested a protective role for calcium intake against insulin resistance and consequent diabetes (7), some other studies did not support this notion (8). Although calcium supplementation may not affect systemic inflammation as judged by serum C-reactive protein (CRP) (9), calcium and vitamin D together may synergistically have a protective effect against diabetes (10) and probably its related systemic inflammation.

Recently, we reported the glycemic optimizing effect of both vitamin D and vitamin D + calcium intake via fortified Persian yogurt drink (doogh) in subjects with T2D (11). Here, the effects of daily intake of vitamin D either with or without extra calcium on certain selected biomarkers of systemic inflammation and immunity (including adipokines) were investigated using the same study population and protocol.

**Subjects and Methods**

The study design and inclusion criteria have been described elsewhere (11). Briefly, 90 known cases of type 2 diabetic patients aged 30–60 yr were enrolled only if they met these inclusion criteria: 1) fasting blood glucose concentration above 126 mg/dl on the first visit; 2) not taking steroidal antiinflammatory or anticoagulant medications; 3) not taking dietary supplements including calcium, vitamin D, or omega-3 within the past 3 months before the intervention; 4) not receiving medications that could potentially influence vitamin D metabolism or insulin; and 5) not having any other clinical disease that could influence vitamin D metabolism (e.g., renal, hepatic, other endocrinological disorders and malignancies).

After complete description of the study protocol and objectives, all participants signed a written informed consent. After a 2-wk run-in period, all subjects were randomly assigned in a double-blind manner to one of the three groups to receive two 250-ml bottles a day of plain Persian yogurt drink or doogh (PD), containing 150 mg calcium and no detectable vitamin D3/250 ml; vitamin D-fortified doogh (DD), containing 500 IU vitamin D3 and 150 mg calcium/250 ml; or calcium + vitamin D-fortified doogh (CDD), containing 500 IU vitamin D3 and 250 mg calcium/250 ml. The duration of intervention was 12 wk. Subjects’ compliance and dietary intake were evaluated as described earlier (11). A summary of the study protocol is demonstrated in Fig. 1.

This study was part of the calcium + vitamin D-fortified yogurt drink (doogh) and diabetes project, which has been approved by the Ethical Committee of the National Nutrition and Food Technology Research Institute (NNFTRI).

**Laboratory investigations**

The procedures of blood handling and serum separation have been described elsewhere (11). Insulin resistance was evaluated using homeostasis model assessment of insulin resistance (12), as described earlier (11). Serum 25-hydroxyvitamin D [25(OH)D] concentration was determined using reverse-phase HPLC (13). Serum highly sensitive C-reactive protein (hsCRP), IgM, and IgG were measured by immunoturbidimetric assay (Pars-Azmoon Inc., Tehran, Iran) and an autoanalyzer (Selecta E; Vitalab, Holliston, The Netherlands).

Fibrinogen was measured using a coagulometer (STart; Diagnostica Stago, Paris, France).

Serum adiponectin (Merckia, Uppsala, Sweden), LCN-2 (BioVendor, Munich, Germany), RBP-4 (Casabio, Wuhan, China) and plasma OPN (IBL, Tokyo, Japan) were assessed using enzyme-linked immunosassay method.

**Peripheral blood mononuclear cells (PBMC) separation and culture and cytokine assay**

PBMC were separated and cultured in RPMI 1640 medium (Sigma-Aldrich, Inc., St. Louis, MO) supplemented with 100 U/ml penicillin G, 10 ng/ml streptomycin (both, Sigma-Aldrich, Inc.), 1% phytohemagglutinin, and 100 ng/ml lipopolysaccharide (both, Fluka, Tokyo, Japan), as described previously (14) with some minor modifications. Human PBMC were plated at a density of 2 × 10^6 cells/well in 12-well plates, and cells were kept for 24 h at 37°C in a 5% humidified CO2 incubator. Then, cytokines [IL-1β, IL-6, TNF-α, and interferon (IFN)-γ] in the cell culture supernatants were measured using commercially available enzyme-linked immunosassay kits (all from Bender-Medsystem, Vienna, Austria) and a microplate reader (Statfax 3200; Awareness Technology, Inc., Palm City, FL).
Statistical analyses

Normality of data distribution was checked using Kolmogrov-Smirnov. Continuous variables were expressed as mean ± SD. Time and group interactions were analyzed by repeated measures ANOVA, with time and treatment as factors. Then, ANOVA was employed to compare changes of values among the groups. To control the possible effect of a potential confounder, analysis of covariance was employed. Moreover, we evaluated time effects within each study group using the paired t-test. Correlation between two sets of data were evaluated using Pearson’s equation. P value < 0.05 (two-tailed test) was considered statistically significant. All statistical analyses were performed using Statistical Package for Social Sciences (version 16.0, 2007; SPSS Inc., Chicago, IL).

Results

Baseline characteristics of the participants have been fully described elsewhere (11). However, some initial anthropometric and biochemical values of the three groups are demonstrated in Table 1. Baseline data did not differ significantly among the groups except for IL-1β, which was significantly lower in PD than in DD (P = 0.006), but there was no difference between PD and CDD (P = 0.090) or between DD and CDD (P = 0.538). To eliminate the possible effects of the initial values on the final ones, changes of the variables were compared among the groups.

After the 12-wk intervention, both DD and CDD groups showed improvement of vitamin D status, confirming high bioavailability of vitamin D in doogh (11).

Compared with the baseline values, hsCRP, IL-1β, IL-6, RBP-4, and fibrinogen concentrations significantly decreased in both DD and CDD groups. The between-group differences for IL-1β and fibrinogen changes remained significant even after controlling for changes of homeostasis model assessment of insulin resistance (P = 0.001 and P = 0.031, respectively). As for hsCRP, the decrement in the CDD group was more than in the DD group, but the difference between these two

<table>
<thead>
<tr>
<th>TABLE 1. Comparison of the initial values of certain characteristics in the three groups (11)</th>
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<tbody>
<tr>
<td>PD</td>
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<tr>
<td>Age (yr)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>WC (cm)</td>
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<td>WHR</td>
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<tr>
<td>FM (%)</td>
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<td>FSG (mg/dl)</td>
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<td>HbA1c (%)</td>
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<td>TC (mg/dl)</td>
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<td>TG (mg/dl)</td>
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<tr>
<td>LDL (mg/dl)</td>
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<td>HDL (mg/dl)</td>
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BMI, Body mass index; FSG, fasting serum glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHR, waist to hip ratio.
groups was insignificant \((P = 0.147)\). In addition, there was a significant increase in serum IgM and adiponectin in both treatment groups. However, the significant difference in serum IgM changes among the groups disappeared after controlling for body mass index \((P = 0.104)\) or body fat mass (FM) changes \((P = 0.091)\) but remained significant after controlling for age \((P = 0.032)\). Mean adiponectin changes in the CDD group were significantly higher than in the PD group \((P = 0.021)\), and interestingly, this difference remained significant even after controlling for both FM changes and age \((P = 0.015)\). Concentrations of TNF-\(\alpha\), IFN-\(\gamma\), LCN-2, IgG, and plasma OPN did not differ significantly in DD and CDD groups after 12 wk, compared with the baseline values (Table 2). Changes of plasma fibrinogen correlated with those of hsCRP \((r = 0.252; P = 0.017)\).

**Discussion**

In the present clinical study, we showed for the first time that a daily intake of 1000 IU vitamin D either with or without extra calcium for 12 wk resulted in a significant decrease of serum hsCRP and plasma fibrinogen concentrations and also in improved serum adipokines including adiponectin and RBP-4 and decreased cellular secretion of the inflammatory cytokines IL-6 and IL-1\(\beta\) in type 2 diabetic subjects. Our findings are in general agreement with those from *in vitro* experiments that showed the suppressor effect of vitamin D on production of inflammatory cytokines by human macrophages and fibroblasts (15).

There are only a few studies on the association of serum levels of 25(OH)D with cytokines *in vivo*, and the results are occasionally conflicting. For example, whereas one study showed vitamin D supplementation decreased serum IL-1 in postmenopausal women (16), another study reported no effect of cholecalciferol supplements on serum cytokines and inflammatory markers in overweight and obese subjects (17). It is noteworthy that most reports of the beneficial effects of vitamin D on inflammatory cytokines have been predominantly on subjects with chronic diseases (16, 18), the findings of which have not been supported by data obtained from healthy subjects (19). It is possible that the immunomodulatory effect of vitamin D can be more clearly observed when the immune system is stimulated (17).

Two recent observational studies have reported higher CRP concentrations among subjects with hypovitaminosis D (20, 21), whereas two trials of vitamin D supplementation *vs.* placebo showed conflicting results; after vitamin D supplementation, CRP declined among patients in the intensive care unit (22), whereas it remained unchanged in outpatients with congestive heart failure (18). The current study expanded our knowledge of this putative role of vitamin D because of the various inflammatory biomarkers investigated, many of which have not been previously studied with respect to vitamin D.

The mechanisms by which vitamin D influences inflammatory biomarkers are not clearly known. However, an *in vitro* line of evidence has demonstrated that the antiinflammatory effect of the active metabolite of vitamin D, 1,25-dihydroxyvitamin D\(_3\), and its analogs could be mediated by

<table>
<thead>
<tr>
<th>Variable</th>
<th>PD Before</th>
<th>PD After</th>
<th>PD (P^a)</th>
<th>DD Before</th>
<th>DD After</th>
<th>DD (P^a)</th>
<th>CDD Before</th>
<th>CDD After</th>
<th>CDD (P^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D(_3) (nmol/liter)</td>
<td>41.6 ± 44.5</td>
<td>37.2 ± 44</td>
<td>0.136</td>
<td>44.4 ± 28.7</td>
<td>77.7 ± 28.6</td>
<td>&lt;0.001</td>
<td>44.5 ± 43.7</td>
<td>74.6 ± 39.5</td>
<td>&lt;0.001</td>
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<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td>57.3 ± 7.8</td>
<td>36.9 ± 7.2</td>
<td>0.07</td>
<td>33.01 ± 9.5</td>
<td>30.9 ± 9.0</td>
<td>0.001</td>
<td>35.1 ± 9.8</td>
<td>34.1 ± 9.8</td>
<td>0.06</td>
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<tr>
<td>HOMA</td>
<td>3.4 ± 1.5</td>
<td>5.5 ± 3.7</td>
<td>0.001</td>
<td>3.3 ± 1.8</td>
<td>2.7 ± 1.5</td>
<td>0.032</td>
<td>3.7 ± 3.3</td>
<td>3.0 ± 1.5</td>
<td>0.282</td>
</tr>
<tr>
<td>CRP (mg/liter)</td>
<td>3.7 ± 5.7</td>
<td>2.9 ± 4.4</td>
<td>0.166</td>
<td>3.3 ± 4.5</td>
<td>1.9 ± 2.1</td>
<td>0.013</td>
<td>5.7 ± 9.4</td>
<td>1.6 ± 1.5</td>
<td>0.015</td>
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<tr>
<td>Adiponectin ((\mu)g/liter)</td>
<td>105.3 ± 83.1</td>
<td>125.6 ± 56.3</td>
<td>0.06</td>
<td>105.5 ± 68.7</td>
<td>156.9 ± 89.6</td>
<td>&lt;0.001</td>
<td>92.8 ± 41.2</td>
<td>150.0 ± 59.5</td>
<td>&lt;0.001</td>
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<tr>
<td>LCN ((\mu)g/liter)</td>
<td>2.81 ± 1.52</td>
<td>2.61 ± 1.06</td>
<td>0.054</td>
<td>2.53 ± 1.0</td>
<td>2.7 ± 0.9</td>
<td>0.012</td>
<td>2.99 ± 1.65</td>
<td>2.27 ± 0.98</td>
<td>0.049</td>
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<tr>
<td>RBP-4 ((\mu)g/liter)</td>
<td>50.3 ± 19.3</td>
<td>45.2 ± 21.0</td>
<td>0.102</td>
<td>61.3 ± 18.7</td>
<td>43.2 ± 13.2</td>
<td>&lt;0.001</td>
<td>55.7 ± 27.6</td>
<td>42.1 ± 18.0</td>
<td>0.003</td>
</tr>
<tr>
<td>OPN ((\mu)g/liter)</td>
<td>61.4 ± 18.5</td>
<td>62.6 ± 21.5</td>
<td>0.739</td>
<td>55.7 ± 19.8</td>
<td>53.0 ± 16.1</td>
<td>0.491</td>
<td>66.4 ± 55.3</td>
<td>58.6 ± 21.8</td>
<td>0.421</td>
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<tr>
<td>IL-1 (ng/liter)</td>
<td>264.2 ± 231.0</td>
<td>192.3 ± 171.7</td>
<td>0.275</td>
<td>601.1 ± 306.8</td>
<td>144.2 ± 60.4</td>
<td>&lt;0.001</td>
<td>489.0 ± 427.5</td>
<td>155.9 ± 54.2</td>
<td>0.003</td>
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<tr>
<td>IL-6 (ng/liter)</td>
<td>153.6 ± 69.5</td>
<td>138.1 ± 48.7</td>
<td>0.347</td>
<td>174.8 ± 76.4</td>
<td>115.8 ± 9.7</td>
<td>0.003</td>
<td>155.5 ± 61.2</td>
<td>116.0 ± 25.5</td>
<td>0.008</td>
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<tr>
<td>TNF-(\alpha) (ng/liter)</td>
<td>616.6 ± 234.1</td>
<td>797.3 ± 329.8</td>
<td>0.009</td>
<td>820.4 ± 485.4</td>
<td>707.4 ± 352.6</td>
<td>0.242</td>
<td>794.4 ± 689.5</td>
<td>755.6 ± 406.5</td>
<td>0.808</td>
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<tr>
<td>IFN-(\gamma) (ng/liter)</td>
<td>254.4 ± 69.0</td>
<td>283.9 ± 99.2</td>
<td>0.113</td>
<td>271.8 ± 69.2</td>
<td>262.3 ± 119.3</td>
<td>0.758</td>
<td>282.8 ± 143.2</td>
<td>260.9 ± 118.8</td>
<td>0.468</td>
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<tr>
<td>Fibrinogen (g/liter)</td>
<td>2.95 ± 0.68</td>
<td>3.00 ± 0.69</td>
<td>0.708</td>
<td>2.79 ± 0.63</td>
<td>2.59 ± 0.59</td>
<td>0.042</td>
<td>3.11 ± 0.65</td>
<td>2.71 ± 0.70</td>
<td>0.006</td>
</tr>
<tr>
<td>IgM (g/liter)</td>
<td>1.00 ± 0.69</td>
<td>0.99 ± 0.66</td>
<td>0.515</td>
<td>0.72 ± 0.43</td>
<td>0.89 ± 0.46</td>
<td>0.016</td>
<td>0.95 ± 0.76</td>
<td>1.10 ± 0.81</td>
<td>0.015</td>
</tr>
<tr>
<td>IgG (g/liter)</td>
<td>11.53 ± 3.15</td>
<td>11.22 ± 3.12</td>
<td>0.618</td>
<td>11.24 ± 2.49</td>
<td>11.00 ± 2.37</td>
<td>0.338</td>
<td>11.43 ± 2.83</td>
<td>11.00 ± 2.59</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Data are expressed as means ± sd. 25(OH)\(_3\), 25-hydroxyvitamin D\(_3\); HOMA, homeostasis model assessment.

\(P^a\) Significance of within-group changes (paired-samples t test).

\(P^b\) Significance between the PD (plain) and vitamin D-fortified (DD) groups (one-factor ANOVA).

\(P^c\) Significance between the calcium + vitamin D-fortified (DD) and PD groups (one-factor ANOVA).

\(P^d\) Significance between the CDD and DD groups (one-factor ANOVA).

\(P^e\) Time-group interaction (two-factor ANOVA).
coupling to the vitamin D receptor and the subsequent down-regulation of several cytokines, namely IL-2, IL-12, TNF-α, IFN-γ, and granulocyte-macrophage colony-stimulating factor (23), as well as IL-1β and IL-6 (24). We previously reported improved insulin resistance after daily vitamin D intake in diabetic subjects (11). The mechanism of this effect is less understood. Altered adipokine profile can be a possible hypothesis. Adiponectin favors glucose uptake and free fatty acid oxidation in muscle and inhibits gluconeogenesis in liver, thereby improving insulin sensitivity (25). It is of note that the association between vitamin D status and circulating adiponectin was demonstrated mostly in subjects with abnormal glucose tolerance. It has been suggested that abnormal glucose tolerance is a proinflammatory state causing reduced adiponectin levels probably through the action of proinflammatory cytokines such as TNF-α and IL-1 (26). Antiinflammatory action of vitamin D may thus modulate the effect of proinflammatory cytokines on circulating adiponectin. Moreover, because vitamin D receptor has been identified in preadipocytes (27), it is likely that 1,25-dihydroxyvitamin D may regulate the adiponectin gene expression. An in vitro study lends further support to this notion (28).

The significant difference in serum adiponectin in CDD compared with PD even after adjustment for FM changes is noteworthy. This adiponectin-enhancing effect may be attributed to the extra calcium intake in the CDD group, which might have acted synergistically with vitamin D. In several studies, the high molecular weight isoform of adiponectin was the predominant form in human serum and correlated best with protection from features of metabolic syndrome and improved insulin sensitivity (29). In addition, it has been suggested that calcium promotes both the formation and biological activity of high molecular weight adiponectin (30).

A significant increase in serum adiponectin and a concomitant decrease in serum RBP-4 in the DD and CDD groups in the present study suggested adipokines as the mediators for the effect of vitamin D on insulin resistance. Moreover, the extra calcium at the dosage used did not confer additional benefit on RBP-4 concentrations. RBP-4 is a recently identified adipokine whose elevation has been reported in people with T2D (31).

In the current study, daily intake of 1000 IU vitamin D either with or without extra calcium for 12 wk did not affect plasma OPN concentrations significantly. OPN is a multifactorial protein that is overexpressed in chronic inflammatory conditions (32). It has also been demonstrated that OPN expression is enhanced in human diabetic arteries, as a consequence of high glucose levels (33). Considering the ameliorating effect of vitamin D intake on several inflammatory markers found in this study, it is speculated that higher intakes of vitamin D would be needed to suppress plasma OPN concentrations.

Raised serum IgM in both the DD and CDD groups, compared with the PD group, and disappearance of the difference after adjustment for FM changes indicates a possible role of adipose tissue in the humoral immunity. The inverse association of serum IgM with FM in the subjects with T2D has been reported earlier (34). Natural antibodies of IgM isotype have been shown to be protective against atherosclerosis (35). Notwithstanding, the importance of this finding remains to be clarified.

Our finding of a significant decrease in plasma fibrinogen in both DD and CDD groups after a 12-wk intervention is remarkable. Fibrinogen, indeed, is an inflammatory biomarker that has been independently associated with coronary heart disease and stroke (36). Having a key role in thrombus formation and platelet aggregation, fibrinogen is a major contributor to plasma viscosity. It is also an acute-phase reactant that is overexpressed in inflammatory states and, as found in the current study, is closely associated with CRP (37).

Whatever the mechanism, decreased plasma fibrinogen in diabetes may have preventive implications against diabetic long-term complications, notably cardiovascular disease and stroke. It has been shown that in a hyperglycemic milieu, fibrinogen can become hyperglycosylated (38). When this abnormal fibrinogen clots, the resulting fibrin structure is composed of small-diameter fibers that are markedly resistant to degradation by plasmin (38).

Finally, to evaluate the overall effect of the intervention on systemic inflammation, multivariate ANOVA was employed with hsCRP, fibrinogen, adiponectin, IL-1, IL-6, and TNF-α concentrations as dependent variable, and treatment (control or intervention) as independent variable. The results revealed a significant improvement on this set of inflammatory markers ($P=0.020$).

Some limitations of the current study must be acknowledged. Most of the subjects in our study (73.3%) had vitamin D deficiency and insufficiency, and an increase of 30.8 nmol/liter in circulating 25(OH)D in the fortified groups was too low to optimize all vitamin D-dependent functions. It has been suggested that serum 25(OH)D concentrations of 80 to 225 nmol/liter can be considered adequate (39). The long-term effects of the intervention remain to be elucidated by further studies.

In conclusion, 12-wk daily intake of vitamin D-fortified doogh improved inflammatory markers in type 2 diabetic patients, and extra calcium conferred additional benefit for increasing the antiinflammatory adipokine, i.e., adiponectin. Our results may therefore offer interesting ther-
apeutic options for those diseases that are associated with up-regulated proinflammatory cytokines.

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Address all correspondence and requests for reprints to: Dr. Tirang R. Neyestani, Laboratory of Nutrition Research, National Nutrition and Food Technology Research Institute (NNFTRI), Shahid Beheshti University of Medical Sciences, 1981619573 Tehran, Iran. E-mail: t.neyestani@nnftri.ac.ir or neytr@yahoo.com.

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Authors’ Contributions: T.R.N. designed and supervised the study and prepared the final manuscript. B.N. was involved in all stages of the research, including the laboratory bench work and writing the preliminary manuscript. H.A.-M. supervised all the statistical analyses. N.S. and A.K. were involved in the anthropometric and laboratory evaluations. N.T., S.H., S.S., and M.Z. recruited the subjects, arranged the visits, instructed the patients, and conducted the follow-ups.

This study was registered at www.ClinicalTrials.gov (no. NCT01229891).

Disclosure Summary: The authors have nothing to declare.

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