Calcium-vitamin D co-supplementation influences circulating inflammatory biomarkers and adipocytokines in vitamin D insufficient diabetics: a randomized controlled clinical trial

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Context: To the best of our knowledge, no study has examined the effects of vitamin D-calcium co-supplementation on inflammatory biomarkers and adipocytokines in vitamin D insufficient type 2 diabetics.

Objective: This study was performed to assess the effects of vitamin D and calcium supplementation on inflammatory biomarkers and adipocytokines in vitamin D insufficient people with type 2 diabetes.

Methods: Totally, 118 diabetic patients were enrolled in this randomized placebo controlled clinical trial. After matching for age, sex, body mass index, type and dose of hypoglycemic agents and duration of diabetes, subjects were randomly assigned into four groups receiving: 1) 50000 IU/wk vitamin D + calcium placebo; 2) 1000 mg/d calcium+vitamin D placebo; 3) 50000 IU/wk vitamin D + 1000 mg/d calcium; 4) vitamin D placebo+calcium placebo for 8 weeks. Blood sampling was done for quantification of inflammatory biomarkers and adipocytokines at study baseline and after 8 weeks of intervention.

Results: Calcium (changes from baseline: -75±19 ng/ml, P=0.01) and vitamin D alone (-56±19 ng/ml, P=0.01) and joint calcium-vitamin D supplementation (-92±19 ng/ml, P=0.01) resulted in a significant reduction in serum leptin levels compared with placebo (-9±18 ng/ml). This was also the case for serum IL-6; such that calcium (-2±1 pg/ml, P<0.001) and vitamin D alone (-4±1 pg/ml, P<0.001) and their combination (-4±1 pg/ml, P<0.001) led to significant reductions compared with placebo (3±1 pg/ml). After adjustment for potential confounders, individuals in calcium (-3.1±1.3, P<0.05), vitamin D (-3.1±1.3, P<0.05) and joint Ca-D groups (-3.4±1.3, P<0.05) had greater reductions in serum TNF-α concentrations compared with placebo (0.1±1.2). Individuals who received joint calcium-D supplements tended to have a decrease in serum hs-CRP levels compared with placebo after controlling for baseline levels (-1.14±0.25 vs. 0.02±0.24 ng/ml, P=0.09).

Conclusion: Joint calcium-vitamin D supplementation might improve systemic inflammation through decreasing IL-6 and TNF-α concentrations in vitamin D insufficient people with type 2 diabetes.

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Abbreviations:

Diabetes is a leading cause of morbidity and mortality worldwide (1). It has been reported that the prevalence of diabetes is around 8.5%, worldwide (2). National estimates in Iran revealed that 7.7% of adults were affected by diabetes (3). Systemic inflammation has been linked primarily to insulin resistance, beta cell dysfunction and type 2diabetes (4, 5). In addition, increased inflammation is associated with diabetes complications (6). Therefore, finding a strategy to reduce inflammation in diabetic patients is of great importance.

Prior observational studies have demonstrated that vitamin D and calcium intake might influence systemic inflammation (7–11). Few data are also available linking calcium intake to inflammation. An inverse association was reported between consumption of dairy products and inflammation in healthy adults (12, 13). Calcium intake (≥600 mg/d) was also inversely associated with serum levels of leptin, adiponectin, and inflammatory biomarkers (14). Despite such information from observational studies, few data from clinical trials are available indicating the effects of calcium and vitamin D on inflammation. Vitamin D replenishment of type 2 persons with diabetes has been shown to improve inflammatory biomarkers and glycemic control (15). However, findings in nondiabetic adults are conflicting (16–18).

Calcium and vitamin D has been hypothesized to act jointly rather than independently (19). It seems that their combined effects on inflammation, and consequently on glycemic control in persons with diabetes, might be better than their individual effects. Few clinical trials, mostly in nondiabetic adults, have examined the combined effects of calcium-vitamin D supplementation on inflammatory biomarkers (20–22). Findings from these studies are conflicting. We are aware of no study that examined the effects of calcium-vitamin D cosupplementation on inflammatory biomarkers in vitamin D insufficient people with diabetes who are on oral hypoglycemic agents. The aim of this study was to assess the effects of vitamin D and calcium supplementation alone and in combination on inflammatory biomarkers in vitamin D insufficient diabetic patients.

Subjects and Methods

Participants. We recruited participants in this study from Isfahan Endocrine and Metabolism Research Center (IEMRC) between March 2012 and September 2012. Based on the formula for parallel design randomized controlled trials, considering serum hs-CRP concentrations as a key variable and given the type I error of 5% and the study power of 90%, we needed 104 patients to be enrolled. Nonsmoker individuals aged > 30 years with type 2 diabetes [fasting blood glucose ≥ 126 mg/dl (≥6.9 mmol/l) or 2 hour post glucose load ≥ 200 mg/dl (≥11.1 mmol/l) or both] and insufficient 25(OH)D levels [<30 ng/ml (<75 nmol/l)] were included in this study. Patients with a history of renal failure, cancer, liver or thyroid diseases or any other inflammatory diseases were not included in the study. Individuals with a history of allergy and those taking corticosteroids or injecting insulin were not included as well. We also did not include those taking any kind of vitamin D or calcium supplements as well as those who were pregnant or lactating and those who had > 4 kg weight change during the last 3 months. Participants who were taking any kind of medication except hypoglycemic agents were not included in this study.

Overall, 120 patients who met all the inclusion criteria and expressed their willingness were enrolled in the study. During the intervention, two persons were excluded from the study because of their personal reasons (**Figure 1**). All participants provided informed written consent. The study was approved by the bioethics committee of Isfahan University of Medical Science and IEMRC. This study has been registered in clinicaltrials.gov with registration number of NCT01662193.

Study design. This is a double blind parallel randomized placebo-controlled clinical trial that was conducted in Isfahan, Iran, between March 2012 and April 2013. Totally, 118 diabetic patients who met the inclusion criteria completed the trial. Before randomization, we stratified participants based on age (±5 years), sex, body mass index (BMI) (±0.5 kg/m^2), type and dosage of hypoglycemic agents use and duration of diabetes (±6 month). All investigators and participants as well as laboratory technicians were blinded to the random assignments, except for the study technician that did the randomization. Then, patients were allocated randomly into four arms using stratified block randomization: 1) Individuals in the vitamin D group received 50000 IU vitamin D3 per week (equivalent to an amount of 1250 μg) plus a daily placebo for calcium; 2) Subjects in calcium group received 1000 mg calcium carbonate per day plus a weekly placebo for vitamin D; 3) Participants in calcium+vitamin D group received 50000 IU vitamin D3 per week plus 1000 mg calcium carbonate per day; and 4) Individuals in the placebo group received separate placebos for calcium daily and for vitamin D weekly. Participants received supplements and placebos for 8 weeks. Calcium supplements and placebos were manufactured by Jalinus Pharmaceutical Company and vitamin D supplements and placebos were manufactured by Dana Pharmaceutical Company. The use of calcium supplements and placebos throughout the study was checked through asking participants to bring the medication containers. Compliance to the vitamin D supplementation was assessed through quantification of serum vitamin D levels. Participants were requested to consume their usual diets throughout the study. They were also asked not to change their routine physical activity levels. To make sure of these recommendations during intervention, three days of dietary records (one weekend day and two weekdays) and three days of physical activity records (at the same days of dietary records) were obtained. Both dietary and physical activity records were taken at week 2, 4 and 6 of intervention. To determine energy and nutrient intakes, all dietary data were converted to gram scale and then were entered to the Nutritionist 4 software. Physical activity was expressed as METs-h/d. To compute the metabolic equivalents (METs) for each person, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient using standard tables (23). Blood sampling was done at study baseline (wk0) and after 8 weeks of intervention (wk8)

to quantify serum 25(OH)D, inflammatory biomarkers and adipocytokines.

Assessment of biomarkers. To examine circulating levels of inflammatory biomarkers and adipocytokines, 10 cc blood samples were taken after 12 hours of overnight fasting at the study baseline and after 8 weeks of intervention. All blood samples were immediately centrifuged for 10 minutes and serum was stored at −70°C until analyses. Serum adiponectin, leptin, TNF-α and IL-6 levels were quantified by enzyme-linked immunosorbent assay (ELISA) method using Booster kits (Orgenium, Helsinki, Finland). The intra- and interassay CVs for adiponectin was $\leq 10\%$ and $\leq 12\%$, for TNF-α $\leq 6\%$ and $\leq 4\%$ and for IL-6 9.4% and 8.6%, respectively. Serum hs-CRP concentrations were assessed using ELISA through Hitachi 911 chemistry and immunoassay analyzer (Sentinel, Milan, Italy). The intra- and interassay CVs for serum leptin and hs-CRP levels were $< 10\%$. Serum 25(OH)D concentrations were examined by radioimmunoassay (RIA) method.

Statistical methods. We applied Kolmogrov-Smirnov test to ensure the normal distribution of variables. Log transformation was applied for non-normally distributed variables. The analyses were done based on intention-to-treat approach. Missing values were treated based on Last-Observation-Carried-Forward method. Baseline general characteristics were examined using one-way analysis of variance (ANOVA) for continuous variables and χ^2 for categorical variables. Data on dietary intakes and physical activity were compared by one-way ANOVA. To determine the effects of supplementation on inflammatory biomarkers and adipocytokines, first we computed the changes from baseline by subtracting the baseline values from the end-of-trial values. Then we applied analysis of covariance (ANCOVA). To identify if the significant differences were influenced by the baseline levels of inflammatory biomarkers, we adjusted for the baseline values of each biomarker. We also controlled for age, gender and physical activity in these analyses. Additional adjustments were made for BMI to determine if the changes are mediated through obesity. Linear regression analysis was used to identify the association between changes in serum 25 (OH) D levels and changes in the concentration of each biomarker. P-values < 0.05 were considered significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

Results

Totally 118 participants were recruited in this study. Number of persons taking only metformin was 22, 23, 22

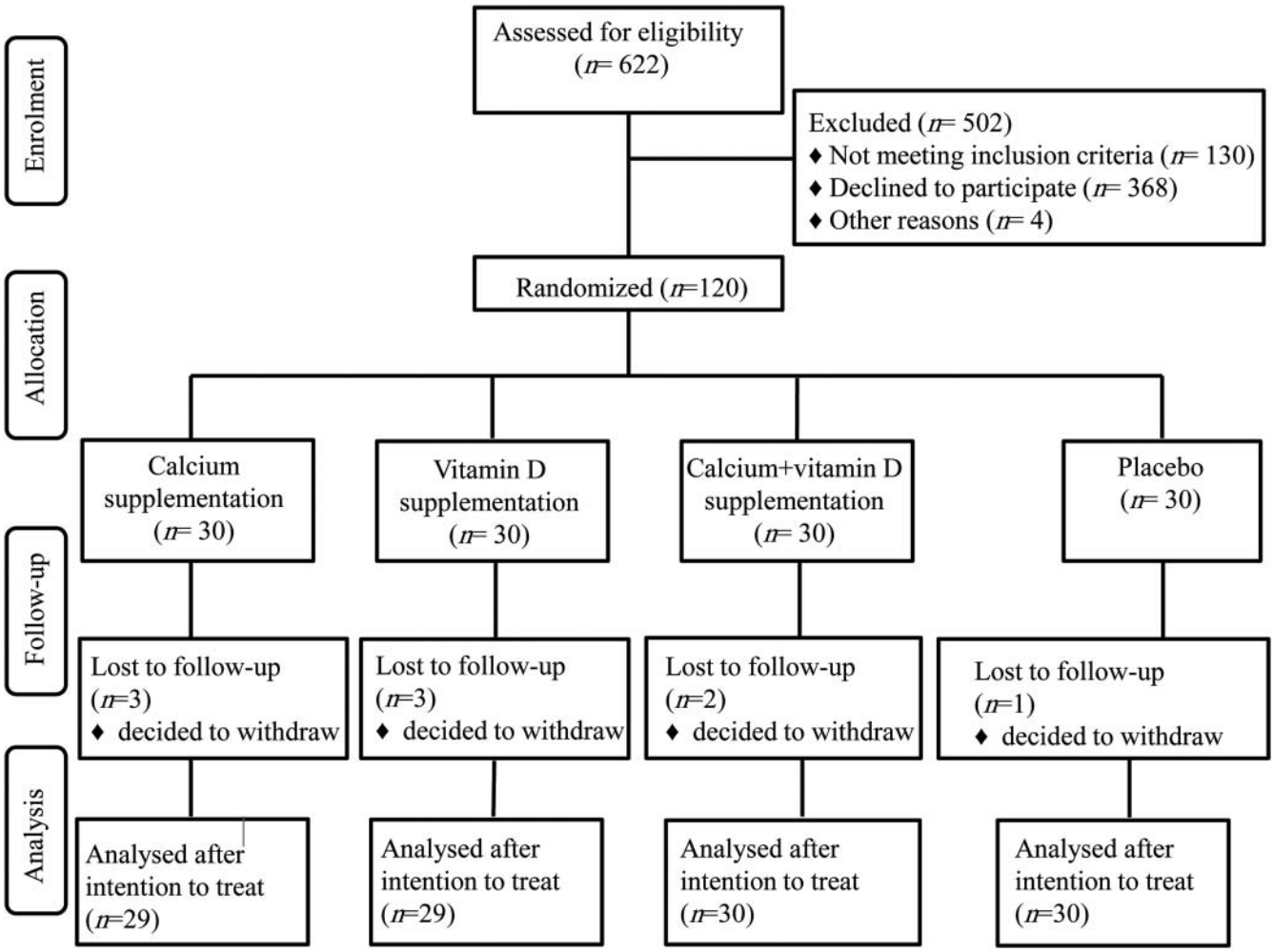

Figure 1. Flow diagram of participants

and 21 in calcium, vitamin D, joint calcium and vitamin D, and placebo groups, respectively. Number of patients who were taking both metformin and glybanclamide were 8, 7, 8 and 9 in calcium, vitamin D, joint calcium and vitamin D, and placebo groups, respectively. General characteristics of participants by intervention groups are shown in Table 1. Subjects in the calcium group were older than those in the joint calcium-vitamin D group. Distribution of participants in terms of sex, menopausal status, obesity and central obesity was not significantly different between four intervention arms. Mean duration of diabetes in all participants was between 4 and 5 years. Moreover, there were no significant differences among intervention groups in terms of BMI at study baseline. Mean dietary intakes of study participants, obtained from three days of dietary records throughout the intervention, are provided in Table 2. No significant differences in dietary energy, macro- and micronutrients intakes were seen between the four intervention groups. Based on three days of nonconsecutive physical activity records throughout the intervention, we found no significant differences in physical activity levels among four intervention groups during intervention.

Baseline serum levels of 25(OH)D across the four groups was not significantly different ($P = .08$) (Table 3). We found a significant increase in mean serum 25(OH)D concentrations among those in vitamin D group (11.2 ± 5.6 ng/ml at study baseline vs. 35.1 ± 14.3 ng/ml after intervention; $P < .001$) as well as those in joint calcium-vitamin D group (12.2 ± 6.6 ng/ml at study baseline vs. 35.4 ± 9.6 ng/ml after intervention; $P < .001$). No significant changes in this biomarker were seen in individuals in calcium (22.3 ± 6.1 ng/ml at study baseline vs. 22.2 ± 7.9 ng/ml after intervention; $P = .93$) or placebo groups (18.3 ± 6.6 ng/ml at study baseline vs. 19.3 ± 7.7 ng/ml after intervention; $P = .37$). These data indicate good adherence of study participants to vitamin D supplementation. Compliance of participants with calcium supplementation was 88% based on the remained tablets at the containers. The weight change in all groups was less than 0.5 kg, which was not statistically significant comparing the four groups ($P = .31$). Calcium-vitamin D cosupplementation resulted in reduced HbA1c ($P = .02$), LDL-cholesterol ($P = .04$) and increased HDL-cholesterol levels ($P = .03$) compared with other groups.

Serum levels of inflammatory biomarkers and adipocytokines at study baseline are shown in Table 3. We observed no significant differences in serum concentrations of adiponectin, leptin, TNF-α, IL-6 and hs-CRP between the four intervention groups at study baseline.

The effects of calcium, vitamin D, and joint calcium-vitamin D supplementation on circulating levels of inflammatory biomarkers and adipocytokines are indicated in Table 4. After adjustment for baseline levels of inflammatory biomarkers, we observed that compared with placebo, supplementation with calcium and vitamin D had no significant effects on serum adiponectin concentrations. However, calcium (changes from baseline: –75 ± 19 ng/ml, $P = .01$) and vitamin D alone (-56 ± 19 ng/ml, $P = .01$) as well as joint calcium-vitamin D supplementation (-92 ± 19 ng/ml, $P = .01$) resulted in a significant reduction in serum leptin levels compared with placebo (-9 ± 18 ng/

Table 1. Baseline general characteristics of study participants[1]

	Calcium[4]	Vitamin D[5]	Ca+D[6]	Placebo	P[7]
	(*n* = 29)	(*n* = 29)	(*n* = 30)	(*n* = 30)	
Age (y)	53.7 ± 5.7	50.2 ± 6.6	49.8 ± 6.1	51.0 ± 6.1	0.06
Women; n (%)	15 (52)	14 (48)	15 (50)	16 (53)	0.98
Postmenopause; n (%)	10 (34)	9 (31)	9 (30)	10 (33)	0.99
Diabetes duration (mo)	53 ± 54	56 ± 38	52 ± 36	57 ± 44	0.96
Obesity[2]; n (%)	15 (52)	14 (48)	13 (43)	14 (47)	0.93
Central obesity[3]; n (%)	17 (59)	12 (41)	19 (63)	19 (63)	0.27
Serum triglyceride (mg/dL)	177 ± 62	168 ± 61	186 ± 89	178 ± 71	0.77
Serum HDL-cholesterol (mg/dL)	42 ± 7	44 ± 8	47 ± 8	46 ± 7	0.11
Serum LDL-cholesterol (mg/dL)	93 ± 23	81 ± 23	92 ± 31	85 ± 19	0.21
Total cholesterol (mg/dL)	170 ± 39*	139 ± 20	143 ± 27	147 ± 31	0.001
HbA_{1c} (%)	6.6 ± 0.8	6.6 ± 0.8	6.7 ± 1.1	6.9 ± 0.9	0.61

[1] All values are means± SD unless indicated

[2] Defined as having BMI≥30 kg/m^2

[3] Defined as having waist circumference of >88 cm for women and >102 cm for men

[4] Receiving 1000 mg calcium carbonate per day plus a weekly placebo for vitamin D

[5] Receiving 50000 IU vitamin D3 per week plus a daily placebo for calcium

[6] Receiving 1000 mg calcium carbonate per day plus 50000 IU vitamin D3 per week

[7] Obtained from ANOVA

Table 2. Nutrient intakes of study participants throughout the intervention[1]

	Calcium[2]	Vitamin D[3]	Ca+D[4]	Placebo	P[5]
	(n = 29)	(n = 29)	(n = 30)	(n = 30)	
Energy (kcal/d)	2052 ± 589	2050 ± 595	2109 ± 666	2147 ± 646	0.97
Fat (g/d)	89 ± 45	90 ± 46	91 ± 41	99 ± 49	0.81
Calcium (mg/d)	1018 ± 409	915 ± 355	968 ± 386	951 ± 349	0.77
Vitamin D (μg/d)	1.7 ± 1.3	1.5 ± 1.1	1.5 ± 1.3	1.5 ± 1.0	0.92
Vitamin C (mg/d)	144 ± 115	151 ± 110	139 ± 91	121 ± 93	0.72
Vitamin E (mg/d)	24 ± 25	23 ± 22	26 ± 21	30 ± 27	0.73
Magnesium (mg/d)	278 ± 87	262 ± 81	278 ± 90	261 ± 98	0.79
Cholesterol (mg/d)	231 ± 88	237 ± 128	208 ± 153	194 ± 94	0.47
Vitamin B2 (mg/d)	2 ± 0.7	2 ± 0.6	2 ± 1	2 ± 0.6	0.71
Iron (mg/d)	12 ± 4	12 ± 5	13 ± 4	12 ± 6	0.78
Zinc (mg/d)	8 ± 2	8 ± 3	8 ± 3	8 ± 3	0.70
Selenium (mg/d)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.84
Omega 3 (g/d)	0.2 ± 0.3	0.2 ± 0.3	0.2 ± 0.3	0.1 ± 0.1	0.38
MUFA (g/d)	28 ± 13	29 ± 15	28 ± 12	31 ± 14	0.86

[1] All values are means± SD unless indicated

[2] Receiving 1000 mg calcium carbonate per day plus a weekly placebo for vitamin D

[3] Receiving 50000 IU vitamin D3 per week plus a daily placebo for calcium

[4] Receiving 1000 mg calcium carbonate per day plus 50000 IU vitamin D3 per week

[5] Obtained from ANOVA

MUFA: Mono unsaturated fatty acids

Table 3. The baseline 25(OH)D, inflammatory and adipocytokines levels of study participants[1]

	Calcium[2]	Vitamin D[3]	Ca+D[4]	Placebo	P[5]
	(n = 29)	(n = 29)	(n = 30)	(n = 30)	
25(OH)D (ng/ml)	22.3 ± 6.1	11.2 ± 5.6	12.2 ± 6.6	18.3 ± 6.6	0.08
Adipocytokines					
Leptin (ng/ml)	166 ± 142	209 ± 134	229 ± 163	234 ± 188	0.34
Adiponectin (ng/ml)	2.5 ± 0.4	2.8 ± 0.5	3.7 ± 0.7	3.0 ± 0.5	0.23
Inflammatory Biomarkers					
IL-6 (pg/ml)	4.5 ± 7.1	7.9 ± 6.0	7.5 ± 5.1	6.7 ± 3.0	0.10
TNF-α (pg/ml)	11.1 ± 10.8	11.5 ± 6.3	13.0 ± 8.6	10.2 ± 3.6	0.55
hs-CRP (ng/ml)	2.0 ± 1.4	2.3 ± 1.6	1.9 ± 1.1	2.2 ± 1.0	0.68

[1]All values are means± SD unless indicated

[2] Receiving 1000 mg calcium carbonate per day plus a weekly placebo for vitamin D

[3] Receiving 50000 IU vitamin D3 per week plus a daily placebo for calcium

[4] Receiving 1000 mg calcium carbonate per day plus 50000 IU vitamin D3 per week

[5] Obtained from ANOVA

IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; hs-CRP: *High sensitivity C-reactive protein*

ml). This was also the case for serum IL-6; such that calcium (-2 ± 1 pg/ml, $P < .001$) and vitamin D alone (-4 ± 1 pg/ml, $P < .001$) and their combination (-4 ± 1 pg/ml, $P < .001$) led to significant reductions compared with placebo (3 ± 1 pg/ml). After adjustment for potential confounders, individuals in calcium (-3.1 ± 1.3, $P < .05$), vitamin D (-3.1 ± 1.3, $P < .05$) and joint Ca-D groups (-3.4 ± 1.3, $P < .05$) had greater reductions in serum TNF-α concentrations compared with placebo (0.1 ± 1.2). Individuals who received joint calcium-D supplements tended to have a decrease in serum hs-CRP levels compared with placebo after controlling for baseline levels (-1.14 ± 0.25 vs. 0.02 ± 0.24 ng/ml, $P = .09$). Further adjustments for BMI did not remarkably alter the above-mentioned findings; however, the significant effect of calcium, vitamin D and calcium-vitamin D cosupplementation on serum TNF-α concentrations became nonsignificant after adjustment for BMI, indicating that the effect might be mediated through obesity.

Findings from linear regression analysis revealed that increased serum 25(OH)D concentrations was associated with a significant reduction in serum leptin (β= −0.19, $P < .001$), IL-6 (β= −011., $P = .01$) and TNF-α (β= −0.10, $P = .02$).

Table 4. The effect of vitamin D and calcium supplementation on inflammation and adipocytokines biomarkers[1]

	Calcium[4]	Vitamin D[5]	Ca+D[6]	Placebo	P[7]
	(n = 29)	(n = 29)	(n = 30)	(n = 30)	
Adiponectin (ng/ml)					
Crude	−1.3 ± 0.3	−1.2 ± 0.5	−0.1 ± 1.0	−0.1 ± 0.5	0.32
Model 1[2]	−1.1 ± 0.6	−1.3 ± 0.6	0.2 ± 0.6	−0.3 ± 0.6	0.29
Model 2[3]	−1.2 ± 0.6	−1.3 ± 0.6	0.1 ± 0.6	−0.3 ± 0.6	0.31
Leptin (ng/ml)					
Crude	−40 ± 28	−72 ± 31	−88 ± 30	−29 ± 39	0.53
Model 1	−75 ± 19	−56 ± 19	−92 ± 19*	−9 ± 18	0.01
Model 2	−75 ± 20	−56 ± 20	−91 ± 19	−9 ± 19	0.01
IL-6 (pg/ml)					
Crude	−3 ± 1	−5 ± 1	−5 ± 1*	3 ± 1	<0.001
Model 1	−2 ± 1	−5 ± 1	−4 ± 1	3 ± 1	<0.001
Model 2	−2 ± 1	−4 ± 1	−4 ± 1	3 ± 1	<0.001
hs-CRP (ng/ml)					
Crude	0.01 ± 0.35	−0.48 ± 0.40	−0.75 ± 0.36	−0.06 ± 0.26	0.28
Model 1	−0.07 ± 0.25	−0.24 ± 0.24	−1.14 ± 0.25	0.02 ± 0.24	0.09
Model 2	−0.09 ± 0.25	−0.25 ± 0.24	−1.19 ± 0.25	0.08 ± 0.24	0.09
TNF-α (pg/ml)					
Crude	−2.7 ± 1.9	−2.1 ± 2.3	−5 ± 1.6*	0.26 ± 1.3	0.02
Model 1	−3.1 ± 1.3	−3.1 ± 1.3	−3.4 ± 1.3	0.1 ± 1.2	0.04
Model 2	−3.1 ± 1.3	−3.1 ± 1.3	−3.2 ± 1.3	−1.0 ± 1.3	0.23

[1] All values are means± SD unless indicated

[2] Adjusted for baseline levels, age, sex and physical activity

[3] Further adjusted for BMI

[4] Receiving 1000 mg calcium carbonate per day plus a weekly placebo for vitamin D

[5] Receiving 50000 IU vitamin D3 per week plus a daily placebo for calcium

[6] Receiving 1000 mg calcium carbonate per day plus 50000 IU vitamin D3 per week

[7] Obtained from ANOVA

* Compared with others, $P < 0.05$

IL-6: Interleukin-6; hs-CRP: *High sensitivity C-reactive protein;* TNF-α: Tumor necrosis factor-α

Discussion

In this randomized placebo-controlled clinical trial of vitamin D and calcium supplementation in patients with type 2 diabetes, calcium-vitamin D cosupplementation resulted in a significant reduction in circulating leptin, IL-6 and TNF-α concentrations compared with other groups. Moreover, individuals in the calcium group tended to have greater reductions in serum hs-CRP levels than other groups. However, supplementation with calcium and vitamin D had no significant effects on serum adiponectin levels after adjustment for baseline levels. To the best of our knowledge, this study is among the first investigations that examined the effects of joint calcium-vitamin D supplementation on inflammatory biomarkers and adipocytokines of type 2 diabetic patients who were on oral hypoglycemic agents. Although metformin might have some anti-inflammatory effects, the groups were matched in terms of type and dosage of OHA use. Therefore, the effects we found would be independent of OHA use.

It is currently recognized that inflammation is involved in the pathophysiology of type 2 diabetes (6, 24). Previous studies have shown that separate vitamin D or calcium supplementations may improve insulin sensitivity and promote β-cell survival (25–27). However, there are very limited data from human studies that examined the effect of vitamin D or calcium supplementation on systemic inflammation in diabetes (15, 28). We found that calcium plus vitamin D supplementation led to decreased levels of serum leptin, IL-6 and TNF-α concentration. Previously reported trials about the effect of single vitamin D or calcium supplementation on inflammatory biomarkers in nondiabetic subjects have shown conflicting results (20, 21). In a study on British adults of Bangladeshi origin, fifty-four vitamin D-deficient subjects were randomized and given either a 'high' dose (50 000 IU) or a 'low' dose (500 IU) of a depot (oily) injection of cholecalciferol, every 3 months, during 1 year. The investigators came to the conclusion that reductions in hs-CRP levels were greater in the 'high' than in the 'low' treatment group (8). Others found that a daily supplement of 83.3 μg vitamin D for 12 months among 200 healthy overweight subjects might beneficially influence circulating levels of TNF-α (25). In a 1-year supplementation study with cholecalciferol in 332 persons, reductions in serum IL-6 levels were found

(29). Calcium supplementation has been shown to decrease tumor-promoting proinflammatory markers in sporadic colorectal adenoma patients (20). However, some studies did not find any beneficial effect of vitamin D or calcium supplementation on inflammation (18, 30). Improving vitamin D status through supplementation with different dosages of vitamin D3 in 305 healthy postmenopausal women did not affect inflammatory biomarkers (18). Another trial has shown that supplementation with 50000 IU/wk vitamin D for 12 weeks cannot decrease proinflammatory cytokines in 90 subjects with coronary artery disease (CAD) and vitamin D deficiency (30). These studies were short in duration and had included few subjects. All above mentioned studies have used vitamin D or calcium supplementation alone and we are aware of only few studies that examined joint calcium-vitamin D supplementation (20, 21). In a study on 90 type 2 diabetic patients with mean 25(OH)D levels of 44 nmol/l, it has been indicated that daily intake of vitamin D-fortified Doogh (a yogurt-based beverage) for 12 weeks improved inflammatory markers and extra calcium conferred additional benefit only for adiponectin (28). Findings from a clinical trial in colorectal adenoma patients, whose serum 25(OH)D levels were < 30 ng/ml, revealed that 2 g/d calcium and/or 800 IU/d vitamin D supplementation for 6 months did not significantly change inflammatory biomarkers (20). Moreover, in another randomized controlled trial (RCT), daily supplementation with 500 mg calcium citrate and 700 IU vitamin D3 for 3 years had no significant effects on markers of systemic inflammation among those with normal fasting glucose (21). It is worth mentioning that the latter was a post hoc analysis of a RCT designed for assessing bone mineral density (BMD). This might explain why the investigators could not reach the beneficial effects of joint supplementation on inflammation. Furthermore, participants of that study were apparently healthy individuals that might have near the normal levels of inflammatory biomarkers. This is in opposite to our study, in which participants were persons with diabetes with elevated levels of inflammation. In addition, the different dosages of calcium and vitamin D can provide further reasons for discrepant findings. It seems that using appropriate doses of calcium and vitamin D supplements in vitamin D insufficient type 2 diabetic patients might help controlling the elevated levels of inflammatory biomarkers and adipocytokines. As systemic inflammation is closely involved in the pathogenesis of type 2 diabetes and associated complications such as dyslipidemia and atherosclerosis, lowering inflammatory biomarkers and adipocytokines through the use of calcium-vitamin D supplements might result in better glycemic and metabolic control of diabetic patients.

Several mechanisms may explain the beneficial effects of calcium and vitamin D supplementation on inflammatory biomarkers and adipocytokines in type 2 diabetic patients. Vitamin D interacts with vitamin D response elements in the promoter region of cytokine genes to interfere with nuclear transcription factors implicated in cytokine generation and action. Moreover, vitamin D can down-regulate activation of nuclear factor-KB, which is an important regulator of genes encoding proinflammatory cytokines implicated in insulin resistance. Also, vitamin D interferes with cytokine generation by up-regulating the expression of calbindin, a cytosolic calcium-binding protein found in many tissues including pancreatic β-cells (31). The mechanism of effects of calcium on inflammation is still unclear. However, it has been claimed that calcitriol regulates macrophage production of inflammatory factors via calcium-dependent mechanism. Therefore, reducing circulating calcitriol levels via increasing dietary calcium intake or calcium supplementation may regulate the macrophage and thereby attenuate inflammation (32). In addition, it seems that calcium intake might influence inflammation through suppression of parathyroid hormone (PTH). Circulating levels of interleukin-6 and tumor necrosis factor (TNF)-alpha were elevated in primary hyperparathyroidism (33). Therefore, it seems that PTH can regulate circulating levels of IL-6 and TNF-α, which stimulate production of hs-CRP.

Strengths of this study include the double blind randomized placebo controlled design, quantification of serum 25(OH)D levels to examine the adherence to the vitamin D supplementation, relatively good compliance with the supplement use, and taking the variety of confounders including baselines levels of biomarkers into account. Moreover, due to the effect of season on vitamin D status, we confined the intervention into one season. Some limitations must also be considered. This study was conducted during summer. Therefore, the vitamin D status of subjects might not accurately reflect the effect of supplements. However, all patients were vitamin D insufficient at study baseline, even in summer. Furthermore, to assess compliance, we measured serum vitamin D levels at the end, not throughout, the study (eg, week 4). As the study has been done among those that were using oral hypoglycemic agents, the findings cannot easily be extrapolated to other diabetic patients (for example those that are injecting insulin). This study cannot suggest the appropriate dosages for vitamin D and calcium supplements for diabetic patients. To reach this, other studies with different dosages of calcium and vitamin D are required. In addition, short duration of intervention might prohibit us to observe the effects of supplementation on some biomarkers of inflammation.

In conclusion, joint calcium-vitamin D supplementation resulted in an improved status of inflammation in vitamin D insufficient type 2 diabetic patients. Further studies to determine the appropriate doses of calcium and vitamin D for these patients are warranted.

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