Does vitamin D supplementation alter plasma adipokines concentrations?

A systematic review and meta-analysis of randomized controlled trials.

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Conflict of Interest Disclosures: None

No. of words: 4826

ABSTRACT:

We aimed to elucidate the role of vitamin D supplementation on adipokines through a systematic review and a meta-analysis of randomized placebo-controlled trials (RCTs). The search included PUBMED, Scopus, Web of Science and Google Scholar through July 1st, 2015. Finally we identified 9 RCTs and 484 participants. Meta-analysis of data from 7 studies did not find a significant change in plasma adiponectin concentrations following vitamin D supplementation (mean difference [MD]: 4.45%, 95%CI: -3.04, 11.93, p=0.244; Q=2.18, I^2=0%). In meta-regression, changes in plasma adiponectin concentrations following vitamin D supplementation were found to be independent of treatment duration (slope: 0.25; 95%CI: -0.69, 1.19; p=0.603) and changes in serum 25-hydroxy vitamin D [25(OH)D] levels (slope: -0.02; 95%CI: -0.15, 0.12; p=0.780). Meta-analysis of data from 6 studies did not find a significant change in plasma leptin concentrations following vitamin D supplementation (MD: -4.51%, 95%CI: -25.13, 16.11, p=0.668; Q=6.41, I^2=21.97%). Sensitivity analysis showed that this effect size is sensitive to one of the studies; removing it resulted in a significant reduction in plasma leptin levels (MD: -12.81%, 95%CI: -24.33, -1.30, p=0.029). In meta-regression, changes in plasma leptin concentrations following vitamin D supplementation were found to be independent of treatment duration (slope: -1.93; 95%CI: -4.08, 0.23; p=0.080). However, changes in serum 25(OH)D were found to be significantly associated with changes in plasma leptin levels following vitamin D supplementation (slope: 1.05; 95%CI: 0.08, 2.02; p=0.033). In conclusion, current data did not indicate a significant effect of vitamin D supplementation on adiponectin and leptin levels.

Keywords: vitamin D, adiponectin, leptin, meta-analysis, systematic review.

No. of words: 248

INTRODUCTION

Vitamin D, a fat-soluble vitamin, plays an important role in bone metabolism and calcium homeostasis [1]. However it has been increasingly recognized that vitamin D deficiency may play a role in several health conditions including cardiovascular disease (CVD) risk. Vitamin D can be present in two hormone precursor forms: ergocalciferol (vitamin D2), found in plants and cholecalciferol (vitamin D3) found in oily fish or synthesized by the skin from 7-dehydrocholesterol during exposure to ultraviolet (UV) rays in sunlight [2]. Both D2 and D3 can be ingested in the form of supplementation or fortified foods. Ergocalciferol and cholecalciferol are then hydroxylated in the liver to form 25-hydroxycholecalciferol (calcidiol or 25(OH)D), which is the best biomarker of vitamin D stores and used to determine sufficiency/deficiency states. Calcidiol is transported to the proximal tubules of the kidneys, where it is hydroxylated by a 1α-hydroxylase enzyme (gene: CYP27B1) to form calcitriol (1,25-dihydroxycholecalciferol and abbreviated to $1,25(OH)_2D$), the biologically active form of vitamin D [3]; this process is a tightly regulated by parathyroid hormone levels and serum calcium and phosphorous levels.

The biological actions of $1,25(OH)_2D$) are mediated through the vitamin D receptor (VDR), a member of the nuclear receptor superfamily, which are widely expressed throughout human tissues: adipocytes, smooth muscles, skin, cells of the immune system, colon, pancreatic β-cells, keratinocytes, osteoblasts and the vasculature [4]. Emerging evidence has suggested a link between vitamin D deficiency and CVD risk, diabetes mellitus (DM), hypertension and dyslipidemia [5]. Lower concentrations of circulating 25(OH)D were associated with higher fasting glucose levels, reduced insulin sensitivity, and increased risk of type 2 DM [6]. Furthermore, many clinical studies showed that serum 25(OH)D concentrations are inversely correlated with body mass index (BMI), fat mass or percentage of body fat and waist circumference [4]. Recently, the role of vitamin D in the regulation of adipogenesis and thereby in the control of energy homeostasis has become another highly debated issue [7].

Adipose tissue secretes bioactive peptides, termed adipokines, including leptin and adiponectin, which play an important role in the regulation of energy homeostasis by influencing several biological processes, such as food intake, insulin action, lipid, and glucose metabolism, regulation of energy balance, coagulation, angiogenesis and vascular remodeling [8]. Altered levels of adipokines have been implicated in the pathogenesis of insulin resistance, dyslipidemia and atherosclerosis [9]. Adiponectin is a protein hormone that modulates a number of metabolic processes, especially carbohydrate and fatty acid metabolism [10]. It has anti-atherosclerotic, as well as anti-inflammatory and anti-diabetic properties [11]. According to recent findings, hypoadiponectinemia can increase the risk of metabolic syndrome and coronary artery disease [12, 13]. Moreover, the secretion of adiponectin decreases with obesity and increases with body mass reduction [14]. It has been shown that the reduction of adiponectin concentrations is correlated with insulin resistance and hyperinsulinemia [15]. Another adipokine, leptin, acts on specific receptors in the hypothalamus to decrease appetite and to increase energy expenditure when body fat store is increased. Elevated serum leptin levels signal excessive energy storage to the central nervous system to suppress food intake and increase energy consumption [16].

Vitamin D supplementation might have different effects on adipokine homeostasis. Experimental data suggested that vitamin D supplementation regulates the expression of adipokines in visceral fat [17]. Moreover, it has been shown that serum 25(OH)D levels are positively correlated with the plasma levels of adiponectin [18]. Some studies reported that vitamin D may decrease the plasma levels of leptin, while others demonstrated an increase production of leptin after vitamin D supplementation. Thus, the data regarding the effects of vitamin D on adipokines are controversial. Therefore, we performed a systematic review of the literature and a meta-analysis of randomized placebo-controlled trials to elucidate the impact of vitamin D supplementation therapy on plasma adipokines concentrations.

METHODS

Search strategy

This study was designed according to the guidelines of the 2009 preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement [19]. PUBMED, Scopus, Web of Science (WoS) and Google Scholar databases were searched using the following search terms in titles and abstracts: (vitamin D OR ergocalciferol OR cholecalciferol) AND (leptin) AND/OR (adiponectin) AND (randomized controlled trial). The wild-card term ‘‘*’’ was used to increase the sensitivity of the search strategy. The search was limited to articles published in English language. The literature was searched from inception to July 1st, 2015. The search was limited to studies in humans. Selected articles were searched to identify further relevant studies. Two reviewers (MD and MCS) evaluated each article separately. Disagreements were resolved by agreement and discussion with a third party (MB).

Study Selection

Original studies were included if they met the following inclusion criteria: (i) being a clinical trial with either parallel or cross-over design, (ii) investigating the impact of vitamin D, either as monotherapy or combination therapy with calcium, on serum/plasma concentrations of adiponectin and/or leptin, and, (iii) presentation of sufficient information on adipokine concentrations at baseline and at the end of follow-up in each group or providing the net change values.

Exclusion criteria were: (i) non-interventional studies, (ii) uncontrolled studies, (iii) observational studies with case-control, cross-sectional or cohort design, and, (iv) lack of sufficient information on baseline or follow-up adipokine concentrations.

Data extraction

Eligible studies were reviewed and the following data were abstracted (MD and MCS): 1) first author's name, 2) year of publication, 3) country were the study was performed, 4) study design, 5) number of participants in the vitamin D and control groups, 6) intervention assigned to the control group, 7) route of administration (single bolus dose or daily dose), 8) type and dose of vitamin D, 9) treatment duration, 10) age, gender and body mass index (BMI) of the participants, 10) baseline and end-trial values for weight and BMI, 11) systolic and diastolic blood pressures, and, 12) data regarding baseline and follow-up serum concentrations of 25(OH)D.

Data extraction was performed independently by 2 reviewers; disagreements were resolved by a third reviewer.

Risk of bias assessment

According to the Cochrane Collaboration [20], a specific tool for assessing risk of bias in each involved study comprises judgment of specific features of the study. This involves evaluating the risk of bias as ‘low risk’, ‘high risk or ‘unclear risk’. The last category presents either lack of information or uncertainty over the potential for bias. There are seven checked areas including: sequence generation (selection bias), allocation sequence concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other potential sources of bias.

Risk-of-bias assessment was performed independently by 2 reviewers; disagreements were resolved by a third reviewer.

Quantitative Data Synthesis

Meta-analyses were conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ) [21]. Net changes in measurements (change scores or mean differences [MD]) were calculated as follows: measure at end of follow-up − measure at baseline. All values were collated as percent change from baseline in each group. Standard deviations (SDs) of the MD were calculated using the following formula: SD = square root $[(SD_{pre\text{-}treatment})^2 + (SD_{post\text{-}treatment})^2 - (2R \times SD_{pre\text{-}treatment} \times SD_{post\text{-}treatment})]$, assuming a correlation coefficient (R) = 0.5. Where standard error of the mean (SEM) was only reported, standard deviation (SD) was estimated using the following formula: SD = SEM × sqrt (*n*), where *n* is the number of subjects.

Net changes in measurements (change scores) were calculated for parallel trials, as follows: (measure at the end of follow-up in the treatment group − measure at baseline in the treatment group) − (measure at the end of follow-up in the control group − measure at baseline in the control group). A random-effects model and the DerSimonian-Laird method were used to compensate for the heterogeneity between studies in terms of study design, treatment duration, and the characteristics of populations being studied [22]. Inter-study heterogeneity was assessed using Cochran Q test and I^2 index. In order to evaluate the influence of each study on the overall effect size, sensitivity analysis was conducted using leave-one-out method, i.e. iteratively removing one study each time and repeating the analysis.

Meta-regression

A weighted random-effects meta-regression using unrestricted maximum likelihood model was performed to assess the association between the overall estimate of effect size with potential

moderator variables including duration of supplementation, baseline serum 25(OH)D and changes in serum 25(OH)D concentrations.

Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, and Begg's rank correlation and Egger's weighted regression tests. Duval and Tweedie "trim and fill" method was used to adjust the analysis for the effects of publication bias [23].

RESULTS

Search results and trial flow

The initial screening for potential relevance removed the articles whose titles and/or abstracts were obviously irrelevant. After assessment, 9 RCTS with 10 treatment arms achieved the inclusion criteria and were selected for the final meta-analysis [24-32]. 7 RCTs (7 treatment arms) determined plasma adiponectin concentrations and 5 RCTs with 6 treatment arms evaluated plasma leptin concentrations. A study flow chart is presented in **Figure 1.**

In the selected studies, 248 participants were allocated to vitamin D supplementation and 236 as controls. The number of participants in these trials ranged from 30 to 81. The studies included were published between 2012 and 2015, and were conducted in the USA (3 studies), Iran (3 studies), Italy, Australia and Israel. All the studies used the oral route of administration. Six studies used multiple daily doses and 3 studies, a single bolus dose. The doses of vitamin D ranged from 400 to 4000 IU/day in multiple daily doses administration. Duration of supplementation with vitamin D ranged between 12 weeks and 12 months when daily doses were given. When single bolus dose was used the doses of vitamin D ranged between 50,000 IU/week

for 8 weeks to 300,000 IU administered once with 24 weeks follow-up. All trials were designed as parallel-group studies. Demographic and baseline parameters of the included studies are shown in **Table 1.** No adverse events related to the supplementation were reported.

Risk of bias assessment

We observed an unclear risk of bias regarding sequence generation, allocation concealment and blinding of outcome assessment in some of the studies, but studies were low-risk in terms of other sources of bias. The systematic assessment of bias in the included trials is presented in **Table 2**.

Effect of vitamin D supplementation on plasma adiponectin concentrations

Meta-analysis of data from 7 treatment arms did not suggest a significant alteration in plasma adiponectin concentrations following vitamin D supplementation (MD: 4.45%, 95% CI: -3.04, 11.93, p=0.244; Q=2.18, I^2=0%) (**Figure 2A**). The pooled effect size was robust and remained significant in the leave-one-out sensitivity analysis (**Figure 2B**). When the studies were categorized according to the treatment modality, there was no significant effect in either of the subsets administering vitamin D as a single bolus dose (MD: 3.18%, 95% CI: -11.32, 17.68, p = 0.667; Q=0.76, I^2=0%) or multiple daily dose (MD: 4.91%, 95% CI: -3.83, 13.64, p=0.271; Q=1.38, I^2=0%) (**Figure 3A**). In meta-regression, changes in plasma adiponectin concentrations following vitamin D supplementation were found to be independent of treatment duration (slope: 0.25; 95% CI: -0.69, 1.19; p=0.603), baseline serum 25(OH)D (slope: -1.09; 95% CI: -4.39, 2.20; p=0.515) and changes in serum 25(OH)D levels (slope: -0.02; 95% CI: -0.15, 0.12; p=0.780) (**Figures 4A, 4B and 4C**).

Effect of vitamin D supplementation on plasma leptin concentrations

Meta-analysis of data from 6 treatment arms did not suggest a significant alteration in plasma leptin concentrations following vitamin D supplementation (MD: -4.51%, 95% CI: -25.13, 16.11, p=0.668; Q=6.41, I^2=21.97%) (**Figure 5A**). Sensitivity analysis showed that this effect size is sensitive to one of the studies; removing of which resulted in a significant reduction in plasma leptin levels (MD: -12.81%, 95% CI: -24.33, -1.30, p=0.029; Q=1.50, I^2=0%) (**Figure 5B**). When the studies were categorized according to the treatment modality, there was no significant effect in either of the subsets administering vitamin D as a single bolus dose (MD: 25.09%, 95% CI: -52.15, 102.33, p=0.524; Q=0, I^2=0%) or multiple daily dose (MD: -5.70%, 95% CI: -28.39, 17.00, p=0.623; Q=5.55, I^2=27.92%) (**Figure 3B**). In meta-regression, changes in plasma leptin concentrations following vitamin D supplementation were found to be independent of treatment duration (slope: -1.93; 95% CI: -4.08, 0.23; p=0.080) and baseline serum 25(OH) D (slope: 2.00; 95% CI: -4.11, 8.10; p=0.522) (**Figure 6A and 6B**). However, changes in serum 25(OH)D were found to be significantly associated with changes in plasma leptin levels following vitamin D supplementation (slope: 1.05; 95% CI: 0.08, 2.02; p=0.033) (**Figure 6C**).

Publication bias

Visual inspection of funnel plots suggested asymmetry in the meta-analyses of vitamin D and adipokines that was addressed by imputing one (for adiponectin) and three (for leptin) potentially missing studies using “trim and fill” method. The corrected effect sizes were calculated to be 4.17 (95% CI: -3.28, 11.62) (for adiponectin) (**Figure 7A**) and -13.97 (95% CI: -37.88, 9.93) (for leptin) (**Figure 7B**).

The results of Egger’s linear regression did not show any sign of publication bias for the adiponectin (intercept = 0.67, standard error = 0.31; 95% CI = -0.14, 1.47, t = 2.13, df = 5, two-

tailed p = 0.087) and leptin (intercept = 1.08, standard error = 0.54; 95% CI = -0.41, 2.56, t = 2.01, df = 4, two-tailed p = 0.115) meta-analyses. Although Begg's rank correlation test did not suggest any publication bias in the adiponectin meta-analysis (Kendall's Tau with continuity correction = 0.57, z = 1.80, two-tailed p-value = 0.072), there was a significant publication bias detected in the leptin meta-analysis (Kendall's Tau with continuity correction = 0.80, z = 2.25, two-tailed p-value = 0.024).

DISCUSSION

To our knowledge, the current systematic review and meta-analysis is the first to evaluate the current evidence from RCTs on the efficacy of supplementation with vitamin D on plasma adiponectin and leptin concentrations. The results of this meta-analysis of 9 RCTs did not indicate a significant effect of vitamin D supplementation on adiponectin and leptin levels. In meta-regression, changes in plasma adiponectin and leptin concentrations following vitamin D supplementation were found to be independent of treatment duration. The changes in serum 25(OH)D were found to be significantly associated with changes in plasma leptin levels, but independent of plasma adiponectin concentrations following vitamin D supplementation.

The preliminary results obtained in a prior meta-analysis on 6 RCTs suggested a potential increase of serum adiponectin after vitamin D supplementation [33]. Those results had indicated a significant dose-response relationship between vitamin D supplementation and adiponectin levels [33]. However, that meta-analysis collected data only from one database (PubMed), had very small number of participants, and the final results are still not available. In comparison, our meta-analysis is larger, used multiple datasets, and performed rigorous statistical analyses including sensitivity analyses and assessment of risk of bias.

The exact mechanisms, by which vitamin D may influence leptin expression and secretion, are still unclear. To address this question, Kong *et al.* tested *in vivo and ex vivo* the effect of vitamin D on leptin expression [34]. It has been noticed that serum leptin levels and adipose leptin mRNA transcript significantly increase after one-week treatment with vitamin D analog RO-27-5646 [34]. These data showed that $1,25(OH)_2D$ stimulated the production of adipose leptin production in a vitamin D receptor-dependent manner, suggesting that vitamin D may modify the energy homeostasis *via* direct regulation of leptin expression [34]. In contrast, an *in vitro* study showed that the treatment of human adipose tissue cultures with $1.25(OH)_2D_3$ for up to 96 h inhibited leptin secretion at 72-92 h, suggesting an indirect effect of vitamin D on leptin regulation [35]. Supplementation with vitamin D significantly elevated serum leptin concentrations in asymptomatic vitamin D-deficient subjects [36]. These results were in agreement with those published by Maggi *et al.* [28] and Ghavamzadeh *et al.* [31].

It might be assumed that a low intake of vitamin D may be associated with low leptin concentrations, increased appetite and weight. Indeed, the mechanism by which vitamin D supplementation may increase the serum leptin levels and reduce weight seems to be related to the bidirectional adipoinsular axis involving appetite suppression, regulation of insulin secretion and increased glucose uptake [37, 38]. Besides that, a growing number of epidemiological studies have shown an inverse correlation between BMI and serum levels of 25(OH)D in humans. In the same manner, Vilarrasa *et al.* also obtained an inverse correlation between BMI, body fat, waist, hip circumference and serum levels of 25(OH)D and leptin in a healthy population [39]. However, no significant associations were observed between serum levels of 25(OH)D and other adipokines measured such as resistin, interleukin-18 and adiponectin [39]. Vitamin D may also be involved in the regulation of adiponectin levels through modification of insulin sensitivity. Baziar *et al.* [24] reported significantly increased levels of 25(OH)D and decreased levels of

fasting serum glucose, insulin and insulin resistance (p = 0.04, 0.02 and 0.007, respectively) after vitamin D supplementation. However, supplementation with vitamin D in a therapeutic dose had no effect on serum adiponectin concentrations. It appears likely there the measurement of total adiponectin level might be a significant limitation for this study. It should be noted that two forms of circulating adiponectin were measured – low-molecular weight (LMW) and high-molecular weight (HMW) adiponectin. The second one is the active form and the ratio of HMW to total adiponectin is considered more accurate for the assessment of the insulin resistance and adiponectin association [40]. Similar results were obtained by Breslavsky *et al.* (1000 IU/day vitamin D for 12 months) [26] and Patel *et al.* (400 IU and 1200 IU/day for 4 months) [41]. However, in the first study, supplementation with vitamin D did not normalized serum adiponectin levels, while the second study had a small sample size and a control group was absent.

The positive association between vitamin D supplementation and serum adiponectin concentrations observed in cross-sectional studies may be caused by increased expression of adiponectin gene or changes of activity of the renin-angiotensin-aldosterone system [42]. Activated vitamin D is a negative inhibitor of the renin-angiotensin-aldosterone system. It has been shown that increased activity of the renin-angiotensin-aldosterone system is associated with increased angiotensin production, leading to decreased serum adiponectin concentrations. Therefore, vitamin D may increase serum adiponectin concentrations through decreasing the angiotensin production [43]. Another possible mechanism explaining the association between vitamin D and adiponectin is the regulation of the adiponectin gene expression through $1,25(OH)_2D_3$, the active form of vitamin D, which has receptors located in preadipocytes [44]. Furthermore, $1,25(OH)_2D_3$ might play an important role in regulation of tumor necrosis factor-α production which is involved in adiponectin synthesis [45-48].

The present meta-analysis has some limitations. *First*, the qualified RCTs generally had very modest populations (none recruited more than 100 patients) and limited follow-up. *Second*, the studies involved were heterogeneous concerning the population similarities (age), the duration of the study as well as type and dose of vitamin D. *Third*, the relationship between all adipokines with vitamin D could not be performed because of the relatively small number of available studies. There is also no information in the included studies on the seeasonal's vitamin D changes.

In conclusion, this meta-analysis did not find a significant effect of supplementation and treatment with vitamin D on plasma adipokine concentrations. However, recent studies suggest that adipokines and adipose tissue may be a direct target for vitamin D. The expression of both the vitamin D receptor and 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) genes has been demonstrated in murine and human adipocytes. Some recent studies have also shown that vitamin D metabolites might influence an increased adipokines production. Since adipokines are associated with a better glycemic control and other metabolic indicators, a possible explanation why vitamin D supplementation may have a significant impact on the adipokine levels is needed, especially in patients with type 2 DM, metabolic syndrome or obesity.

ACKNOWLEDGMENT:

The authors declare no competing financial interests. This meta-analysis was written independently; no company or institution supported it financially. No professional writer was involved in the preparation of this meta-analysis. The meta-analysis has been prepared within Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group (www.lbpmcgroup.umed.pl). The authors declare no competing financial interests.

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Table 1. Demographic characteristics and baseline parameters of the studies selected for analysis.

Study		**Baziar *et al.*[24]**	**Belenchia *et al.*[25]**	**Breslavsky *et al.* [26]**	**Gagnon *et al.* [27]**	**Maggi *et al.*[28]**	**Neyestani *et al.*[29]**	**Shah *et al.* [30]**	**Ghavamzadeh *et al.* [31]**	**Chai *et al.* [32]**
Year		2014	2013	2013	2014	2013	2012	2015	2014	2012
Location		Iran	USA	Israel	Australia	Italy	Iran	USA	Iran	USA
Design		Randomized double-blind placebo-controlled parallel group trial	Randomized double-blind placebo-controlled parallel group trial	Randomized double-blind placebo-controlled parallel group trial	Randomized double-blind placebo-controlled parallel group trial	Randomized double-blind placebo-controlled parallel group trial	Randomized double-blind controlled parallel group trial	Randomized double-blind placebo-controlled parallel group trial	Randomized double-blind placebo-controlled parallel group trial	Randomized double-blind, placebo-controlled parallel group trial
Trial duration		2 months	6 months	12 months	6 months	6 months	3 months	6 months	3.5 months	6 months
Inclusion criteria		Type 2 diabetic patients aged 31-65, BMI > 25 and < 30 kg/m^2, serum 25(OH)D 10-30 ng/mL, glycated hemoglobin (HbA1c) < 8%, no alcohol consumption and smoking, insulin therapy, consuming thiazolidinediones, pregnancy or	Obese adolescent patients aged between 9 and 19 yrs and at least at the 85th percentile for BMI	Patients with type 2 diabetes mellitus recruited from the hypertension outpatient clinic	Vitamin D-deficient men and women aged ≥18 yrs and at risk of type 2 diabetes (BMI between 25 and 40 kg/m^2, a serum 25(OH)D concentration ≤50 nmol/L [<20.1 ng/mL] and fasting plasma glucose 6.1–6.9 mmol/L and/or 2-h plasma glucose post 75 g glucose load 7.8–11.0 mmol/L [140-200 mg/dL])	Patients who were 60 yrs or older with type 2 diabetes mellitus and diabetic foot complications	Type 2 diabetes subjects (fasting blood glucose concentration >126 mg/dl; not taking steroidal anti-inflammatory or anticoagulant medications; not taking dietary supplements including calcium, vitamin D, or omega-3 within the past 3	Overweight and obese adolescents (ages ≥11and <18 yrs, BMI of ≥85th percentile for age and gender, and serum 25(OH)D concentration between 10 and 60 ng/mL)	Type 2 diabetic patients on glucose lowering agents, – but not insulin-not suffering from other illnesses (such as cardiovascular diseases, renal failure, and/or inflammatory diseases) non supplemented with vitamin D and/or Ca	Adults aged 30 to 75 yrs, in general health, with a history of at least one pathology-confirmed adenomatous colorectal polyp within the past 36 months, no contraindications to calcium or vitamin D supplementation or rectal biopsy procedures and no medical conditions, habits, or medication usage that would otherwise interfere with the study

		menopause, consuming vitamin D-interfering drugs (corticosteroids, antiepileptics and contraceptives) and also calcium and vitamin D supplementation in last six months, no history of myocardial infarction, angina pectoris and stroke in last year or suffering from cardiovascular, liver, kidney, thyroid gland diseases and chronic inflammation.					months before the intervention; not receiving medications that could potentially influence vitamin D metabolism or insulin; not having any other clinical disease that could influence vitamin D metabolism)			

Type and dose of vitamin D		50000 IU vitamin D_3 soft gelatin spherical pearls single dose/week	2x2000 IU vitamin D_3 pills/day	1000 IU vitamin D_3 capsules/day	Combination 2000 IU vitamin D_3 capsules+1200 mg Ca/day for the first 2 months; Combination 4000 IU vitamin D_3 capsules+1200 mg Ca/day for the next 2 months; Combination 6000 IU vitamin D_3 capsules+1200 mg Ca/day for the last 2 months	A single dose of 300,000 IU vitamin D_3	Vitamin D-fortified doogh (Persian yogurt drink), containing 500 IU vitamin D3 and 150 mg calcium /250 ml, 2 times daily	150,000 IU vitamin D_2 capsule at baseline and another 150,000 IU vitamin D_2 capsule after 12 weeks	Vitamin D_3 (cholecalciferol) 400 IU /ml (10 mcg/ml) plus thin vegetable oil (purified component of coconut and palm oil)	800 IU vitamin D_3
										800 IU vitamin D_3 +2 g Ca elemental
Participants	VitD	41	18	24	35	14	30	14	26	23
										23
	Control	40	17	23	45	16	30	17	25	23
Age (years)	VitD	50.34±6.71	14.6± 2.3	66.8±9.2	53.8±11.9	69*	51.5±5.4	15.1±0.4**	52.26±2.09**	60.2±8.1
										62.1±7.5
	Control	52.75±6.34	13.9± 2.4	65.8±9.7	55.3±11.1		50.8±6.7	13.6±0.4**	49.28±2.00**	58.5±8.2
Male (%)	VitD	68.29	52.0	45.83	29.0	64.28	NA	40.0	41.18	70.0
										70.0
	Control	65.0	48.0	47.83	33.0	87.5	NA	30.0		70.0
BMI (kg/m^2)	VitD	27.33±1.64	39.5±5.1	27.9±5.2	31.1±5.7	29*	29.2±4.4	36.0±1.6**	28.9±0.86	28.9±5.6
										31.6±6.0
	Control	27.25±1.35	38.9±6.7	30.6±5.1	31.9±6.2		29.9±4.7	31.0±1.1**	27.9±0.93	30.6±7.2
Adiponectin, (ng/mL)	VitD	3480±1570	5860±200	10.37±6.57	6070±3613†	14,045.4±12,698.9	105.5±68.7	$4.8 \cdot 10^{-3}$ ($3.4 \cdot 10^{-3}$-$6.7 \cdot 10^{-3}$)‡	NA	NA
										NA

	Control	4040±3980	5880±210	15.81±11.31	6424±4408†	12,205±8,286.7	105.3±83.1	$7.2\cdot10^{-3}$($3.6\cdot10^{-3}$-$8.8\cdot10^{-3}$) ‡	NA	NA
Leptin (ng/mL)	VitD	NA	43.5± 1.8	27.88±27.97	NA	18.83±16.66	NA	NA	11.96± 2.18**	14.9±4.4**
										26.3±4.5**
	Control	NA	43.5±1.9	30.54±28.96	NA	12.36±14.03	NA	NA	11.20± 1.96**	17.2±4.5**
Serum 25(OH)D ng/ml	VitD	14.33±5.85	19.2± 6.3	11.8±10.9	18.8±5.2	11.2±4.36	17.6±11.6	19.6±1.4**	21.46±4.65**	19.4±1.9**
										18.8±1.9**
	Control	15.50 ±5.55	19.6±7.9	11.7±6.5	17.2±5.2	13.6±6.91	16.8±18.0	25.8±2.6**	22.16±5.32**	18.8±1.9**
SBP (mmHg)	VitD	NA	NA	154.2±21.5	120.8±14.0	NA	NA	NA	NA	NA
	Control	NA	NA	151.8±18.0	126.8±12.1	NA	NA	NA	NA	NA
DBP (mmHg)	VitD	NA	NA	76.2±8.8	73.1±10.0	NA	NA	NA	NA	NA
	Control	NA	NA	72.2±10.8	76.8±8.9	NA	NA	NA	NA	NA

Values are expressed as mean±SD; *only mean; **values are expressed as mean ± SEM; † - logarithmically-transformed variables; ‡ - median (interquartile range)

Abbreviations: SD: standard deviation; SEM: standard error of the mean; BMI: body mass index; NA: not available; 25 (OH) D: 25-hydroxyvitamin D; SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 2. Assessment of risk of bias in the included studies using Cochrane criteria

Study	Ref	SEQUENCE GENERATION	ALLOCATION CONCEALMENT	BLINDING OF PARTICIPANTS AND PERSONNEL	BLINDING OF OUTCOME ASSESSMENT	INCOMPLETE OUTCOME DATA	SELECTIVE OUTCOME REPORTING	OTHER POTENTIAL THREATS TO VALIDITY
Baziar *et al.* 2014	**[24]**	**U**	**U**	**L**	**U**	**L**	**L**	**L**
Belenchia *et al.* 2013	**[25]**	**L**	**L**	**L**	**U**	**L**	**L**	**L**
Breslavsky *et al.* 2013	**[26]**	**U**	**U**	**L**	**L**	**L**	**L**	**L**
Gagnon *et al.* 2014	**[27]**	**L**	**L**	**L**	**U**	**L**	**L**	**L**
Maggi *et al.* 2013	**[28]**	**L**	**L**	**L**	**U**	**L**	**L**	**L**
Neyestani *et al.* 2012	**[29]**	**U**	**U**	**L**	**U**	**L**	**L**	**L**
Shah *et al.* 2015	**[30]**	**L**	**L**	**L**	**U**	**L**	**L**	**L**
Ghavamzadeh *et al.* 2014	**[31]**	**U**	**U**	**L**	**U**	**L**	**L**	**L**
Chai *et al.* 2012	**[32]**	**U**	**U**	**L**	**L**	**L**	**L**	**L**

L: low risk of bias; H: high risk of bias; U: unclear risk of bias.

FIGURE LEGENDS:

Figure 1. Flow chart of the number of studies identified and included into the meta-analysis.

Figure 2. Forest plot displaying mean difference and 95% confidence intervals for the impact of vitamin D supplementation on plasma adiponectin concentrations (Figure 2A). Lower plot shows leave-one-out sensitivity analysis (Figure 2B).

Figure 3. Forest plot displaying mean difference and 95% confidence intervals for the impact of single bolus dosing and multiple daily dosing of vitamin D on plasma adiponectin (Figure 3A) and leptin (Figure 3B) concentrations.

Figure 4. Meta-regression plots of the association between mean changes in plasma adiponectin concentrations with duration of supplementation (Figure 4A), baseline serum 25(OH)D (Figure 4B) and changes in serum 25(OH)D concentrations (Figure 4C).

Figure 5. Forest plot displaying mean difference and 95% confidence intervals for the impact of vitamin D supplementation on plasma leptin concentrations (Figure 5A). Lower plot shows leave-one-out sensitivity analysis (Figure 5B).

Figure 6. Meta-regression plots of the association between mean changes in plasma leptin concentrations with duration of supplementation (Figure 6A), baseline serum 25(OH)D (Figure 6B), and changes in serum 25(OH)D concentrations (Figure 6C),.

Figure 7. Funnel plot displaying publication bias in the studies reporting the impact of vitamin D supplementation on plasma adiponectin (Figure 7A) and leptin (Figure 7B) concentrations.