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Vitamin D_3 and the risk of CVD in overweight and obese women: a randomised controlled trial

Amin Salehpour[1], Farzad Shidfar[1]*, Farhad Hosseinpanah[2], Mohammadreza Vafa[1], Maryam Razaghi[1], Anahita Hoshiarrad[3] and Mahmoodreza Gohari[4]

[1]*Department of Nutrition, School of Public Health, Tehran University of Medical Sciences, No. 52, Alvand Street, Arjantin Square, Tehran, Iran*
[2]*Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Floor 4th, Number 24, Parvaneh Street, Yemen Street, Chamran Exp, Tehran, Iran*
[3]*National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, No. 42, Arghavan Street, Farahzadi Boulevard, Shahrak-e Gharb, Tehran, Iran*
[4]*Department of Biostatistics, Tehran University of Medical Sciences, No. 52, Alvand Street, Arjantin Square, Tehran, Iran*

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Abstract

Evidence indicates that vitamin D deficiency contributes to CVD. We investigated the effect of vitamin D_3 supplementation on cardiovascular risk factors in women. Healthy premenopausal overweight and obese women (*n* 77; mean age 38 (SD 8·1) years) were randomly allocated to the vitamin D (25 μg/d as cholecalciferol) or the placebo group in a double-blind manner for 12 weeks. Blood pressure, serum lipoproteins, apolipoproteins and anthropometric parameters were recorded. Dietary intake was recorded using 24 h food recall and FFQ. Physical activity was assessed by the International Physical Activity Questionnaire. Mean total cholesterol concentrations increased in the vitamin D group (0·08 (SD 0·56) mmol/l) but declined in the placebo group (0·47 (SD 0·58) mmol/l), and a significant effect was observed ($P \leq 0·001$). In the vitamin D group, mean HDL-cholesterol concentration increased, whereas it decreased in the placebo group (0·07 (SD 0·2) *v.* −0·03 (SD 0·2) mmol/l; $P = 0·037$). Mean apoA-I concentration increased in the vitamin D group, although it decreased in the placebo group (0·04 (SD 0·39) *v.* −0·25 (SD 0·2) g/l; $P \leq 0·001$). Mean LDL-cholesterol:apoB-100 ratio augmented in the vitamin D group, while this ratio declined in the placebo group (0·11 (SD 0·6) *v.* −0·19 (SD 0·3); $P = 0·014$). Body fat mass was significantly decreased in the vitamin D group more than the placebo group (−2·7 (SD 2) *v.* −0·4 (SD 2) kg; $P \leq 0·001$). The findings showed that supplementation with vitamin D_3 can significantly improve HDL-cholesterol, apoA-I concentrations and LDL-cholesterol:apoB-100 ratio, which remained significant in the multivariate model including anthropometric, dietary and physical activity measures.

Key words: **Vitamin D: CVD: Obesity**

Vitamin D is recognised as the vitamin of sunshine[1]. A relationship between vitamin D and CVD has been proposed by observing more incidence of CVD in winter compared with summer in many countries[2,3], which might be attributable to the protective effect of vitamin D on CVD[4]. Ecological studies have indicated the higher rate of CHD and high blood pressure increment with more distance from the equator, which is also related to low sun exposure and higher prevalence of vitamin D deficiency[5,6]. It has been shown that a range of markers including geographic latitude, altitude and season affect vitamin D status which are negatively correlated with cardiovascular morbidity and mortality. Evidence suggests that low levels of vitamin D may contribute to the development of CVD[7].

On the other hand, a range of CVD risk factors including dyslipoproteinaemia, high blood pressure, reduced glucose tolerance, diabetes and increased inflammatory markers are related to obesity, which has been increasing dramatically during recent decades[8–10]. Serum 25-hydroxyvitamin D (25(OH)D) levels negatively correlate with BMI[11]. It is likely that alteration in vitamin D homeostasis affects CVD development in obese individuals[12]. Low levels of 25(OH)D cause higher levels of parathyroid hormone (PTH), which is a known non-traditional CVD risk factor[13,14].

Investigations have shown that hypovitaminosis D has an undesirable effect on total and LDL-cholesterol concentrations[15], therefore resulting in high total cholesterol and low apoA-I concentrations. An independent and positive

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; HR, hazard ratio; PTH, parathyroid hormone.

* **Corresponding author:** Dr F. Shidfar, fax +98 21 88779487, email f-shidfar@tums.ac.ir

significant correlation has been reported among serum 25(OH)D levels and apoA-I and HDL-cholesterol concentrations. It seems that the effects of vitamin D on the lipid profile are independent of Ca and other unfavourable effects caused by high type 2 diabetes risk[15,16].

Scragg *et al.*[17] and Jorde *et al.*[18] have shown the association between 25(OH)D and blood pressure. Vitamin D affects blood pressure via paracrine and endocrine mechanisms in tissues that are specifically related to high blood pressure including vascular smooth muscle cells[19], the endothelium and cardiomyocytes[20]. Also, 1,25-hydroxyvitamin D is considered as a negative endocrine regulator of the renin–angiotensin system, and sunlight exposure as an indirect marker of vitamin D synthesis in the skin has an inverse correlation with the prevalence of high blood pressure, reducing blood pressure levels[20–22].

As the prevalence of vitamin D deficiency is high in Iranian women[23,24] and the burden of overweight and obesity is the fifth leading risk for global deaths[25], which increases the risk of non-communicable diseases including CVD, we evaluated the effect of vitamin D_3 supplementation on cardiovascular risk factors in healthy overweight and obese women.

Materials and methods

Participants

The present study was performed between November 2009 and April 2010 in the Heart and Vascular Laboratory at the Pharmacology Department of Tehran University of Medical Sciences, Tehran, Iran. We distributed advertisements in the campus and requested all women to participate in the study. The inclusion criteria were as follows: good public health status; age 18–50 years; BMI $\geq 25\,kg/m^2$; free of known osteoporosis; gastrointestinal disease; diabetes mellitus; CVD; renal disease; high blood pressure (>160/90 mmHg). Participants were excluded from the study if they were following a weight-reduction programme, taking weight-loss drugs, having a change in weight more than 3 kg during the last 3 months, pregnant, lactating, smoking, drinking alcohol, taking nutritional supplements, cholesterol and TAG-lowering agents as well as anti-hypertensive agents. At the beginning of the study, sunscreen use was discontinued in all subjects.

A total of eighty-five participants who met the above inclusion criteria were recruited into a double-blind clinical trial. We randomly assigned participants to one of two groups: vitamin D (*n* 42) or placebo (*n* 43). Written informed consent was obtained from all subjects. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Ethics Committee of the Tehran University of Medical Sciences and Iranian Registry of Clinical Trial (registration no. IRCT138809092709N2). This trial was registered at ClinicalTrials.gov as NCT01344161. Of the eighty-five participants who were eligible for the present intervention study, eight subjects dropped out of the follow-up: in the vitamin D group, one patient became pregnant and one patient followed a weight-loss programme. In the placebo group, one patient used oral contraceptive pills and five other patients were unwilling to continue the 12-week examination for personal reasons. Finally, seventy-seven participants completed the study and, of these, thirty-nine were in the vitamin D group and thirty-eight were in the placebo group.

Design

A vitamin D_3 supplement (25 μg/d as cholecalciferol; Merck Pharma GmbH) or placebo (25 μg/d as lactose; Merck Pharma GmbH) was given to the participants per month. The period of the intervention was 90 d and the participants took one tablet of vitamin D_3 supplement or placebo every day. Compliance with the supplementation was 87 %.

Blood samples were collected from the antecubital vein of each patient after an overnight fast of at least 12 h. After centrifugation for 20 min (3000 ***g***), the serum samples were frozen simultaneously and stored at −80°C until analysed. In order to eliminate the probable effects of sex hormones on blood lipids, blood sampling was not performed between days 1 and 5 of the menstrual cycle.

Anthropometry measurements

Body weight and height were measured on a digital scale (model 763; Seca GmbH & Co, KG) with participants wearing light indoor clothing. Waist and hip circumference were measured using an Ergonomic Circumference Measuring Tape (model 201; Seca GmbH & Co, KG). BMI was calculated by dividing weight (kg) by height (m^2). Body composition was assessed by Bioelectrical Impedance Analysis (model 4000, Body Stat Quad Scan; Bodystat). All anthropometric indices were measured by following the WHO standard procedures[26]. We assessed physical activity levels using the International Physical Activity Questionnaire[27] and determined the average of metabolic equivalents (MET)-min/week.

Dietary assessments

Energy, macronutrient, Ca, vitamin D, saturated fat, MUFA and PUFA intakes were estimated using 24 h food recall and validated FFQ[28]. On a monthly basis, a nutritionist completed the questionnaires by a direct interview. Because the Iranian food composition table is incomplete (limited to only raw materials and a few nutrients)[29], each food and beverage, only unfortified US food equivalents, was analysed for nutrient intake using Nutritionist IV software (version 4.1; First Databank Division, The Hearst Corporation) to assess macronutrient and micronutrient contents of the foods. The Iranian food composition table was used as an alternative for traditional Iranian food items, such as kashk, which is not included in the Food Composition Tables for Use (United States Department of Agriculture food composition table)[30].

Blood pressure

Systolic and diastolic blood pressure was measured in the right or the left arm supported at the heart level of seated

participants (Model Gamma G-7; Heine). Means of two measurements taken at 5 and 10 min of rest were used.

Biochemical assay

25(OH)D was measured using an enzyme immunoassay (Immunodiagnostic Systems Limited). Intra- and inter-assay CV for 25(OH)D were 6·9 and 8·1 %, respectively. Intact PTH was measured using an immunoenzymometric assay (Immunodiagnostic Systems Limited). Intra- and inter-assay CV for intact PTH were 5·5 and 8·3 %, respectively. TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol were measured by a direct colorimetric enzymatic method (Greiner). Intra- and inter-assay CV for TAG were 2·6 and 2·9 %, for total cholesterol were 1·4 and 2·1 %, for LDL-cholesterol were 2·2 and 2·5 %, and for HDL-cholesterol were 2·4 and 2·6 %, respectively. Lipoprotein(a) was measured by an enzyme immunoassay (Mercodia). Intra- and inter-assay CV for lipoprotein(a) were 6·6 and 7·8 %, respectively. Also, we measured apoA-I and apoB-100 using a sandwich technique (ELISA; AlerCHEK). Intra- and inter-assay CV for apoA-I and apoB-100 were 5·6, 6·9 % and 6·1, 7·4 %, respectively.

Statistical analysis

Continuous variables are expressed as means and standard deviations, and categorical variables as percentage proportions. The change in variables was analysed with ANCOVA to adjust mean differences in variables, in which baseline value, fat mass and waist circumference were used as covariates. P values $<0·05$ were considered statistically significant. Relationships between change in 25(OH)D concentrations and cardiovascular markers were evaluated using simple Pearson's correlations. To adjust the analyses for confounding variables, partial correlations were computed for all dependent variables, after adjusting for fat mass and waist circumference

Table 1. Baseline characteristics of the participants who received vitamin D_3 supplements (25 μg/d) or placebo*

(Mean values and standard deviations)

	Vitamin D group		Placebo group		
Characteristics	Mean	SD	Mean	SD	*P*
Age (years)	38†	7	37	8	0·29
Body weight (kg)	73·9	10·2	75·1	11·9	0·61
BMI (kg/m^2)	30·1	3·9	29·5	4·4	0·54
Fat mass (kg)	30·2	6·9	29	8·7	0·53
Waist circumference (cm)	89·9	8·7	91·2	12·1	0·59
Physical activity (MET-min/week)	902	1245	702	996	0·43
Energy intake					0·33
kcal/d	1866	927	2060	834	
kJ/d	7807	3878	8619	3489	
Carbohydrate intake (g/d)	280	134	329	140	0·12
Fibre intake (g/d)	16	9	18	10	0·23
Protein intake (g/d)	64	29	76	35	0·10
Fat intake (g/d)	55	44	49	24	0·43
Dietary Ca intake (mg/d)	873	586	677	386	0·08
Dietary vitamin D intake (μg/d)	0·53	0·6	0·39	0·37	0·22
Dietary SFA intake (g/d)	18·8	15·4	15·8	9·1	0·30
Dietary MUFA intake (g/d)	19·2	18·5	16·7	9·5	0·45
Dietary PUFA intake (g/d)	13	17·1	12·2	7·8	0·78
Systolic blood pressure (mmHg)	110·5	17·5	116·7	11·4	0·07
Diastolic blood pressure (mmHg)	67·9	10·1	71·9	9·1	0·07
25(OH)D (nmol/l)‡	36·8	30	46·9	32	0·15
PTH (pmol/l)‡	1·4	0·7	1·4	0·7	0·84
TAG (mmol/l)	1·51	0·4	1·62	0·5	0·31
Total cholesterol (mmol/l)	4·71	0·6	4·89	0·8	0·29
LDL-C (mmol/l)	3·1	0·59	3·2	0·77	0·28
HDL-C (mmol/l)	0·91	0·2	0·87	0·2	0·45
ApoA-I (g/l)	1·63	0·2	1·78	0·2	0·006
ApoB-100 (g/l)	1·35	0·2	1·38	0·2	0·53
TAG:HDL-C	1·78	0·6	2·0	0·98	0·25
Total cholesterol:HDL-C	5·45	1·5	5·88	1·7	0·25
Total cholesterol:LDL-C	1·54	0·14	1·52	0·14	0·59
LDL-C:HDL-C	3·64	1·2	3·96	1·3	0·28
LDL-C:apoB-100	2·34	0·5	2·39	0·5	0·68
ApoA-I:apoB-100	1·26	0·3	1·33	0·2	0·36
Lipoprotein(a) (μmol/l)	0·62	0·62	0·56	0·52	0·62

MET, metabolic equivalents; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol.

* Mean values were not significantly different from week 0 between the groups ($P>0·05$; unpaired *t* test).

† Mean values were not significantly different from month 0 between the groups ($P>0·05$; unpaired *t* test).

‡ To convert 25(OH)D values to ng/ml, divide by 2·5. To convert PTH values to pg/ml, divide by 0·11.

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as independent variables. The statistical program SPSS (version 16; SPSS, Inc.) was used to perform the analyses.

Results

Participant characteristics are shown in Table 1. Baseline physical characteristics in both groups were similar. The intake of SFA and MUFA decreased in both groups from the beginning of the study up to week 12. The intake of PUFA increased in the vitamin D group and decreased in the placebo group. Despite this, changes in dietary fatty acid intake were not significant between the two groups.

Baseline systolic blood pressure was lower in the intervention group (67·9 (SD 10·1) *v.* 71·9 (SD 9·1) mmHg). Systolic blood pressure increased in the vitamin D group, but declined in the placebo group (0·51 (SD 12·7) *v.* −2·2 (SD 10·2) mmHg; Table 2). Diastolic blood pressure increased in both groups (2·3 (SD 6·7) *v.* 0·13 (SD 8·3) mmHg). The changes were not statistically significant between the two groups.

At baseline, the percentage of subjects with hypovitaminosis D (25(OH)D <75 nmol/l) and vitamin D sufficiency (25(OH)D ≥75 nmol/l) in the vitamin D group was 89·7 and 10·3 %, reaching 53·8 and 46·2 % after supplementation, respectively. Related values in the placebo group were 89·5 and 10·5 % at baseline, reaching 86·8 and 13·2 %, respectively ($P<0·001$). Serum intact PTH concentrations declined in the vitamin D group up to 1·2 (SD 0·5) pmol/l. However, serum PTH concentrations increased in the placebo group up to 1·7 (SD 0·8) pmol/l during 12 weeks ($P<0·001$). Body fat mass decreased in the vitamin D and placebo groups (−2·7 (SD 2) *v.* −0·4 (SD 2) kg; $P<0·001$, respectively).

Serum TAG concentrations decreased in both groups after 12 weeks, but these alterations were higher in the placebo group compared with in the vitamin D group (0·26 (SD 0·28) *v.* 0·29 (SD 0·3) mmol/l, respectively). This result was not

Table 2. Anthropometric, dietary and biochemical variables in the participants who received vitamin D_3 supplements (25 μg/d) or placebo after the intervention and change in variables between the measurement periods

(Mean values and standard deviations)

	Vitamin D group				Placebo group				
	Week 12		Change*		Week 12		Change		
Characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*†
Body weight (kg)	73·5	10·4	−0·3	1·5	75	12·3	−0·1	1·7	0·71
BMI (kg/m^2)	30	4	−0·13	0·6	29·5	4·6	−0·04	0·6	0·5
Fat mass (kg)	28·2	7·5	−2·7	2	28·6	8·9	−0·4	2	<0·001
Waist circumference (cm)	89·5	8·8	−0·3	4·3	91·6	13	0·4	4·1	0·38
Physical activity (MET-min/week)	892	1488	−10	1627	1081	1372	379	1137	0·23
Energy intake									0·32
kcal/d	2010	1289	143·7	1358·4	1852	992	−208	920·9	
kJ/d	8409	5393	601	5683	7748	4150	−870	3853	
Carbohydrate intake (g/d)	312	186	31·84	194·6	294	164	−34·32	143	0·23
Fibre intake (g/d)	16	12	1	11·7	14	7	−4·3	11·3	0·1
Protein intake (g/d)	72	53	7·8	54·3	66	32	−9·3	35·6	0·29
Fat intake (g/d)	53	43	−2·3	52·2	45	36	−4·2	39·3	0·48
Dietary Ca intake (mg/d)	829	533	−43·9	674·4	625	454	−51·8	509·5	0·18
Dietary vitamin D intake (μg/d)	0·4	0·47	−0·09	0·77	0·37	0·35	−0·04	0·52	0·7
Dietary SFA intake (g/d)	18·1	12·3	−0·67	16·3	13·9	11·4	−1·8	13·4	0·73
Dietary MUFA intake (g/d)	16·8	13·2	−2·3	18·2	15·2	18·2	−1·4	20·1	0·83
Dietary PUFA intake (g/d)	13·7	16·7	0·67	23·2	11·9	8·4	−0·31	10	0·81
Systolic blood pressure (mmHg)	111	11·3	0·51	12·7	114·4	13	−2·2	10·2	0·3
Diastolic blood pressure (mmHg)	70·2	8·8	2·3	6·7	72·1	10·6	0·13	8·3	0·207
25-Hydroxyvitamin D (nmol/l)	75	22	38·2	32	51·5	31	4·6	14	<0·001
PTH (pmol/l)	1·2	0·5	−0·2	0·5	1·7	0·8	0·2	0·5	<0·001‡
TAG (mmol/l)	1·25	0·36	−0·26	0·28	1·32	0·4	−0·29	0·3	0·588
Total cholesterol (mmol/l)	4·79	0·8	0·08	0·56	4·41	0·8	−0·47	0·58	<0·001‡
LDL-C (mmol/l)	3·2	0·79	0·13	0·5	2·9	0·75	−0·3	0·5	<0·001
HDL-C (mmol/l)	0·99	0·1	0·07	0·2	0·84	0·1	−0·03	0·2	0·037‡
ApoA-I (g/l)	1·67	0·2	0·04	0·39	1·53	0·2	−0·25	0·2	<0·001‡
ApoB-100 (g/l)	1·31	0·2	−0·03	0·2	1·34	0·2	−0·03	0·1	0·942
TAG:HDL-C	1·3	0·4	−0·48	0·5	1·64	0·8	−0·36	0·5	0·356
Total cholestrol:HDL-C	4·94	1·0	−0·51	1·2	5·33	1·1	−0·55	1·2	0·902
Total cholestrol:LDL-C	1·51	0·15	−0·02	0·15	1·51	0·13	−0·005	0·13	0·531
LDL-C:HDL-C	3·34	0·9	−0·29	1	3·58	0·9	−0·38	1	0·718
LDL-C:apoB-100	2·45	0·4	0·11	0·6	2·2	0·4	−0·19	0·3	0·014‡
ApoA-I:apoB-100	1·3	0·3	0·04	0·3	1·16	0·2	−0·17	0·3	0·009‡
Lipoprotein(a) (μmol/l)	0·66	0·63	0·03	0·19	0·61	0·55	0·05	0·1	0·557

MET, metabolic equivalents; PTH, parathyroid hormone; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol.

* After 12 weeks.

† An ANCOVA was used to adjust mean differences in all dependent variables.

‡ Mean values remained significantly different between the groups after adjusting for fat mass and waist circumference ($P<0·05$).

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statistically significant. Baseline serum total cholesterol concentrations in the vitamin D group were lower than those in the placebo group (4·7 (SD 0·6) *v.* 4·89 (SD 0·8) mmol/l, respectively). After 12 weeks, total cholesterol concentrations increased in the vitamin D group and declined in the placebo group (0·08 (SD 0·56) *v.* −0·47 (SD 0·58) mmol/l; $P<0·001$), respectively. Similarly, serum LDL-cholesterol concentrations increased in the vitamin D group and decreased in the placebo group (0·13 (SD 0·5) *v.* −0·3 (SD 0·5) mmol/l; $P<0·001$), respectively. HDL-cholesterol concentrations increased in the vitamin D group and declined in the placebo group (0·07 (SD 0·2) *v.* −0·03 (SD 0·2) mmol/l; $P=0·037$), respectively. At baseline, serum apoA-I concentrations in the vitamin D group were lower than those in the placebo group (1·63 (SD 0·2) *v.* 1·78 (SD 0·2) g/l; $P=0·006$), respectively. After the intervention, apoA-I concentration increased in the vitamin D group, though it decreased in the placebo group (0·04 (SD 0·39) *v.* −0·25 (SD 0·2) g/l; $P<0·001$). Serum apoB-100 concentrations declined in both groups, but the changes were not statistically significant. The LDL-cholesterol:apoB-100 ratio increased in the vitamin D group, which indicates less atherogenic properties of LDL-cholesterol particles, whereas this ratio declined in the placebo group indicating that LDL-cholesterol particles were smaller and had higher density (0·11 (SD 0·6) *v.* −0·19 (SD 0·3); $P=0·014$). The apoA-I:apoB-100 ratio increased in the vitamin D group, but decreased in the placebo group (0·04 (SD 0·3) *v.* −0·17 (SD 0·3); $P=0·009$).

A positive correlation was observed between changes in serum 25(OH)D concentrations and systolic blood pressure (r 0·24, $P=0·032$), which remained statistically significant after adjusting for fat mass and waist circumference (r 0·23, $P=0·044$). A positive and significant trend was seen between changes in serum 25(OH)D concentrations and total cholesterol concentrations (r 0·27, $P=0·014$; Fig. 1), which did not remain significant after adjusting for fat mass and waist circumference. There was a positive and significant correlation between changes in serum 25(OH)D concentrations and HDL-cholesterol concentrations (r 0·26, $P=0·022$; Fig. 2) and between serum 25(OH)D concentrations and apoA-I concentrations (r 0·25, $P=0·023$; Fig. 3). The correlation between changes in serum 25(OH)D concentrations and HDL-cholesterol concentrations was statistically significant even after adjusting for fat mass and waist circumference (r 0·25, $P=0·03$), but did not remain significant between changes in serum 25(OH)D concentrations and apoA-I concentrations.

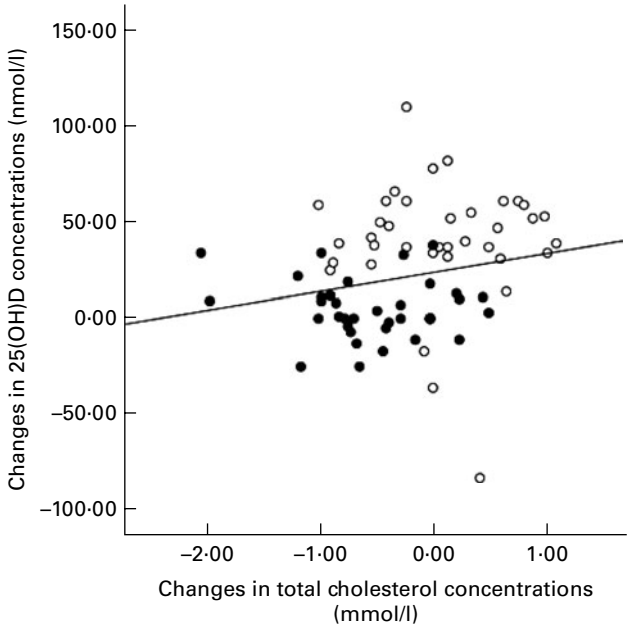

Fig. 1. Relationship between changes in serum 25-hydroxyvitamin D (25(OH)D) and total cholesterol concentrations. ○, Vitamin D group; ●, placebo group. r 0·279; $P=0·014$.

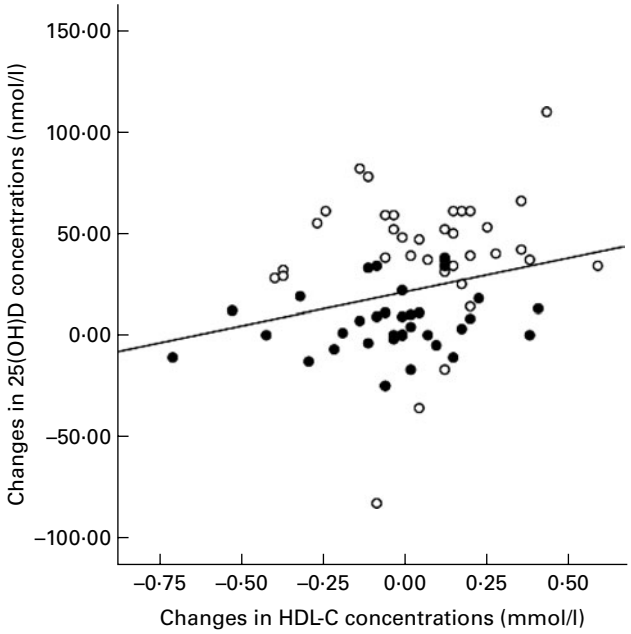

Fig. 2. Relationship between changes in serum 25-hydroxyvitamin D (25(OH)D) and HDL-cholesterol (HDL-C) concentrations. ○, Vitamin D group; ●, placebo group. r 0·260; $P=0·022$.

Discussion

The present study is one of the first reports about the effect of vitamin D_3 supplementation solely on blood lipids and lipoproteins in healthy overweight and obese women. The present study has shown that although the daily intake of a 25 μg vitamin D_3 supplement increases total and LDL-cholesterol concentrations, it has a beneficial effect on HDL-cholesterol, apoA-I concentrations, apoA-I:apo B-100 and LDL-cholesterol:apoB-100 ratios in overweight and obese women.

In the present study, although vitamin D_3 supplementation significantly increased 25(OH)D concentrations, some participants in the vitamin D group did not reach sufficient 25(OH)D concentrations. It seems that they may need higher doses or a longer period of time to be supplemented[31]. 25(OH)D concentrations are known to be an independent predictor of CVD[32]. 25(OH)D levels <37·5 nmol/l, compared with levels higher than 75–100 nmol/l, are related to an increase in

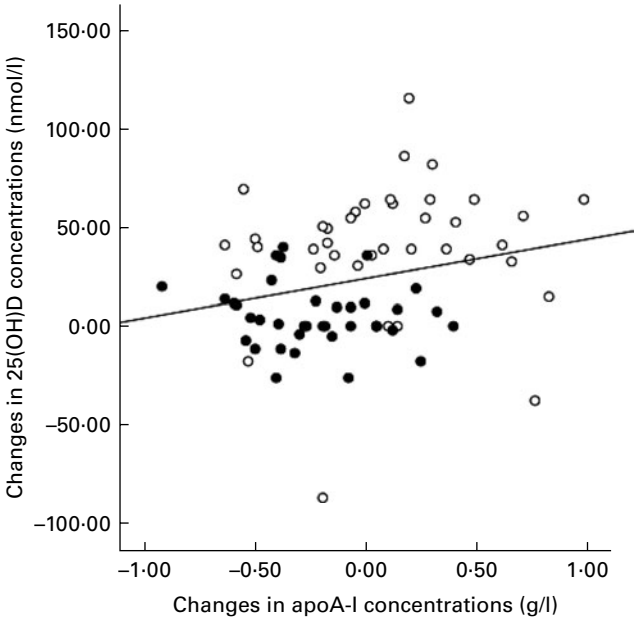

Fig. 3. Relationship between changes in serum 25-hydroxyvitamin D (25(OH)D) and apoA-I concentrations. ○, Vitamin D group; ●, placebo group. r 0·25; P=0·023.

cardiovascular events. In a previous study, men with 25(OH)D levels ≤37·5 nmol/l had a higher risk of myocardial infarction (R^2 2·42), compared with those with 25(OH)D levels ≥75 nmol/l[33]. Moreover, in a prospective cohort study with an average follow-up duration of 7·7 years in 3285 patients, Dobnig *et al.*[34] reported that hazard ratios (HR) for all-cause mortality (HR 2·08, 95 % CI 1·60, 2·70; HR 1·53, 95 % CI 1·17, 2·01, respectively) and those for cardiovascular mortality causes (HR 2·22, 95 % CI 1·57, 3·13; HR 1·82, 95 % CI 1·29, 2·58, respectively) in the two lowest quartiles of 25(OH)D with medians of 19 and 33·2 nmol/l were higher compared with patients in the highest quartiles of 25(OH)D with a median of 71 nmol/l. In the Framingham Offspring Cohort Study with an average follow-up duration of 5·4 years in individuals with high blood pressure, the risk of cardiovascular events increased two times in participants with 25(OH)D concentrations ≤37·5 nmol/l compared with those with 25(OH)D concentrations ≥37·5 nmol/l[35]. Scragg *et al.* reported that among white patients without high blood pressure from the Third National Health and Nutrition Examination Survey (NHANES III 1988–1994), systolic blood pressure and pulse pressure values in the highest quartile of 25(OH)D (≥85·7 nmol/l) were lowered by 1·8 and 1·6 mmHg, respectively, compared with the lowest quartile of 25(OH)D (≤40·4 nmol/l). Also, systolic blood pressure was decreased up to 1·8 and 4·6 mmHg in patients aged >50 and <50 years, respectively, when 25(OH)D concentrations increased from 20 to 100 nmol/l[17]. In contrast, in a cross-sectional study, the Longitudinal Aging Study Amsterdam, by Snijder *et al.*[36], no correlation has been reported between 25(OH)D concentrations and systolic blood pressure or diastolic blood pressure. We did not find any significant change in blood pressure after 12 weeks. Zittermann *et al.*[37] demonstrated that after 12 months of supplementation with 83 μg vitamin D/d coupled with a weight-reduction programme in overweight patients, systolic and diastolic blood pressure decreased, but only significant effects of time were observed. Additionally, Major *et al.*[38] and Pfeifer *et al.*[39] reported similar results. PTH and its related peptides can affect cardiovascular cells via receptors[40–42]. PTH is known as a risk factor for CVD, and patients with hyperparathyroidism are more exposed to cardiovascular morbidity and mortality[43,44]. PTH enhances myocyte hypertrophy[45] and vascular remodelling[46]. Moreover, PTH probably has a pre-inflammatory effect by stimulating cytokine release in vascular smooth muscle cells[47]. In the present study, PTH concentrations decreased significantly in the vitamin D group, but increased in the placebo group.

On the other hand, the correlation between vitamin D and serum TAG concentrations has been reported in patients with the end stage of renal disease[48]. Zittermann *et al.*[37] and Major *et al.*[38] demonstrated that TAG concentrations reduced in the vitamin D group. It has been suggested that vitamin D can reduce hepatic TAG synthesis or secretion by increasing intestinal Ca absorption[49]. By this way, vitamin D increases lipolytic activity of heparin and also increases TAG uptake by peripheral tissues[50]. Furthermore, Ca increment in hepatocytes stimulates microsomal TAG transfer protein, which is correlated with VLDL-cholesterol synthesis and secretion. Vitamin D induces intestinal Ca absorption that results in the suppression of Ca increment in hepatocytes and a decrease in VLDL-cholesterol synthesis and/or secretion[49].

In the present study, supplementation with vitamin D_3 increased total and LDL-cholesterol concentrations. Chiu *et al.*[51] reported that 25(OH)D levels had a positive correlation with total and LDL-cholesterol concentrations. Zittermann *et al.*[37] also found a significant vitamin D-dependent increase in LDL-cholesterol. In contrast, in 170 British Bangladeshis, John *et al.*[52] reported a negative correlation between 25(OH)D concentrations and total and LDL-cholesterol concentrations. It seems that vitamin D through an increase in intestinal Ca absorption[53] reduces the formation of insoluble Ca–fat soaps[54], therefore resulting in low Ca in lumen content, a low rate of Ca bonding to bile acids[55,56], conversion of cholesterol to bile acids and, finally, cholesterol excretion[57]. Several studies have demonstrated that serum 25(OH)D concentrations are a strong, independent predictor of apoA-I concentrations[51,58,59]. Auwerx *et al.*[59] reported that apoA-I concentrations had a positive correlation with 25(OH)D levels in Belgian men. It has been postulated that in the promoter of the *apoA-I* gene, there are vitamin D response elements through which 1,25(OH)$_2$D enhances the transcription of the *apoA-I* gene in human hepatoma cells[60].

In conclusion, daily supplementation with 25 μg vitamin D_3 by repletion of the body's vitamin D storage results in significant improvement in some cardiovascular risk factors in overweight and obese women.

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