International Journal of Food Sciences and Nutrition,
2011; Early Online: 1–10

informa
healthcare

Effects of vitamin D supplementation on 25-hydroxyvitamin D, high-density lipoprotein cholesterol, and other cardiovascular disease risk markers in subjects with elevated waist circumference

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Abstract
The objective of the present trial was to assess the effects of vitamin D supplementation on serum 25-hydroxyvitamin D [25(OH)D] and high-density lipoprotein cholesterol (HDL-C) in subjects with high waist circumference. Subjects were randomly assigned a daily multivitamin and mineral (MVM) supplement or a MVM supplement plus vitamin D 1,200 IU/day (MVM + D) for 8 weeks. There was a significant difference in mean change for 25(OH)D between the MVM and MVM + D treatment groups (-1.2 ± 2.5 nmol/l vs. 11.7 ± 3.0 nmol/l, respectively; $P = 0.003$). Vitamin D 1,200 IU/day did not increase 25(OH)D to a desirable level (≥ 75 nmol/l) in 61% of participants. There were no significant changes in cardiovascular disease risk markers. Thus, vitamin D supplementation with 1,200 IU/day was insufficient to achieve desirable serum 25(OH)D in most participants and did not affect cardiovascular disease risk markers.

Keywords: *Vitamin D, high-density lipoprotein cholesterol, cardiovascular disease risk*

Introduction

A number of observational studies have shown inverse relationships between vitamin D status, as assessed by the circulating concentration of 25-hydroxyvitamin D [25(OH)D], and the incidence of several chronic diseases, especially cardiovascular disease (Giovannucci 2007, Wang et al. 2008). Moreover, low vitamin D status has been associated with several markers of cardiovascular disease risk, including the metabolic syndrome and its components (Martini and Wood 2006, Dobnig et al. 2008, Giovannucci et al. 2008, Maki et al. 2009).

In recent years, an increasing number of researchers have concluded that vitamin D intakes and 25(OH)D concentrations in many people are too low for optimum health (Bischoff-Ferrari et al. 2006, Holick and Chen 2008). Although an optimal 25(OH)D concentration has not been established, a level of 75 nmol/l (≥ 30 ng/ml) is considered a desirable target (Holick and Chen 2008); however, an estimated 30–50% of the US population has circulating 25(OH)D below this level (Holick and Chen 2008, Lee et al. 2008). The Institute of Medicine recommendations at the time this study was conducted were 200 IU/day dietary vitamin D for children and adults up to the age of 50 years, 400 IU/day for those ≥ 50 years, and 600 IU/day for those ≥ 70 years (Dietary Reference Intakes 1997). However, results from recent studies indicate that substantially higher intakes are often required to achieve desirable 25(OH)D concentrations, particularly in locations where sun exposure is limited and for high-risk groups such as the elderly and dark-skinned individuals (Giovannucci 2007, Vieth et al. 2007, Holick and Chen 2008). Since few foods are naturally rich dietary sources of vitamin D, vitamin D-fortified foods and dietary supplements may be necessary to achieve and maintain a desirable 25(OH)D concentration (Lee et al. 2008).

Recently, our group reported results from a cross-sectional investigation in which a strong relationship was observed between serum concentrations of 25(OH)D

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ISSN 0963-7486 print/ISSN 1465-3478 online
DOI: 10.3109/09637486.2010.536146

and high-density lipoprotein cholesterol (HDL-C) (Maki et al. 2009). Each 25 nmol/l increment in 25(OH)D was associated with an increment of 0.1 nmol/l in HDL-C after adjustment for established determinants of the HDL-C concentration. This may be of considerable public health importance given that each 0.03 nmol/l increment in HDL-C is associated with a reduction in coronary heart disease (CHD) risk of 3% or more (Gordon et al. 1989). The mechanism(s) accounting for the association between serum concentrations of 25(OH)D and HDL-C concentrations are not clear and very limited data have been reported on the effects of supplemental vitamin D on HDL-C and other aspects of the serum lipoprotein lipid profile.

The aim of the present trial was to assess the efficacy of a multivitamin and mineral (MVM) supplement, with or without vitamin D, on serum 25(OH)D, HDL-C and other cardiovascular disease risk markers. We chose to examine subjects with waist circumference $\geq$ 88 cm (women) or $\geq$ 102 cm (men), since high waist circumference is a component of the metabolic syndrome and is inversely associated with circulating concentrations of both HDL-C and 25(OH)D (Maki et al. 1997, Grundy et al. 2004). The dosage of vitamin D (1,200 IU/day) was selected because it represents twice the daily intake recommended by The Institute of Medicine (Dietary Reference Intakes 1997) for any population subgroup and is a quantity that is practical for inclusion in a MVM supplement. A secondary aim was to assess possible benefits to cardiovascular disease risk markers of incorporating additional ingredients into the supplement (omega-3 fatty acids and probiotics) in an uncontrolled, 8-week extension conducted in subjects who received the MVM + vitamin D during the initial 8 weeks.

Materials and methods

Study procedures

This was a randomized, double-blind, placebo controlled trial conducted at Provident Clinical Research (Addison, IL, USA) in the period from mid-summer through the fall of 2009. The study was conducted according to Good Clinical Practice Guidelines, the Declaration of Helsinki (2000), and US 21 Code of Federal Regulations. Informed consent was obtained from all subjects before protocol-specific procedures were carried out. Subjects were informed of their right to withdraw from the study at any time. The study consisted of three periods: a 2-week screening/baseline period, followed by an 8-week double-blind placebo-controlled treatment period, and an 8-week open-label, single-arm extension period.

Subjects

Participants included men and women 18–79 years of age, inclusive, each with waist circumference $\geq$ 88 cm for women and $\geq$ 102 cm for men. Other inclusion criteria included willingness throughout the study period to maintain habitual diet, physical activity patterns, stable body weight, and willingness to avoid sunbathing and use of tanning beds. Eligible subjects were required to be ambulatory and judged to be in good general health on the basis of medical history and routine laboratory tests and to have no plans to change smoking habits during the study period. Exclusion criteria included use of any medications intended to alter the fasting lipid profile, use of over-the-counter or prescription weight-loss medications, or use of a dietary vitamin D supplement (other than a multivitamin/mineral supplement with no more than 200 IU vitamin D) for at least 4 weeks prior to screening, or use of any functional foods or dietary supplements that might alter lipid metabolism for at least 2 weeks prior to screening. In addition, if a subject exhibited any of the following at the screening visit, he or she would be excluded from the study: CHD or a CHD risk equivalent (Adult Treatment Panel III 2001), abnormal laboratory test results of clinical significance, poorly controlled hypertension ($\geq$ 160 mmHg systolic or $\geq$ 100 mmHg diastolic blood pressure), known allergy or sensitivity to the study product or any ingredients of the study products.

Clinic visits

After the screening visit, eligible subjects were randomly assigned to two treatment groups: MVM supplement without vitamin D, or the same MVM supplement with vitamin D 1,200 IU/day in the form of cholecalciferol (MVM + D). Subjects in each condition were instructed to consume three tablets of the study product daily for 8 weeks. At the conclusion of the double-blind treatment period, subjects from the MVM + D group were allowed to participate in an 8-week open-label single-arm extension period. Neither subjects nor clinic staff members were aware of the treatment received during the initial double-blind treatment period and eligibility for the extension was built into the initial randomization scheme. This subset of subjects received a supplement regimen that, in addition to the MVM supplement with 1,200 IU/day vitamin D, included 280 mg/day eicosapentaenoic acid (EPA), 180 mg/day docosahexaenoic acid (DHA), and 250 million colony-forming units (CFU) each of the probiotic strains *Bifidobacterium longum* and *Lactobacillus acidophilus* (MVM + D Extension). All study supplements were provided by the Shaklee Corporation (Pleasanton, CA, USA). Subjects were asked to maintain their normal dietary patterns throughout the extension period.

Study compliance and blinding

Compliance with study product consumption was evaluated by subject interview and the counting of unused study product returned to the clinic.

Compliance was recorded as a percentage of scheduled intakes of study product consumed. Non-compliance was defined as consumption of $< 80\%$ of the scheduled intake. Study personnel remained blinded to the treatment assigned to subjects throughout the double-blind treatment period. A set of sealed unblinding envelopes were retained for use in an emergency situation where knowledge of the treatment assignment would have been essential for the subject's immediate medical care.

Laboratory measurements

All laboratory measurements were conducted by Elmhurst Memorial Hospital Laboratory (Elmhurst, IL, USA). Blood samples were collected under fasting conditions (≥ 9 h). Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were measured using the Beckman Coulter's LX20 PRO (Fullerton, CA, USA). The concentration of LDL-C (in mg/dl) was calculated according to the Friedewald equation as follows: LDL-C = TC − HDL-C − TG / 5 (Friedewald et al. 1972). Since this equation is not valid when the TG concentration is above 4.5 mmol/l, LDL-C values were not calculated for the few instances where subjects had values in this range. The concentration of total 25(OH)D was determined using the DiaSorin Liason® Total-D™ chemiluminescence immunoassay (Stillwater, MN, USA). The concentration of high-sensitivity C-reactive protein (hs-CRP) was determined using Beckman Coulter's LX20 chemiluminescence immunoassay.

Plasma 25(OH)D was assessed with a single blood draw at baseline and the end of both the double-blind treatment period and the extension period. All lipid measurements and hs-CRP were assessed with two blood draws at baseline (1 week apart) and at the end of the double-blind treatment (weeks 7 and 8) and the extension (weeks 15 and 16) periods, with the results for each study period based on a single value [25(OH)D] or the average of the measurements from the two blood draws (lipids and hs-CRP).

Anthropometric measures and blood pressure

Waist circumference was measured at the screening visit. Measurements were obtained on a horizontal plane at the level of the iliac crest using a non-stretch anthropometric tape at the end of a normal expiration, according to the recommendations of the National Cholesterol Education Program Adult Treatment Panel III (Adult Treatment Panel III 2001). Three measurements were taken and averaged. If the range of values exceeded 0.5 cm, a fourth measurement was taken and the outlying value discarded. Body weight was measured using a digital scale to the nearest 0.1 kg (Model 349KLX; Health-O-Meter, Boca Raton, FL, USA).

Resting hemodynamic variables were obtained at each clinic visit. All measurements were obtained in duplicate, with values averaged, after the subject had been seated for at least 5 min. Systolic and diastolic pressures were measured using an automated blood pressure measurement device (Vital Signs Monitor 300 Series; Welch Allyn®, Beaverton, OR, USA), and the appropriate sized cuff (bladder within the cuff must encircle $\geq 80\%$ of the arm), separated by 2 min.

Nutrient analysis and study questionnaires

Subjects were counseled to maintain constant daily energy intake throughout the trial. Three-day diet records were completed at baseline, the end of the double-blind treatment period, and the end of the extension period to evaluate consistency of dietary intake. Diet records were analyzed using the Food Processor® Nutrition Analysis & Fitness Software (version 10.4; ESHA Research, Salem, OR, USA). Daily intakes of energy and key nutrients were calculated including, total daily intakes of and, where applicable, percentages of energy from: carbohydrate, protein, fat; saturated, polyunsaturated, and monounsaturated fatty acids; alcohol; dietary fiber; soluble dietary fiber; and selected vitamins and minerals.

Subjects completed the Stanford 7-day Physical Activity Recall Questionnaire at baseline and at the end of each study period. In addition, all subjects completed a questionnaire to assess typical sun exposure and lifestyle habits, including duration of outdoor activities and sun protection practices (clothing, sun screen, etc.). Subjects completed this questionnaire at baseline and at the end of each study period. Based on the sun exposure questionnaire, subjects were classified into three categories; low exposure (< 1 h/week), medium exposure, (1–2 h/week) and high exposure (> 2 h/week).

Statistical analyses

Statistical analysis was performed on a modified intent-to treat (MITT) population for each treatment phase, which included all subjects who entered the treatment phase and had at least one post-randomization blood sample. For each outcome variable, descriptive statistics (number of subjects, mean, standard error of the mean, standard deviation, median, interquartile limits, minimum and maximum) were calculated for values at each time point. For variables that are based on the average of multiple values, all available data points were used in the average, or in place of the average, in cases where not all data points were available. Baseline comparability of treatment groups for demographic, anthropometric, blood pressure, and laboratory values was assessed by analysis of variance (ANOVA) for continuous variables and chi-square or Fisher's exact tests for categorical variables.

Pearson correlation coefficients were employed to show the strengths of the relationships between serum 25(OH)D and markers of metabolic syndrome in all

Table I. Subject disposition for the double-blind treatment period and extension period.

Category[a]	MVM	MVM + D	MVM + D Extension
Randomized or entered extension[b]	29 (48.3%)	31 (51.7%)	29 (48.3%)
Completed study	29 (50.9%)	30 (52.6%)	27 (45.0%)
Did not complete study	–	1 (33.3%)	2 (3.3%)
Eligible for:			
Modified intent-to-treat population	29 (48.3%)	31 (51.7%)	27 (45.0%)
Per-protocol population	27 (48.2%)	29 (51.8%)	27 (45.0%)
Discontinued due to:			
Adverse event (AE) or serious AE	1 (1.7%)	–	–
Withdrawal of consent	–	–	–
Lost to follow-up	–	–	2 (3.3%)

Data presented as *n* (%). [a] There were no significant differences between groups; [b] Eighty subjects were screened.

subjects at baseline. For variables that did not conform to a normal distribution, correlation coefficients were completed with and without rank or natural logarithm transformations. Because the results were not materially different, only the non-transformed results are presented.

Analyses of covariance models were used to compare changes or percentage changes from baseline to end-of-treatment for the double-blind treatment phase. Initial models contained terms for baseline, treatment group and a baseline by treatment interaction term. Models were reduced until only significant ($P < 0.05$) terms or treatment remained. For the extension period, repeated-measures ANOVA models were used to compare baseline, end-of-double-blind treatment and end of extension period values. A Sidak correction was used to adjust *P* values for pairwise comparisons in order to maintain a family-wise error rate ≤ 0.05 (Šidák 1971). For all models of continuous dependent variables, the Shapiro-Wilk test was utilized to assess normality of the residuals. If normality was rejected ($P < 0.01$), the final analysis was performed on ranked values.

Results

Participants and demographics

Of 80 subjects screened, 60 met all inclusion criteria and no exclusion criteria and were randomly assigned to the two treatment groups (Table I). Of the 60 subjects randomized, 59 completed the double-blind study. One subject in the MVM + D group withdrew participation because of an adverse event (nausea). Demographic and baseline characteristics were similar across groups (Table II). No statistically significant differences between groups were present for gender, race, age, smoking status, height or body mass index. The proportions of overweight and obese subjects were 44.8% and 55.2% in the MVM group, and 45.2% and 51.6% in the MVM + D group, respectively.

Of the 29 subjects who entered the extension, 27 completed the study and the remaining two subjects were lost to follow-up. Since these individuals did not provide any efficacy data during the extension period, they were dropped from all extension study analyses.

Study compliance

Study compliance assessed by subject interview and the unused pill count for the double-blind treatment period was 96.0% and 98.5% for the MVM and MVM + D groups, respectively. Study compliance during the extension period was 98.8%.

25(OH)D correlations and response to treatment

Pearson correlation coefficients were examined to determine the relationship between baseline 25(OH)D concentrations and components of the metabolic syndrome for which relationships had been observed in previous investigations (Maki et al. 2009). There were significant inverse relationships between 25(OH)D and fasting glucose ($r = -0.279$; $P = 0.030$), TG ($r = -0.283$; $P = 0.028$), and hs-CRP ($r = -0.345$; $P = 0.008$) concentrations. There was no significant relationship between 25(OH)D and waist circumference ($r = -0.203$; $P = 0.119$) and there was a near-significant trend for a positive relationship between 25(OH)D and fasting HDL-C ($r = 0.236$; $P = 0.067$).

Table II. Subject characteristics for the double-blind treatment period.

Characteristic[a]	MVM ($n = 29$)	MVM + D ($n = 31$)
Gender		
Male	7 (24.1%)	8 (25.8%)
Female	22 (75.9%)	23 (74.2%)
Race/ethnicity		
Non-Hispanic White	27 (93.1%)	30 (96.8%)
Other	2 (6.9%)	1 (3.2%)
Smoking status		
Current smoker	15 (51.7%)	16 (51.6%)
Age (years)	54.3 (2.0)	50.3 (2.5)
Body mass index (kg/m^2)	31.7 (1.0)	31.7 (1.1)
Waist circumference (cm)	103.8 (2.1)	105.7 (2.2)

Data presented as *n* (%) or mean (standard error of the mean). [a] There were no significant differences between groups. All *P* values were > 0.20.

Table III. Indicators for cardiovascular disease risk for the double-blind treatment period.

Parameter[a]	MVM ($n = 29$)	MVM + D ($n = 31$)	P value
25-Hydroxyvitamin D (nmol/l)			
Baseline	67.9 (3.7)	64.4 (3.7)	0.508
Change	−1.2 (2.5)	11.7 (3.0)	0.003
Total cholesterol (mmol/l)			
Baseline	5.54 (0.13)	5.24 (0.20)	0.220
% Change	−0.6 (1.9)	1.6 (1.5)	0.359
LDL-C (mmol/l)			
Baseline	3.55 (0.13)	3.41 (0.16)	0.505
% Change	0.1 (2.6)	1.4 (1.9)	0.836
HDL-C (mmol/l)			
Baseline	1.20 (0.05)	1.18 (0.06)	0.831
% Change	1.7 (1.4)	2.8 (2.0)	0.653
Non-HDL-C (mmol/l)			
Baseline	4.34 (0.14)	4.05 (0.19)	0.237
% Change	−0.9 (2.3)	1.3 (1.7)	0.660
TC/HDL-C ratio			
Baseline	4.88 (0.24)	4.72 (0.26)	0.433
% Change	−2.2 (1.9)	−0.5 (1.9)	0.601
Triglycerides (mmol/l)			
Baseline	1.83 (0.19)	1.41 (0.16)	0.044
% Change	−5.3 (5.4)	3.5 (4.7)	0.138
hs-CRP (mg/l)			
Baseline	3.34 (0.87)	3.95 (1.22)	0.731
Change	0.23 (0.36)	0.09 (0.37)	0.988
Body weight (kg)			
Baseline	89.3 (3.1)	92.3 (3.4)	0.496
Change	−0.4 (0.3)	0.5 (0.2)	0.009
Systolic blood pressure (mmHg)			
Baseline	120.3 (1.9)	115.8 (1.8)	0.090
Change	−3.5 (1.3)	−0.5 (1.1)	0.294
Diastolic blood pressure (mmHg)			
Baseline	74.1 (1.5)	71.9 (1.4)	0.227
Change	−1.8 (0.9)	0.1 (0.8)	0.244
Heart rate (bpm)			
Baseline	66.3 (1.7)	68.6 (1.4)	0.276
Change	2.2 (0.8)	2.0 (1.4)	0.481

Data presented as mean (standard error of the mean). [a]Baseline was defined as the average of visits 1 and 2; weeks −1 and 0, and end of treatment is defined as an average of visits 3 and 4; weeks 7 and 8.

There was a significant difference in the change from baseline to the end of the double-blind treatment period in 25(OH)D concentration between the MVM and MVM + D treatment groups (−1.2 ± 2.5 nmol/l vs. 11.7 ± 3.0 nmol/l, respectively; $P = 0.003$; Table III). In the MVM + D group, the percentage of subjects with 25(OH)D < 75 nmol/l declined from 64.5% to 61.3%, while that in the MVM group increased from 69.0% to 75.9%. Neither the percentage of subjects with 25(OH)D < 75 nmol/l at the end of treatment, nor the percentage of subjects who shifted from a value < 75 to ≥ 75 nmol/l was statistically significant ($P > 0.20$ for both).

For subjects who continued with the extension period, the mean change from baseline in 25(OH)D declined slightly from what was observed at the end of the double-blind treatment period, but maintained significance compared with baseline (7.2 ± 3.5 nmol/l, $P = 0.002$; Table IV).

Plasma markers of cardiovascular disease risk

There were no statistically significant changes in any elements of the fasting lipid profile or in hs-CRP in either group during the double-blind treatment period (Table III). For the subjects that entered the extension period, mean HDL-C significantly increased from baseline values (7.2 ± 2.3%; $P = 0.026$). In addition, subjects in the extension period exhibited significant decreases from baseline values in mean non-HDL-C (−5.7 ± 1.3%; $P < 0.001$), TC (−2.9 ± 1.2%; $P = 0.048$), TC/HDL ratio (−8.7 ± 1.5%; $P < 0.001$), and a near significant reduction in LDL-C (−4.3 ± 2.0%; $P = 0.069$). There were no significant changes in hs-CRP during the extension period.

Dietary intake and sun exposure

There were no statistically significant changes in daily intakes of energy or macronutrient composition,

Table IV. Indicators of cardiovascular disease risk for the extension period.

Parameter[a]	MVM + D Extension ($n = 27$)	*P* value
Total cholesterol (mmol/l)		
Baseline	5.27 (0.22)	0.047
% Change at end of treatment	0.6 (1.4)	
% Change at end of extension	−2.9 (1.2)⋆	
LDL-C (mmol/l)		
Baseline	3.40 (0.17)	0.069
% Change at end of treatment	−0.1 (1.9)	
% Change at end of extension	−4.3 (2.0)	
HDL-C (mmol/l)		
Baseline	1.21 (0.07)	0.026
% Change at end of treatment	2.2 (2.1)	
% Change at end of extension	7.2 (2.3)⋆	
Non-HDL-C (mmol/l)		
Baseline	4.06 (0.21)	0.001
% Change at end of treatment	0.2 (1.6)	
% Change at end of extension	−5.7 (1.3)⋆	
TC/HDL-C ratio		
Baseline	4.63 (0.29)	< 0.0001
% Change at end of treatment	−0.7 (2.0)	
% Change at end of extension	−8.7 (1.5)⋆	
Triglycerides (mmol/l)		
Baseline	1.44 (0.18)	0.514
% Change at end of treatment	4.9 (5.1)	
% Change at end of extension	−6.6 (4.2)	
25-Hydroxyvitamin D (nmol/l)		
Baseline	63.9 (4.0)	0.002
Change at End of treatment	12.5 (3.2)⋆	
Change at end of extension	7.2 (3.5)	
hs-CRP (mg/l)		
Baseline	2.65 (0.46)	0.803
Change at end of treatment	−0.10 (0.29)	
Change at end of extension	0.68 (0.73)	
Body weight (kg)		
Baseline	91.1 (2.9)	0.002
Change at end of treatment	0.4 (0.3)	
Change at End of extension	1.0 (0.3)⋆	
Systolic blood pressure (mmHg)		
Baseline	115.7 (1.9)	0.555
Change at end of treatment	−0.5 (1.2)	
Change at end of extension	−1.3 (1.2)	
Diastolic blood pressure (mmHg)		
Baseline	72.0 (1.4)	0.467
Change at end of treatment	−0.1 (0.9)	
Change at end of extension	−1.1 (1.0)	
Heart rate (bpm)		
Baseline	68.6 (1.5)	0.083
Change at end of treatment	1.9 (1.5)	
Change at end of extension	2.9 (1.5)	

Data presented as mean (standard error of the mean). [a] Baseline was defined as average of visits 1 and 2; weeks −1 and 0; end of treatment is defined as an average of visits 3 and 4; weeks 7 and 8; and end of extension is defined as an average of visits 5 and 6; weeks 15 and 16. Change at end of treatment = Change from baseline (average of weeks −1 and 0) to End of main study (average of weeks 7 and 8). Change at end of extension = Change from baseline (average of weeks −1 and 0) to End of extension (average of weeks 15 and 16). Treatment and Extension *P* values were obtained from *t*-tests for changes different from zero. *P* values are adjusted due to corrections for multiple comparisons. ⋆Significant ($P < 0.05$) pairwise difference from baseline value.

including carbohydrate, protein, total fat, monounsaturated fatty acids, polyunsaturated fatty acids, and fiber during the double-blind treatment period or extension period. There was a significant difference in the change in mean dietary vitamin D consumption between the MVM and MVM + D groups (0.4 ± 9.3 IU/day vs. 1,132.3 ± 42.7 IU/day; $P < 0.001$). This significant increase in vitamin D intake from baseline was maintained in the extension period (1,174.7 ± 31.6 IU/day; $P < 0.001$). There were no significant changes from baseline in estimated sun exposure or physical activity in either group during the double-blind treatment period or for subjects who entered the extension period (data not shown).

Body weight and blood pressure

There was a significant difference in body weight change between the MVM and MVM + D groups (-0.4 ± 0.3 and 0.5 ± 0.2 kg, respectively; $P = 0.009$; Table III). The change from baseline in body weight for the MVM + D group remained significantly different from baseline during the extension period (1.0 ± 0.3 kg; $P = 0.002$), although the change in body weight from the end of the double-blind period to the end of the extension was not statistically significant (0.4 ± 0.3 kg; $P = 0.206$). No significant differences between groups were observed for hemodynamic measurements.

Safety and tolerability

There were a total of 14 adverse events recorded during the double-blind treatment and extension periods. There were no significant differences in the frequencies of adverse events between groups or study periods. The majority of adverse events were considered by the investigator to be of mild or moderate intensity and unlikely to be related to the study product. None of the adverse events were considered serious. One subject in the MVM + D treatment group withdrew consent during the double-blind treatment period due to an adverse event (nausea).

Discussion

The amount of dietary vitamin D required to achieve adequate circulating concentrations of 25(OH)D is a topic of considerable interest (Vieth et al. 2007). Based on the criterion that a serum 25(OH)D concentration ≥ 75 nmol/l is desirable for optimum health (Holick and Chen 2008), vitamin D insufficiency in the United States appears to be widespread (Holick and Chen 2008, Lee et al. 2008), and the Institute of Medicine recommendations for vitamin D consumption at the time this study was conducted were inadequate for many individuals (Giovannucci 2007, Vieth et al. 2007, Holick and Chen 2008). In the present study, baseline 25(OH)D measures were taken mid-summer when it would be expected that they be at annual peak due to sun exposure, and yet almost two-thirds of subjects had concentrations less than 75 nmol/l. Moreover, supplementing with 1,200 IU/day vitamin D in this sample of predominantly overweight and obese, white, middle-aged men and women was not sufficient to achieve a 25(OH)D concentration ≥ 75 nmol/l in most subjects. Despite supplementation with twice the daily intake currently recommended by the Institute of Medicine for any population subgroup (Dietary Reference Intakes 1997), the median concentration at the end of the double-blind treatment period in the MVM + D group was 72.1 nmol/l, and 61% of the subjects in the MVM + D condition still exhibited 25(OH)D $<$ 75 nmol/l, including two subjects (6.5%) with vitamin D deficiency [25(OH)D $<$ 50 nmol/l]. This finding adds to the growing body of evidence that current recommendations for vitamin D intake are inadequate to maintain desirable 25(OH)D levels in many segments of the population (Giovannucci 2007, Vieth et al. 2007, Holick and Chen 2008), and underscores the point that individuals with increased adiposity may be an at-risk group for adverse outcomes related to low levels of circulating 25(OH)D.

Studies that have examined vitamin D consumption and its effects on blood concentrations of 25(OH)D have shown variable results, but studies conducted during the winter season, when serum concentrations are low due to lack of sun exposure, have been fairly consistent. A supplemental intake of 1,000 IU vitamin D/day for 8 weeks during the winter in healthy young men living in Nebraska raised 25(OH)D by 29.0 nmol/l, or about 0.03 nmol/l 25(OH)D/IU supplemental vitamin D (Barger-Lux et al. 1998). In a trial conducted among postmenopausal women living in Nebraska, 1,100 IU/ay vitamin D over 1 year raised mean 25(OH)D by 24.0 nmol/l, or by about 0.02 nmol/l/IU supplemental vitamin D (Lappe et al. 2007). Among healthy middle-aged Canadian men and women who were predominately white, 1,000 IU/day vitamin D during winter increased mean 25(OH)D by 28.0 nmol/l, or by about 0.027 nmol/l/IU supplemental vitamin D (Vieth et al. 2001). And in a trial among healthy black postmenopausal women in New York, 800 IU/day vitamin D for 3 months increased 25(OH)D by 24.5 nmol/l, or by about 0.03 nmol/l/IU supplemental vitamin D (Talwar et al. 2007).

Thus, with a range of 0.02–0.03 nmol/l/IU supplemental vitamin D during winter conditions when sun exposure is minimal, it would take an estimated 1,250–1,667 IU supplemental vitamin D daily to increase mean serum concentrations from the 37 to 75 nmol/l range, and even more to achieve higher levels or to ensure that everyone achieves the desired higher level. The validity of this estimate is supported by a study where, in predominately white subjects with baseline 25(OH)D concentrations of 40.6 nmol/l, 1,000 IU/day vitamin D for 3 months produced 25(OH)D concentrations of 75 nmol/l in just 35% of subjects, whereas 4,000 IU/day vitamin D increased serum concentrations to levels at or above 75 nmol/l in 88% of subjects (Vieth et al. 2001). Also, in black women, 2,000 IU/day vitamin D during the winter failed to raise 25(OH)D to $>$ 75 nmol/l in 40% of the sample (Talwar et al. 2007). These findings are also generally consistent with a recent report suggesting that individuals of European descent may require as much as 1,000–2,550 IU/day vitamin D while individuals of African-American descent may require as much as 1,000–3,100 IU/day vitamin D depending upon the season and sun exposure (Hall et al. 2010).

There are several other factors, in addition to vitamin D intake, that contribute to the circulating concentration of 25(OH)D. Sun exposure is a key determinant

of the circulating 25(OH)D concentration. In the present investigation, we did not observe differences in sun exposure between groups during the study based on a semi-quantitative questionnaire. The slight decline in 25(OH)D observed during the extension study may have been due to seasonal variation in sun exposure, since most subjects completed the extension in mid to late autumn. Additionally, adiposity also likely contributes to variability in the 25(OH)D response to supplementation. Vitamin D is fat soluble and therefore can be sequestered in adipose tissue. Thus, there is a greater storage capacity for vitamin D in overweight and obese individuals, which may result in a reduced circulating concentration of 25(OH)D (Wortsman et al. 2000, Maki et al. 2009). As a result, to maintain a given 25(OH)D level, overweight and obese individuals may have to consume greater quantities of vitamin D than would be the case for those of normal weight. This might apply particularly to the subjects in the present investigation, since we studied individuals with elevated waist circumference (Maki et al. 1997, 2009, Grundy et al. 2004).

It is clear from the present investigation that 1,200 IU/day vitamin D supplementation for 8–16 weeks was not adequate to raise 25(OH)D to a desirable level in this sample of predominately white, overweight, or obese individuals in this geographic region (Chicago, IL suburbs). Given the potential wide variation in individual vitamin D requirements due to season, sun exposure, body fat, race, and dietary intake, the degree to which these results can be generalized to other groups is uncertain (Hall et al. 2010). However, these results speak to the need for supplemental vitamin D consumption to be tailored to meet individual needs (Baraké et al. 2010, Hall et al. 2010).

Since 25(OH)D concentrations were increased only modestly in the present investigation, this study was unable to test the hypothesis that increasing the circulating level of 25(OH)D increases the concentration of HDL-C. Our prior cross-sectional data demonstrated a strong relationship between serum concentrations of 25(OH)D and HDL-C, with each 25 nmol/l increment in 25(OH)D associated with an increase of 0.10–0.11 mmol/l in HDL-C after adjustment for established determinants of the HDL-C level (Maki et al. 2009). In the subjects consuming the MVM + D in the present investigation, we observed a mean increase of 11.7 nmol in 25(OH)D, which was accompanied by a non-significant mean increase of 0.03 mmol/l in HDL-C. The predicted change of ~0.05 mmol/l in HDL-C based on the observed rise of 25(OH)D is well within the 95% confidence interval for the observed change in HDL-C of 0.02–0.08 mmol/l. Thus, our results neither support nor refute the hypothesis that raising 25(OH)D with vitamin D supplementation might elevate the HDL-C concentration (Dobnig et al. 2008, Liu et al. 2009, Maki et al. 2009). Recently, Jorde and colleagues failed to demonstrate an increase in HDL-C, or changes in other cardiovascular risk markers, after vitamin D supplementation for 1 year in a randomized, double-blind, placebo-controlled study in overweight and obese adults in Norway (Jorde et al. 2010). Subjects consumed placebo, 20,000 or 40,000 IU vitamin D per week for 1 year, and the active treatment groups exhibited increases in mean serum 25(OH)D from 56.7 to 101.1 nmol/l and from 58.7 to 140.0 nmol/l, respectively. Consistent with our earlier findings (Maki et al. 2009), they also noted a significant association between baseline HDL-C level and the circulating 25(OH)D concentration ($r = 0.27$, $P < 0.001$). Thus, at present, while the available data support a direct relationship between 25(OH)D and HDL-C concentration, vitamin D supplementation has not been found to improve the cardiovascular disease risk factor profile.

At the conclusion of the extension study during which participants took 280 mg/day EPA, 180 mg/day DHA, and 250 million CFU each of the probiotic strains *B. longum* and *L. acidophilus* in addition to the MVM + D supplement, a significant increase in HDL-C was observed compared with baseline, as well as significant reductions in non-HDL-C and the TC/HDL-C ratio, and a near-significant reduction in LDL-C. This extension was uncontrolled and therefore it is possible that observed changes were due to some unknown factor. The dosage of EPA 280 mg/day and DHA 180 mg/day alone is unlikely to have produced these effects based on our prior experience (Maki et al. 2008) and that of others (Harris et al. 1991, Hamazaki et al. 1996, Theobald et al. 2004). However, it is possible that the favorable changes observed resulted from the probiotics or additive effects of the probiotics and the EPA + DHA. There are limited data from studies in humans to suggest potential lipid-altering effects of probiotic bacteria (Bukowska et al. 1998). One hypothesis is that probiotic bacteria may enhance colonic fermentation, producing propionate, which appears to inhibit hepatic cholesterol synthesis (Wolever et al. 1991, 1996, Pereira and Gibson 2002). An additional mechanism may involve reducing intestinal cholesterol absorption through inhibition of the Niemann–Pick C1-Like 1 transporter (Huang and Zheng 2010). We cannot rule out a delayed effect of the additional 8 weeks of supplementation with vitamin D, although this appears unlikely given that circulating levels of 25(OH)D did not rise beyond the level observed at 8 weeks, and, in fact, declined slightly. Nonetheless, the results from the extension phase suggest possible favorable effects from the addition of low-dose, long-chain omega-3 fatty acids and/or probiotics to the supplement regimen, a hypothesis that warrants further investigation.

The MVM + D subjects exhibited a small but statistically significant increase in body weight during the study. This change in body weight occurred despite counseling of subjects regarding maintaining

stable exercise and dietary habits. There was a small increase in mean reported daily energy consumption in the MVM + D group (92 kcal/day) that was not statistically significant. The increase in body weight continued during the 8-week extension phase, although the increase from the end of the double-blind treatment period to the end of the extension period was not statistically significant. It is possible that the observed changes in body weight over the course of the double-blind treatment and extension periods were chance findings, although small increases in body weight have been reported in a previous vitamin D supplementation trial (Nagpal et al. 2009). Since vitamin D supplementation has been shown to increase insulin sensitivity (Borissova et al. 2003, Nagpal et al. 2009), one potential explanation might be that increased insulin sensitivity enhances body fat or fluid accumulation, as has been observed with thiazolidinediones (Mitri et al. 2009). This issue should be investigated further in subsequent trials.

In conclusion, the results of the present study indicate that supplementation with 1,200 IU/day vitamin D during the summer and fall seasons was inadequate to bring a majority of subjects to a sufficient 25(OH)D concentration ($\geq$ 75 nmol/l) in this sample of predominantly white overweight and obese subjects. Thus, the intervention did not provide a test of the hypothesis that increasing 25(OH)D elevates the HDL-C concentration. Further research with a higher vitamin D dose is warranted to investigate this hypothesis. Finally, preliminary evidence from the open-label, single-arm extension study suggests that adding probiotics and omega-3 fatty acids may have favorable effects on HDL-C and non-HDL-C concentrations. Randomized controlled trials are needed to investigate these findings.

Declaration of interest: The present study was supported by Shaklee Corporation, Pleasanton, CA, USA. KCM, MRR, and AL have received research grant support from Shaklee Corporation. LGW and JFM are employees of Shaklee Corporation. CDJ has served as a consultant to Shaklee Corporation.

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