

Serum 25-hydroxyvitamin D is independently associated with high-density lipoprotein cholesterol and the metabolic syndrome in men and women

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KEYWORDS:

Vitamin D;
High-density lipoprotein cholesterol;
Metabolic syndrome;
Cardiovascular disease risk

BACKGROUND: Low vitamin D status has been associated with markers of cardiovascular disease risk.

OBJECTIVE: This cross-sectional study assessed the relationships between serum 25-hydroxyvitamin D [25(OH)D] and selected markers for cardiovascular disease risk, including metabolic syndrome and its components, in adult men and women.

METHODS: Fasting blood samples, anthropometric measurements, and blood pressure were assessed in 257 men and women. Dietary intake was assessed with food frequency and dietary supplement questionnaires.

RESULTS: Total vitamin D intake and that from dietary supplements were significantly associated with increasing serum 25(OH)D tertile (both $P < .001$). Mean \pm SEM serum high-density lipoprotein cholesterol (HDL-C) increased in a graded fashion ($P < .001$) from the lowest (48.4 ± 1.8 mg/dL) to the highest (62.3 ± 2.1 mg/dL) 25(OH)D tertile. The relationship between 25(OH)D and HDL-C remained significant ($P < .001$) after adjustment for established determinants of the HDL-C, with each 10-ng/mL increase in 25(OH)D associated with a 4.2-mg/dL increase in HDL-C concentration. Serum triglycerides ($P = .008$), waist circumference ($P < .001$), and body mass index ($P < .001$) showed graded, inverse relationships with 25(OH)D tertile, and the prevalence of metabolic syndrome decreased significantly from the lowest to the highest 25(OH)D tertile (31%, 14%, and 10%, respectively, P for trend = .001).

CONCLUSIONS: Lower serum 25(OH)D is associated with the metabolic syndrome and adverse values for some metabolic syndrome risk factors, particularly the HDL-C concentration. Research is warranted to assess whether increasing vitamin D intake will improve the metabolic cardiovascular risk factor profile.

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In recent years, a number of observational studies^{1–3} 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. Moreover, low vitamin D status has been associated with several markers of cardiovascular disease risk, including the metabolic syndrome and its components.⁴⁻¹⁰

A serum concentration of 25(OH)D ≥ 30 ng/mL is considered sufficient, < 30 ng/mL is considered insufficient, and < 20 ng/mL reflects vitamin D deficiency.¹¹ Vitamin D insufficiency is common in the United States, with population studies suggesting that approximately 30% to 50% of the general population has circulating 25(OH)D levels < 30 ng/mL.^{11,12} The Institute of Medicine recommends 200 IU/day of dietary vitamin D.¹³ However, results from some studies have suggested that 800–1000 IU of dietary vitamin D may be needed to maintain sufficient vitamin D status, particularly in locations where sun exposure is limited and for high-risk groups such as the elderly and dark-skinned individuals.^{2,11}

Given that few foods are rich dietary sources of vitamin D, supplementation of the diet through consumption of vitamin D-fortified foods or dietary supplements may be necessary to maintain sufficient vitamin D status.¹² In recent years, some researchers have suggested that greater intakes of vitamin D, including supplementation when diet is inadequate, should be recommended for the general population because of the low known risk and high potential public health benefits.^{2,11,14,15} In light of the associations of low blood levels of 25(OH)D with cardiovascular disease risk, improved understanding of the relationships between vitamin D intakes from food and dietary supplements with blood levels of 25(OH)D and cardiovascular disease risk markers could have important public health implications.

This cross-sectional study was undertaken in a group that was anticipated to contain a high prevalence of vitamin D supplement users. The objectives were to assess the degree to which vitamin D intake from food and supplements correlates with the serum 25(OH)D level and to evaluate the relationships between 25(OH)D concentration and selected markers for cardiovascular disease risk, particularly the metabolic syndrome and its components, across a wide range of 25(OH)D concentrations.

Methods

An institutional review board (Schulman Associates IRB, Cincinnati, OH) approved the protocol before initiation of the study, and subjects provided written informed consent before any study procedures were performed.

Subjects

This cross-sectional investigation included men and women ≥ 18 years of age ($n = 257$). Most participants ($n = 211$) were attending an annual meeting in New Orleans, Louisiana, for a dietary supplement manufacturer and distributor (Shaklee Corporation, Pleasanton, CA).

Results from a previous study of attendees of this annual meeting suggested that we could anticipate a high average level of 25(OH)D in this group.¹⁶ Additional participants were recruited through advertising to come to a clinic in Bloomington, Indiana, or Addison, Illinois, for testing. Female subjects who were pregnant or lactating and subjects with a history of cancer (other than non-melanoma skin cancer) in the previous 2 years were excluded.

Study questionnaires

Questionnaires assessed medical history, sun exposure, physical activity, and medication and dietary supplement use. The sun exposure questionnaire was based on studies published previously and provided a semiquantitative estimate of exposure based on hours outdoors, clothing typically worn while outdoors, and use of sunscreen.^{17,18} Physical activity was assessed by use of the Stanford 7-Day Physical Activity Recall questionnaire, and a physical activity score was calculated as previously described.¹⁹ Dietary intake was evaluated by use of the Harvard Food Frequency questionnaire, which assesses dietary intake during the past year.²⁰

Anthropometric measures and blood pressure

Waist circumference was measured on a horizontal plane at the level of the iliac crest with a nonstretch anthropometric tape at the end of a normal expiration, according to the recommendations of the National Cholesterol Education Program Adult Treatment Panel III.²¹ Three measurements were taken and then averaged. If the range of values exceeded 0.5 cm, a fourth measurement was taken and the outlying value discarded. Blood pressure was obtained after the subject had been seated for at least 5 minutes. Systolic and diastolic pressures were measured twice, with values averaged, by the use of an automated blood pressure measurement device, and the appropriate sized cuff (bladder within the cuff must encircle $\geq 80\%$ of the arm), separated by 2 minutes. Body weight was measured with a digital scale to the nearest 0.1 kg (Health-O-Meter, Model 349KLX, Boca Raton, FL).

Laboratory measurements

Clinical laboratory measurements included serum levels of 25(OH)D, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, triglycerides (TG), and glucose. Blood samples were collected under fasting conditions (≥ 9 hours) by the use of serum separator tubes. Lipoprotein lipid and glucose measurements were conducted by Elmhurst Memorial Hospital Laboratory (Elmhurst, IL), and 25(OH)D measurements were completed by the Mayo Clinic Laboratory (Rochester, MN). TC and TG concentrations were measured by use of the Beckman Coulter's LX20 PRO (Fullerton, CA). LDL-C concentration in mg/dL was calculated according to the Friedewald equation²²

as follows: $LDL-C = TC - HDL-C - TG/5$. Because this equation is not valid when the TG concentration is >400 mg/dL, LDL-C values were not calculated for the few subjects that had values in this range. Glucose concentration was analyzed by use of the glucose oxidase method with enzymatic reagents.²³ The analysis for serum 25(OH)D used an extraction by liquid chromatography and mass spectrometry.²⁴

Statistical analyses

Statistical analyses were conducted with SAS version 9.1.3 (SAS Institute, Cary, NC). Descriptive statistics are presented for characteristics for the study sample categorized by vitamin D supplement use and tertiles of serum 25(OH)D. Multivariate linear and logistic regression analyses were completed to evaluate associations between serum 25(OH)D concentration, the metabolic syndrome, and its components. Pearson correlation coefficients were calculated to show the strength of the relationship between serum 25(OH)D and HDL-C values in men and women. For variables that did not conform to a normal distribution, modeling was completed with and without rank or natural logarithm transformations. Because the results were not materially different, only the nontransformed results are presented. *P* values $< .05$ were considered statistically significant. Values in the text are reported as mean \pm standard error of the mean, unless otherwise specified.

Results

Subject characteristics

Data were collected during a period of 6 weeks in August and September of 2008. The study sample included 257 subjects, including 71 men and 186 women. Subjects were predominantly non-Hispanic white (93.4%). All were ambulatory; 16 were taking medications for lipid management and 23 for hypertension. Six subjects reported a history of type 2 diabetes mellitus.

A majority of study participants ($n = 222$, 86.4%) were vitamin D supplement users, including those who took a multivitamin containing vitamin D. Thirteen subjects used supplements that did not contain vitamin D, and 22 did not use any dietary supplements. Data for dietary intake of vitamin D were not available for 13 subjects for whom food frequency questionnaire responses were incomplete.

Thirty subjects (11.6%) had 25(OH)D <30 ng/mL (vitamin D insufficiency) and 2 (0.8%) were deficient, with 25(OH)D <20 ng/mL. Compared with the remainder of the sample, subjects taking vitamin D supplements had significantly ($P = .001$) greater mean serum 25(OH)D (41.7 ± 0.7 ng/mL) than nonusers (35.4 ± 1.5 ng/mL). Subjects were divided into tertiles of sun exposure based on responses to the sun exposure questionnaire. There were no significant

differences observed in serum 25(OH)D values across tertiles of estimated sun exposure: 39.4 ± 1.1 ng/mL, 41.4 ± 1.1 ng/mL, and 41.4 ± 1.2 ng/mL, respectively, for the first, second, and third tertiles of sun exposure.

Serum 25(OH)D tertiles

Subject characteristics within each tertile of serum 25(OH)D are shown in Table 1. No significant trend across tertiles was observed for the prevalence of female sex or current cigarette smoking. A significant trend was present for ethnicity across 25(OH)D tertiles ($P = .026$), with the prevalence of non-Hispanic white ethnicity/race increasing from the lowest to the highest tertile. The prevalence of metabolic syndrome decreased significantly from the first to the third tertile ($P = .001$).

A significant trend for age was observed ($P = .039$), which can be attributed to lower mean age in the first tertile compared with tertiles 2 and 3. Waist circumference and body mass index decreased progressively from the first to the third tertile (both $P < .001$). Alcohol intake and vitamin D from food did not differ significantly across 25(OH)D tertiles. Vitamin D from supplements ($P < .001$) and food plus supplements ($P < .001$) increased progressively from the first to the third 25(OH)D tertile. No significant relationship was present across 25(OH)D tertiles for physical activity score or blood pressure. The serum glucose concentration tended to decline with increasing 25(OH)D tertile, but this relationship was of marginal significance ($P = .059$). Increasing serum 25(OH)D tertile was directly associated with the HDL-C concentration ($P < .001$) and inversely associated with the TG concentration ($P = .008$). The relationship between serum 25(OH)D and HDL-C concentration (as continuous variables) in men and women is shown graphically in Figure 1. Concentrations of LDL-C and non-HDL-C did not differ across tertiles of 25(OH)D.

Multivariate models

Multivariate modeling was used to further assess the relationships between the serum 25(OH)D concentration (as a continuous variable) and components of the metabolic syndrome for which significant trends were present in univariate analyses (HDL-C, TG, waist circumference), as well as for the metabolic syndrome itself. These results are shown in Table 2.

HDL-C was strongly associated with serum 25(OH)D. After adjustment for age and sex, each 1-ng/mL increment in 25(OH)D concentration was associated with an increase of 0.52 mg/dL in serum HDL-C. The strength of this relationship was diminished somewhat by adjustment for other determinants of the HDL-C concentration (such as age, sex, waist circumference, physical activity score, alcohol consumption, smoking status), but remained highly significant (regression coefficient = 0.42 mg/dL, $P < .001$). Inclusion of triglyceride concentration as an independent variable

Table 1 Characteristics of the participants by tertile of serum 25-hydroxyvitamin D concentration

	Serum 25-hydroxyvitamin D			P for trend
	Tertile 1 ≤ 34 ng/mL (n = 80)	Tertile 2 35-45 ng/mL (n = 90)	Tertile 3 ≥ 46 ng/mL (n = 87)	
Participants, n (%) [*]	80 (31.1)	90 (35.1)	87 (33.9)	—
Women, n (%)	55 (68.8)	65 (72.2)	66 (75.9)	.305
Non-Hispanic white, n (%)	71 (88.8)	84 (93.3)	85 (97.5)	.026
Current smoker, n (%)	3 (3.8)	2 (2.2)	1 (1.1)	.280
Current metabolic syndrome, n (%) [†]	25 (31.3)	13 (14.4)	9 (10.3)	<.001
	Mean ± SEM			
Serum 25(OH)D, ng/mL	29.7 ± 0.4	39.8 ± 0.3	52.1 ± 0.8	<.001
Age, yr	48.3 ± 1.6	53.1 ± 1.6	53.0 ± 1.6	.039
Waist circumference, cm	94.5 ± 2.0	89.9 ± 1.5	85.6 ± 1.2	<.001
Body mass index, kg/m ²	29.1 ± 0.8	26.8 ± 0.6	25.3 ± 0.5	<.001
Alcohol intake, drinks/wk	2.9 ± 0.4	3.2 ± 0.6	3.3 ± 0.4	.588
Vitamin D intake, IU/d				
From food	275.9 ± 19.4	327.3 ± 24.1	307.1 ± 18.4	.324
From supplements	632.3 ± 60.9	799.3 ± 67.5	1019.4 ± 77.1	<.001
From food + supplements	887.5 ± 44.4	1104.7 ± 74.9	1315.9 ± 79.9	<.001
Physical activity score, met-hrs/wk	278.6 ± 5.6	284.8 ± 5.8	290.8 ± 5.8	.139
Systolic blood pressure, mm Hg	119.5 ± 1.4	119.0 ± 1.5	118.3 ± 1.5	.583
Diastolic blood pressure, mm Hg	74.3 ± 1.3	72.5 ± 0.9	73.2 ± 1.0	.508
Glucose, mg/dL	94.8 ± 2.7	91.4 ± 1.3	89.8 ± 1.2	.059
Total cholesterol, mg/dL	191.5 ± 4.2	194.2 ± 3.8	201.2 ± 3.9	.085
Non-HDL cholesterol, mg/dL	143.1 ± 4.0	139.9 ± 3.8	138.9 ± 3.6	.457
LDL cholesterol, mg/dL	121.0 ± 3.6	121.2 ± 3.5	122.0 ± 3.2	.838
HDL cholesterol, mg/dL	48.4 ± 1.8	54.3 ± 1.9	62.3 ± 2.1	<.001
Triglycerides, mg/dL	113.1 ± 9.3	93.6 ± 7.1	84.5 ± 5.8	.008

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^{*}Because of a large number of tied values, the number of participants in each tertile is not equal.

[†]Metabolic syndrome defined according to the updated definition described by Grundy et al.²⁵

in the fully adjusted model did not alter the association between 25(OH)D and HDL-C (regression coefficient = 0.42 mg/dL, $P < .001$, data not shown in table).

The association between 25(OH)D and triglyceride concentration observed in the tertile analyses was no longer significant after adjustment for age and sex ($P = .146$). After adjustment for age and sex, each 1-ng/mL increment in 25(OH)D was associated with a 0.31-cm smaller waist circumference ($P < .001$). Adjustment for additional variables increased the strength of the association to a reduction of 0.38 cm in waist circumference for each 1 ng/mL increment in 25(OH)D ($P < .001$). Substitution of body mass index for waist circumference as the dependent variable produced similar results (data not shown), suggesting a robust inverse association between serum 25(OH)D level and anthropometric indicators of adiposity.

Logistic regression models indicate that each 1-ng/mL increment in 25(OH)D was associated with a 5% reduction in the relative odds for metabolic syndrome in a model adjusted for age and sex ($P = .006$). Adjustment for

additional variables (not including metabolic syndrome components) increased the strength of the relationship to a 7% reduction in the relative odds for metabolic syndrome for each 1 ng/mL increment in 25(OH)D ($P = .003$).

Sensitivity analyses

To assess possible confounding of the relationships of 25(OH)D concentration with metabolic cardiovascular risk factors related to use of dietary supplements other than vitamin D, multivariate models were run that included the variables shown in the final models in Table 2 as well as total intakes of selected nutrients (vitamins C and E, beta-carotene, zinc, magnesium, and potassium) estimated from the Harvard Food Frequency Questionnaire responses. Including intakes of these nutrients did not significantly reduce the unexplained variance or materially alter the regression coefficients for 25(OH)D in any case when entered into the model individually or as a group. Additional analyses were completed in which subjects who

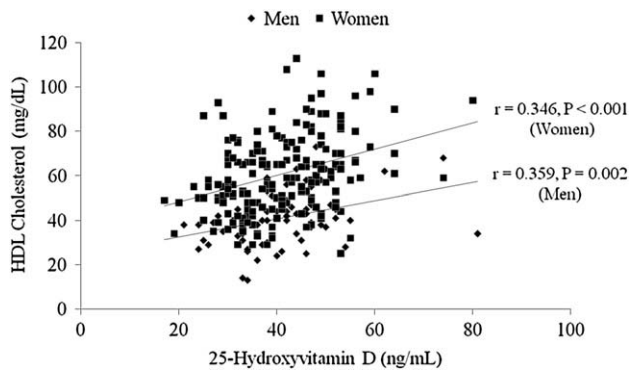


Figure 1 Relationship between serum 25-hydroxyvitamin D and high-density lipoprotein (HDL) cholesterol concentrations in men and women.

were taking any medications intended to modify lipids, glucose, or blood pressure and those taking dietary supplements with known potential to alter the lipid profile (eg, fish oil, niacin >200 mg/d) were excluded. Associations of 25(OH)D with HDL-C, waist circumference and metabolic syndrome remained highly statistically significant, although the multivariate regression coefficient for 25(OH)D as a predictor of HDL-C was reduced from 0.42 to 0.38 mg/dL, $P < .001$.

Discussion

The results of the present study indicate that serum 25(OH)D concentration is significantly inversely associated with prevalence of the metabolic syndrome in a group of generally healthy men and women, a large majority of whom had 25(OH)D levels in the normal range. Although 25(OH)D was inversely associated with glucose and TG concentrations, its relationship with the metabolic syndrome appeared to be driven primarily by strong associations with HDL-C (direct) and waist circumference (inverse). Neither systolic nor diastolic blood pressures were associated with 25(OH)D concentration. Although previous surveys also have reported associations between low 25(OH)D concentration and metabolic syndrome components, to our knowledge, the present investigation is the first to report this finding in a sample with a high prevalence of vitamin D dietary supplement users in which frequencies of vitamin D insufficiency and deficiency were low. Even among nondietary supplement users, mean 25(OH)D concentration was >30 ng/mL, which we attribute to the study having been conducted at the end of the summer months.

In our sample, neither vitamin D from food nor sun exposure was a significant predictor of the serum 25(OH)D concentration. Vitamin D dietary supplement users had a greater mean 25(OH)D concentration than nonusers, and a graded relationship was present between daily vitamin D intake from supplements and the 25(OH)D concentration. Individuals in the top tertile for 25(OH)D had a mean daily intake of vitamin D from food and supplements of 1316 IU, compared with 888 IU among subjects in the lowest tertile.

This difference of 428 IU in mean daily intake was associated with a difference of 22.4 ng/mL in mean 25(OH)D concentration.

Optimal vitamin D intake and serum 25(OH)D concentration have not been defined. Data from cohort studies suggest that low circulating levels of 25(OH)D are associated with greater risks for chronic diseases and mortality, although it is uncertain whether increases in 25(OH)D above the level of sufficiency (30 ng/mL) are associated with further risk reduction.^{2,8} Serum 25(OH)D levels of 50 – 90 ng/mL (approximately the level observed in the top tertile in our sample) are common in individuals with high sun exposure.²⁶

The most notable finding from the present study was the strong relationship between serum concentrations of 25(OH)D and HDL-C concentrations. Each 10-ng/mL increment in 25(OH)D was associated with an increase of 3.8 to 4.2 mg/dL in HDL-C after adjustment for established determinants of the HDL-C concentration. This is of considerable potential importance given that each 1-mg/dL increment in HDL-C is associated with a 4 – 6% reduction in coronary heart disease (CHD) risk.^{21,27} This finding differs somewhat from those of some other studies in which no relationship was reported^{28,29} or the relationship did not retain statistical significance in multivariate models.^{9,10,30} Dobnig et al⁷ reported a graded association between the HDL-C concentration and 25(OH)D concentration in a group of men undergoing angiography. However, their data suggest an increment of only ~1.5 mg/dL in HDL-C for each 10-ng/mL increase in 25(OH)D concentration, considerably less than observed in our sample.

The reasons for the stronger association in our study are not entirely clear. However, our sample differed from most others in that it was enriched with vitamin D dietary supplement users and all data were collected at the end of the summer months when recent sun exposure was likely to have been above-average. Thus, our sample had markedly higher mean levels of 25(OH)D than has been the case in most population studies. Very limited data have been reported on the effects of supplemental vitamin D on HDL-C and other aspects of the serum lipoproteins lipid profile. We believe that such investigations should be undertaken.

A potential explanation for our observation of an inverse association between 25(OH)D and indicators of adiposity (waist and body mass index) may be that vitamin D is fat soluble and is therefore easily 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.³¹ As a result, to maintain a given circulating 25(OH)D concentration, overweight and obese individuals may have to consume greater quantities of vitamin D than would be the case for normal weight populations.

Results from our study are generally concordant with those from several investigators who have reported inverse associations between 25(OH)D concentration and increased prevalence of the metabolic syndrome (4-6,9,10). A greater

Table 2 Multivariate linear and logistic regression analyses for the relationships between serum 25-hydroxyvitamin D [25(OH)D] in ng/mL and selected cardiovascular risk markers (dependent variables)

	β	SE (β)	P value for 25(OH)D
High-density lipoprotein cholesterol, mg/dL			
Model 1*	0.52	0.09	<.001
Model 2†	0.42	0.10	<.001
Triglycerides, mg/dL			
Model 1*	-0.59	0.40	.146
Waist circumference, cm			
Model 1*	-0.31	0.08	<.001
Model 2‡	-0.38	0.09	<.001
Metabolic syndrome (0 = no, 1 = yes, logistic regression)§			
Model 1*	-0.05	0.02	.006
Model 2‡	-0.07	0.02	.003

*Adjusted for age and sex.

†Adjusted for age, sex, waist circumference, physical activity score, alcohol consumption (average drinks per week), smoking status (no, yes), and vitamin D supplement use (no, yes).

‡Adjusted for age, sex, physical activity score, alcohol consumption, smoking status (no, yes), and vitamin D supplement use (no, yes).

§The relationship between an increase of 1 ng/mL in 25-hydroxyvitamin D and relative odds for metabolic syndrome may be calculated from e^β . For example, for $\beta = -0.05$ as in Model 1, $e^{-0.05} = 0.95$, indicating a 5% reduction in the relative odds for metabolic syndrome for each 1-ng/mL increment in 25(OH)D.

prevalence of metabolic syndrome in those with low 25(OH)D has been reported previously in adults.^{9,10} children,⁶ and morbidly obese individuals.⁵ Increased abdominal adiposity and the presence of the metabolic syndrome are typically associated with insulin resistance. To date, little information has been published regarding the influence of interventions to increase 25(OH)D on insulin sensitivity.

A proposed mechanism through which greater blood levels of 25(OH)D might improve insulin sensitivity includes effects on the renin-angiotensin system. There is evidence to suggest that angiotensin II can induce insulin resistance through a mechanism related to the stimulation of nuclear factor kappa-light-chain-enhancer of activated B cells.^{32,33} Vitamin D may be involved in the regulation of this system because induction of vitamin D deficiency is followed by increased renin-angiotensin II expression.³⁴ Thus, adequate vitamin D status may prevent angiotensin II-induced stimulation of nuclear factor kappa-light-chain-enhancer of activated B cells, resulting in greater insulin sensitivity. Another possible mechanism may be related to insulin like growth factor-1 (IGF-1) receptors. For example, 1,25(OH)₂D, which is synthesized by hydroxylation of 25(OH)D, appears to promote the action of IGF-1 by increasing IGF-1 receptor expression.³⁵ An increase in IGF-1 receptor expression could result in greater insulin sensitivity, primarily attributable to the structural homology and cross reactivity between IGF-1 receptors and insulin receptors.^{9,35}

It has also been suggested that calcium is an important regulator of energy metabolism and adiposity.^{36,37} Calcium plays a key role in the regulation of circulating 25(OH)D; 1,25(OH)₂D; and parathyroid hormone, all of which may exert regulatory effects on energy metabolism. Results from in vitro investigations in human adipocytes suggest

that parathyroid hormone may stimulate increases in intracellular calcium and repartitioning of dietary energy to favor lipid storage.^{36,37} Individuals with 25(OH)D insufficiency often have elevated levels of parathyroid hormone.³⁸ Whether the association between low 25(OH)D and adiposity is cause, effect, or an epiphenomenon is poorly understood at present and deserving of additional investigation.

Two surprising findings in the present study were the lack of association between 25(OH)D and blood pressure and our failure to observe a relationship between sun exposure and 25(OH)D concentration. Previous studies have suggested that low 25(OH)D may be associated with blood pressure elevation³⁹ and that increasing the 25(OH)D concentration may lower blood pressure.⁴⁰ It is possible that 25(OH)D insufficiency increases blood pressure, but that once a state of sufficiency has been reached, no further changes in blood pressure will occur.³⁹ Because a large majority of our sample had 25(OH)D levels >30 ng/mL, this may have prevented detection of such a relationship in our dataset.

One potential explanation for our failure to find a relationship between estimated sun exposure and serum 25(OH)D is that the investigation took place in late summer, when many individuals may have been experiencing above-average sun exposure. In addition, the semiquantitative questionnaire used to assess sun exposure is a crude tool that may not have had sufficient precision to reflect important differences. Alternatively, because a large proportion of subjects were vitamin D supplement users, supplement use may have obscured a relationship between sun exposure and 25(OH)D.

A strength of this investigation is the fact that the data were collected during a short time frame, thus minimizing seasonal variations in exposure to the sun and serum lipid values. In addition, the sample was enriched with dietary supplement users, allowing the assessment of relationships

among those at the higher end of the 25(OH)D distribution. To the authors' knowledge, none of the previous investigations of these relationships have included significant numbers of habitual dietary supplement users.

This study also has several limitations that make the generalizability of these findings uncertain. First, this was a relatively small sample that was predominantly non-Hispanic white and generally healthy with few chronic diseases. Only a small percentage of subjects were non-supplement users, limiting the ability to assess the relationship between dietary supplement use and 25(OH)D status. Furthermore, dietary supplement users may differ from the general population in many respects, including education level, income, and better self-reported health in comparison with individuals who don't take dietary supplements.⁴¹ Although we could find no evidence of material confounding of the reported relationships between 25(OH)D and metabolic cardiovascular risk factors by supplements other than vitamin D that many subjects were taking, such confounding cannot be completely ruled out because the measurement tools used may not be sensitive enough to rule out residual confounding. Similarly, the food frequency and sun exposure questionnaires provide only rough estimates for these known determinants of 25(OH)D status. Thus, our failure to find associations of sun exposure and dietary (nonsupplement) vitamin D with 25(OH)D concentration should be viewed with caution.

Conclusion

In summary, in the present study, serum 25(OH)D concentration was directly correlated with the HDL-C level and inversely associated with waist circumference and prevalence of the metabolic syndrome. Serum 25(OH)D was greater in vitamin D supplement users and vitamin D from dietary supplements was significantly associated with the circulating 25(OH)D concentration. These results suggest that clinical trials should be undertaken to assess the impact of increasing vitamin D intake on the metabolic cardiovascular risk factor profile.

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