

Use of a questionnaire to assess vitamin D status in young adults

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Abstract

Objective: We hypothesized that young adults would commonly have vitamin D deficiency and that a questionnaire could help identify subjects with the condition.

Design: Between January and May 2004, we administered a questionnaire to a convenience sample of young adults. We measured each participant's serum level of 25-hydroxyvitamin D (25(OH)D) using a chemiluminescent assay and defined deficiency as serum 25(OH)D < 16 ng/ml.

Setting and subjects: We recruited young adults living in Madison, Wisconsin without pre-existing conditions affecting vitamin D and/or Ca metabolism.

Results: One hundred and eighty-four adults (mean age 24 years, 53% women, 90% Caucasian) participated in the study. Nearly three in four adults (71%) had 25(OH)D level < 30 ng/ml and 26% were vitamin D-deficient. In multivariate analysis, persons reporting a tanning booth use (OR = 0.24, 95% CI 0.09, 0.63, $P = 0.004$), tanning booth use (OR = 0.09, 95% CI 0.02, 0.43, $P = 0.002$) and daily ingestion of two or more servings of milk (OR = 0.21, 95% CI 0.09, 0.48, $P < 0.001$) were less likely to be deficient. These three questions provided a sensitivity and specificity of 79% and 78%, respectively, for the presence of deficiency.

Conclusions: The questionnaire is moderately useful to identify young adults likely to be vitamin D-deficient. Additional revisions of the questionnaire may improve its ability to predict vitamin D deficiency.

Keywords
Deficiency
Odds ratio
Questionnaire
Risk factors
Survey
Vitamin D

Vitamin D deficiency is common in older adults, with recent studies describing deficiency in 18–25% of adult postmenopausal women^(1,2). Vitamin D deficiency results from several factors including inadequate sun exposure, reduced cutaneous vitamin D synthesis, poor nutrition, and certain medications and co-morbid diseases such as anticonvulsants and coeliac sprue⁽³⁾. The increasing measurement of serum 25-hydroxyvitamin D (25(OH)D) in older people arises from an increasing awareness of the prevalence of hypovitaminosis D and the role of vitamin D in both the prevention and management of osteoporosis⁽⁴⁾. However, the prevalence of hypovitaminosis D and its impact on health is less certain in adults under 50 years of age.

Healthy young adults may develop vitamin D deficiency for several reasons. First, the vitamin D intake of young adults is often below the recommended intake of

200 IU/d^(5,6). Second, young adults today spend less time outside than young adults one decade ago^(7–9). Third, the increasing use of sunscreen to reduce skin damage or cancer may decrease or eliminate cutaneous vitamin D synthesis^(8,10,11). Finally, many young adults drink carbonated beverages in place of milk, thereby decreasing the intake of both Ca and vitamin D and potentially increasing the risk of fracture⁽¹²⁾.

Despite mounting evidence that young adults are at risk for vitamin D deficiency, no specific recommendations exist regarding evaluation of their vitamin D status. Such lack of guidelines may result from limited information on either the impact of vitamin D on the development of peak bone mass^(13–15) or the long-term safety of increasing serum 25(OH)D levels in young adults. Although epidemiological data suggest that improved vitamin D status may decrease the risk of certain cancers and autoimmune diseases^(16–18), a true cause–effect relationship has not been established. Measurement of serum 25(OH)D is costly, with charges ranging from \$US 45 to \$US 100. A questionnaire to identify persons at

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high or low risk of vitamin D deficiency would be clinically useful, particularly as there is no consensus regarding the indications for measurement of 25(OH)D in young adults.

Other groups have used questionnaires to detect hypovitaminosis D. One group queried subjects on the use of multivitamins, milk and other foods containing vitamin D⁽¹⁹⁾ and revealed a positive correlation between serum 25(OH)D levels and multivitamin intake. However, subjects did not record sun exposure. A study in Icelandic women demonstrated an association between higher serum 25(OH)D levels and sun-seeking and dietary habits, but the questionnaire itself was not published⁽²⁰⁾. Utilizing questions to assess diet and sun exposure, a third group reported associations between serum 25(OH)D levels and season of measurement, BMI, age, time spent indoors, living in three southern states, vitamin D intake and creatinine. However, the study was limited to elderly subjects⁽²¹⁾.

We hypothesized that a simple questionnaire could identify young adults with a high and low likelihood of vitamin D deficiency. We designed a series of questions to assess use of vitamin D-containing supplements, milk and sun exposure in order to test this hypothesis.

Materials and methods

Between January and May 2004 we recruited 184 men and women between the ages of 18 and 40 years into the current study. The purposes of the study were twofold. First, we wished to estimate the prevalence of vitamin D deficiency in young adults. Second, we queried whether a questionnaire could identify subjects at high or low risk of vitamin D deficiency. We excluded individuals with pre-existing conditions affecting vitamin D and/or Ca metabolism including liver or kidney disease, eating disorders, skin diseases and use of oral corticosteroids, anticonvulsants, insulin or bisphosphonates. We paid volunteers \$US 20 for the single study visit and notified them of vitamin D test results by mail.

The Human Subjects Committee of the University of Wisconsin approved the study protocol. Participants received verbal and written descriptions of the study, signed the consent form and retained a copy for their records. We recorded the age, gender and self-reported race of each person at the study visit. Each subject completed a questionnaire designed to quantify intake of vitamin D through diet and sun exposure and to record the presence of conditions or medications known to affect vitamin D stores (Table 1).

To measure 25(OH)D, we collected blood from non-fasting participants⁽²²⁾ and transported samples, without exposure to light, to a central laboratory at the University of Wisconsin. Samples were stored at -70°C until analysis. Subsequently, we measured serum 25(OH)D using a

Table 1 The Vitamin D Questionnaire

- | |
|---|
| 1. Have you received a suntan in the past 12 months?
Yes or No |
| 2. Do you use sunscreen? Yes or No |
| 3. On average, how much sun exposure have you had in the past week?
less than 5 minutes per day, 5–15 minutes per day, 15–30 minutes per day, more than 30 minutes per day |
| 4. Have you used a tanning booth in the past year?
Yes or No |
| 5. How many servings of milk do you get daily? _____ |
| 6. Do you take multivitamins? Yes or No
If yes, how many multivitamin tablets do you take daily? _____ |
| 7. Do you take vitamin D supplements or calcium with vitamin D?
Yes or No
If so, how many IU per day? _____ |
| 8. Do you take cod-liver oil or omega-3 fatty acids (fish oil)?
Yes or No |
| 9. What is your ethnic background? |
| 10. Have you been diagnosed with Crohn's disease, ulcerative colitis or coeliac sprue? Yes or No |
| 11. Have you had diarrhoea in the past two weeks? Yes or No |

Liaison chemiluminescence assay (DiaSorin Inc., Stillwater, MN, USA). The chemiluminescence assay is an accurate, rapid and precise method for vitamin D measurement, correlating well with traditional radioimmunoassay but overestimating levels by 3.9 ng/ml compared with HPLC^(23–25). In a study of 329 clinical samples, the intra- and inter-assay CV for this assay were 8–13% and 8–15%, respectively⁽²⁵⁾.

The precise cut-off points used to define vitamin D adequacy, insufficiency and deficiency vary, depending on the assay utilized and the investigator. However, many experts use a 25(OH)D level <30 ng/ml (75 nmol/l) to define vitamin D insufficiency and a level <16 ng/ml (40 nmol/l) to define deficiency⁽³⁾. For the purposes of the present study, we used serum 25(OH)D <16 ng/ml to categorize individuals as vitamin D-deficient.

Statistical analysis

We summarized data as the mean and standard deviation for continuous variables and as frequencies and percentages for categorical variables. We compared vitamin D-sufficient and -deficient subjects using the Wilcoxon rank-sum test for continuous variables and the χ^2 or Fisher's exact test for categorical variables. We used univariate and multivariate logistic regression models to evaluate the effects of questionnaire responses on odds ratios for vitamin D deficiency. We controlled for age and gender in multivariate analysis. This is because age and gender are significantly associated with the likelihood of vitamin D deficiency and both likely affect nutritional and sun-seeking habits. We assessed the sensitivity and specificity of combinations of questions in their ability to identify subjects with vitamin D deficiency. We completed analyses using SAS version 9.1 (SAS Institute, Cary, NC, USA) and R version 2.4.0 (The R Project for Statistical Computing, <http://www.r-project.org>) statistical software packages.

Results

We recruited 184 subjects for the study. Two-thirds of subjects (n 124) participated during winter and 33% (n 60) during the spring. Subjects' mean (sd) age was 24 (4) years while the median age and range were 22.4 years and 18 to 40 years, respectively. Over half of the subjects (53%, n 98) were female and 90% (n 165) were Caucasian (Table 2). Mean (sd) serum 25(OH)D levels were 25 (11) ng/ml (range 4 to 52 ng/ml). Nearly three in four subjects (71%, n 130) had serum 25(OH)D level <30 ng/ml and one in four (26%, n 48) subjects were vitamin D-deficient.

Table 2 summarizes the entire group's answers to the questionnaire. Participants reported drinking an average of 1.9 (1.5) servings of milk daily. Nearly half of the subjects (46%, n 84) ingested a daily multivitamin but only 6% (n 11) took an additional vitamin D supplement and 3% (n 5) reported daily use of cod-liver oil. Subjects' mean vitamin D intake through milk was 188 (148) IU and through supplements was 77 (238) IU daily. Eighty-five per cent of responders (n 157) reported sun tanning, 88% (n 161) reported sunscreen use, 29% (n 53) reported over 30 min of sun exposure daily and 35% (n 64) reported tanning booth use in the past year.

Several characteristics identified subjects more likely to have vitamin D deficiency (Table 3). Individuals with vitamin D deficiency reported lower milk intake (1.2 *v.* 2.1 servings/d, $P < 0.001$). Subjects with deficiency were less likely to report a suntan (71% *v.* 90%, $P < 0.001$) or use a tanning booth (4% *v.* 46%, $P < 0.001$). Individuals with vitamin D deficiency were slightly older (mean age 26.5 years *v.* 22.9 years, $P < 0.001$) and more often male (65% *v.* 40%, $P = 0.005$). Finally, non-Caucasian individuals were more likely to be vitamin D-deficient than Caucasians (deficiency in 67% of non-Caucasians *v.* 23% of Caucasians, $P < 0.001$).

We performed univariate and multivariate logistic regression analyses to determine the odds ratio for vitamin D deficiency based on individual items in the questionnaire (Table 4). In univariate results, older age and male gender conferred a greater odds ratio for vitamin D deficiency. We controlled for age and gender in multivariate analyses for two reasons. First, age and gender may themselves be associated with differing nutritional and sun-seeking habits. Second, other studies disagree on whether older age and male gender are risk factors for vitamin D deficiency^(21,26,27). Results from adjusted and unadjusted analyses were similar. In multivariate analyses, adults under the median age of 22.4 years (OR = 0.25, 95% CI 0.12, 0.53, $P < 0.001$), those reporting a suntan (OR = 0.24, 95% CI 0.09, 0.63, $P = 0.004$), tanning booth use (OR = 0.09, 95% CI 0.02, 0.43, $P = 0.002$) and daily ingestion of two or more servings of milk (OR = 0.21, 95% CI 0.09, 0.48, $P < 0.001$) were less likely to be deficient. In contrast, individuals more likely to be deficient were non-Caucasians (OR = 5.50, 95% CI 1.35,

Table 2 Participants' demographics and answers to the vitamin D questionnaire: young adults (n 184) living in Madison, Wisconsin, January–May 2004

Characteristic	Mean or n	SD or %
Serum 25(OH)D (ng/ml)*	25	11
Vitamin D deficiency	48	26
Age (years)* (median 22.4, range 18 to 40)	24	4
Female gender	98	53
Ethnicity		
Asian	11	6
African American	1	<1
Caucasian	165	90
Hispanic	1	<1
Native American	2	1
Other/no answer	4	2
Season of 25(OH)D collection		
Spring	60	33
Winter	124	67
Suntan		
Yes	157	85
No	25	14
No answer	2	1
Sunscreen		
Yes	161	88
No	21	11
No answer	2	1
Sun exposure		
<5 min/d	16	9
5–15 min/d	38	21
15–30 min/d	75	40
>30 min/d	53	29
No answer	2	1
Tanning booth		
Yes	64	35
No	118	64
No answer	2	1
Milk (servings/d)*	1.9	1.5
Multivitamin use		
Yes	84	46
No	98	53
No answer	2	1
Vitamin D supplement use		
Yes	11	6
No	170	92
No answer	3	2
Cod-liver oil or fish oil use		
Yes	5	3
No	175	95
No answer	4	2
Total vitamin D intake (IU/d)*	265	258
From milk*	188	148
From supplements*	77	238
Chronic intestinal disorder		
Yes	1	1
No	179	97
No answer	4	2
Recent diarrhoea		
Yes	8	4
No	171	93
No answer	5	3

25(OH)D, 25-hydroxyvitamin D.

Data are shown as mean values with their standard deviation (indicated by *) or as numbers and percentage.

22.41, $P = 0.02$) and men (OR = 3.44, 95% CI 1.60, 7.37, $P = 0.002$). We performed additional univariate and multivariate analyses without non-Caucasian subjects (n 19); these analyses showed virtually identical findings (Table 5).

Table 3 Characteristics of subjects with and without vitamin D deficiency: young adults (*n* 184) living in Madison, Wisconsin, January–May 2004

Characteristic	Vitamin D deficient (<i>n</i> 48)		Vitamin D sufficient (<i>n</i> 136)		<i>P</i> value
	Mean or <i>n</i>	sd or %	Mean or <i>n</i>	sd or %	
Serum 25(OH)D (ng/ml)*	12	3	29	10	<0.001
Age (years)*	26.5	5.6	22.9	3.1	<0.001
Male gender	31	65	55	40	0.005
Ethnicity					
Caucasian	38	81	127	97	0.001
Non-Caucasian	9	19	4	3	
Season of 25(OH)D collection					
Spring	18	38	42	31	0.44
Winter	30	63	94	69	0.40
Suntan	34	71	123	90	<0.001
Sunscreen	40	83	121	90	0.20
Sun exposure					
<5 min/d	5	10	11	8	0.99
5–15 min/d	11	23	27	20	0.96
15–30 min/d	18	38	57	42	0.96
>30 min/d	14	29	39	29	0.96
Tanning booth	2	4	62	46	<0.001
Milk (servings/d)*	1.2	1.6	2.1	1.5	<0.001
Multivitamin use	17	35	67	50	0.08
Vitamin D supplement use	3	6	8	6	0.95
Cod-liver oil or fish oil use	3	6	2	2	0.09
Use of any tablet with vitamin D	19	40	69	51	0.19
Total vitamin D intake (IU/d)*	242	333	273	226	0.47
From milk*	121	103	211	154	<0.001
From supplements*	121	342	62	187	0.14
Chronic intestinal disorder	0		1	1	0.54
Recent diarrhoea	0		8	6	0.08

25(OH)D, 25-hydroxyvitamin D.

Data are shown as mean values with their standard deviation (indicated by*) or as numbers and percentage.

Table 4 Univariate and multivariate odds ratios for vitamin D deficiency: young adults (*n* 184) living in Madison, Wisconsin, January–May 2004

	Univariate model			Multivariate model*		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
Demographic variables						
Age <22.4 years	0.30	0.14, 0.63	<0.001	0.25	0.12, 0.53	<0.001
Male gender	2.67	1.29, 5.69	0.004	3.44	1.60, 7.37	0.002
Non-Caucasian	7.41	1.94, 34.82	<0.001	5.50	1.35, 22.41	0.02
Sun-seeking habits						
Winter season	0.75	0.36, 1.59	0.47	1.22	0.53, 2.79	0.64
Suntan	0.22	0.08, 0.57	<0.001	0.24	0.09, 0.63	0.004
Sunscreen	0.54	0.19, 1.61	0.20	0.72	0.24, 2.16	0.56
Sun exposure						
<5 min/d	1.30	0.33, 4.34	0.77	0.85	0.24, 3.07	0.81
5–15 min/d	1.18	0.48, 2.75	0.68	1.54	0.62, 3.83	0.35
15–30 min/d	0.81	0.39, 1.67	0.61	0.96	0.45, 2.06	0.92
>30 min/d	1.00	0.45, 2.17	1.0	0.84	0.36, 1.94	0.68
Tanning booth	0.05	0.006, 0.21	<0.001	0.09	0.02, 0.43	0.002
Dietary habits						
Milk (2+ servings/d)	0.28	0.13, 0.59	<0.001	0.21	0.09, 0.48	<0.001
Multivitamin use	0.55	0.26, 1.14	0.09	0.51	0.23, 1.11	0.09
Vitamin D supplement use	1.04	0.17, 4.58	1.0	1.39	0.29, 6.72	0.68
Fish oil use	4.29	0.48, 52.9	0.12	3.31	0.42, 25.90	0.26
Use of any tablet with vitamin D	0.62	0.30, 1.27	0.18	0.51	0.23, 1.12	0.09
Intestinal conditions						
Chronic intestinal disorder	0	0, 107	0.55	0	0, infinity	0.99
Recent diarrhoea	0	0, 1.62	0.11	0	0, infinity	0.99

*Multivariate analyses are adjusted for age and gender.

Table 5 Univariate and multivariate odds ratios for vitamin D deficiency in Caucasian subjects: young adults (*n* 165) living in Madison, Wisconsin, January–May 2004

	Univariate model			Multivariate model*		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
Demographic variables						
Age <22.4 years	0.33	0.14, 0.76	0.005	0.28	0.13, 0.63	0.002
Male gender	2.67	1.19, 6.24	0.01	3.19	1.44, 7.05	0.004
Sun-seeking habits						
Winter season	0.88	0.39, 2.09	0.84	4.18	0.67, 4.18	0.27
Suntan	0.25	0.08, 0.78	0.007	0.21	0.07, 0.64	0.006
Sunscreen	0.73	0.19, 3.38	0.74	0.72	0.20, 2.66	0.62
Sun exposure						
<5 min/d	1.24	0.27, 4.53	0.75	0.63	0.15, 2.64	0.53
5–15 min/d	0.88	0.29, 2.34	1.0	1.43	0.52, 3.94	0.49
15–30 min/d	0.82	0.35, 1.82	0.71	0.87	0.38, 2.00	0.75
>30 min/d	1.26	0.53, 2.90	0.55	1.08	0.45, 2.61	0.85
Tanning booth	0.06	0.007, 0.27	<0.001	0.11	0.02, 0.52	0.005
Dietary habits						
Milk (2+ servings/d)	0.30	0.13, 0.69	0.003	0.25	0.10, 0.59	0.002
Multivitamin use	0.69	0.30, 1.52	0.36	0.61	0.27, 1.41	0.25
Vitamin D supplement use	1.72	0.27, 8.57	0.43	2.29	0.42, 12.4	0.34
Fish oil use	5.29	0.58, 65.7	0.08	3.76	0.47, 29.8	0.21
Intestinal conditions						
Chronic intestinal disorder	0	0, 130	1.0	0	0, infinity	0.99
Recent diarrhoea	0	0, 1.99	0.20	0	0, infinity	0.99

*Multivariate analyses are adjusted for age and gender.

Table 6 Sensitivity and specificity of three questions* for vitamin D deficiency: young adults (*n* 184) living in Madison, Wisconsin, January–May 2004

	Sensitivity (%)	Specificity (%)
Three questions answered 'no'	19	98
Two or more questions answered 'no'	79	78
One or more questions answered 'no'	96	25
Any questions answered 'no'	100	0

*Questions: (i) Have you received a suntan in the past 12 months? Yes or No; (ii) Have you used a tanning booth in the past year? Yes or No; (iii) How many servings of milk do you get daily? Two or more servings = Yes.

We evaluated the sensitivity and specificity of the questionnaire as a screening test for vitamin D deficiency (Table 6). Three self-reported habits (suntan, tanning booth use and drinking two or more servings of milk daily) discriminated between young adults with and without vitamin D deficiency. Using a threshold of two out of three negative responses for these habits, we obtained a sensitivity of 79% and a specificity of 78% for identifying persons with vitamin D deficiency. We obtained a similar sensitivity (76%) and specificity (79%) when excluding non-Caucasian subjects (Table 7).

Discussion

We hypothesized that, like other age groups, young adults would commonly have vitamin D deficiency. In this study of 184 healthy young subjects, 71% had serum

Table 7 Sensitivity and specificity of three questions* for vitamin D deficiency in Caucasian subjects: young adults (*n* 165) living in Madison, Wisconsin, January–May 2004

	Sensitivity (%)	Specificity (%)
Three questions answered 'no'	13	98
Two or more questions answered 'no'	76	79
One or more question answered 'no'	95	25
Any questions answered 'no'	100	0

*Questions: (i) Have you received a suntan in the past 12 months? Yes or No; (ii) Have you used a tanning booth in the past year? Yes or No; (iii) How many servings of milk do you get daily? Two or more servings = Yes.

25(OH)D level <30 ng/ml and 26% were clearly vitamin D-deficient, with 25(OH)D level below 16 ng/ml. People with vitamin D deficiency may develop osteomalacia, a disease characterized by unmineralized osteoid leading to bone pain and skeletal fragility. Higher vitamin D levels are associated with increased bone mass^(13–15,28). Indeed, studies suggest that preventing vitamin D deficiency may optimize Ca homeostasis and facilitate peak bone mass in young adults^(13,14). The high prevalence of deficiency in young adults highlights the need for further research to identify the precise vitamin D level needed to optimize musculoskeletal health. Such knowledge will facilitate patient education and public policy, with the goal of achieving vitamin D adequacy.

We hypothesized that a questionnaire could identify young people at high and low risk of vitamin D deficiency. A single question did not reliably distinguish between these groups. However, those subjects who

received a suntan, used a tanning booth or drank at least two servings of milk daily were significantly less likely to be deficient than subjects not reporting these habits. In combination, these three items were useful in differentiating between those with and without vitamin D deficiency. For subjects responding in the negative to any two of these three questions, we obtained a sensitivity of 79% and specificity of 78% for predicting vitamin D deficiency. Although the questionnaire needs further revision to improve its performance, it appears that three questions may help clinicians decide whether to pursue laboratory testing for vitamin D deficiency.

It is not surprising that sun exposure and milk ingestion may protect against vitamin D deficiency. Vitamin D fortification of milk is required in the USA, based on research carried out decades ago at the University of Wisconsin. An 8 ounce glass of milk contains ~100 IU of vitamin D. Likewise, cutaneous sun exposure increases 25(OH)D levels, unless sunscreen with a sun protection factor >15 is used⁽²⁹⁾. Additionally, many tanning beds emit UV-B light, which increases vitamin D synthesis^(20,30,31). Although sun-induced summer increments in serum 25(OH)D gradually decline over the winter, women with low vitamin D intake but high summer sun exposure may maintain higher serum 25(OH)D levels in the winter as well⁽³²⁾. While the explanation for this observation is unknown, summer sun exposure was associated in one study with improved vitamin D status year-round⁽³²⁾.

Few studies have used questionnaires to predict low serum 25(OH)D levels. In a study by Tangpricha *et al.*, serum 25(OH)D levels were higher in subjects taking multivitamins, but not higher in milk drinkers⁽¹⁹⁾. The authors analysed these associations between habits and serum 25(OH)D levels⁽¹⁹⁾, rather than stratifying subjects as sufficient or deficient as we did. Our vitamin D-deficient subjects were less likely to take multivitamins compared with sufficient subjects (35% *v.* 50%, $P=0.08$) but further analyses showed no effect of multivitamin use on the odds of vitamin D deficiency. Milk consumption in Tangpricha's study was lower (mean 1.6 (SD 1) servings/d)⁽¹⁹⁾ than that reported by our subjects, which might explain why there was no difference in vitamin D levels between their subjects who drank and did not drink milk. In a questionnaire-based Icelandic study, older women consuming fish oil or multivitamins had higher serum 25(OH)D levels ($P<0.01$) than younger women who did not report these habits⁽²⁰⁾. Women whose used tanning beds ($P=0.06$) or travelled to warmer climates ($P<0.01$) also had higher 25(OH)D levels⁽²⁰⁾. A third study demonstrated associations between serum 25(OH)D and season, BMI, age, time spent indoors, living in southern states, vitamin D intake and creatinine⁽²¹⁾. Taken as a whole, many of these findings are very similar to ours and suggest that questions about sun exposure and supplemental and dietary vitamin D intake may be universally useful to identify individuals at risk for vitamin D deficiency.

Based on larger cross-sectional studies⁽²⁶⁾, we do not believe that questions about age will prove useful to exclude or suspect vitamin D deficiency. Indeed, ours is not the first study to report vitamin D inadequacy in young adults. In Iceland, younger women had lower vitamin D levels than older women⁽²⁰⁾. Nearly half of adolescent girls in Maine had hypovitaminosis D at least once during a three-year observation⁽³³⁾. Two-thirds of internal medicine residents had 25(OH)D levels below 20 ng/ml during spring months in Oregon⁽³⁴⁾. Together these studies indicate that hypovitaminosis D is common in young adults.

One unexpected finding in the present study was that men were more likely than women to be vitamin D-deficient. In large epidemiology studies, men typically have higher serum 25(OH)D levels than women^(21,26,27). However, other studies have reported no gender difference in 25(OH)D levels^(21,35). Based on our and other studies, we do not believe gender is a useful means of identifying persons at higher risk of vitamin D deficiency.

Strengths of our study include testing of individuals of both genders from ages 18 to 40 years and uniform measurement of serum 25(OH)D by a single assay. Study weaknesses also exist. The first is the recruitment of relatively few, predominantly Caucasian, study subjects. Aside from milk, we did not query intake of other foods that might contain vitamin D; however, very few other foods contain meaningful doses of vitamin D^(3,36). Additionally, the chemiluminescent assay used for the study may slightly overestimate serum 25(OH)D compared with the gold standard HPLC assay. We did not record time of day in the sun, although it is known that both season and time of day influence cutaneous vitamin D synthesis⁽³⁷⁾. We measured 25(OH)D levels in late winter and early spring, a time of low sun exposure in Wisconsin⁽³⁷⁾. Thus, 25(OH)D levels were measured at a nadir in our subjects, increasing the likelihood of vitamin D deficiency.

Additional research is needed to refine the current questionnaire and provide cost-effective algorithms to identify individuals who benefit from serum 25(OH)D measurement. Ideally, a larger study performed in one season would query subjects about sun exposure, milk ingestion and intake of foods (fatty fish, liver, eggs) and supplements containing vitamin D. The study should also assess BMI and smoking, given the higher risk of deficiency reported in obese patients and smokers^(21,38,39). Additionally, symptoms or signs of vitamin D deficiency, such as proximal muscle weakness or tibial tenderness, might allow better identification of subjects at high risk of deficiency⁽⁴⁰⁾. Ideally, a questionnaire modified from the one herein would provide a 'score' with higher sensitivity and specificity for vitamin D deficiency. Such a tool would prove useful in clinical practice.

Peak bone mass occurs around 30 years of age^(41,42). Models indicate that interventions to increase peak bone mass are more effective at preventing osteoporosis than

interventions later in life⁽⁴³⁾. Early research suggests that improved vitamin D status promotes peak bone mass^(13,14). If researchers confirm the importance of vitamin D on peak bone mass, young adults would benefit from global vitamin D fortification of food and beverages.

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References

- Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT, Poetruschke RA, Chen E & dePapp AE (2005) Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab* **90**, 3215–3224.
- Lips P, Hosking DJ, Lippuner K, Norquist JM, Wehren L, Maalouf G, Ragi-Els S & Chandler J (2006) The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *J Intern Med* **260**, 245–254.
- Vieth R (1999) Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* **69**, 842–856.
- Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T & Dawson-Hughes B (2005) Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA* **293**, 2257–2264.
- Gartner LM & Greer FR (2003) Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. *Pediatrics* **111**, 908–910.
- Misra M, Tsai P, Anderson EJ, Hubbard JL, Gallagher K, Soyka LA, Miller KK, Herzog DB & Klibanski A (2006) Nutrient intake in community-dwelling adolescent girls with anorexia nervosa and in healthy adolescents. *Am J Clin Nutr* **84**, 698–706.
- Hartman JJ (2000) Vitamin D deficiency rickets in children: prevalence and need for community education. *Orthop Nurs* **19**, 63–67.
- Cokkinides V, Weinstock M, Glanz K, Albano J, Ward E & Thun M (2006) Trends in sunburns, sun protection practices, and attitudes toward sun exposure protection and tanning among US adolescents, 1998–2004. *Pediatrics* **118**, 853–864.
- Schofield PE, Freeman JL, Dixon HG, Borland R & Hill DJ (2001) Trends in sun protection behaviour among Australian young adults. *Aust N Z J Public Health* **25**, 62–65.
- Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA & Holick MF (1987) Sunscreens suppress cutaneous vitamin D₃ synthesis. *J Clin Endocrinol Metab* **64**, 1165–1168.
- Peacey V, Steptoe A, Sanderman R & Wardle J (2006) Ten-year changes in sun protection behaviors and beliefs of young adults in 13 European countries. *Prev Med* **43**, 460–465.
- Tucker KL, Morita K, Qiao N, Hannan MT, Cupples LA & Kiel DP (2006) Colas, but not other carbonated beverages, are associated with low bone mineral density in older women: The Framingham Osteoporosis Study. *Am J Clin Nutr* **84**, 936–942.
- Valimaki VV, Alfthan H, Lehmuskallio E, Loyttyniemi E, Sahi T, Stenman UH, Suominen H & Valimaki MJ (2004) Vitamin D status as a determinant of peak bone mass in young Finnish men. *J Clin Endocrinol Metab* **89**, 76–80.
- Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irtala KM, Leino AE & Viikari JS (2002) Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. *Am J Clin Nutr* **76**, 1446–1453.
- Bischoff-Ferrari HA, Dietrich T, Orav EJ & Dawson-Hughes B (2004) Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. *Am J Med* **116**, 634–639.
- Barger-Lux MJ & Heaney RP (1994) The role of calcium intake in preventing bone fragility, hypertension, and certain cancers. *J Nutr* **124**, 8 Suppl., 1406S–1411S.
- Garland CF, Garland FC & Gorham ED (1999) Calcium and vitamin D. Their potential roles in colon and breast cancer prevention. *Ann N Y Acad Sci* **889**, 107–119.
- Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA & Saag KG (2004) Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. *Arthritis Rheum* **50**, 72–77.
- Tangpricha V, Pearce EN, Chen TC & Holick MF (2002) Vitamin D insufficiency among free-living healthy young adults. *Am J Med* **112**, 659–662.
- Sigurdsson G, Franzson L, Porgeirsdottir H & Steingrimsdottir L (1999) Vitamin D intake and serum 25-OH-vitamin D concentration in different age groups of Icelandic women. *Laeknabladid Med J* **85**, 398–405.
- Jacques PF, Felson DT, Tucker KL, Mahnken B, Wilson PW, Rosenberg IH & Rush D (1997) Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. *Am J Clin Nutr* **66**, 929–936.
- Juttman JR, Visser TJ, Buurman C, de Kam E & Birkenhager JC (1981) Seasonal fluctuations in serum concentrations of vitamin D metabolites in normal subjects. *Br Med J (Clin Res Ed)* **282**, 1349–1352.
- Lensmeyer GL, Wiebe DA, Binkley N, Drezner MK, Singh R & Darcy TP (2005) Clinically reliable assay for routine monitoring of 25-hydroxyvitamin D₃ and 25-hydroxyvitamin D₂ in serum using a semi-automated extraction and HPLC. *Clin Chem* **51**, Suppl. A, A192.
- Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, DeLuca HF & Drezner MK (2004) Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. *J Clin Endocrinol Metab* **89**, 3152–3157.
- Ersfeld DL, Rao DS, Body JJ, Sackrison JL Jr, Miller AB, Parikh N, Eskridge TL, Polinske A, Olson GT & MacFarlane GD (2004) Analytical and clinical validation of the 25 OH vitamin D assay for the LIAISON automated analyzer. *Clin Biochem* **37**, 867–874.
- Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW & Sahyoun NR (2002) Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* **30**, 771–777.
- Zadshir A, Tareen N, Pan D, Norris K & Martins D (2005) The prevalence of hypovitaminosis D among US adults: data from the NHANES III. *Ethn Dis* **15**, Suppl. 5, S5-97–S5-101.

28. Dawson-Hughes B, Dallal GE, Krall EA, Harris S, Sokoll LJ & Falconer G (1991) Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. *Ann Intern Med* **115**, 505–512.
29. Webb AR & Holick MF (1988) The role of sunlight in the cutaneous production of vitamin D₃. *Annu Rev Nutr* **8**, 375–399.
30. Devgun MS, Johnson BE & Paterson CR (1982) Tanning, protection against sunburn and vitamin D formation with a UV-A 'sun-bed'. *Br J Dermatol* **107**, 275–284.
31. Tangpricha V, Turner A, Spina C, Decastro S, Chen TC & Holick MF (2004) Tanning is associated with optimal vitamin D status (serum 25-hydroxyvitamin D concentration) and higher bone mineral density. *Am J Clin Nutr* **80**, 1645–1649.
32. Salamone LM, Dallal GE, Zantos D, Makrauer F & Dawson-Hughes B (1994) Contributions of vitamin D intake and seasonal sunlight exposure to plasma 25-hydroxyvitamin D concentration in elderly women. *Am J Clin Nutr* **59**, 80–86.
33. Sullivan SS, Rosen CJ, Halteman WA, Chen TC & Holick MF (2005) Adolescent girls in Maine are at risk for vitamin D insufficiency. *J Am Diet Assoc* **105**, 971–974.
34. Haney EM, Stadler D & Bliziotis MM (2005) Vitamin D insufficiency in internal medicine residents. *Calcif Tissue Int* **76**, 11–16.
35. Rucker D, Allan JA, Fick GH & Hanley DA (2002) Vitamin D insufficiency in a population of healthy western Canadians. *CMAJ* **166**, 1517–1524.
36. Utiger RD (1998) The need for more vitamin D. *N Engl J Med* **338**, 828–829.
37. Webb AR, Kline L & Holick MF (1988) Influence of season and latitude on the cutaneous synthesis of vitamin D₃: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D₃ synthesis in human skin. *J Clin Endocrinol Metab* **67**, 373–378.
38. Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ & Shaw S (1985) Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest* **76**, 370–373.
39. Isaia G, Giorgino R, Rini GB, Bevilacqua M, Maugeri D & Adami S (2003) Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. *Osteoporos Int* **14**, 577–582.
40. Van Veldhuizen PJ, Taylor SA, Williamson S & Drees BM (2000) Treatment of vitamin D deficiency in patients with metastatic prostate cancer may improve bone pain and muscle strength. *J Urol* **163**, 187–190.
41. Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR & Kimmel DB (1992) Bone gain in young adult women. *JAMA* **268**, 2403–2408.
42. Lin YC, Lyle RM, Weaver CM, McCabe LD, McCabe GP, Johnston CC & Teegarden D (2003) Peak spine and femoral neck bone mass in young women. *Bone* **32**, 546–553.
43. Hernandez CJ, Beaupre GS & Carter DR (2003) A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporos Int* **14**, 843–847.