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Genetic and non-genetic correlates of vitamins K and D

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Abstract

Objective—To assess the genetic and nongenetic correlates of circulating measures of vitamins K and D status in a community-based sample of men and women.

Subjects/Methods—A cross-sectional study of 1762 participants of the Framingham Offspring Study (919 women; mean age 59 years). Vitamin K status was measured as plasma phylloquinone and serum percent undercarboxylated osteocalcin (ucOC), and vitamin D was measured using plasma 25-hydroxyvitamin D (25(OH)D). Associations between vitamin K status and vitamin D status with biologically plausible nongenetic factors were assessed using stepwise regression. Heritability and linkage were determined using Sequential Oligogenic Linkage Analysis Routines (SOLAR).

Results—Nongenetic factors accounted for 20.1 and 12.3% of the variability in plasma phylloquinone in men and women respectively, with triglycerides and phylloquinone intake being the primary correlates. In men 12.2% and in women 14.6% of the variability in %ucOC was explained by nongenetic factors in our models. Heritability estimates for these vitamin K status biomarkers were nonsignificant. Season, vitamin D intake, high-density lipoprotein (HDL) cholesterol and waist circumference explained 24.7% (men) and 24.2% (women) of the variability in plasma 25(OH)D. Of the three vitamins examined, only 25(OH)D was significantly heritable (heritability estimate=28.8%, $P<0.01$), but linkage analysis of 25(OH)D did not achieve genome-wide significance.

Conclusions—Variability in biomarkers of vitamin K status was attributed to nongenetic factors, whereas plasma 25(OH)D was found to be significantly heritable. Further studies are warranted to investigate genetic loci influencing vitamin D status.

Keywords

vitamin K; phylloquinone; undercarboxylated osteocalcin; vitamin D; heritability; genetics

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Introduction

Vitamins K and D are two nutrients involved in bone health (Cockayne *et al.*, 2006). Vitamin K functions as a cofactor in the γ-carboxylation of specific proteins, of which osteocalcin (OC) is found in bone (Ferland, 1998). The transcription and translation of OC is regulated by 1,25-dihydroxyvitamin D (Arbour *et al.*, 1995). When circulating concentrations of vitamin K are low, a greater proportion of OC is not carboxylated, the measure of which (percentage of total undercarboxylated OC, %ucOC) is used as an estimate of decreased availability of vitamin K to bone. Elevated %ucOC has been associated with lower bone mineral density in elderly men and women (Booth *et al.*, 2004). Low vitamin D status has also been associated with an elevated %ucOC (Szulc *et al.*, 1993), although this association remains unconfirmed.

Whereas dietary vitamin K intake and age have been identified as important determinants of vitamin K, less is known about the association between other nongenetic factors and vitamin K status (Sokoll and Sadowski, 1996; McKeown *et al.*, 2002). The determinants of vitamin D status have been described, with season and dietary intake being identified as the primary contributors (Burnand *et al.*, 1992; Jacques *et al.*, 1997). The heritability of vitamin D status has been estimated at 43% in a sample of primarily female twins (Hunter *et al.*, 2001). The heritability of vitamin K status is unknown, and to our knowledge there are no reports of the nongenetic determinants and heritability of vitamin K and vitamin D status in the same population.

Using data from the Framingham Offspring Cohort, we assessed the nongenetic correlates and heritability of vitamin K status (as measured by plasma phylloquinone and %ucOC) and vitamin D status (plasma 25(OH)D) in a community-based sample of men and women.

Participants and methods

Study sample

The Framingham Heart Study is a longitudinal community-based study initiated to study the epidemiology of cardiovascular disease (CVD) (Dawber *et al.*, 1951). Beginning in 1971, offspring of the original cohort and their spouses were invited to participate in the Framingham Offspring Study (Kannel *et al.*, 1979). Every 4–8 years, Offspring participants undergo examinations, which include physical examinations, blood biochemistries, assessment of cardiovascular risk factors and questionnaires. Samples for vitamins K and D, and OC measurements were collected between 1997 and 1999 (the end of examination cycle 6 (1995–1998) and the beginning of cycle 7 (1998–2001)). Usual intakes of phylloquinone and vitamin D were assessed using the Willett Food Frequency Questionnaire (FFQ). This FFQ queries frequency of intake of reference portions of 126 foods over the previous year (Willett *et al.*, 1985). Questionnaires were considered invalid and were excluded from analysis if they had reported energy intakes <2.51 and >16.74 MJ per day (600 and 4000 kcal per day, respectively) or if they had >12 food items blank (n=59). A total of 1762 participants (843 men and 919 women) had a valid FFQ, in addition to biochemical measures. Since anticoagulant medications are vitamin K antagonists (Bach *et al.*, 1996), we excluded men (n=30) and women (n=8) who reported current anticoagulant medication use from the analyses of plasma phylloquinone and serum %ucOC.

Participant characteristics

Information was collected about medical history, medication use, smoking status, alcohol use and dietary intake, and, for women, menopause status and hormone replacement therapy (HRT) use at the examination at which the vitamin samples were collected. Height, weight and waist circumference measurements were taken according to a standardized protocol. Waist

circumference was chosen as a measure of abdominal adiposity since it has been associated with poor status of certain fat-soluble vitamins (Ohrvall *et al.*, 1993; Snijder *et al.*, 2005). Season was included as a covariate since seasonal influences of vitamins D and K status have been reported (Dawson-Hughes *et al.*, 1997; Woitge *et al.*, 1998; McKeown *et al.*, 2002).

Vitamins K and D measurements

Fasting morning blood samples were collected and plasma/serum was stored at −70 °C until analysis. Vitamin K status was assessed by measures of plasma phylloquinone and serum % ucOC. Plasma phylloquinone concentrations were determined using high-pressure liquid chromatography (Davidson and Sadowski, 1997). Low and high control specimens had average values of 0.56 and 3.15 nmol l^{-1}, with coefficients of variation (total CVs) of 15.2 and 10.9%, respectively. Serum total OC and ucOC were measured by radioimmunoassay (RIA), using the method of Gundberg (Gundberg *et al.*, 1984). Since this binding varies with the amount of total OC in the sample, the ucOC is expressed as a %ucOC (Gundberg *et al.*, 1998). The total CVs for the three control serums with an average total OC result of 3.4, 7.1 and 11.9 μg l^{-1} were 22.3, 12.8 and 7.8%, respectively. Vitamin D status was estimated by measuring plasma 25(OH)D concentration using RIA (DiaSorin, Stillwater, MN REF 68100E). The total CVs for the con trol values of 36 and 137 nmol l^{-1} were 8.5 and 13.2%, respectively.

Statistical analyses

To normalize skewed distributions, plasma phylloquinone and %ucOC were logarithmically transformed. Pearson's correlation coefficients were used to assess associations between biochemical measures with age, waist circumference, body mass index (BMI), dietary intakes, triglycerides, cholesterol (total, high-density lipoprotein, HDL and low-density lipoprotein, LDL), and serum creatinine. Spearman's correlations were used to assess associations with smoking status, medication use, and (for women) menopause status and current HRT use. Because of a significant interaction between age and sex with respect to plasma phylloquinone and 25(OH)D, all analyses were subsequently sex specific.

We used stepwise multivariable linear regression, with a $P<0.10$ threshold for inclusion, to identify factors associated with biochemical measures. Candidate covariates were chosen based on correlation coefficients and biological plausibility. For vitamin K status, we examined age, phylloquinone intake, triglycerides, cholesterol (total, HDL and LDL), waist circumference, serum creatinine, smoking status, lipid-lowering medication use, season of blood draw and, for women, menopause status and HRT use. For the determinants of plasma 25(OH)D, the covariates included age, vitamin D intake, triglycerides, cholesterol (total, HDL and LDL), waist circumference, serum creatinine, smoking status, current use of osteoporosis medication, season of blood draw and, for women, menopause status and HRT use. Season was defined as: June–August; summer: September–November; fall: December–February; winter: March–May; spring, with fall, winter and spring being entered into the stepwise models as dichotomous variables and summer as the reference. Association between plasma phylloquinone and 25 (OH)D was checked using a partial correlation coefficient, adjusted for the same covariates included in the stepwise models. Since vitamin deficiency has been associated with several chronic diseases (Holick, 2004; Erkkila *et al.*, 2006), we repeated all analyses on individuals free of CVD, diabetes and hypertension. All analyses were performed using SAS, version 9.1. A *P*-value of <0.05 was considered to be statistically significant.

Heritability

Before testing for a significant heritable component, we first tested the association with nongenetic covariates as detailed in the above section and for each of the three log-transformed biochemical measures we obtained sex-specific models that included only the significant nongenetic covariates. The residuals of each model were percentiled (ranked) and the

equivalent percentile value from a normal distribution was substituted (that is, the residuals were ranked normalized) to fit a normal distribution (since violation of this assumption may lead to false-positive genetic evidence). The normalized variables for each of the biochemical measures were then used as the dependent variables for estimation of heritability (h^2). The heritability estimates, defined as the proportion of total variation explained by additive genetic effect, were computed using the Sequential Oligogenic Linkage Analysis Routines (SOLAR, version 2.1.4) (Almasy and Blangero, 1998), with between 264 and 267 sibpairs (n=595–597).

Since only plasma 25(OH)D was found to be significantly heritable, variance component linkage analysis was carried out on the residual phenotypes for plasma 25(OH)D using 601 genome scan microsatellite markers distributed across the 22 autosomal chromosomes with an average intermarker spacing of 5.8 cM. There was no linkage analysis performed on biochemical measures of vitamin K status because they were not significantly heritable. The underlying principle of variance component linkage analysis is that relatives who share more genetic material identical by descent (IBD) at a quantitative trait locus (QTL), should have phenotype values that are more correlated than relatives with less IBD sharing. Variance component models incorporating genetic marker information at a putative QTL, in the form of IBD sharing between relatives, are compared with models only incorporating polygenic effects, without genetic marker information, to test the null hypothesis of no linkage to a particular genome location. Multipoint (that is, using several genetic markers) probabilities of IBD between relative pairs were estimated at every cM using the program LOKI (Heath, 1997). The log of the odds ratio (LOD) score (logarithm base 10 of the likelihood ratio of the models with and without QTL effects) was computed every 1cM using SOLAR (version 2.1.4) (Almasy and Blangero, 1998) and measures the evidence that a QTL is located at a specific genetic location.

Results

Participant characteristics are summarized in Table 1. Mean (±s.d.) concentrations of biochemical measures were within previously reported ranges for these assays (Sadowski *et al.*, 1989;Gundberg *et al.*, 1998;Looker *et al.*, 2002).

In men, statistically significant correlates of plasma phylloquinone were age (inverse), phylloquinone intake (direct), triglyceride concentrations (direct), LDL cholesterol (direct) and smoking status (inverse) (Table 2). Together with winter season (inverse, compared to summer, P=0.05) and use of lipid-lowering medication (direct, P=0.09), these factors explained 20.1% of the variability in plasma phylloquinone. In women, statistically significant correlates of plasma phylloquinone were phylloquinone intake (direct), triglycerides (direct), total cholesterol (direct) and use of lipid-lowering medication (direct), which explained 12.3% of the variability.

If analyses were repeated on men and women free of CVD (n=678 men and 835 women) and free of diabetes (fasting glucose <126mgdl^{-1}, and not taking oral hypoglycemic medications or insulin; n=681 men and 812 women), determinants of plasma phylloquinone were not changed (data not shown). Among men without hypertension (systolic blood pressure <140mm Hg, diastolic blood pressure <90mm Hg and not on hypertension medications; n=427), determinants of plasma phylloquinone were attenuated such that only triglyceride concentration and smoking status were statistically significant (P<0.05). In women free of hypertension (n=550), along with vitamin K intake and triglyceride concentration, increasing age became positively associated (P=0.04) and serum creatinine became inversely associated (P=0.02) with plasma phylloquinone.

Correlates of %ucOC in men were waist circumference (inverse), and season (highest in summer), which, with smoking (inverse, P=0.06) and HDL cholesterol (direct, P=0.09), accounted for 12.2% of the variability (Table 3). In women, correlates of %ucOC included phylloquinone intake (inverse), smoking status (inverse), menopause status (direct), use of HRT (inverse) and season (highest in summer) (P<0.05), which together with HDL cholesterol (inverse, P=0.08), explained 14.6% of the variability. If BMI was included as a covariate measure of body composition, it was significantly associated with %ucOC in women only (P=0.04, data not shown). Determinants of %ucOC were not changed in men without CVD and in women without hypertension. In men without hypertension or diabetes, the association with waist circumference was attenuated (P>0.05). Among women free of CVD or diabetes, menopause status was no longer significantly associated with %ucOC. Plasma phylloquinone was inversely associated with %ucOC in both men and women (parameter estimate=−0.17 (men) and −0.14 (women) both P<0.01). Plasma 25(OH)D was not associated with %ucOC or plasma phylloquinone in men or women.

In men, vitamin D intake (including supplements; direct), waist circumference (inverse), HDL cholesterol (direct), serum creatinine (direct) and season (highest in summer) were correlates (all P<0.05) of plasma 25(OH)D (Table 4). Together with age (direct) (P=0.08), these factors accounted for 24.7% of the variability in plasma 25(OH)D in men. In women, the significant correlates of plasma 25(OH)D were vitamin D intake (direct), HDL cholesterol (direct), waist circumference (inverse), HRT use (direct) and season (highest in summer) (P<0.05), which together explained 24.2% of the variability. Use of vitamin D supplements was associated with vitamin D status in women only (P=0.03) (data not shown). If BMI was included as a covariate instead of waist circumference, it too was inversely associated with plasma 25(OH)D (P<0.01), and the other nongenetic factors in the models for men and women were unchanged (data not shown).

Determinants of plasma 25(OH)D were not substantively changed in men free of hypertension. If analyses were repeated on women free of hypertension, serum creatinine became positively associated and LDL cholesterol became inversely associated with plasma 25(OH)D (both P=0.04), while the association with HDL cholesterol became non-significant (P=0.08). If analyses were repeated on men free of diabetes and CVD, the association of plasma 25(OH) D with serum creatinine was no longer significant, whereas the determinants were not changed in women free of CVD and diabetes.

The heritability (h^2) of plasma phylloquinone, estimated at 13.4±11.1% (mean±standard error) in multivariable-adjusted analyses respectively, was nonsignificant (P=0.11). The heritability of %ucOC was also nonsignificant in multivariable-adjusted analyses (15.9±11.8%, P=0.08). The heritability of plasma 25(OH)D, estimated at 28.8±11.3% in multivariable-adjusted analysis, was significant (P=0.003). The maximum LOD score for plasma 25(OH)D was found on chromosome 14 (LOD=1.16) at 56 cM, which did not achieve genome-wide significance using the methods of Tang and Siegmund (2001).

Discussion

In this community-based sample of men and women, two biochemical measures of vitamin K status were associated with several nongenetic factors, but were not found to be significantly heritable. Although plasma phylloquinone was significantly inversely associated with %ucOC, as was expected, the only significant nongenetic correlate common to both measures was phylloquinone intake in women. Col lectively these findings suggest that these biochemical measures are influenced by different physiological processes, and much of their interindividual variability have yet to be explained. Vitamin D status was neither associated with the measure

of vitamin K status, nor were there common nongenetic correlates among these three biochemical measures.

The primary correlates of plasma phylloquinone were triglycerides and dietary phylloquinone intake. That the variability in plasma phylloquinone explained by circulating triglycerides was two times greater in men, compared to women, might be attributable to the wider range of circulating triglycerides observed in men ($142\pm97 mgdl^{-1}$) than women ($130\pm72 mgdl^{-1}$) (Booth *et al.*, 2004). Consistent associations have been reported between plasma phylloquinone and triglycerides (Sadowski *et al.*, 1989; McKeown *et al.*, 2002; Booth *et al.*, 2004). Although we report associations between plasma phylloquinone with total cholesterol and LDL, as have been reported elsewhere (Thane *et al.*, 2006), the lack of association between %ucOC and triglyceride concentration suggests that the carboxylation of OC in bone is not directly related to the amount of phylloquinone in circulation (Booth *et al.*, 2004).

The association between age and vitamin K status is inconsistent across studies, which may be reflective of an age-related increase in triglycerides and/or in the case of women, the role of estrogen as a determinant of %ucOC. Plasma phylloquinone concentrations in individuals ≥50 years are consistently greater compared to those <40 years (Sadowski *et al.*, 1989; Sokoll and Sadowski, 1996), but age-related differences among older adults are less evident (Thane *et al.*, 2002). Postmenopausal women had higher %ucOC (that is, indicative of lower vitamin K status) than premenopausal women in our sample, while in the post-menopausal women, the use of HRT was associated with a reduction in %ucOC, as has been reported elsewhere (Lukacs *et al.*, 2006). This may suggest estrogen status to be an important determinant of vitamin K status, independent of diet.

In contrast to vitamin K, vitamin D status, as measured by plasma 25(OH)D concentrations, had a heritability of 28.8% in multivariable-adjusted analysis, consistent with other reports (28–57%) (Hunter *et al.*, 2001). Approximately 24% of the interindividual variability in plasma 25(OH)D also was explained by nongenetic determinants, such as season, vitamin D intakes, waist circumference and HDL cholesterol in men, as well as current use of HRT in women. These correlates of vitamin D status are consistent with previous reports (Hintzpeter *et al.*, 2007). Among women in our study, current use of HRT was also positively associated with vitamin D status, as has been reported elsewhere (Hintzpeter *et al.*, 2007), but 58% of women who were taking HRT also took vitamin D supplements, which may have confounded the interpretation of the results.

This study has several limitations. First, the cross-sectional analyses limit the ability to draw causal inferences between the nongenetic correlates and the measures of vitamin status. While the use of a single plasma phylloquinone as a clinical indicator of long-term vitamin K status is unreliable, it is an acceptable measure for ranking individuals across a range of levels (McKeown *et al.*, 2002). Additionally, the high level of measurement error associated with the FFQ, which is a semiquantitative estimate of dietary intakes, may lead to an underestimation of the variability in the biomarkers of vitamin status that is explained by dietary intakes (Day *et al.*, 2001; Kipnis *et al.*, 2003). The heritability estimates were also based on these same biochemical measures obtained from a single blood draw. The variability in the reported heritability of triglycerides, which is between 36 and 80% in studies using a single blood measurement, has been attributed to biological variation in circulating triglycerides (Beekman *et al.*, 2002; Middelberg *et al.*, 2006). Since phylloquinone is transported on triglyceride-rich lipoproteins and is subject to the same biological variation, we cannot discount potential for variability in the heritability of plasma phylloquinone. We acknowledge that we had limited power to observe genome-wide significance in our linkage scan of vitamin D concentrations because of our modest sample size and a genome scan with 601 microsatellite markers. Furthermore, the heritability of %ucOC was borderline significant in this sample, which may

be the result of a low sample size. We cannot exclude the possibility that some of the heritability estimates could be inflated by unmeasured environmental factors, or interactions between genetic and environmental factors. The Offspring cohort is primarily of white European ancestry and middle-aged to elderly; hence the ability to generalize the correlates and heritability of vitamin K and vitamin D status to different ethnic/racial populations or younger individuals is limited.

In conclusion, vitamin K status, as estimated by plasma phylloquinone and serum %ucOC is associated with non-genetic factors, but is not significantly heritable. Nongenetic factors explain approximately 12–20% of the variability in plasma phylloquinone, while 12–14% of the variability in serum %ucOC is explained by nongenetic factors. The limited number of common nongenetic factors may reflect different physiological processes underlying the two measures of vitamin K status. Similarly, much of the interindividual variability in these two measures was not attributed to known biologically plausible correlates. In contrast, more of the residual variability in plasma 25(OH)D was explained by additive genetic effects. Additional studies are warranted to investigate genetic loci influencing plasma 25(OH)D concentrations.

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Table 1

Participant characteristics

	Men (n=843) Mean (s.d.)	Women (n=919) Mean (s.d.)
Age (years)	59 (9)	58 (7)
Waist circumference (cm)[a]	103(11)	96 (15)
BMI (kgm^{-2})[a]	28.8 (4.6)	27.6 (5.7)
Triglycerides ($mg\ dl^{-1}$)[a]	142(97)	130 (72)
Total cholesterol ($mg\ dl^{-1}$)[a]	192 (35)	206 (36)
HDL cholesterol ($mg\ dl^{-1}$)[a]	46 (13)	60 (16)
LDL cholesterol ($mgdl^{-1}$)	119 (31)	120 (33)
Serum creatinine ($mg\ dl^{-1}$)[a]	1.19 (0.22)	0.98 (0.25)
Alcohol use (oz per month)[a]	205 (365)	64 (132)
Anticoagulant use (%)[a]	3.6	0.9
Smoking (%)	11.7	13.4
Diabetes (%)[a]	15.6	9.7
Hypertension (%)[a]	47.9	39.3
Prevalent CVD (%)[a]	17.3	7.4
Lipid-lowering medication (%)[a]	24.9	16.0
Osteoporosis treatment (%)[a]	0.8	10.4
Postmenopausal (%)	NA	83.3
HRT use, postmenopausal (%)	NA	40.6

Vitamin status	*Mean (s.d.)*	*Range*	*Mean (s.d.)*	*Range*
Plasma phylloquinone ($nmol\ l^{-1}$)[a]	1.6 (2.2)	0.1–35.0	1.3 (1.3)	0.1–16.1
%ucOC[a]	17 (17)	0–91	19 (18)	0–83
Plasma 25 (OH)D ($nmol\ l^{-1}$)	49.0 (17.7)	11.0–146.3	49.2 (19.4)	5.5–128.0
Phylloquinone intake (μg per day)[a]	151 (130)	19–2058	165 (98)	15–799
Vitamin D intake (IU per day)[a]	409 (325)	13–2589	439 (305)	14–2186
(μg per day)	(10 (8))	(0.3–65)	(11 (7))	(0.3–55)

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; HDL, highdensity lipoprotein; HRT, hormone replacement therapy; LDL, low-density lipoprotein; ucOC, undercarboxylated osteocalcin.

[a]Genders significantly different ($P<0.05$).

Table 2

Correlates of log plasma phylloquinone in men and women

	Men			Women		
	Parameter estimate	**P-value**	**Percent variability explained**	**Parameter estimate**	**P-value**	**Percent variability explained**
Age (years)	−0.004	<0.01	0.7		*	
Phylloquinone intake (mg per day)	0.09	<0.01	1.9	0.12	<0.01	7.4
Triglycerides (mg dl^{-1})	0.002	<0.01	15.7	0.001	<0.01	2.8
LDL cholesterol ($mgdl^{-1}$)	0.001	0.01	0.4		*	
Total cholesterol ($mgdl^{-1}$)		*		0.002	<0.01	1.5
Smoking (no/yes)	−0.10	<0.01	0.7		*	
Lipid-lowering medication use (no/yes)	0.06	0.09	0.3	0.09	0.01	0.6
Winter season (compared to summer)	−0.05	0.05	0.4		*	
Total variability explained by clinical characteristics			20.1			12.3

Abbreviation: LDL, low-density lipoprotein.

*Did not meet inclusion threshold of 0.10 in stepwise analyses.

Table 3

Correlates of log serum %ucOC in men and women

	Men			Women		
	Parameter estimate	**P-value**	**Percent variability explained**	**Parameter estimate**	**P-value**	**Percent variability explained**
Phylloquinone Intake (mg per day)		*		−0.07	<0.01	0.8
Waist circumference (cm)	−0.002	0.02	0.6		*	
HDL cholesterol ($mgdl^{-1}$)	0.002	0.09	0.3	−0.001	0.08	0.3
Smoking (no/yes)	−0.08	0.06	0.4	−0.10	<0.01	0.8
Menopause status (no/yes)		NA		0.07	0.04	0.5
HRT use (no/yes)		NA		−0.15	<0.01	3.4
Season						
Summer (reference)						
Fall	−0.18	<0.01	1.5	−0.14	<0.01	0.9
Winter	−0.31	<0.01	4.3	−0.33	<0.01	4.8
Spring	−0.41	<0.01	5.1	−0.35	<0.01	3.1
Total variability explained by clinical characteristics			12.2			14.6

Abbreviations: HDL, high-density lipoprotein; HRT, hormone replacement therapy.

*Did not meet inclusion threshold of 0.10 in stepwise analyses.

Table 4

Correlates of plasma 25(OH)D (nmol l^{-1}) in men and women

	Men			Women		
	Parameter estimate	**P-value**	**Percent variability explained**	**Parameter estimate**	**P-value**	**Percent variability explained**
Age (years)	0.11	0.08	0.3		*	
Vitamin D intake (IU per day)	5.25	<0.01	4.7	6.95	<0.01	8.4
HDL cholesterol ($mgdl^{-1}$)	0.11	0.01	0.5	0.11	<0.01	0.7
Waist circumference (cm)	−0.21	<0.01	3.1	−0.23	<0.01	5.5
Serum creatinine ($mgdl^{-1}$)	5.23	0.04	0.5		*	
HRT use (no/yes)		NA		3.97	<0.01	1.8
Season						
Summer (reference)						
Fall	−10.17	<0.01	5.5	−12.91	<0.01	1.3
Winter	−21.50	<0.01	1.4	−17.19	<0.01	2.8
Spring	−20.79	<0.01	8.7	−17.44	<0.01	3.7
Total variability explained by clinical characteristics			24.7			24.2

Abbreviations: HDL, high-density lipoprotein; HRT, hormone replacement therapy.

* Did not meet inclusion threshold of 0.10 in stepwise analyses.