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Vitamin K and Vitamin D Status: Associations with Inflammatory Markers in the Framingham Offspring Study

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Abstract

In vitro data suggest protective roles for vitamins K and D in inflammation. To examine associations between vitamins K and D and inflammation *in vivo*, we used multiple linear regression analyses, adjusted for age, sex, body mass index, triglyceride concentrations, use of aspirin, lipid lowering and hormone replacement medications, season, and menopausal status. Participants were from the Framingham Offspring Study (n=1381; mean age 59 years; 52% women). Vitamin K status, measured by plasma phylloquinone and phylloquinone intake, was inversely associated with circulating inflammatory markers as a group, and with several individual inflammatory biomarkers ($p< 0.01$). Percent undercarboxylated osteocalcin, a functional measure of vitamin K status, was not associated with overall inflammation, but was associated with C-reactive protein ($p<0.01$). Although plasma 25-hydroxyvitamin D was inversely associated with urinary isoprostanes, an oxidative stress indicator ($p<0.01$), overall associations between vitamin D status and inflammation were inconsistent. The observation that high vitamin K status was associated with lower concentrations of inflammatory markers suggests that a protective role for vitamin K in inflammation merits further investigation.

Keywords

inflammation; vitamin K; vitamin D; epidemiology

Cardiovascular disease and osteoporosis are major age-related health concerns that contribute to morbidity and mortality in the elderly (1). Inflammation is characteristic of these two chronic diseases (2, 3). Several pro-inflammatory cytokines, such as interleukin-6,

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osteoprotegerin, and tumor necrosis factor-α, are implicated in the process of vascular calcification and the regulation of bone remodeling (4, 5). The reciprocal effect of inflammatory cytokines on vascular and bone tissue may partially explain the simultaneous manifestation of bone loss with vascular calcification (6, 7).

Vitamins K and D are fat-soluble vitamins that have been implicated in both cardiovascular and bone health, and more recently in the activity of proinflammatory cytokines. Vitamin K is an established cofactor in the γ-carboxylation of vitamin K dependent proteins. Two vitamin K-dependent proteins, osteocalcin and matrix-gla protein, are present in skeletal and vascular tissue, respectively (8), and a role for vitamin K in cardiovascular and skeletal health has been reported (9, 10). Vitamin K also is associated with a decreased production of proinflammatory cytokines in *in vitro* studies (11, 12, 13). With the exception of one small study done in patients with chronic kidney disease (14), reports of the relations between vitamin K and inflammatory cytokines to date are primarily *in vitro* (11, 13)

The importance of vitamin D for optimal calcium homeostasis and bone metabolism is well recognized (15), and there is some suggestion of a role for vitamin D in reducing cardiovascular disease risk (16, 17). Furthermore, *in vitro* data suggest that the biologically active form of vitamin D [$1,25(OH)_2D$, or calcitriol] has several immunomodulatory functions, including suppression of pro-inflammatory cytokine expression and regulation of immune cell activity (18). *In vivo*, vitamin D supplementation has been associated with a reduction in proinflammatory cytokines in patients with osteoporosis (19) and heart failure (20), but not in healthy individuals (21).

We hypothesized that vitamin K and vitamin D status are inversely associated with measures of inflammation in older adults. To test this hypothesis, we examined cross-sectional associations between dietary and biochemical measures of vitamin K status (plasma phylloquinone, serum percent undercarboxylated osteocalcin, and phylloquinone intake) and vitamin D status (plasma 25-hydroxyvitamin D), and a panel of circulating proinflammatory biomarkers (C-reactive protein, CD40 ligand, P-selectin, osteoprotegerin, tumor necrosis factor-α, tumor necrosis factor receptor-2, intercellular adhesion molecule-1, interleukin-6, monocyte chemoattractant protein, myeloperoxidase, urinary isoprostanes, fibrinogen, and lipoprotein phospholipase A2 mass and activity) in the Framingham Offspring Study, a community-based sample of men and women.

MATERIALS AND METHODS

The design and selection criteria for the Framingham Offspring Study have been described elsewhere (22). Every four to eight years, Offspring participants undergo extensive examinations, which include medical history, medication use, physical examinations, blood biochemistries, and assessment of cardiovascular risk factors. Participants were excluded from the present investigation if they were currently taking steroidal anti-inflammatory medication (n=219), anticoagulant medication (n= 41), did not have a valid FFQ (n=59), or did not have all of the measures for the inflammatory markers (n=271), excluding tumor necrosis factor-α or urinary isoprostanes. Of the 1850 eligible participants, data on 1381 individuals (669M, 712F) were available for analysis.

Self-reported information was routinely collected about alcohol use, dietary intake, and, for women, menopausal status and hormone replacement therapy. Intakes from foods and supplements, including phylloquinone (vitamin K_1) and vitamin D, were assessed using the Willett Food Frequency Questionnaire (23). Questionnaires were considered invalid and excluded from analysis if the participant reported energy intake <2.51 and >16.74 MJ/day (600 and 4,000 kcal/day, respectively) or if they had >12 food items blank (n=59). This

study was approved by the Institutional Review Boards at Tufts University and Boston University Medical Center. All participants signed informed consent.

Fasting blood samples (>10 hour) were collected between 1997 and 1999, and plasma/serum was stored at −80°C until analysis. The measures for assessment of vitamin status straddled the end of cycle six (1995-1998) (n=694) and the beginning of cycle seven (1998-2001) (n=687) because of the funding award date of the vitamin assay research grant. Vitamin K status was assessed by measures of plasma phylloquinone and serum percent undercarboxylated osteocalcin. A high percent undercarboxylated osteocalcin is indicative of low vitamin K status in bone. Plasma phylloquinone concentrations were determined using reverse phase high performance liquid chromatography as described elsewhere (24). Low and high control specimens had average values of 0.56 and 3.15 nmol/L, with coefficients of variation (CVs) of 15.2 and 10.9% respectively. Serum total osteocalcin and undercarboxylated osteocalcin were measured by radioimmunoassay, using Gundberg's method (25). The undercarboxylated osteocalcin, a functional marker of vitamin K status, was determined by the amount of osteocalcin that did not bind to hydroxyapatite *in vitro*. Since this binding varied with the amount of total osteocalcin in the sample, the undercarboxylated osteocalcin was expressed as percentage of total osteocalcin (26). The CVs for the three control serums with an average total OC result of 3.4, 7.1, and 11.9 μg/L were 22.3, 12.8, and 7.8% respectively. Vitamin D status was estimated by measuring plasma 25(OH)D concentration, the standard measure of vitamin D status which reflects both sun-induced synthesis in the skin and dietary intakes, using RIA (DiaSorin, Stillwater MN). The CVs for the control values of 36 and 137 nmol/L were 8.5% and 13.2%, respectively.

The following inflammatory biomarkers were measured in duplicate from samples taken during the 7th exam cycle (1998-2001) using commercially available enzyme-linked immunoassay kits: plasma CD40 ligand [Bender MedSystems, Burlingame CA; intra-assay CV of 5.2(6.4) percent], plasma P-selectin [R&D systems Minneapolis MN; intra-assay CV of 3.2(2.4) percent], plasma osteoprotegerin [BioMedica Gesellschaft mbH, Vienna Austria, distributed by ALPCO Diagnostics; intra-assay CV of 3.7(2.9) percent], plasma tumor necrosis factor-α [R&D system; 8.8 percent], plasma tumor necrosis factor receptor-2 [R&D systems; intra-assay CV of 2.3(1.6) percent], serum soluble intercellular adhesion molecule-1 [R&D systems; intra-assay CV of 3.9(2.9) percent], serum interleukin-6 [R&D systems; intra-assay CV of 3.1(2.2) percent], serum monocyte chemoattractant protein [R&D systems; intra-assay CV of 3.8(3.3) percent], serum myeloperoxidase [Oxis, Foster City, CA; intra-assay CV of 3.2(2.7) percent], and urinary isoprostanes (27) [Cayman, Ann Arbor, MI; intra-assay CV of 9.6(6.8 percent). Single determinants of serum C-reactive protein were made using a high sensitivity assay (Dade Behring, Deerfield IL; intra-assay CV of 3.2 percent) (28). Fibrinogen was measured in duplicate using the clot time method of Claus (29) [Diagnostica Stago reagents; intra-assay CV of 1.1(1.1) percent]. Lipoprotein phospholipase A2 mass and activity were measured by diaDexus and GlaxoSmithKline, respectively [intra-assay CV of 4.3 and 4.3 (7.8) percent respectively].

Statistical analyses

To improve the symmetry of skewed distributions, all of the inflammatory markers and markers of vitamin K status, including intake, were logarithmically transformed for analysis. An inflammation index was generated to create an indicator of overall inflammation by summing the normalized deviates of the individual markers of inflammation (30). The inflammation index was correlated with the log of each individual marker (Pearson r = 0.12-0.62; all $p < 0.01$). The measures of vitamin K status and vitamin D status were used as continuous regressor variables (each in separate models) and the biomarkers of inflammation were used as the dependent variables (one at a time) in multivariable-adjusted

linear regression models. Although we assessed associations between measures of vitamin K status and vitamin D status with plasma tumor necrosis factor-α and urinary isoprostanes, these two inflammatory markers were not included in the inflammation index because the number of individuals with these measures (n=992 and 1087 respectively) was less than the other measures (specimen collection for their assays occurred later in the examination cycle); including them in the inflammation index would have reduced the sample size for the inflammation index models.

Additional covariates, which were taken from the same exam cycle (six or seven) as the measures of vitamin K and D status, included triglycerides, body mass index, aspirin use, lipid lowering medication use, menopause status, and hormone replacement therapy. The covariates selected were those that were determined to be statistically significant correlates with the corresponding biochemical measures of vitamin status (Shea et al, submitted; unpublished data). Since seasonal differences in vitamin D status and percent undercarboxylated osteocalcin have been reported previously (31, 32), season was included as a covariate in the models to assess associations between vitamin D status and percent undercarboxylated osteocalcin and the markers of inflammation. We chose to report changes in inflammation associated with a two-fold increase in plasma phylloquinone, phylloquinone intake, serum percent of undercarboxylated osteocalcin, and plasma 25-hydroxyvitamin D, since these increments of change were deemed plausible based on the means and ranges of the vitamin status measures for this sample. Our primary analyses focused on the associations between measures of vitamin status and the inflammation index. Associations between measures of vitamin status and individual markers of inflammation were considered in secondary analyses. In subsequent analyses we excluded individuals with prevalent cardiovascular disease at examination seven. A p-value ≤0.01 was considered to be statistically significant. All analyses were performed using SAS 9.1.

Effect modification by sex and age was tested for each inflammatory marker by entering product terms (age X vitamin status or sex X vitamin status) into the multiple linear regression models. We also checked effect modification by exam cycle because the measures of vitamin K status and vitamin D status were not consistently taken from the same exam as the measures of inflammation. To reduce likelihood of type 1 error, we used a Bonferroni adjustment and considered interactions to be significant if $p<0.003$. None of interaction terms we tested were significant at this level, and therefore were not included in the final statistical models.

RESULTS

Study participants characteristics are typical of a community based cohort (table 1). Participants were middle-aged to elderly (mean age 59, minimum-maximum 35-89 yrs) and were 51.6% female. Reported use of osteoporosis medication was 6.2 percent and use of lipid lowering medication was 20.5 percent. Mean (± SD) concentrations of plasma phylloquinone, plasma 25(OH)D, and serum percent undercarboxylated osteocalcin were within previously reported reference ranges for these assays (26, 33, 34).

Vitamin K status, as measured by plasma phylloquinone and phylloquinone intake, was significantly inversely associated with the overall inflammation index, which represented the sum of the normalized deviates of the individual markers (table 2). Secondary analyses of the individual markers demonstrated significant ($p<0.01$) associations with 5 of the 14 markers (table 2). A two-fold higher plasma phylloquinone concentration was associated with 15 percent lower CD40 ligand, three percent lower intracellular adhesion molecule-1, eight percent lower interleukin-6, four percent lower serum osteoprotegerin, and four percent lower tumor necrosis factor receptor-2 concentrations in multivariable-adjusted

analyses. Usual dietary phylloquinone intake was also significantly (p=0.01) inversely associated with C-reactive protein, fibrinogen, interleukin-6, lipoprotein phospholipase A2 mass, myeloperoxidase, osteoprotegerin, and urinary isoprostanes (table 2). The percent undercarboxylated osteocalcin and plasma 25-hydroxyvitamin D were not significantly associated with overall inflammation, as indicated by the inflammation index (tables 2 and 3). However, plasma 25-hydroxyvitamin D was significantly inversely associated with urinary isoprostanes (p<0.01), a measure of oxidative stress, which was not included in the inflammation index.

Exclusion of individuals with prevalent cardiovascular disease did not change associations between plasma phylloquinone and markers of inflammation. The association between percent undercarboxylated osteocalcin and C-reactive protein was attenuated (p=0.02). However, in these same individuals, the inverse association between phylloquinone intake and C-reactive protein reached statistical significance (*p*=0.009), and plasma 25-hydroxyvitamin D was significantly inversely associated with interleukin 6 (*p*=0.009) (data not shown).

DISCUSSION

In our community-based sample, there was an inverse association between vitamin K status, as measured by plasma phylloquinone and phylloquinone intake, and overall circulating markers of inflammation. In the same cohort, vitamin D status, as measured by plasma 25-hydroxyvitamin D was not consistently associated with systemic inflammatory markers.

Our findings are in general agreement with *in vitro* studies that report a decrease in production of inflammatory markers, including interleukin-6, by human macrophage and fibroblast cells with vitamin K treatment (11, 13). The current study expands our knowledge on this putative role of vitamin K because our panel consisted of 14 biomarkers of inflammation, many of which have not been previously studied with respect to vitamin K. In contrast, our findings in this community sample did not support the *in vitro* data that suggest treatment with different forms of vitamin D may reduce production of inflammatory cytokines (35, 36)., although we acknowledge that in *in vitro* studies the active vitamin D metabolite (calcitriol) was more effective in influencing cytokine production, while *in vivo* 25-hydroxyvitamin D was used as the estimate of vitamin D status, since circulating concentrations of calcitriol are tightly controlled. Most studies that report a beneficial effect of vitamin D on inflammatory cytokines were based on individuals who were diagnosed with chronic diseases (19, 20, 37). The Framingham Offspring study is a generally healthier, older cohort. Our results are similar to those of a single study by Gannage-Yared, which reported that supplementation with 25-hydroxyvitamin D did not influence concentrations of circulating cytokines in a small sample (n=47) of healthy post-menopausal women (21).

The mechanisms by which vitamin K influences biomarkers of inflammation are not known, although there is some suggestion that vitamin K suppresses inflammation by decreasing expression of genes for individual cytokines, such as interleukin-6 and osteoprotegerin (13,38). Interleukin-6 and osteoprotegerin were the two markers that were inversely associated with both plasma phylloquinone and phylloquinone intake in our study. Vitamin K, interleukin-6 and osteoprotegerin are all implicated in bone resorption and the regulation of vascular calcification (39-41). Lower osteoprotegerin concentrations in skeletal and vascular tissue are associated with an increase in bone resorption and vascular calcification (42), whereas patients with osteoporosis and cardiovascular disease are reported to have increased concentrations of circulating osteoprotegerin (43, 44). These observed increases in circulating osteoprotegerin concentrations in patients with skeletal and/or vascular pathology

are proposed to be an incomplete compensatory response to factors leading to increased bone resorption and/or atherosclerosis (45).

It has been assumed that any role of vitamin K in bone or cardiovascular health is mediated through its action as a co-factor in the carboxylation of vitamin K-dependent proteins, including osteocalcin. Whereas we observed an association between phylloquinone intake and concentrations and overall inflammation, there was no association between percent undercarboxylated osteocalcin and overall inflammation. The percent undercarboxylated osteocalcin is used as a measure of the amount of vitamin K available for carboxylation in extra-hepatic tissues. It is possible that vitamin K modulates inflammation by a mechanism that does not involve its role in γ-carboxylation. The *in vitro* studies suggest a direct effect of vitamin K on gene expression that is not related to carboxylation of vitamin K-dependent proteins. Further, it has been shown that vitamin K has a protective effect against oxidative stress that is independent of carboxylation, which may be an alternative anti-inflammatory mechanism associated with vitamin K (46). The variation in biochemical measures of vitamin K status is determined by both dietary and non-dietary factors (Shea et al, submitted; unpublished data), which may explain why the significance of associations between biochemical measures of vitamin K status and phylloquinone intake with individual markers of inflammation were not always consistent.

Calcitriol has been postulated to regulate immune function through the nuclear vitamin D receptor, which is expressed by most cells of the immune system. *In vivo*, the reported immunoregulatory effects of active vitamin D occur at very high concentrations (18). Since the conversion of 25-hydroxyvitamin D to calcitriol is tightly regulated, and range of circulating calcitriol concentrations is narrow in the absence of chronic disease (47), the reported effects of vitamin D on inflammation may not have been detected by our measures of vitamin D status in this community sample of healthy individuals, similar to the findings of others (21). Vitamin D status, as measured by plasma 25-hydroxyvitamin D, was inversely associated with urinary isoprostanes, a measure of oxidative stress. This inverse association is in general agreement with Lin et al. who have reported a protective role of vitamin D in reducing oxidative stress by acting to terminate the lipid peroxidation chain reaction (48).

Strengths and Limitations

There are several limitations to this study. Importantly, we tested associations between multiple biomarkers of inflammation and measures of vitamin K and D status, and we can not discount that significant associations may be due to chance because of multiple testing. By using a $p \leq 0.01$ as the level of significance, we hoped to decrease this possibility. Certain health conditions, such as prevalent cardiovascular disease, are associated with increases in inflammatory biomarkers and with unhealthy lifestyle patterns that may influence vitamin K and D status. However, results were not substantively changed in analyses excluding individuals with diagnosed cardiovascular disease. Conversely, phylloquinone is in foods generally consumed as part of a healthy diet (49), which may partially account for the reduction in inflammatory markers associated with higher intakes of phylloquinone. Furthermore, although *in vitro* data suggest that calcitriol, the biologically active form of vitamin D, is involved in immunoregulation, we did not measure this form of vitamin D since circulating levels are tightly regulated. The Offspring cohort participants are primarily older, of northern European descent, and predominantly reside in the Northeastern United States; we can not generalize our findings to other ethnic/racial groups, younger individuals, or individuals residing in sunnier climates. We acknowledge that the measurement of some of the vitamin concentrations at examination six (49 percent), about three years prior to the examination seven inflammatory markers may have led to some misclassification of the association; however, it should be noted that we did not observe significant effect

modification by examination. The cross-sectional study design precludes causal inferences in interpreting our results. Although we modeled the relation of vitamins to inflammatory markers (dependent variables), we acknowledge that inflammatory markers may influence vitamins concentrations, or both may be related via other unmeasured intermediate factors. Finally, although the associations were statistically significant, the clinical significance of the very modest changes observed is uncertain. Balanced against these limitations are the novelty of the analyses, the routine ascertainment of vitamins, inflammatory biomarkers and covariates, in community-based individuals.

Clinical and research implications

Our findings provide one potential alternative mechanism for a putative, protective effect of vitamin K in the progression of cardiovascular disease and osteoporosis, since both diseases are characterized by inflammation. Limited *in vitro* data support the inverse association between vitamin K and interleukin-6, and this may influence the association between vitamin K and other cytokines, such as osteoprotegerin. Further research to better elucidate mechanisms underlying the associations between vitamin K and inflammatory cytokines is warranted.

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TABLE 1

Participant characteristics (n=1381)

Clinical Characteristics	mean [SD] or n [%]
Age (years)	59 [8]
Females – n [%]	712 [51.6]
Body mass index (kg/m^2)	28.1 [5.2]
Triglycerides (mg/dL)	135 [86]
Waist circumference (cm)	39.1 [5.5]
Alcohol use (oz/month)	139 [289]
Smoking -n [%]	173 [12.5]
Diabetes - n [%]	159 [11.5]
Hypertension -n [%]	584 [42.3]
Post-menopausal - n [%]	593 [83.4]
Hormone replacement therapy (post-menopausal) - n [%]	239 [33.6]
Lipid lowering treatment - n [%]	283 [20.5]
Osteoporosis treatment n [%]	86 [6.2]
Prevalent cardiovascular disease n [%])	161 [11.7]

Vitamin K status	mean [SD]	minimum-maximum
Plasma phylloquinone (nmol/L)	1.5 [1.9]	0.1-25.6
Percent undercarboxylated osteocalcin	17.4 [16.8]	0-79.7
Phylloquinone intake (μg/d)	156 [118]	17-2059
Vitamin D status		
Plasma 25 (OH)D (nmol/L)	49.4 [18.6]	5.5-146.3
Vitamin D intake (IU/d)	426 [317]	23-2589
Measures of Inflammation		
Inflammation Summary Statistic	−0.3 [4.9]	−12.1-28.4
CD40 ligand (ng/mL)	3.4 [4.8]	0.1-29.5
C-reactive protein (mg/L)	3.8 [5.3]	0.2-66.2
Fibrinogen (mg/dl)	375 [71]	181-676
Intercellular adhesion molecule-1 (mg/mL)	259 [83]	130-1328
Interleukin-6 (pg/mL)	3.6 [3.8]	0.4-51.2
Lipoprotein phospholipase A2 activity (nmol/mL/min)	144 [36]	41-364
Lipoprotein phospholipase A2 mass (ng/mL)	302 [95]	78-886
Monocyte chemoattractant protein (pg/mL)	322 [121]	31-2140
Myeloperoxidase (mg/mL)	47.9 [32.5]	4.9-377.0
Osteoprotegerin (pmol/L)	5.5 [1.8]	0.6-26.9
P-selectin (ng/mL)	36.1 [14.1]	2.5-175.8
Tumor necrosis factor-α (pg/mL)	1.4 [1.3]	0.3-21.1
Tumor necrosis factor receptor-2 (pg/mL)	2158[769]	892-8215
Urinary isoprostanes (pg/mL)	1559 [1369]	31-11125

TABLE 2

Association between log measures of vitamin K status and markers of inflammation in men and women

	Estimated change in inflammation marker per 2-fold change in vitamin K status					
	Increase in plasma phylloquinone (nmol/L)		Increase in phylloquinone intake (μg/d)		Increase in serum percent undercarboxylated osteocalcin†	
	Estimated change*	*p* value	Estimated change	*p* value	Estimated change †	*p* value
Inflammation Summary Statistic	0.62	**<0.001**	0.78	**<0.001**	1.06	0.53
CD40 ligand	0.85	**0.007**	0.96	0.25	1.01	0.90
C-reactive protein	0.90	0.03	0.92	0.014	0.85	**0.007**
Fibrinogen	0.98	0.08	0.99	0.03	1.01	0.89
Intercellular adhesion molecule-1	0.97	**0.006**	1.00	0.51	0.98	0.16
Interleukin-6	0.92	**0.009**	0.94	**0.008**	0.96	0.18
Lipoprotein phospholipase A2 activity	1.01	0.47	0.99	0.14	1.01	0.41
Lipoprotein phospholipase A2 mass	1.01	0.48	0.98	0.01	1.04	0.02
Monocyte chemoattractant protein	0.97	0.13	0.98	0.07	1.04	0.03
Myeloperoxidase	0.97	0.26	0.94	**0.001**	0.99	0.65
Osteoprotegerin	0.96	**0.008**	0.97	**0.004**	1.00	0.75
P-selectin	0.99	0.75	0.99	0.24	1.03	0.12
Tumor necrosis factor-α	1.00	0.99	0.99	0.65	1.04	0.13
Tumor necrosis factor receptor-2	0.96	**0.004**	0.99	0.10	0.98	0.10
Urinary isoprostanes	0.88	0.02	0.89	**<0.001**	0.98	0.68

*Covariates: sex, age, body mass index, triglyceride concentration, aspirin use, lipid lowering medication use, menopause status, hormone replacement therapy use

†Adjusted for season as well as covariates above.

TABLE 3

Association between measures of vitamin D status and markers of inflammation in men and women

	Estimated change in inflammation marker per 2-fold increase in vitamin D status*			
	Plasma 25-hydroxyvitamin D (nmol/L)		Vitamin D intake (IU/d)	
	Estimated change†	*p* value	Estimated change†	*p* value
Inflammation Summary Statistic	0.98	0.06	0.98	0.73
CD40 ligand	1.00	0.83	1.09	**0.002**
C-reactive protein	1.01	0.23	0.98	0.63
Fibrinogen	1.00	0.19	1.00	0.16
Intercellular adhesion molecule-1	1.00	0.25	1.00	0.96
Interleukin-6	1.00	0.02	1.00	0.90
Lipoprotein phospholipase A2 activity	1.00	0.45	0.99	0.45
Lipoprotein phospholipase A2 mass	1.00	0.14	0.99	0.35
Monocyte chemoattractant protein	1.00	0.07	0.99	0.69
Myeloperoxidase	1.00	0.10	0.98	0.19
Osteoprotegerin	1.00	0.74	1.00	0.93
Plasma tumor necrosis factor receptor-2	1.00	0.79	1.00	0.83
P-selectin	1.00	0.97	0.97	0.30
Tumor necrosis factor α	1.00	0.39	0.98	0.29
Urinary isoprostanes	0.997	**0.007**	0.79	**0.01**

*Serum measures, unless otherwise indicated

†Covariates: sex, age, body mass index, triglyceride concentration, aspirin use, lipid lowering medication use, season, menopause status, hormone replacement therapy use