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Circulating Uncarboxylated Matrix Gla Protein Is Associated with Vitamin K Nutritional Status, but Not Coronary Artery Calcium, in Older Adults[1,2,3,4]

M. Kyla Shea[5], Christopher J. O'Donnell[6,7], Cees Vermeer[8], Elke J. P. Magdeleyns[8], Michael D. Crosier[9], Caren M. Gundberg[10], José M. Ordovas[11,12], Stephen B. Kritchevsky[5], and Sarah L. Booth[11,*]

[+] Author Affiliations

*To whom correspondence should be addressed. E-mail: sarah.booth@tufts.edu.

Abstract

Matrix Gla protein (MGP) is a calcification inhibitor in vascular tissue that must be carboxylated by vitamin K to function. Evidence suggests circulating uncarboxylated MGP (ucMGP) is elevated in persons with disease characterized by vascular calcification. The primary purpose of this study was to determine cross-sectional and longitudinal associations between plasma ucMGP, vitamin K status, and coronary artery calcium (CAC) in older adults without coronary heart disease. Genetic determinants of ucMGP were also explored. Cross-sectional associations among baseline plasma ucMGP, vitamin K status biomarkers [plasma phylloquinone, uncarboxylated prothrombin (PIVKA-II), serum uncarboxylated osteocalcin (%ucOC)], CAC, and plausible genetic polymorphisms were examined in 438 community-dwelling adults (60–80 y, 59% women). The effect of phylloquinone supplementation (500 μg/d) for 3 y on plasma ucMGP was determined among 374 participants. At baseline, plasma phylloquinone was lower and %ucOC and PIVKA-II were greater across higher plasma ucMGP quartiles (all $P < 0.001$, age-adjusted). Major allele homozygotes for MGP *rs*1800801 and *rs*4236 had higher plasma ucMGP than heterozygotes or minor allele homozygotes. ($P \leq 0.004$). The decrease in plasma ucMGP was greater in the 190 participants who received phylloquinone (mean ± SD) (−345 ± 251 pmol/L) than in the 184 who did not (−40 ± 196 pmol/L) ($P < 0.0001$). CAC did not differ according to ucMGP quartile ($P = 0.35$, age-adjusted). In the phylloquinone-supplemented group, the 3-y change in ucMGP was not associated with the 3-y change in CAC [unstandard β (SE) = −0.02 (0.02); $P = 0.44$]. Plasma ucMGP was associated with vitamin K status biomarkers and was reduced following phylloquinone supplementation, suggesting it may be a useful marker of vitamin K status in vascular tissue. Plasma ucMGP did not reflect CAC in healthy older adults.

Introduction

Coronary artery calcium (CAC)[13] predicts cardiovascular disease morbidity and mortality independent of established risk factors (1, 2). Proteins that inhibit calcification have been identified as important negative regulators of this pathology. Matrix Gla protein (MGP) is a calcification inhibitor found in vascular and other soft tissue (3, 4). In mice, targeted deletion of the MGP gene results in rapid and complete arterial calcification, resulting in death by 6 wk (5). For MGP to function, it must be partially γ-carboxylated, which requires vitamin K. MGP is synthesized in the uncarboxylated form (ucMGP), and without sufficient vitamin K, MGP remains uncarboxylated and does not inhibit calcification (6, 7). The degree of γ-carboxylation required for MGP to inhibit calcification in humans is not known. ucMGP is elevated in human sclerotic arterial tissue and the carboxylated MGP form is more abundant in healthy vascular tissue (8), which provides evidence that a lack of functional MGP increases risk for vascular calcification. In addition to being carboxylated, MGP can also undergo a post-translational phosphorylation, which is also thought to contribute to its functionality (9). The phosphorylated ucMGP accumulates in the vessel wall, whereas the dephosphorylated form is detectable in plasma (10).

Although the role of MGP in vascular calcification has been elucidated in animal models, there have been few human studies. Data are conflicting as to whether total MGP in circulation, which reflects the total pool of MGP regardless of its carboxylation status, differs between patients with known cardiovascular disease and healthy controls (11–13). Distinguishing the uncarboxylated and carboxylated fractions of MGP in the circulation may better clarify the role of the functional forms of MGP in CAC (4). Evidence suggests the amount of ucMGP in the circulation is increased among patient populations characterized by pathologic soft-tissue calcification (9, 10, 14). However, the studies that have examined the association between plasma ucMGP and vascular calcification thus far are limited to case–control comparisons or specific disease populations (9, 10, 14). To evaluate the utility of ucMGP as a predictive marker of CAC, it is necessary to examine its association with CAC in a population free of clinical disease.

In a randomized controlled trial of vitamin K supplementation, we found that older community-dwelling adults who adhered to phylloquinone (vitamin K1) supplementation had less CAC progression over 3 y (15). The assessment of ucMGP from archived baseline and postintervention blood samples from this study provided an opportunity to determine the correlates of circulating ucMGP, the effect of phylloquinone supplementation on plasma ucMGP, and the association between plasma ucMGP and CAC progression after 3 y. We hypothesized that increased circulating ucMGP would be associated with poorer vitamin K status and positively associated with CAC in older, community-dwelling adults.

The impact of MGP on regulation of calcification in humans appears to have a genetic component (16). Genotype data were available for participants in the parent study (16, 17), so the genetic determinants of plasma ucMGP were explored in a secondary analysis.

Methods

Study design and participants.

A total of 452 community-dwelling men and women (age range 60–80 y; 421 whites, 14 blacks, 4 Hispanics, 11 Asians, and 2 Native Americans) participated in a randomized controlled trial designed to determine the effect of vitamin K supplementation (500 μg/d for 3 y) on CAC and age-related bone loss, as previously described (18). Prior to enrollment, all participants completed a detailed medical history questionnaire. Participants were generally in good health and free from clinical cardiovascular disease and laboratory evidence of kidney or liver disease or osteoporosis, and not taking warfarin. All participants provided written informed consent.

Equal numbers of men and women were randomized to receive a daily multivitamin with 500 μg of phylloquinone or the same multivitamin without phylloquinone. All participants also received a second supplement that contained 600 mg of elemental calcium and 10 μg (400 IU) of cholecalciferol (15, 18). The nutrient composition of all supplements is described in detail elsewhere (15, 18). Supplements were manufactured specifically for this study by Hermes Arzeneimittel. Upon receipt, the phylloquinone content of the phylloquinone-containing supplements was (mean ± SD) 564 ± 77 μg; at 19 mo, the final phylloquinone content was 428 ± 32 μg (18).

Of the 452 participants enrolled, 438 (258 women) had measures of ucMGP and CAC at baseline and were included in cross-sectional analyses. Of these, 374 also had measures of ucMGP and CAC at follow-up and were included in longitudinal analyses (51 did not complete the study and 27 had missing CAC scans at follow-up). The participants excluded from the longitudinal analysis had significantly higher CAC, measured according to agatston score (AS; the standard scoring method for CAC) (19), at baseline [median (IQR) AS = 85 (348) vs. 27 (181); P = 0.007; based on Wilcoxon's Rank Sum test] and slightly lower HDL cholesterol [(mean ± SD) 1.4 ± 0.3 vs. 1.5 ± 0.4 mmol/L; P = 0.05 (independent samples t test)]. Otherwise, these participants did not differ from the 374 who were included in longitudinal analysis (all $P \geq 0.10$).

Biochemical measurements.

All blood samples were drawn after a 12-h fast and dedicated aliquots were stored at −80°C until the time of analysis.

MGP.