Brief Review

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Vitamin K–Dependent Matrix Gla Protein as Multifaceted Protector of Vascular and Tissue Integrity

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● **Online Data Supplement**

Cardiovascular disease remains the leading cause of mortality and is worldwide directly responsible for ≈18 million deaths, representing over 30% of all-cause mortality globally.[1] Calcification of the conduit arteries is a hallmark of cardiovascular disease[2,3] and an independent risk factor for myocardial infarction, stroke, and cardiovascular death.[2,3] Vascular smooth muscle cells and the endothelium synthesize a small secretory protein (11 kD), which is named MGP (matrix Gla protein), because it contains 5 γ-carboxyglutamate (Gla) amino-acid residues (Figure 1).[4] Activation of MGP requires 2 posttranslational modifications: serine phosphorylation and vitamin K–dependent γ-glutamate carboxylation (Figure 1).[4,5]

Active MGP, once released into the extracellular space, acts as a local inhibitor of calcification (Figure 2). Mice lacking the *MGP* gene die within 2 months because of widespread arterial calcification that leads to disintegration and rupture of the arterial wall and massive bleeds.[6] Selectively reintroducing MGP expression in the liver of the MGP-deficient mice resulted in circulating MGP levels 6- to 10-fold higher than in wild-type animals.[7] The MGP originating from the transgene conserved its biological activity in vitro but did not inhibit arterial calcification.[7] Studies in rodents[6,8] showed MGP expression at the RNA and protein level in multiple organs. The widespread expression of MGP points to a role of MGP that by far exceeds its well-known function as local inhibitor of calcification. Recent research confirmed this concept, usually by measuring plasma dp-ucMGP (desphospho-uncarboxylated MGP), a biomarker reflecting poor vitamin K status.[9] This Brief Review summarizes the growing evidence implicating activated MGP in maintaining microvascular integrity and preserving the structure and function of vital organs, including the retina,[10–13] kidney,[14–17] and heart.[18–20] A PubMed search limited to literature sources published in English after 1988, using as key words in title or abstract matrix Gla protein combined with one of the following key words calcification OR arter* OR heart OR kidney OR retin* OR mortality OR bone informed this review and revealed the involvement of MGP in a wide spectrum of age-related chronic diseases extending beyond the cardiovascular field.

dp-ucMGP as Biomarker of Vitamin K Status

VKDPs (vitamin K–dependent proteins) can be categorized into hepatic and extrahepatic VKDPs.[21] Hepatic VKDPs are mainly involved in blood coagulation. Extrahepatic VKDPs have various functions because their Gla residues have high affinity for calcium. The extrahepatic VKDP osteocalcin regulates bone formation[22] and mineralization.[23] Once carboxylated, the negatively charged γ-carboxyglutamic acid residues bind positively charged calcium ions at the surface of bone mineral. The plasma level of osteocalcin, therefore, reflects bone turnover.[24] MGP is also an extrahepatic VKDP. Vascular stress upregulates MGP transcription as reflected by circulating t-ucMGP (total uncarboxylated MGP).[25] t-ucMGP mainly consists of phosphorylated MGP and is sequestered at sites of arterial calcification.[26,27] In healthy volunteers (Figure S1 in the online-only Data Supplement), MGP circulates in 3 conformations: dp-ucMGP, desphospho-carboxylated MGP, and phosphorylated-carboxylated MGP. ucMGP coprecipitates with unphosphorylated MGP but not with phosphorylated MGP; carboxylated MGP coprecipitates with both unphosphorylated and phosphorylated MGP. However, these experiments do not explain the 10 000-fold difference in circulating dp-ucMGP and t-ucMGP.[28] dp-ucMGP is the best single biomarker of vitamin K deficiency, outperforming ratios of various MGP moieties.[28] In the general population, circulating dp-ucMGP increases with age and with worsening of renal function (Figure 3), which might be explained in part by vitamin K deficiency.

Dietary sources of vitamin K include leafy vegetables (phylloquinone; vitamin K_1) and fermented foods (menaquinones; vitamin K_2), such as cheese and soybeans fermented with *Bacillus subtilis var. natto* (natto).[29] In humans, gut bacteria also synthesize vitamin K.[30] In contrast to dietary vitamins, which are absorbed in the proximal tract of the small intestine, the predominant uptake of microbiotically synthesized vitamins occurs in the colon.[31] Abuse of antibiotics impairs the synthesis of vitamin K by the gut flora.[15]

Measurement of circulating levels of vitamin K is rarely done in clinical practice, because of the complexity of the assay and the lack of a high-throughput method[32] and

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This article was sent to Toshiro Fujita, Consulting Editor, for review by expert referees, editorial decision, and final disposition.

The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.119.12412.

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(*Hypertension*. 2019;73:1160-1169. DOI: 10.1161/HYPERTENSIONAHA.119.12412.)

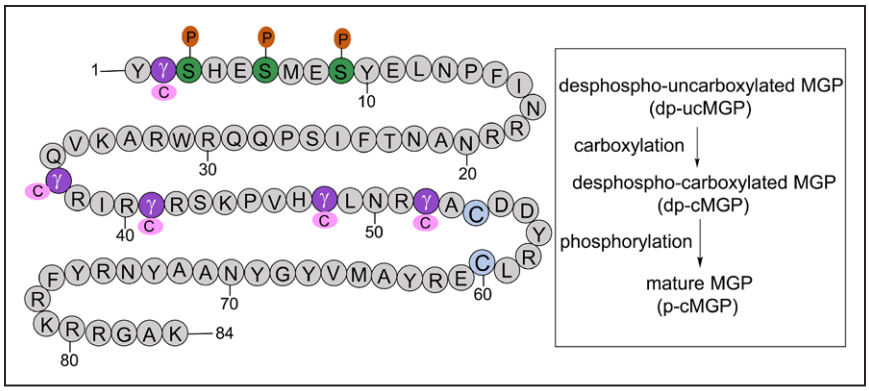

Figure 1. Full activation of MGP (matrix Gla protein) requires 2 posttranslational modifications, that is, vitamin K–dependent carboxylation of glutamate at positions 2, 37, 41, 48, and 52 and serine phosphorylation at positions 3, 6, and 9 by a Golgi-casein kinase. MGP therefore occurs in 4 conformations: dp-ucMGP (desphospho-uncarboxylated MGP), dp-cMGP (desphospho-carboxylated MGP), p-ucMGP (phosphorylated-uncarboxylated MGP), and p-cMGP (phosphorylated-carboxylated MGP). Adapted from Hackeng et al[4] with permission. Copyright ©2008, John Wiley and Sons.

because plasma levels only reflect dietary intake (vitamin K_1 and K_2) and production by the intestinal microflora (vitamin K_2) without providing any information on the activity of MGP. In research settings, the concentration of plasma dp-ucMGP was usually assessed using the inaKtif MGP iSYS kit (Immunodiagnostic Systems Ltd, Boldon), which is a dual-antibody test based on a sandwich ELISA approach.[28]

Macrocirculatory Traits

Macrocirculatory properties, which have been associated with circulating dp-ucMGP, include arterial calcifications and arterial stiffness. Moreover, plasma dp-ucMGP is a predictor of mortality and adverse cardiovascular outcomes in longitudinal studies of patients and populations.

Vascular Calcification

Arterial calcification is a hallmark of vascular disease and imminent cardiovascular complications.[2,3] Studies using multislice spiral computed tomography showed association between arterial calcification and circulating dp-ucMGP.[33–35] In a single regression analysis of 107 patients with chronic kidney disease (CKD; 40% women; mean age, 67 years), the aortic calcification score increased by 10% for a 100 pmol/L (1.06 µg/L) increment in dp-ucMGP (r^2=0.143; P<0.0001).[33] This association retained significance (P=0.003) when adjusted for age, previous cardiovascular disease, and the stage of CKD.[33] In a cross-sectional study of 195 postmenopausal women, the coronary calcification score was 10.7% higher for a 100 pmol/L (1.06 µg/L) increment in plasma dp-ucMGP (P=0.035), if adjusted for age and smoking, but this association weakened to 9.1% (P=0.065), if additionally adjusted for hypertension and diabetes mellitus.[34] Findings in a longitudinal study of 571 postmenopausal women were similar.[36] Among 198 patients with type-2 diabetes mellitus and normal or slightly impaired renal function, the odds of having a below-knee arterial calcification score[37] above versus below the median was 1.88 (95% CI, 1.14–3.11; P=0.014) for a 2.72-fold increment in plasma dp-ucMGP.[35] This association was independent of sex, age, previous cardiovascular disease, and total uncarboxylated MGP plasma levels.[35]

Warfarin is a vitamin K antagonist, widely prescribed to reduce coagulation by inhibiting vitamin K–dependent coagulation factors. Patients on warfarin treatment are prone to develop vascular calcification.[18,19] Specimens of aortic valves were obtained from 45 patients (57.8% women; mean age, 71 years) undergoing heart transplantation with clinically

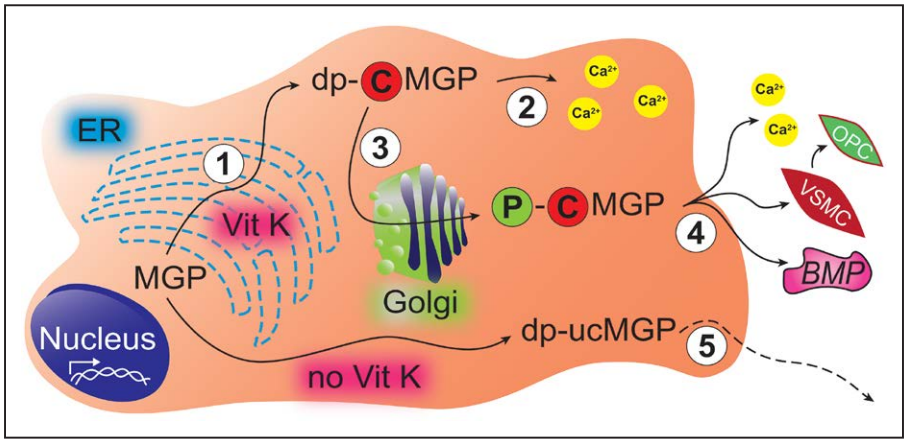

Figure 2. Synthesis, activation, secretion, and downstream actions of MGP (matrix Gla protein). Endothelial and vascular smooth muscle cells express MGP. Step 1: After translation in the endoplasmatic reticulum (ER), vitamin K activates MGP by stimulating γ-carboxylation. Step 2: dp-cMGP (desphospho-carboxylated MGP) can sequester intracellular calcium, thereby providing protection against injury caused by calcium deposition. Step 3: A Golgi-associated casein kinase phosphorylates the serine residues of dp-cMGP to p-cMGP (phosphorylated-carboxylated MGP), thereby facilitating secretion. Step 4: p-cMGP is secreted into the extracellular matrix or the circulation to inhibit soft tissue calcification, VSMC (vascular smooth muscle cell) trans-differentiation into OPC (osteochondrogenic progenitor cells) and signaling via the BMP (bone morphogenetic protein) pathway. Step 5: Inactive dp-ucMGP (desphospho-uncarboxylated MGP), a biomarker reflecting poor vitamin K status, escapes from cells into the blood stream but does not inhibit calcification.

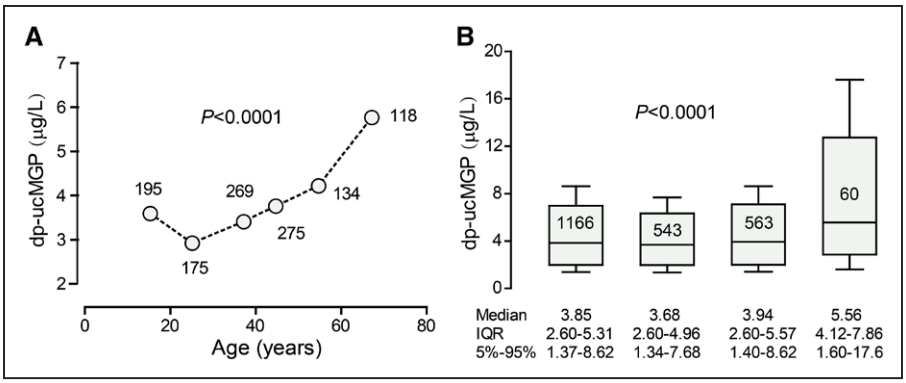

Figure 3. Dependency of circulating dp-ucMGP (desphospho-uncarboxylated matrix Gla protein) on age and stage of chronic kidney disease in 1166 participants enrolled in the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO). **A**, The number of participants contributing to the plotted value is given alongside the plotted value. **B**, Box plots represent the median, interquartile range (IQR) and fifth to 95th percentile interval of the dp-ucMGP level in all participants and in participants with stage 1 (n=543), 2 (n=563), or stage 3 (n=60) of chronic kidney disease according to the National Kidney Foundation (KDOQI) guideline. *P* values indicate the significance of the associations.

manifest aortic valve stenosis or insufficiency, among whom 10 patients received preoperative treatment with vitamin K antagonists.[18] The grade of aortic valve calcification in patients with preoperative fenprocoumon treatment was 2-fold greater than in matched controls without such treatment.[18] A post hoc patient-level meta-analysis of 8 prospective randomized trials compared the changes in coronary percent atheroma volume and the calcium index in matched arterial segments of patients with coronary artery disease who were treated (n=171) or not (n=4129) with warfarin during an 18- to 24-month period.[19] A significantly greater annualized increase in calcium index was observed in warfarin-treated compared with nonwarfarin-treated patients (median 0.03 versus 0.02; *P*<0.001). A patient-matched cohort (n=164 per group) produced confirmatory results; the multivariable-adjusted odds ratio of having greater calcium index in relation to warfarin treatment was 1.16 (CI, 1.05–1.28; *P*=0.003).[19]

Arterial Stiffness

Carotid-femoral pulse wave velocity is the gold standard for the assessment of arterial stiffness. In patients with hypertension,[38] diabetes mellitus,[39] renal dysfunction,[17] or heart failure,[40] this index was associated with circulating dp-ucMGP. These observations were replicated in 2 population studies.[41,42] In 1001 participants enrolled in the Swiss Kidney Project on Genes in Hypertension (53% women; mean age, 46.5 years), for per 1-SD increment in plasma dp-ucMGP (200 pmol/L [2.12 µg/L]), carotid-femoral pulse wave velocity was 0.198-m/s higher (CI, 0.111–0.277 m/s; *P*<0.001) with adjustments applied for age, body mass index, systolic and diastolic blood pressure, heart rate, plasma glucose, diabetes mellitus, and history of cardiovascular disease.[41] In 1087 individuals examined in the framework of the Czech post-Monitoring Trends and Determinants in Cardiovascular Disease study (52.8% women; age range, 25–75 years), carotid-femoral pulse wave velocity increased across fourths of the distribution of plasma dp-ucMGP (*P*<0.001). After adjustment for all potential confounders, carotid-femoral pulse wave velocity remained independently (*P*=0.031) associated with plasma dp-ucMGP with an association size amounting to 1 m/s for a 11.6 pmol/L (0.123 µg/L) increment in plasma dp-ucMGP.[42] In patients with heart failure with preserved ejection fraction (n=96) and heart failure patients with reduced ejection fraction (n=53) and controls without heart failure (n=199), carotid-femoral pulse wave velocity with adjustment for confounders was positively associated with circulating dp-ucMGP (standardized β, 0.18; CI, 0.03–0.34; *P*=0.023).[40] In analyses restricted to participants with heart failure, the association remained significant (standardized β, 0.32; CI, 0.04–0.61; *P*=0.026). Carotid-femoral pulse wave velocity also increased with warfarin use (standardized β, 0.13; CI, 0.004–0.26; *P*=0.043), but this association lost significance with additional adjustment for circulating dp-ucMGP,[40] indicating that dp-ucMGP incorporates information on vitamin K antagonism.

Mortality and Cardiovascular and Renal Outcomes

The substantial evidence relating mortality and fatal plus nonfatal cardiovascular outcomes to plasma dp-ucMGP is summarized in Table 1. It predominantly originates from studies in patients with calcified aortic stenosis,[43] heart failure,[44,45] type-2 diabetes mellitus,[46] chronic vascular disease,[47] or CKD[33] or studies in recipients of a kidney transplant.[48] Only 3 studies[49–51] were population based.

The primary end point in most patient studies[33,43–48] was total mortality. Sample size ranged from 107[33] to 799[47] and the average or median follow-up from 1.9[43] to 11.2[46] years. Two early studies[33,43] reported that the risk of death was higher in patients with CKD[33] or severe aortic valve calcification,[43] if their plasma dp-ucMGP level was higher than the median (≈950 pmol/L [10.1 µg/L]). In one[33] of these 2 studies,[33,43] this association lost significance when multivariable adjusted. Four later studies[44,46–48] demonstrated association of total[44,47,48] and cardiovascular mortality,[47] heart failure,[44,46] or malfunction of a renal allograft[48] with plasma dp-ucMGP across quantiles of its distribution[47,48] or with a 1-SD increment[44,46] in its plasma level. These associations between adverse health outcomes and dp-ucMGP withstood multivariable adjustment (Table 1) with the exception of the association of fatal heart failure (13 cases) with dp-ucMGP, which retained significance if only adjusted for aspirin use.[44]

Table 1. Longitudinal Studies Relating Death or Cardiovascular Disease to Plasma dp-ucMGP

Study	Participants (Country)	No. of Participants (% Women) Age	Outcome (Follow-Up, y)	Main Results Covariables
Schurgers et al, 2010[33]	CKD (France)	107 (40.0) mean, 67 y	TM (2.2)	34 deaths; HR for dp-ucMGP >921 pmol/L (median), 2.85 (CI, 1.36–5.90; *P*=0.006); *P*<0.05, if adjusted for age or CKD stage or hemoglobin; significance lost in multivariable-adjusted models.
Ueland et al, 2010[43]	AS (Norway)	147 (45) mean, 74 y	TM (1.9)	25 deaths; HR for dp-ucMGP >950 pmol/L (median), 9.16 (CI, 2.74–30.6; *P*<0.001) if unadjusted; HR, 4.04 (CI, 1.02–16.2; *P*=0.047) with cumulative adjustment for sex, age, BMI, eGFR, NT-proBNP, CRP, HT, DM, and LVEF.
Ueland et al, 2011[44]	HF (Norway)	179 (22%) mean, 56 y	TM/HTx (2.9)	TM (44 deaths+4 HTx) was unrelated to dp-ucMGP; HR (+1 SD) for fatal HF, 5.62 (CI, 2.05–15.5; *P*=0.001) with adjustment for use of aspirin (HR, 0.12; CI, 0.02–0.96; *P*=0.046).
Dalmeijer et al, 2013[46]	T2DM (Netherlands)	518 (82.2%) mean, 58.1 y	CVD/CHD/PAD/HF (11.2)	160 CVD, 99 CHD, 38 PAD, 28 HF; HRs (+1 SD): 1.21 (CI, 1.06–1.38; *P*=0.01) for CVD; 1.12 (CI, 0.94–1.34; *P*=0.21) for CHD; 1.32 (1.07–1.65; *P*=0.02) for PAD; and 1.75 (CI, 1.42–2.17; *P*<0.001) for HF; adjusted for sex, age, BMI, waist-to-hip ratio, SBP and DBP, total cholesterol, smoking, physical activity, and education.
Mayer et al, 2014[47]	VD (Czech Republic)	799 (28.9%) mean, 65.1 y	TM/CVM (5.6)	159 deaths (107 cardiovascular); HRs Q4 vs Q1–3: 1.89 (CI, 1.32–2.72; *P*=0.001) for TM and 1.88 (CI, 1.22–2.90; *P*=0.004) for CVM; adjusted for sex, age, waist circumference, smoking, BNP, history of HF or stroke, and warfarin treatment (10.6%).
Van den Heuvel et al, 2014[51]	Community (Netherlands)	577 (55.8%) mean, 59.9 y	CVD (5.6)	40 cases; HR T3 vs T1, 2.69 (CI, 1.09–6.62; *P*=0.032); adjusted for sex, age, BMI, smoking and drinking, HT, DM, serum total cholesterol, albumin and 25-hydroxyvitamin D, physical activity, and education.
Keyzer et al, 2015[48]	KTx (Netherlands)	518 (44%) mean, 51 y	TM/graft failure (9.8)	152 deaths; 54 graft failure; HRs for a 2.72-fold increase: 1.56 (CI, 1.14–2.12; *P*=0.005) for TM; 2.28 (CI, 1.40–3.69; *P*=0.001) for graft failure; HRs Q4 vs Q1: 1.88 (CI, 1.08–3.26; *P*=0.03) for TM; 2.62 (CI, 1.13–6.03; *P*=0.007) for graft failure; adjusted for sex, age, BMI, eGFR, smoking, serum triglycerides, and use of mycophenolate mofetil.
Liu et al, 2015[49]	FLEMENGHO (Belgium)	2318 (51.2%) mean, 43.5 y	TM/CVM/CVD/CHD (14.1)	197 death; 70 CVM; 180 CVD; 85 CHD; HRs for 2-fold increase: 1.06/1.02 for linear/squared term (CI, 1.01–1.11/1.01–1.03; *P*≤0.014) for TM; 1.14 (CI, 1.01–1.28; *P*=0.027) for CVM; 0.99 (CI, 0.94–1.05; *P*=0.87) for CVD; and 0.93 (CI, 0.88–0.99; *P*=0.021) for CHD; adjusted for family clusters, sex, age, BMI, SBP, heart rate, smoking and drinking, serum total cholesterol, DM, antihypertensive drug treatment, and history of CVD.
Riphagen et al, 2017[50]	PREVEND (Netherlands)	4275 (54.0%) mean, 53 y	TM/CVM (8.5)	279 death; 74 CVM; HRs for 2-fold increase: 0.33/1.08 for linear/squared term (CI, 0.17–0.66/1.03–1.13; *P*≤0.002) for TM; 0.17/1.11 for linear/squared term (CI, 0.05–0.58/1.03–1.20; *P*≤0.009) for CVM; adjusted for ethnicity, sex, age, BMI, SBP, smoking, eGFR, total-to-HDL serum cholesterol ratio, CRP, albuminuria, use of antihypertensive drugs and warfarin, DM, history of CVD, and education.

Articles are identified by first author, year of publication and reference number. HRs are given for the difference in plasma dp-ucMGP (desphospho-uncarboxylated matrix Gla protein): T3 vs T1, high vs low third of the dp-ucMGP distribution; Q4 vs Q1, highest vs lowest fourth of the dp-ucMGP distribution; Q4 vs Q1-3, highest fourth vs the remainder of the dp-ucMGP distribution; to convert dp-ucMGP from pmol/L to μg/L divide by 94.299. AS indicates severe valvular aortic stenosis; BMI, body mass index; BNP, brain natriuretic peptide; CHD, coronary heart disease; CKD, chronic kidney disease; CRP, C-reactive protein; CVD, cardiovascular disease; CVM, cardiovascular mortality; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, glomerular filtration rate estimated from serum creatinine; FLEMENGHO, Flemish Study on Environment, Genes and Health Outcomes (family-based population study in North Limburg, Belgium); HDL, high-density lipoprotein; HF, heart failure; HR, hazard ratio; HT, hypertension; HTx, heart transplantation; KTx, recipients of kidney transplant; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAD, peripheral arterial disease; PREVEND, Prevention of Renal and Vascular End-Stage Disease, prospective cohort study in Groningen, The Netherlands; SBP, systolic blood pressure; T2DM, type-2 diabetes mellitus; TM, total mortality; and VD, vascular disease.

Our literature search also revealed 3 prospective community- or population-based studies.[49–51] The Longitudinal Aging Study Amsterdam included 577 participants.[51] With adjustments applied for sex, age, body mass index, smoking and drinking, hypertension and diabetes mellitus, serum cholesterol, albumin and 25-hydroxyvitamin D, physical activity and education, the hazard ratio (HR) of a composite cardiovascular end point in the highest versus the lowest third of dp-ucMGP

(12 versus 17 events) was 2.69 (CI, 1.09–6.62; *P*=0.032).[51] In view of the low number of cases, over-adjustment might be an issue in this analysis. Among 2318 people taking part in the FLEMENGHO (Flemish Study on Environment, Genes and Health Outcomes),[49] the risk of all-cause and noncancer mortality curvilinearly increased (*P*≤0.008) by 15.0% (CI, 6.9%–25.3%) and 21.5% (CI, 11.1% to −32.9%), respectively, for a doubling of the nadir of the risk function (1.43 and 0.97 µg/L [134.8 and 91.5 pmol/L]). With higher dp-ucMGP, cardiovascular mortality log-linearly increased (HR for dp-ucMGP doubling, 1.14 [CI, 1.01–1.28]; *P*=0.027).[49] In 4275 people analyzed within the framework of the Prevention of Renal and Vascular End-Stage Disease Study,[50] the association of total and cardiovascular mortality with plasma dp-ucMGP was curvilinear. The multivariable-adjusted HRs associated with a doubling of dp-ucMGP for the linear/squared terms were 0.33/1.08 (CI, 0.17–0.66/1.03–1.13; *P*≤0.002) for total mortality and 0.17/1.11 (CI, 0.05–0.58/1.03–1.20; *P*≤0.009) for cardiovascular mortality.[50]

Microvascular Traits

Microvascular alternations are early markers of disease, driven by the primary pathological process itself, and usually antedate macrovascular lesions.[52–54] More recent studies, therefore, addressed the role of MGP in microvascular disease.[12,15,16] MGP is abundantly expressed in retinal,[12,13] renal,[8,15] and myocardial microcirculation,[8,20] where the active protein contributes to maintaining microvasculatory integrity and organ function.

Retinal Microcirculation

MGP is abundantly expressed in the eye,[10,11,55,56] where it takes part in preserving the structural integrity of the trabecular meshwork,[10,11] the sclera,[55] and the retinal ganglion cells.[56] In mice, MGP is also abundantly expressed in the retinal microvasculature,[13] where MGP exhibits anticalcification and antistiffness properties. Among 935 randomly recruited FLEMENGHO participants (50.3% women; mean age, 40.9 years), plasma dp-ucMGP was measured from 1996 until 2010.[12] At a follow-up examination, on average 11.0 years later, the retinal arteriolar diameter was assessed by nonmydriatic retinal photography. In multivariable-adjusted models, a doubling of dp-ucMGP was associated with 1.40 µm (CI, 0.32–2.48; *P*=0.011) narrower retinal arteriolar diameter.[12] These observations—for the first time collected in a representative population sample—are clinically relevant, because smaller retinal arteriolar diameter[57,58] and lower arteriole-to-venule diameter ratio[59] predict cardiovascular mortality,[57] coronary heart disease,[59] and lacunar stroke.[58]

Renal Function

The renal microcirculation consists of 2 specialized microvasculatory structures, the glomerular capillaries and the peritubular microvascular network, respectively, located in the renal cortex and the renal medulla.[60] Glomerular filtration rate and microalbuminuria are microvascular phenotypes, which are predictive of total and cardiovascular mortality[61] and adverse cardiovascular outcomes.[62] MGP is abundantly expressed in the kidney, with MGP immunoreactivity being associated with the epithelium of Bowman capsule and the proximal tubules.[8] In multivariable-adjusted cross-sectional analyses of 1166 white Flemish and 352 black South Africans, a doubling of dp-ucMGP was associated with a 1.46 and 2.78 mL/min per 1.73 m^2 lower estimated glomerular filtration rate (*P*≤0.023) and, therefore, with a higher probability of having a higher stage of CKD.[15] A subsequent longitudinal study, including 1009 Flemish followed up for 8.9 years, confirmed that a 5-fold higher plasma dp-ucMGP at baseline was associated with a 3.15 mL/min per 1.73 m^2 lower estimated glomerular filtration rate at follow-up (CI, 1.26–5.05; *P*=0.001).[16] The HR expressing the risk of progression to an estimated glomerular filtration rate of <60 mL/min per 1.73 m^2 was 3.49 (CI, 1.45–8.40; *P*=0.005). The HR relating the presence of microalbuminuria at follow-up to baseline circulating dp-ucMGP was 4.70 (CI, 1.57–14.1; *P*=0.006).[16]

In addition to a protective effect of active MGP on the renal microcirculation, other mechanisms might explain our observations. Renal interstitial fibrosis is a universal predictor of a decline in renal function and is characterized by exaggerated deposition of extracellular matrix by an expanding population of fibroblasts and myofibroblasts.[63] In the context of fibrosis, MGP antagonizes signaling via the BMP (bone morphogenetic protein) pathway (Figure 2).[64,65] BMPs belong to the transforming growth factor-β superfamily.[64] Once activated, BMP type-1 and type-2 receptors induce endothelial dysfunction,[66] disruption of the integrity of the arterial wall and the extracellular matrix,[67] promote untoward deposition of calcium,[68] and activate profibrotic pathways.[69]

Left Ventricular Function

A novel paradigm drew attention on inflammation of the coronary microcirculation as a potential mechanism underlying diastolic left ventricular dysfunction,[70] in addition to higher left ventricular loading conditions and dysregulation of ventricular-arterial coupling, for instance as a consequence of stiffening of the central elastic arteries.[71] This hypothesis justified examining the association between the E/e′ ratio, an index reflecting left ventricular filling pressure, and plasma dp-ucMGP in representative population samples recruited in Flanders and Switzerland. With adjustments applied for potential confounders and with the association size expressed for a doubling of dp-ucMGP, E/e′ was 0.26 higher in 668 Flemish, 0.33 higher in 386 Swiss, and 0.31 higher in both cohorts combined (*P*≤0.026).[20] These epidemiological findings were backed up by tissue staining studies. Cardiac biopsies from patients with ischemic or dilated cardiomyopathy and healthy hearts (n=4 for each) were stained with conformation-specific MGP antibodies.[20] The active MGP moieties, carboxylated MGP and phosphorylated MGP, were predominantly distributed in the media and intima of muscular left ventricular microvessels in normal and diseased hearts.[20] Inactive uncarboxylated MGP was abundant in fibrotic areas of diseased hearts, around the nuclei of interstitial cells and in the perivascular matrix. Inactive unphosphorylated MGP was almost absent in vessel walls and in fibrotic areas but was abundant in cardiomyocytes of all hearts and colocalized with active carboxylated MGP.[20]

Table 2. Summary of Observation Studies and Clinical Trials of Vitamin K Supplementation on dp-ucMGP and Cardiovascular Health

Study	Participants (Country)	NO. of Participants (% Women), Age	Design	Treatment	Outcome Follow-Up	Main Results
Dalmeijer et al, 2012[9]	Healthy Netherlands	60 (60%) aged 40–65 y	Randomized, double-blind, placebo-controlled	(1) placebo (n=20); (2) 180 μg MK-7 (n=22); and (3) 360 μg MK-7 (n=18)	NA 12 wk	Plasma dp-ucMGP decreased by 31% and 46% in 180 μg MK-7 and 360 μg MK-7 supplementation groups.
Knapen et al, 2015[85]	Postmenopausal Netherlands	244 (100%) aged 55–65 y	Randomized, double-blind, placebo-controlled	(1) placebo (n=124); and (2) 180 μg MK-7 (n=120)	Arterial stiffness 3 y	(1) dp-ucMGP decreased by 50% in MK-7 group compared with placebo. (2) Absolute changes in cfPWV −0.36 vs +0.021 m/s (*P*=0.040) and in stiffness index β −0.67 vs +0.15 (*P*=0.018) between MK-7 and placebo group.
Kurnatowska et al, 2016[82]	CKD Poland	38 (44.7%) aged 18–70 y	Observational	(1) 10 μg cholecalciferol (n=12); and (2) 10 μg cholecalciferol+90 μg MK-7 (n=26)	Cardiovascular risk factors 270 days	(1) dp-ucMGP decreased by 10.7%; and (2) no difference in cardiovascular risk factors between MK-7 group and control group.
Mansour et al, 2017[83]	KTx Lebanon	60 (43.3%) mean, 49.7 y	Observational	360 μg MK-7	Arterial stiffness 8 wk	(1) dp-ucMGP decreased by 55.1% by MK-7 supplementation. (2) Improvement in cfPWV was associated with the reduction in dp-ucMGP (*P*=0.014).
Aoun et al, 2017[84]	Hemodialysis Lebanon	50 (40%) median, 71.5 y	Observational	360 μg MK-7	NA 4 wk	The average drop in dp-ucMGP was 86% by MK-7 supplementation.

Articles are identified by first author, year of publication and reference number. cfPWV indicates carotid-femoral pulse wave velocity; CKD, chronic kidney disease; dp-ucMGP, desphospho-uncarboxylated matrix Gla protein; KTx, recipients of kidney transplant; and MK-7, menaquinone-7.

Tissue Integrity

The expression of MGP in a large number of tissues points to the multifaceted role of this protein, thereby moving the focus beyond its involvement in maintaining vascular integrity.[72]

Nephrolithiasis

Nephrolithiasis represents a nonvascular process of unwanted calcification with high recurrence rates.[73] In rat models of nephrolithiasis, MGP is polarly expressed at the apical membrane of tubular epithelial cells in the ascending thick limbs of Henle's loop and the distal convoluted tubule and in stone-forming rats also in the medullary collecting duct.[74] Multilaminated crystals developed in injurious renal tubules that lacked MGP expression.[74] Two case-control studies found association between nephrolithiasis and genetic variation in the *MGP* gene.[75,76] In 122 Japanese patients with kidney stones and 125 controls, Gao et al[75] investigated 19 SNPs in *MGP*, including rs4236 and rs1800802. Compared with minor allele rs4236 carriers (*G*; prevalence, 24.3%), major allele homozygotes (*AA*; prevalence, 75.7%) had a 1.82-fold increased risk of kidney stones (CI, 1.00–3.22; *P*=0.047).[75] A subsequent Chinese case-control study confirmed association of nephrolithiasis with rs4236 but not with rs1800802.[76] Among 1748 randomly recruited Flemish, 144 had a history of nephrolithiasis at baseline; over 12.0 years (median), 37 cases had incident nephrolithiasis.[77] With adjustments applied for potential confounders, the odds ratio for having prevalent nephrolithiasis associated with a doubling dp-ucMGP was 1.31 (CI, 1.04–1.64; *P*=0.022).[77] Circulating dp-ucMGP levels were associated (*P*≤0.001) with *MGP* variants rs2098435, rs4236, and rs2430692. In a multivariable-adjusted model, the HR of having incident nephrolithiasis in relation to a doubling of circulating dp-ucMGP was 2.48 (CI, 1.71–3.61; *P*<0.001).[77]

Cartilage and Bone

Vitamin K plays a pivotal role in maintaining bone health.[78] Increasing evidence also implicates MGP in maintaining bone health.[79–81] In the Health, Aging and Body Composition study, 791 older community-dwelling adults underwent magnetic resonance imaging to measure bilateral knee structural features.[80] The highest compared with the lowest fourth of the dp-ucMGP distribution had higher odds of having meniscus damage (1.6; CI, 1.1–2.3), osteophytes (1.7; CI, 1.1–2.5), bone marrow lesions (1.9; CI, 1.3–2.8), and subarticular cysts (1.5; CI, 1.0–2.1).[80] In 468 recipients of a kidney transplant, mineral density of the femoral neck was significantly less

(P=0.0006) 14 days after surgery, if plasma dp-ucMGP before surgery was higher.[81] This association was independent of sex, age, body mass index, and bone remodeling activity. During a median follow-up of 5.1 years, 33 patients sustained a fragility fracture. In a multivariable-adjusted Cox model, circulating dp-ucMGP levels above median versus below median were associated with higher risk of osteoporotic fractures with a HR of 2.21 (CI, 1.00–4.91; P<0.05).[81]

Studies of Vitamin K Supplementation

Three observational studies[82–84] and 2 randomized clinical trials[9,85] (Table 2) examined the effects of vitamin K substitution on plasma dp-ucMGP levels, the cardiovascular risk profile,[82] or arterial stiffness.[83,85] The patients enrolled in these studies included either healthy people[9,85] or patients with CKD, receiving[83,84] or not receiving renal replacement therapy.[82] The sample size, dose of menaquinone-7 administered, and follow-up duration ranged from 38[82] to 244[85] patients, from 90 [82] to 360 μg[9,83,84] per day, and from 4 weeks[84] to 3 years.[85] Overall, these studies showed a dose-dependent decrease in circulating dp-ucMGP with an 86% decrease already observed after 4 weeks of substitution by 360 μg menaquinone-7.[84] In a randomized double-blind trial of 244 postmenopausal women followed up for 3 years, arterial stiffness as captured by aortic pulse wave velocity (−0.36 versus +0.021 m/s; P=0.040) or stiffness index β (−0.67 versus +0.15; P=0.018), decreased in the intervention compared with the control group.[85] These results should be considered as hypothesis generating in view of the small sample size and because there were no between-group differences in the vitamin K–induced changes in the elastic properties of the carotid artery (eg, distensibility, compliance, and Young's modulus).

Clinical Perspective

Aging is one of the greatest social and economic challenges worldwide.[86] With this demographic transition, health care costs are escalating, so that health care system must adjust to remain sustainable. In FLEMENGHO, plasma dp-ucMGP levels ranging from 1.4 to 4.6 μg/L were optimal in terms of the risk of mortality and macrovascular cardiovascular illness[49]; the 4.6 μg/L threshold corresponded with the 65th percentile of the dp-ucMGP distribution. Thus, vitamin K supplementation before irreversible organ damage sets in might find its application in the prevention of a wide range of disabling diseases, which increasingly challenge health care system in the second millennium. In aged people and in patients with CKD, diabetes mellitus, or on treatment with warfarin or antibiotics, circulating dp-ucMGP levels might be measured over time to track the risk of vascular complications. However, which levels of plasma dp-ucMGP should be acted on for optimal vascular and microvascular health remains an issue to be resolved. Furthermore, no biomarker should make it to clinical practice without properly powered randomized clinical trials. Coronary heart disease,[49] heart failure,[87,88] and CKD[89] represent appropriate end points in such trials, in which safety remains to be addressed as well. In patients with atherosclerotic disease, elevated plasma dp-ucMGP was associated with less plaque hemorrhage, suggestive of more stable lesions,[90] so that vitamin K substitution might increase the preponderance of soft vulnerable plaques. On the contrary, vitamin K has a wide safety range and does not cause hypercoagulability. In rats, vitamin K supplementation by 3 mg of either vitamin K_1 or K_2 per gram of food, that is, 300 mg per kilogram of body weight, did not increase plasma prothrombin or the thrombin potential.[91] In 2 clinical trials, either 1 mg per day of K_1[92] or 45 mg per day of K_2[93] did not affect coagulation. These considerations highlight the research track to be implemented to translated decennia of research to clinical application.

Sources of Funding

The European Union (HEALTH-F7-305507-HOMAGE), the European Research Council (Advanced Researcher Grant 2011-294713-EPLORE and Proof-of-Concept Grant 713601-uPROPHET), the European Research Area Net for Cardiovascular Diseases (JTC2017-046-PROACT), and Research Foundation Flanders, Ministry of the Flemish Community, Brussels, Belgium (G.0881.13) supported Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO).

Disclosures

C. Vermeer was an employee of the R&D Group VitaK until September 30, 2017. The other authors report no conflicts.

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Hypertension

Data Supplement

Vitamin K-Dependent Matrix Gla Protein as Multifaceted Protector of Vascular and Tissue Integrity

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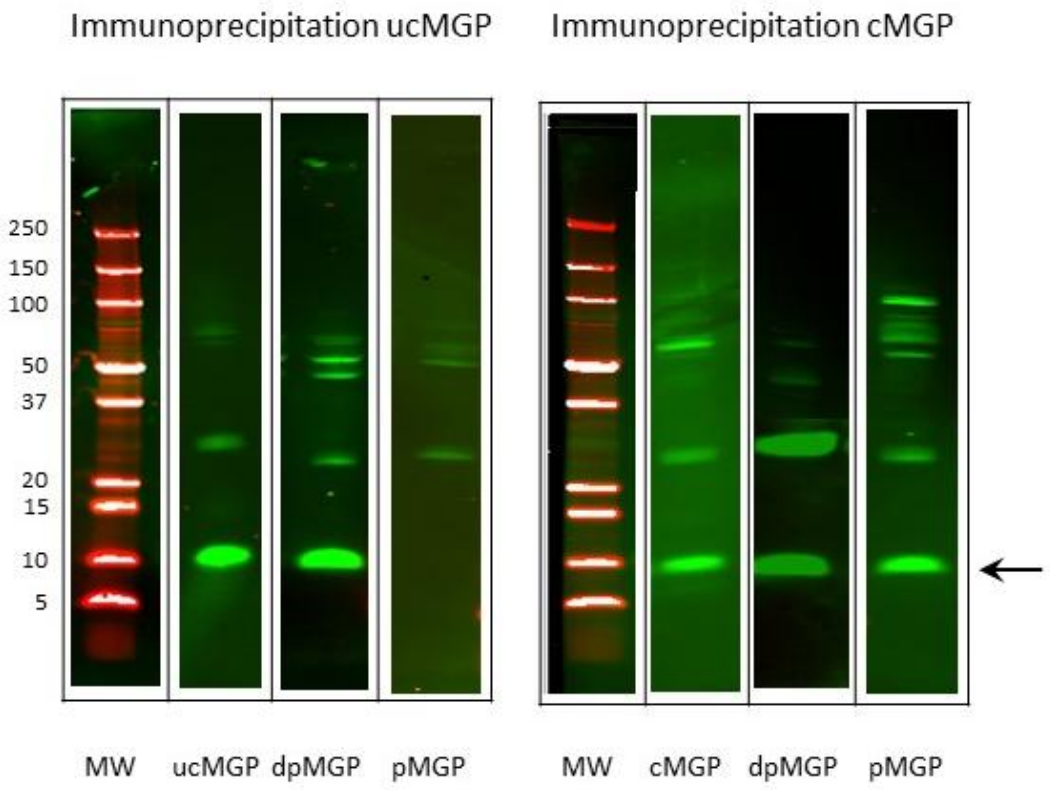

Figure S1.

Western blots of immunoprecipitated fractions of matrix Gla protein (MGP). Left panel: Plasma from healthy volunteers was pooled and extracted with insolubilized antibodies against ucMGP, eluted and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis [1]. After transfer on polyvinylidene difluoride membranes staining was performed with antibodies against ucMGP (lane 2), dpMGP (lane 3) and pMGP (lane 4). MW markers (BioRad Laboratory, Hercules, CA) ran in lane 1. The arrow indicates the position of MGP. Right panel: normal pooled plasma was extracted with insolubilized antibodies against cMGP and membranes were stained with antibodies against cMGP (lane 2), dpMGP (lane 3) and pMGP (lane 4). Abbreviations: MW, molecular weight in kD; ucMGP, uncarboxylated MGP; dpMGP, unphosphorylated MGP; pMGP, phosphorylated MGP, cMGP, carboxylated MGP.

Interpretation of Figure S1:

In healthy volunteers, MGP circulates in three conformations: dp-ucMGP, dp-cMGP and p-cMGP. ucMGP coprecipitates with dpMGP but not pMGP; cMGP coprecipitates with both dpMGP and pMGP. However, these experiments do not explain the ten-thousand-fold difference in circulating dp-ucMGP and t-ucMGP [2].

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