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Vitamin K Status, Warfarin Use and Arterial Stiffness in Heart Failure

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Abstract

Large artery stiffening contributes to the pathophysiology of heart failure (HF) and associated comorbidities. Matrix Gla-Protein (MGP) is a potent inhibitor of vascular calcification. MGP activation is vitamin-K dependent. We aimed to: (1) to compare dephospho-uncarboxylated Matrix Gla-Protein (dp-ucMGP) levels between subjects with HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF), and subjects without HF; (2) to assess the relationship between dp-ucMGP levels and arterial stiffness; (3) to assess the relationship between warfarin use, dp-ucMGP levels, and arterial stiffness in HF. We enrolled 348 subjects with HFpEF (n=96), HFrEF (n=53) or no HF (n=199). Carotid-femoral pulse wave velocity (CF-PWV), a measure of large artery stiffness, was measured with arterial tonometry. Dp-ucMGP was measured with ELISA. Dp-ucMGP levels were greater in both HFrEF (582 pmol/L; 95%CI=444 to 721 pmol/L) and HFpEF (549 pmol/L; 95%CI= 455 to 643 pmol/L) compared to controls (426 pmol/L; 95%CI= 377 to 475 pmol/L; ANCOVA P=0.0067). Levels of dp-ucMGP were positively associated with CF-PWV (Standardized β=0.31; 95%CI=0.19 to 0.42; P<0.0001), which was also true in analyses restricted to patients with HF (Standardized β=0.34; 95%CI=0.16 to 0.52; P=0.0002). Warfarin use was significantly associated with CF-PWV (Standardized β=0.13; 95%CI=0.004-0.26; P=0.043), but this relationship was eliminated after adjustment for dp-ucMGP. In conclusion, levels of dp-ucMGP are increased in HFpEF and HFrEF and are independently associated with arterial stiffness. Future studies should investigate whether vitamin K supplementation represents a suitable therapeutic strategy to prevent or reduce arterial stiffness in HFpEF and HFrEF.

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Keywords

heart failure; HFpEF; MGP; vitamin K; arterial stiffness; pulse wave velocity

Introduction

Large artery stiffening causes an excessive pulsatile load to the heart and to the microvasculature of various target organs. Both effects are involved in the pathophysiology of cardiac dysfunction and heart failure (HF)-associated comorbidities (such as renal dysfunction). The identification of pathways related to arterial stiffness in HF may provide novel therapeutic targets.

Matrix Gla-Protein (MGP) is a protein produced by chondrocytes and vascular smooth muscle cells.[1] The inactive form of MGP (dephospho-uncarboxylated MGP, dp-ucMGP) undergoes serial post-translational γ-glutamate carboxylation and serine phosphorylation to form active MGP. The active form of MGP is a potent inhibitor of vascular calcification. Carboxylation of dp-ucMGP is vitamin K dependent and is thus reduced in vitamin K-deficient states.[1] Dp-ucMGP is secreted into the circulation and an increase in its levels indicates deficient MGP activation. Recent studies in non-HF populations indicated that abnormal MGP maturation correlates with arterial stiffness in humans.[2; 3] Less information is available regarding the relationship between MGP and arterial stiffness in HF. HF is associated with hemodynamic abnormalities, metabolic abnormalities, renal dysfunction and the use of multiple medications, which could impact the relationship between MGP and PWV.

A previous study demonstrated increased levels of dp-ucMGP in a HFrEF population.[4] However, whether HFpEF is associated with increased dp-ucMGP levels, and more importantly, whether MGP levels correlate with arterial stiffness in subjects with HFrEF or HFpEF is unknown. This is important because HFpEF and HFrEF are different disease states, with different pathophysiology and response to therapy; furthermore, it is important to better understand HFpEF given that there are no currently available evidence-based effective pharmacologic interventions for this condition.

Patients with HF often receive warfarin, a vitamin K antagonist that may inhibit MGP activation. In animal models, warfarin administration at high doses produces profound arterial calcification[5] and several lines of evidence link warfarin use to arterial calcification in humans.[6;7;8;9;10] Arterial calcification is an important contributor to arterial stiffness.[11] However, the relationship between warfarin use, MGP activation, and large artery stiffness in HF is unknown.

In this study, we aimed to: (1) Compare levels of plasma dp-ucMGP levels between subjects with HFpEF, HFrEF and subjects without HF; (2) Assess the relationship between dp-ucMGP levels and carotid-femoral pulse wave velocity (CF-PWV), the non-invasive gold standard index of large artery stiffness[12;13], (3) Assess the relationship between warfarin use, dp-ucMGP levels and arterial stiffness in HF.

Methods

We prospectively enrolled a convenience sample of 348 adults at the Corporal Michael J. Crescenz VA Medical Center referred for a cardiac magnetic resonance imaging study. The protocol was approved by the Philadelphia VA Medical Center Institutional Review Board, and all subjects provided written informed consent. The investigation conforms with the principles outlined in the *Declaration of Helsinki*.

The data, analytic methods, and study materials are not publicly available for purposes of reproducing the results or replicating the procedures. Such data may be made available to other researchers for collaborative research, through the establishment of appropriate agreements.

Key exclusion criteria were as follows: (1) Claustrophobia; (2) Presence of metallic objects or implanted medical devices in body; (3) Conditions that could make the measurements of CF-PWV less accurate and/or unreliable (i.e., arrhythmia such as atrial fibrillation); (4) History of sarcoidosis or amyloidosis, or suspected infiltrative heart disease.

In order to optimize case classification according to LVEF and other cardiac parameters detailed below, we measured left ventricular ejection fraction (LVEF) and cardiac structure and function using the current gold-standard method (steady-state free precession cine cardiac MRI). HFrEF was defined as a symptomatic HF in the presence of a <50%. HFpEF was defined as (1) NYHA Class II-IV symptoms consistent with HF; (2) LVEF>50%; (3) a mitral E wave to annular (e') ratio >14; or at least 2 of the following: (a) a mitral E wave to annular e' ratio >8; (b) treatment with a loop diuretic for control of HF symptoms; (c) left atrial volume index >34 mL/m^2 of body surface area (BSA); (d) NT-pro B-type natriuretic peptide level >200 pg/mL; and (e) LV mass index >149 g/m^2 in men and 122 g/m^2 in women. Subjects without HF had an LVEF >50%, and no symptoms and signs consistent with HF.

Carotid-Femoral Pulse Wave Velocity Measurement

Carotid femoral pulse wave velocity (CF-PWV) was measured using the SphygmoCor system (Atcor Medical; Sydney, Australia). Briefly, carotid-to-femoral transit time (ΔT) was computed from the foot-to-foot time difference between sequentially acquired carotid and femoral waveforms, using the intersecting tangents method, and the QRS complex of the ECG as a fiducial point. The distance between the sternal notch and the carotid artery was subtracted from the distance between the sternal notch and the femoral artery (measured with rigid calipers), in order to estimate the path length (L), and CF-PWV was computed as L/ΔT. The coefficient of variation for CF-PWV measurements in our lab is <10%.

Cardiac MRI

We measured LV mass, volume and left atrial volume, using a 1.5 Tesla whole body MRI scanner (Avanto or Espree, Siemens, Malvern, PA, USA) equipped with a phase-array cardiac coil.

LV volumes (end-diastolic and end-systolic volumes) and function (ejection fraction, EF) were measured using steady-state free-precession (SSFP) cine imaging. Typical acquisition parameters were: TR=30.6 ms; TE=1.3 ms; Slice thickness=8 mm; Phases=30; Parallel image (IPAT) factor=2; and Matrix size=192×192. CMR42 software (Circle CVI, Calgary, AB, Canada) was utilized to manually trace the LV short-axis cine images at end of diastole and systole. LV mass (LVM) was calculated as the difference between epicardial and endocardial volumes, multiplied by the myocardial density. Left atrial volume was calculated by averaging the volumes measured end-systole by manually tracing the left atrial endocardial border in the apical 2-chamber and 4-chamber views.

Plasma dp-ucMGP measurement

Citrate tubes were used for collection of venous blood samples at the time of enrollment. Plasma was prepared and stored at −80 °C for batch analysis. A dual-antibody sandwich ELISA technique (VitaK; Maastricht University; The Netherlands) was used to measure dp-ucMGP. Intra-assay coefficients of variation for this assay have previously been reported at 3.1% and 5.4% for lower and upper limit of normal. Inter-assay variation coefficients are 6.9% and 13.6% for lower and upper limits of normal.[2]

Statistical Methods

Our study had 80% power to detect standardized effect sizes of at least 0.15 with a 2-sided alpha error rate of 0.05. Continuous and categorical variables were compared between the groups using analysis of variance (ANOVA) and chi-square tests, respectively. Given previous studies demonstrating an important influence of age, sex, ethnicity and warfarin use on dp-ucMGP,[6; 7; 14] we adjusted for these factors in comparisons of dp-ucMGP levels between the groups, using ANCOVA. *Post-hoc* pairwise comparisons were conducted using the Bonferroni correction method. Bivariable and multivariable linear regression models were utilized to assess independent correlates of dp-ucMGP, with adjustments for multiple potential confounders. Similarly, linear regression models were used to assess the how warfarin use, dp-ucMGP levels, and various other factors relate to arterial stiffness (CF-PWV). As recommended by current guidelines[12], these models were adjusted for mean arterial pressure and heart rate (which can affect CF-PWV independently of the underlying intrinsic material properties of the arterial wall). When required, Box-Cox transformation was applied to normalize regression model residuals. We present standardized regression coefficients for easier comparison of the magnitude of the effect of various independent variables on the dependent variable in regression models.

Results

Baseline characteristics of our participants with HFpEF, and HFrEF and no HF are presented and compared in Table 1. Most of the subjects were male, but the proportion of males was greater in the HFpEF group and lower in the HFrEF group. Compared to the other groups, subjects with HFpEF were significantly older, demonstrated a much greater BMI, lower estimated GFR, lower serum magnesium, and the highest prevalence of diabetes (69.79%) and hypertension (90.62%). The prevalence of coronary artery disease was highest in HFrEF (52.83%). LV mass was increased in both HFpEF and HFrEF, without significant differences

between HFpEF and HFrEF, whereas LV end-diastolic volume was significant greater in HFrEF than in HFpEF. A greater percentage of HFpEF and HFrEF subjects used beta-blockers, aspirin, and furosemide as well, whereas insulin use was approximately twice as prevalent in HFpEF (32.29%) compared to either HFrEF (15.09%) or subjects without HF (14.57%).

Dp-ucMGP Levels in HF

Figure 1 shows mean dp-ucMGP levels in patients without HF, HFpEF and HFrEF. There were significant between-group differences in dp-ucMGP levels (P=0.0067). In *post-hoc* pairwise comparisons, dp-ucMGP levels were significantly greater in HFpEF (549 pmol/L; 95%CI= 455 to 643 pmol/L) and HFrEF (582 pmol/L; 95%CI=444 to 721 pmol/L) compared to controls (426 pmol/L; 95%CI= 377 to 475 pmol/L), without significant differences between the 2 heart failure groups.

Multivariable correlates of dp-ucMGP

We assessed the presence of HFpEF or HFrEF and various other covariates as correlates of dp-ucMGP levels in a linear regression model (Table 2), which also included age, sex, ethnicity, BMI, systolic blood pressure, history of hypertension, coronary artery disease, diabetes, warfarin use, and serum calcium, magnesium and phosphorus. Standardized regression coefficients and 95% CIs for all independent variables are shown in Table 2**.** Values of standardized regression coefficients and 95% CIs for significant independent correlates of dp-ucMGP levels in this model are shown in Figure 2A**.**

In this multivariable model, variables significantly associated with dp-ucMGP included the presence of HFpEF (Standardized β=0.12; 95%CI=0.01 to 0.23; P=0.03328), HFrEF (Standardized β=0.12; 95%CI=0.02 to 0.23; P=0.01617), warfarin use (Standardized β=0.42; 95%CI=0.32 to 0.51; P<0.00001), age (Standardized β=0.19; 95%CI=0.09 to 0.30; P=0.00039), male sex (Standardized β=−0.11; 95%CI=−0.20 to −0.01; P=0.03121) and African-American ethnicity (Standardized β=−0.34; 95%CI=−0.44 to −0.24; P<0.00001).

Since renal dysfunction has been recently shown to be independently associated with dp-ucMGP[15], we also constructed a model additionally adjusted for eGFR (Table 3 **and** Figure 2B). In this model, eGFR was significantly associated with dp-ucMGP (Standardized β= −0.24; 95%CI=−0.34 to −0.13; P<0.0001). HFrEF remained significantly associated with dp-ucMGP (Standardized β=0.13; 95%CI=0.024 to 0.23; P=0.015), whereas HFpEF was no longer significantly associated with dp-ucMGP (Standardized β=0.08; 95%CI=−0.03 to 0.19; P=0.175). In this model, warfarin use, age, male sex, and African-American ethnicity remained significantly associated with dp-ucMGP.

Relationship Between dp-ucMGP and CF-PWV

In unadjusted analyses, dp-ucMGP levels were positively associated with CF-PWV (Standardized β=0.31; 95%CI=0.19 to 0.42; P<0.0001). Similarly, in analyses restricted to patients with heart failure (either HFpEF or HFrEF), dp-ucMGP levels were positively associated with CF-PWV (Standardized β=0.34; 95%CI=0.16 to 0.52; P=0.0002). There was

no interaction between either HFpEF (P=0.37) or HFrEF status (P=0.69) and dpuc-MGP as determinants of CF-PWV.

In a model that adjusted for age, sex, race/ethnicity, mean arterial pressure, heart rate, heart failure group membership, body mass index, history of hypertension, coronary artery disease, diabetes, warfarin use, serum calcium, magnesium and phosphorus and estimated GFR, dp-ucMGP remained significantly associated with CF-PWV (Standardized β=0.18; 95%CI=0.03-0.34; P=0.023).

In analyses restricted to subjects with HF, after adjustment for age, sex, race/ethnicity, mean arterial pressure, heart rate, heart failure group membership, body mass index, history of hypertension, coronary artery disease, diabetes, warfarin use, serum calcium, magnesium and phosphorus and estimated GFR, dp-ucMGP was significantly associated with CF-PWV (Standardized β=0.32; 95%CI=0.04-0.61; P=0.026).

Relationship Between Warfarin Use, dp-ucMGP and CF-PWV

In addition to being associated with dp-ucMGP levels, warfarin use was also independently associated with CF-PWV. In a model that adjusted for age, sex, race/ethnicity, mean arterial pressure, heart rate, heart failure group membership, body mass index, history of hypertension, coronary artery disease, diabetes, warfarin use, serum calcium, magnesium and phosphorus, and eGFR, warfarin use was significantly associated with CF-PWV (Standardized β=0.13; 95%CI=0.006-0.26; P=0.041). After further adjustment for dp-ucMGP levels, warfarin was no longer associated with CF-PWV (Standardized β=0.04; 95%CI=−0.12 to 0.19; P=0.63).

Discussion

In this study, we investigated the association between plasma levels of dp-ucMGP (a marker of vitamin-K dependent activation of MGP, a potent inhibitor of vascular calcification), HF and large artery stiffness (CF-PWV). Our study demonstrates that dp-ucMGP levels are significantly increased in subjects with both HFpEF and HFrEF in comparison to individuals without HF. We found that dp-ucMGP was significantly associated with CF-PWV after adjusting for multiple potential confounders in regression analyses, which was also true in analyses restricted to subjects with HF. Finally, we demonstrate that warfarin use is independently associated with greater large artery stiffness. Our findings are consistent with the known vitamin K-dependent activation of MGP and its inhibitory role on arterial calcification and support the paradigm that poor activation of MGP in the vascular wall is related to large artery stiffening.

Dp-ucMGP, HF and arterial stiffness

Recent reports demonstrated a positive relationship between dp-ucMGP levels and CF-PWV in a general population sample, in patients with type 2 diabetes mellitus and in patients with mild-to-moderate renal dysfunction without HF.[14; 15] A recent study also demonstrated an association between E/e’ and dp-ucMGP in a general population sample without HF, suggesting that a poor vitamin K status could constitute an early risk factor for the development of diastolic dysfunction.[16] To the best of our knowledge, only one previous

study has assessed dp-ucMGP levels in HF patients. This study included subjects with HFrEF and demonstrated increased dp-ucMGP levels in this population.[4] Our study is the first to compare levels of dp-ucMGP in HFpEF and HFrEF and to establish a relationship between dp-ucMGP and large artery stiffness in subjects with HF. We demonstrate increased dp-ucMGP levels in both HFpEF and HFrEF. Interestingly, this association was independent of multiple confounders other than renal dysfunction in both types of HF; however, in HFpEF (but not in HFrEF) it was not independent of renal function. HFpEF and HFrEF constitute different conditions with different pathophysiologic contributors and response to therapy. Comorbidities, such as renal dysfunction, are thought to play an important role in the pathophysiology of HFpEF.[17] Therefore, the fact that the difference in dp-ucMGP levels was not independent of renal dysfunction may be due to the known association of HFpEF with chronic kidney disease (which was also evident in our study sample, and may primarily be responsible for increased dpucMGP levels in HFpEF), or alternatively, to the fact that patients with HFpEF who exhibit deficient MGP activation are more prone to developing progressive renal dysfunction from arterial stiffening. Although our cross-sectional study cannot assess these temporal relationships, both scenarios are plausible. A recent animal model demonstrated intrinsic abnormalities in vitamin K metabolism even in early CKD,[18] and renal dysfunction has recently been shown to be strongly and independently associated with dp-ucMGP levels.[15] Yet, deficient activation of MGP, with subsequent aortic calcification and stiffening, has the potential to promote the progression of renal dysfunction on one hand,[19] and excessive myocardial pulsatile load (which can contribute to myocardial remodeling, dysfunction and the development of HFpEF) on the other.[20; 21] Large artery stiffness and the associated pulsatile arterial hemodynamic dysfunction are implicated in the pathophysiology of HFpEF[20; 21; 22; 23; 24; 25; 26; 27; 28] through effects on LV pulsatile load, the timing of wave reflections and central pressure and flow pulsatility, which in turn may promote comorbidities such as cognitive dysfunction[29] and particularly renal dysfunction.[12; 30; 31; 32; 33; 34] Regardless of the primary cause, our findings demonstrate that patients with HFpEF, like their HFrEF counterparts, exhibit abnormal vitamin-K dependent MGP activation, which in turn correlates with large artery stiffness. To date, effective pharmacologic interventions to improve outcomes in HFpEF are not available. Therefore, the novel identification of specific pathways that may play a role in the underlying pathophysiology of HFpEF is important, particularly if they can be targeted with available pharmacologic agents. Vitamin K2 supplementation has been shown to reduce dp-ucMGP levels, indicating that it exerts a positive effect on MGP maturation/carboxylation, consistent with its known biologic role.[35; 36] Whether vitamin K2 supplementation can reduce or prevent large artery stiffening in subjects with or at risk for HFpEF or HFrEF remains to be studied in randomized trials.

We found a relationship between warfarin use and large artery stiffness (CF-PWV). It is known that warfarin interferes with vitamin K epoxide reductase in the vitamin K cycle, thereby interfering with the activation of vitamin K dependent proteins (VKDPs) such as MGP, which leads to increased levels of inactive MGP (dp-ucMGP).[37; 38] We report an association between warfarin with increased dp-ucMGP levels in HF and demonstrate that warfarin use is associated with increased large artery stiffness (CF-PWV) in heart failure. This relationship was eliminated after adjustment for dp-ucMGP levels. These findings

support the importance of poor activation of MGP in the vascular wall as an underlying factor in large artery stiffening. It is possible that warfarin use may potentiate this sequence of deleterious events by interfering with MGP activation. This off-target effect of warfarin may have important clinical implications, given the millions of patients with HF and other conditions current being treated with warfarin, and the availability of Direct Oral Anticoagulants (DOACs). Not only could the use of DOACs avoid warfarin-induced effects on vitamin-K dependent arterial calcification and stiffening, but the potential benefits of vitamin K2 supplementation could also be extended to patients with heart failure who require chronic anticoagulation. These important clinical issues need to be addressed in future prospective studies.

Strengths and limitations

Our study should be interpreted in the context of its strengths and limitations. Strengths of our study include the inclusion of a multiethnic population of patients and the inclusion of both HFpEF and HFrEF. We used high-fidelity carotid and femoral tonometry for measurements of CF-PWV, which is considered the gold-standard non-invasive approach for the non-invasive assessment of large artery stiffness.[12] We utilized cardiac magnetic resonance imaging to accurately assess cardiac structure and function, and precisely quantify LVEF, which is critical for HF case classification. Our study also has some limitations. Consistent with the demographics of patients in a VA Medical Center, our sample was composed predominantly of males. Finally, this was a cross-sectional investigation, which provides evidence of associations, rather than direct causal inferences. However, our observations and hypotheses were based on, and should be interpreted in the context of the biologic effects of dp-ucMGP that impact arterial stiffness, and the well-established effects of warfarin on vitamin K-dependent protein carboxylation. However, future prospective longitudinal studies in larger cohorts are needed to demonstrate the causal relationship between dp-ucMGP and HF.

Perspectives

In summary, we demonstrate that both HFpEF and HFrEF are associated with greater dp-ucMGP levels, indicating a deficient vitamin K-dependent activation of MGP, an inhibitor of arterial calcification. We further demonstrate that dp-ucMGP levels are independently associated with large artery stiffening in HF and that warfarin use is associated with arterial stiffness. Interpreted in the context of the biologic role of MGP, our observations support a role for deficient vitamin-K dependent MGP activation in large artery stiffening in human HF. Warfarin use may exert deleterious effects on arterial stiffness via inhibition of vitamin-K-dependent MGP activation, which has important clinical implications. Future studies (including randomized trials) should examine the potential therapeutic implications of avoidance of warfarin on arterial calcification and stiffening, and of vitamin K2 supplementation in patients with or at risk for HF.

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Novelty and Significance

What is New?

- Our study is the first to demonstrate increased dp-ucMGP levels in both HFpEF and HFrEF.
- Warfarin use is significantly associated with large artery stiffness in HF, which could relate to its effects on the MGP-dependent pathway.

What is Relevant?

- Large artery stiffness plays an important role in the development of cardiac dysfunction and target organ damage (such as the kidney), thus contributing both to heart failure and to associated comorbidities.
- Warfarin, a widely used anticoagulant agent, is associated with large artery stiffness in HF patients, which may relate to its inhibition of vitamin K-dependent MGP activation.
- Given the increasing use of DOACs, future studies should examine whether warfarin use leads to large artery stiffening in HF patients and whether vitamin K2 supplementation could represent a novel therapy to reduce or prevent the progression of large artery calcification and stiffness in patients with or at risk for heart failure.

Summary

We demonstrate increased levels of dp-ucMGP in HFpEF and HFrEF subjects in comparison to controls without HF. We also demonstrate that warfarin is associated with large artery stiffness, which may be due to the known vitamin K-dependent activation of MGP and its inhibitory role on arterial calcification. This relationship has important implications regarding the potential deleterious effects of warfarin (and potential benefits of vitamin K supplementation) on large artery stiffness in human HF, which should be tested in future trials.

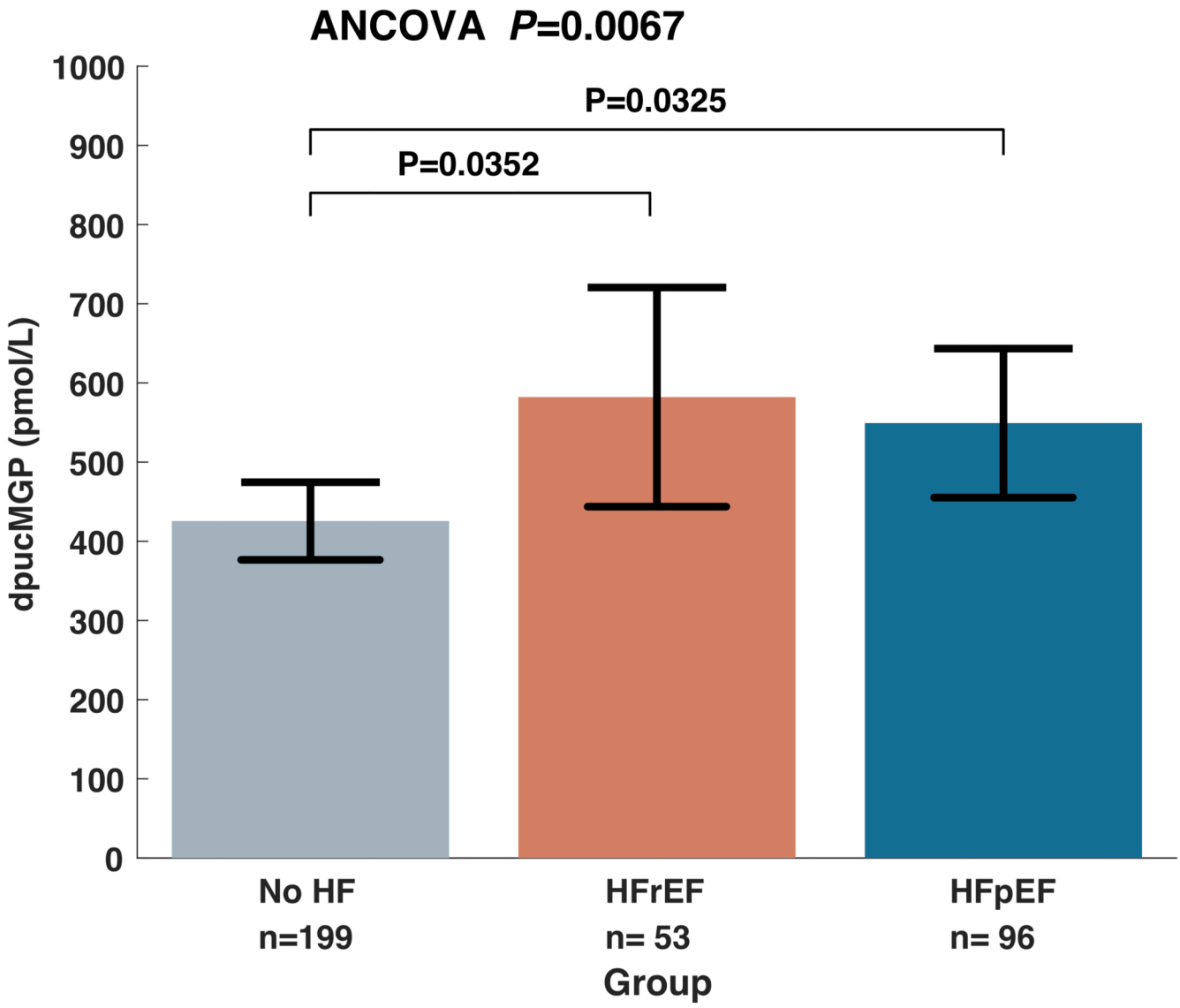

Figure 1.
Comparison of dp-ucMGP levels between subjects with no HF, HFrEF and HFpEF, adjusted for age, gender, ethnicity and warfarin use.

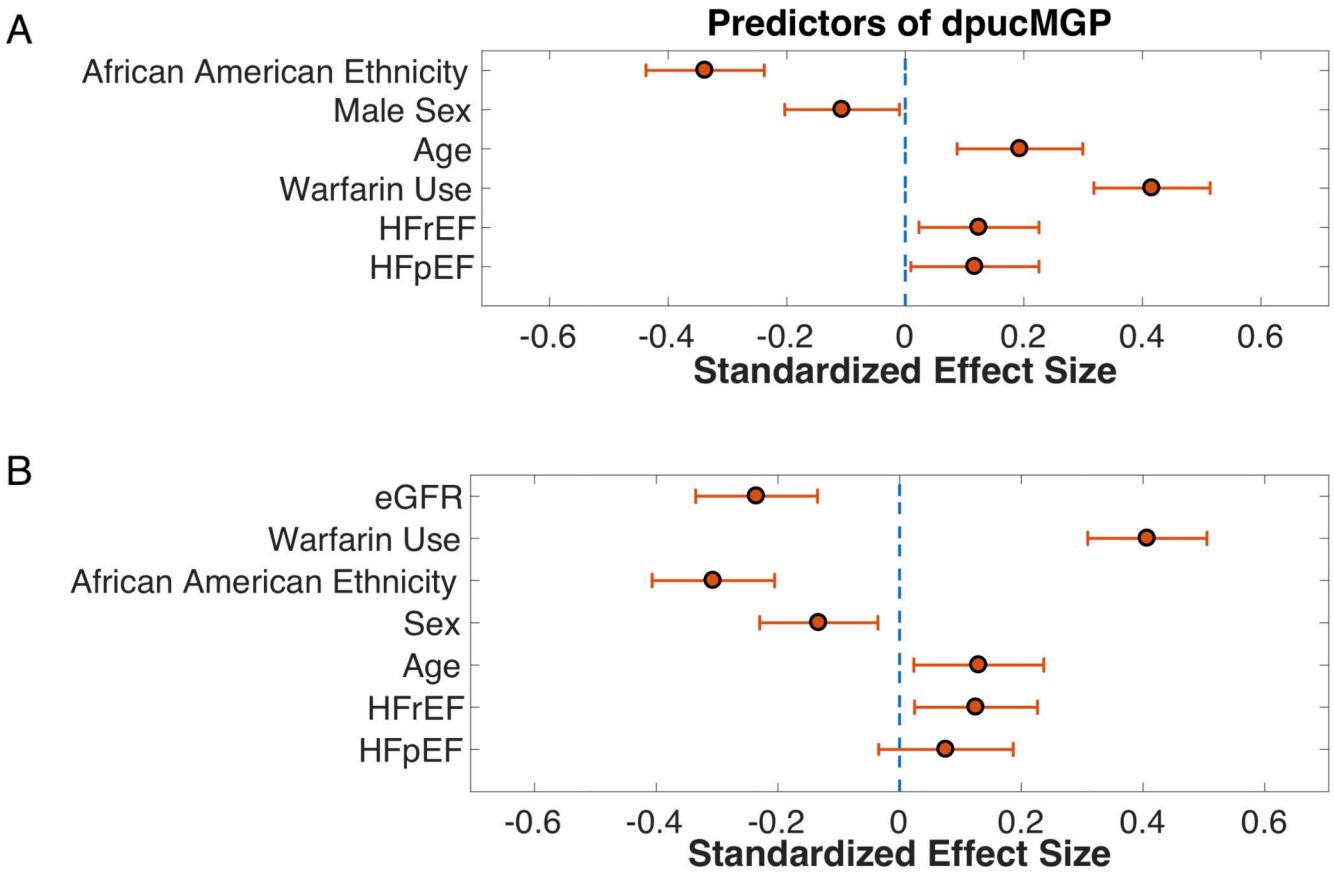

Figure 2.

Multivariable model showing correlates of dp-ucMGP. 2A: without adjustment for estimated glomerular filtration rate (eGFR). 2B: with further adjustment for eGFR. Standardized regression coefficients and 95% confidence intervals are shown.

Table 1.

General Characteristics of Study Population.

Characteristics	No HF	HFrEF	HFpEF	*P* value
Age, years	59.9 (58.2 to 61.5)	65 (61.5 to 68.5)	63.9 (61.3 to 66.4)	0.0033 *#
Male Sex	179 (89.95%)	52 (98.11%)	80 (83.33%)	0.0182
Body mass index, kg/m^2	30 (29.1 to 30.8)	28 (26.4 to 29.7)	36 (34.4 to 37.5)	<0.0001 #$
Hypertension	151 (75.88%)	43 (81.13%)	87 (90.62%)	0.0108
Diabetes Mellitus	82 (41.21%)	27 (50.94%)	67 (69.79%)	<0.0001
Coronary Artery Disease	50 (25.13%)	28 (52.83%)	29 (30.21%)	0.0005
Brachial SBP, mmHg	142 (139 to 145)	141 (136 to 147)	148 (144 to 152)	0.0541
Carotid SBP, mmHg	132 (129 to 136)	136 (129 to 143)	139 (134 to 144)	0.0961
Diastolic Blood Pressure, mmHg	82.2 (80.4 to 84.1)	82.4 (79.1 to 85.8)	82.8 (80.2 to 85.4)	0.9496
Mean Artery Pressure, mmHg	102 (100 to 104)	103 (99 to 107)	104 (101 to 107)	0.5122
Carotid Pulse Pressure, mmHg	49 (46.5 to 51.6)	52.1 (47 to 57.1)	55.3 (51.1 to 59.5)	0.0289 #
Carotid-Radial PWV, mmHg	9.7 (9.3 to 10.1)	10.3 (9.5 to 11.1)	9.6 (9.1 to 10.2)	0.3727
Carotid-Femoral PWV, m/s	9.5 (9 to 10)	10.2 (9.1 to 11.3)	9.9 (9.1 to 10.7)	0.3828
Serum Magnesium (mg/dL)	1.97 (1.91 to 2.03)	1.96 (1.84 to 2.08)	1.84 (1.75 to 1.92)	0.0372 #
Serum Phosphate (mg/dL)	3.38 (3.28 to 3.47)	3.31 (3.1 to 3.5)	3.41 (3.25 to 3.56)	0.7686
Serum Calcium (mg/dL)	8.78 (8.14 to 9.38)	8.7 (7.41 to 9.94)	8.42 (7.54 to 9.34)	0.8188
LV EDV index (ml/m^2)	48.9 (46.8 to 51.1)	76.4 (70.2 to 82.7)	56.4 (52.4 to 60.4)	<0.0001 *#$
LV EF	57.3 (55.2 to 59.4)	31.9 (29.8 to 34.1)	61.3 (57.6 to 64.9)	<0.0001 *$
LV Mass (g)	139 (133 to 145)	184 (169 to 200)	164 (153 to 176)	<0.0001 *#
LV Mass Index (g/height$^{1.7}$)	30.6 (29.3 to 31.8)	38.7 (35.7 to 41.6)	37.2 (34.7 to 39.7)	<0.0001 *#
Medication use				
Estimated GFR (ml/1.73 m^2)	83.2 (78.8 to 87.5)	74.2 (66.5 to 81.9)	68.5 (63.3 to 73.6)	<0.0001 #
Beta Blockers	80 (40.20%)	47 (88.68%)	62 (64.58%)	<0.0001
Aspirin	103 (51.76%)	42 (79.25%)	69 (71.88%)	<0.0001
ACE Inhibitors	86 (43.22%)	39 (73.58%)	49 (51.04%)	0.0004
ARBs	17 (8.54%)	7 (13.21%)	20 (20.83%)	0.0118
Furosemide	4 (2.01%)	37 (69.81%)	58 (60.42%)	<0.0001

Characteristics	No HF	HFrEF	HFpEF	*P* value
Digoxin	3 (1.51%)	2 (3.77%)	2 (2.08%)	0.5787
Spironolactone	4 (2.01%)	7 (13.21%)	7 (7.29%)	0.0026
Hydralazine	6 (3.02%)	8 (15.09%)	10 (10.42%)	0.0024
Warfarin	14 (7.04%)	11 (20.75%)	3 (3.12%)	0.0006
Calcium-channel blockers	56 (28.14%)	11 (20.75%)	34 (35.42%)	0.1542
Insulin	29 (14.57%)	8 (15.09%)	31 (32.29%)	0.0010

Numbers represent mean (95%CI) or count (percentage); GFR=glomerular filtration rate.

*Post-hoc *P*<0.05, No HF vs. HFpEF

#Post-hoc *P*<0.05, HFrEF vs. HFpEF

$Post-hoc P<0.05 No HF vs HFpEF.

Table 2.

Linear Regression Model Showing the Correlates of dp-ucMGP without adjustment for estimated glomerular filtration rate

Correlates	Standardized Estimate	95%CI, Lower Bound	95%CI, Upper Bound	*P* value
HFpEF	**0.12**	**0.01**	**0.23**	**0.03328**
HFrEF	**0.12**	**0.02**	**0.23**	**0.01617**
Warfarin Use	**0.42**	**0.32**	**0.51**	**<0.00001**
Age	**0.19**	**0.09**	**0.30**	**0.00039**
Male Sex	**−0.11**	**−0.20**	**−0.01**	**0.03121**
African American Race	**−0.34**	**−0.44**	**−0.24**	**<0.00001**
Other Race	−0.07	−0.16	0.03	0.16757
BMI	0.04	−0.06	0.15	0.41162
Systolic Blood Pressure	−0.02	−0.12	0.08	0.70679
Hypertension	0.05	−0.06	0.15	0.36387
CAD	−0.05	−0.16	0.05	0.28546
DM	0.01	−0.10	0.11	0.89556
Magnesium	−0.05	−0.18	0.08	0.42580
Phosphorus	0.02	−0.08	0.12	0.70950
Calcium	0.04	−0.08	0.17	0.46720

Table 3.

Linear Regression Model Showing the Correlates of dp-ucMGP after further adjustment for estimated glomerular filtration rate

Correlates	Standardized Estimate	95%CI, Lower Bound	95%CI, Upper Bound	*P* value
HFpEF	0.08	−0.03	0.19	0.17573
HFrEF	**0.13**	**0.02**	**0.23**	**0.01502**
Warfarin Use	**0.41**	**0.31**	**0.50**	**<0.00001**
Age	**0.13**	**0.02**	**0.24**	**0.01699**
Male Sex	**−0.13**	**−0.23**	**−0.04**	**0.00759**
African American Ethnicity	**−0.31**	**−0.41**	**−0.21**	**<0.00001**
eGFR	**−0.24**	**−0.34**	**−0.13**	**<0.00001**
Other Race/Ethnicity	−0.05	−0.14	0.05	0.35316
BMI	0.07	−0.04	0.18	0.21841
Systolic Blood Pressure	−0.01	−0.11	0.08	0.77311
Hypertension	0.02	−0.09	0.12	0.74233
CAD	−0.06	−0.16	0.04	0.25006
DM	0.00	−0.11	0.10	0.96671
Magnesium	−0.10	−0.23	0.03	0.13995
Phosphorus	0.03	−0.07	0.13	0.57629
Calcium	0.10	−0.02	0.22	0.11076