Inactive Matrix Gla-Protein and Arterial Stiffness in Type 2 Diabetes Mellitus

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BACKGROUND
Large artery stiffness is increased in diabetes mellitus and causes an excessive pulsatile load to the heart and to the microvasculature. The identification of pathways related to arterial stiffness may provide novel therapeutic targets to ameliorate arterial stiffness in diabetes. Matrix Gla-Protein (MGP) is an inhibitor of vascular calcification. Activation of MGP is vitamin K dependent. We hypothesized that levels of inactive MGP (dephospho-uncarboxylated MGP; dp-ucMGP) are related to arterial stiffness in type 2 diabetes.

METHODS
We enrolled a multiethnic cohort of 66 participants with type 2 diabetes. Carotid-femoral pulse wave velocity (CF-PWV) was measured with high-fidelity arterial tonometry (Sphygmocor Device). Dp-ucMGP was measured with ELISA (VitaK; The Netherlands).

RESULTS
The majority of the participants were middle-aged (62 ± 12 years), male (91%), and had a history of hypertension (82%). Average hemoglobin A1C was 7.2% (55 mmol/mol). Mean dp-ucMGP was 624 ± 638 pmol/l and mean CF-PWV was 11 ± 4 m/sec. In multivariable analyses, dp-ucMGP was independently related to African American ethnicity (β = −0.24, P = 0.005), warfarin use (β = 0.56, P < 0.001), and estimated glomerular filtration rate (eGFR, β = −0.32, P < 0.001). Dp-ucMGP predicted CF-PWV (β = 0.40, P = 0.011), even after adjustment for age, gender, ethnicity, mean arterial pressure, eGFR, and warfarin use.

CONCLUSIONS
In our cross-sectional analysis, circulating dp-ucMGP was independently associated with CF-PWV in type 2 diabetes. This suggests that deficient vitamin K-dependent activation of MGP may lead to large artery stiffening and could be targeted with vitamin K supplementation in the patients with diabetes.

Keywords: arterial stiffness; blood pressure; diabetes mellitus; hypertension; Matrix Gla-Protein; pulse wave velocity; vitamin K.

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Large artery stiffness is increased in diabetes mellitus and independently predicts cardiovascular morbidity and mortality.4 Carotid-femoral pulse wave velocity (CF-PWV) is considered the current noninvasive “gold standard” test to assess large artery stiffness.5 An increased CF-PWV is independently associated with microvascular complications of diabetes, including nephropathy (albuminuria and glomerular filtration rate),6 retinopathy,7 neuropathy,8 and cognitive decline.9 Despite these proven strong associations of large artery stiffness, limited insight exists into the mechanisms underlying arterial stiffening.

Matrix Gla-Protein (MGP) is a small protein (14k Da) secreted by the vascular smooth muscle cells and chondrocytes in the tunica media of the arterial wall.10 The inactive form of MGP (dephospho-uncarboxylated MGP, dp-ucMGP) undergoes 2 posttranslational modifications for maturation into the active form, with an intermediate form called dephospho-carboxylated MGP. Carboxylation of dp-ucMGP is dependent on the presence of vitamin K:

\[
\text{dp-ucMGP} \xrightarrow{\gamma\text{-Glutamyl Carboxylation}} \text{dp-cMGP} \xrightarrow{\text{Serine Phosphorylation}} \text{Active MGP}
\]

Active MGP is protective against the arterial calcification through inhibition of bone morphogenetic protein-induced osteochondrogenic programming, along with the rate-limiting direct inhibitory action on ectopic mineralization.10 The levels of circulating dp-ucMGP correlate with macrovascular calcifications in type 2 diabetes.11 Moreover, dp-ucMGP has a strong association with cardiovascular morbidity and mortality.12,13

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Two prior cross-sectional population-based studies have proven the association of circulating dp-ucMGP with CF-PWV. However, only a minority of the participants had diabetes in these studies. Furthermore, these 2 studies only included Caucasians Europeans, and whether race/ethnicity may influence dp-ucMGP is unknown.

Large artery stiffness in diabetes has been proposed to result from accelerated advanced glycation end products-mediated collagen crosslinking, hyperglycemia-mediated osteoinduction and insulin resistance-related endothelial dysfunction. However, aortic calcification is also increased in diabetes and likely contributes to increased aortic stiffness. MGP has been shown to protect against arterial calcifications in setting of hyperglycemia. However, whether circulating dp-ucMGP is related to large artery stiffness in diabetes is unknown.

The aim of this study is to assess whether circulating dp-ucMGP is independently associated with CF-PWV (i.e., large artery stiffness) in adults with type 2 diabetes in a US multiethnic sample.

METHODS

We prospectively enrolled a convenience clinical sample of 66 subjects aged 18–80 years, with a history of type 2 diabetes at the Corporal Michael J. Crescenz VA Medical Center. The protocol was approved by the Philadelphia VA Medical Center Institutional Review Board, and all subjects provided written informed consent. Participants underwent a noninvasive CF-PWV assessment and a blood sample collection for measurement of dp-ucMGP. Exclusion criteria were as follows: (i) conditions that would make the study measurements less accurate or unreliable (i.e., arrhythmia such as atrial fibrillation at the time of evaluation); (2) pregnancy; (3) unstable medical conditions that would likely prevent the subject from completing the study procedures; and (4) inability to sign an informed consent form.

CF-PWV measurements

CF-PWV was measured using a Sphygmocor device (Atcor Medical, Sydney, Australia) with sequential tonometric recordings of the carotid and the femoral pulse, using the QRS complex as a fiducial time point.

Plasma dp-ucMGP measurements

Venous blood samples were collected in citrate at the time of enrollment, and plasma was prepared and stored at −80°C for batch analysis. Dp-ucMGP was measured utilizing a dual-antibody sandwich ELISA technique (VitaK; Maastricht University; The Netherlands). Previously reported intra-assay coefficients of variation for this assay are 3.1% and 5.4% for lower and upper limit of normal range. Interassay variation coefficients have been reported at 6.9% and 13.6% for lower and upper limit of normal range.

Statistical methods

Continuous variables are presented as mean ± SD unless otherwise stated. Categorical variables are presented as frequencies and percentages. Linear regression was performed to determine the clinical correlates of dp-ucMGP in univariable and multivariable models. The relationship between dp-ucMGP and CF-PWV was also assessed, in unadjusted analyses and after adjustment for potential confounders. Significant correlates of dp-ucMGP were included in adjusted analyses. Given the importance of age and mean arterial pressure as determinants of CF-PWV, we also included these covariates in adjusted models. For easier comparison of the magnitude of the relationships of different predictors, standardized regression coefficients are presented. All probability values are 2-tailed. Statistical significance was defined alpha <0.05. Statistical analyses were performed using SPSS software (IBM SPSS version 23, Chicago, IL).

RESULTS

Baseline characteristics of the study participants are shown in the Table 1. Mean age in the sample was 62 years; the sample was predominantly composed of males, with approximately...
equal proportions of Caucasian and African American participants. There was a high prevalence of hypertension (82%). The average hemoglobin A1C was 7.2% (55 mmol/mol) and nearly a third of the participants were receiving insulin replacement therapy. Mean circulating dp-ucMGP was 624 ± 638 pmol/l and mean CF-PWV was 11 ± 4 m/sec.

Clinical correlates of dp-ucMGP concentrations

In univariable analysis, older age ($\beta = 0.29; P = 0.017$), a lower estimated glomerular filtration rate (eGFR; $\beta = -0.46; P < 0.001$), and warfarin use ($\beta = 0.64; P < 0.001$) were associated with higher dp-ucMGP concentrations (Table 2), whereas African American ethnicity was associated with lower dp-ucMGP concentrations ($\beta = -0.30; P = 0.014$). In a multivariable model, eGFR ($\beta = -0.32; P < 0.001$), African American ethnicity ($\beta = -0.24; P = 0.005$), warfarin use ($\beta = 0.56; P < 0.001$) were significant predictors of CF-PWV. This model predicted 61% of the variance in dp-ucMGP in the sample.

dp-ucMGP as a predictor of CF-PWV

Table 3 shows regression models in which dp-ucMGP is tested as a predictor of CF-PWV. In unadjusted analyses, dp-ucMGP was a significant predictor of CF-PWV ($\beta = 0.40; P = 0.001$). After adjustment for age, gender, and mean arterial pressure, dp-ucMGP remained a significant predictor of CF-PWV ($\beta = 0.26; P = 0.010$). Similarly, after adjustment for significant correlates of dp-ucMGP shown in Table 2, dp-ucMGP remained a significant independent predictor of CF-PWV ($\beta = 0.40; P = 0.011$).

DISCUSSION

In a multiethnic cohort of the participants with type 2 diabetes, we found that the levels of circulating dp-ucMGP are independently associated with CF-PWV, a measure of large artery stiffness. Our findings suggest that impaired vitamin K-dependent activation of MGP might play an important role in modulating large artery stiffness in type 2 diabetes. These mechanisms can be targeted to reduce arterial stiffness in these patients, for instance by increasing nutritional vitamin K intake.

Multiple studies have shown that large artery stiffness is increased in both type 1 and type 2 diabetes. Increased stiffness is seen early in the course of the disease, and recent data suggests that this might even precede the onset of disease (impaired fasting glucose and impaired glucose tolerance states). Increased aortic stiffness and early arrival of reflected waves lead to excessive after-loading of the left ventricle during systole, respectively. Moreover, an increase in large artery stiffness leads to excessive penetration of pulsatility from large arteries into the microvasculature, which could lead to end-organ microvascular damage.

Table 2. Characteristics of study participants as predictors of dp-ucMGP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ Coefficient</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Age</td>
<td>0.29</td>
<td>0.017</td>
</tr>
<tr>
<td>Male</td>
<td>0.06</td>
<td>0.611</td>
</tr>
<tr>
<td>African American ethnicity</td>
<td>-0.30</td>
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<tr>
<td>Current smoker</td>
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<td>0.789</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.07</td>
<td>0.559</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.01</td>
<td>0.912</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.22</td>
<td>0.071</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.21</td>
<td>0.086</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>0.12</td>
<td>0.328</td>
</tr>
<tr>
<td>CVA or TIA</td>
<td>-0.04</td>
<td>0.770</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>-0.02</td>
<td>0.890</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin use</td>
<td>-0.15</td>
<td>0.223</td>
</tr>
<tr>
<td>ACE inhibitor or ARB use</td>
<td>0.14</td>
<td>0.267</td>
</tr>
<tr>
<td>Aspirin use</td>
<td>0.13</td>
<td>0.296</td>
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<tr>
<td>Beta blocker use</td>
<td>0.22</td>
<td>0.071</td>
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<tr>
<td>Statin use</td>
<td>-0.02</td>
<td>0.896</td>
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<tr>
<td>Warfarin use</td>
<td>0.64</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: ACE, angiotensin convertase enzyme; ARB, angiotensin receptor blocker; CVA, cerebrovascular accident; dp-ucMGP, dephospho-uncarboxylated Matrix Gla-Protein; eGFR, estimated glomerular filtration rate; TIA, transient ischemic attack.

*Standardized coefficients are presented for easier comparison of various predictors.
MGP plays an important role in inhibiting ectopic mineralization of the arterial wall. Dp-ucMGP has poor affinity for calcium and is preferentially released in the circulation. Liabeuf et al., in a cross-sectional study of 198 middle-aged participants with type 2 diabetes, measured plasma dp-ucMGP and total ucMGP. In a multivariable model adjusting for age, gender, cardiovascular disease, and total ucMGP, the authors found an independent correlation of circulating dp-ucMGP with arterial calcifications (below-knee vascular calcifications as detected by computed tomography ($\beta = 0.26, P < 0.0001$). However, whether plasma dp-ucMGP correlates with large artery (i.e., aortic) stiffness in diabetes has not been previously investigated. Although this relationship has recently been reported in general population European Caucasian samples (SKIPOGH and Czech-MONICA studies), the mechanisms for the increase in arterial stiffness in diabetes differs from those in the general population. Yao et al., in their mouse model of diabetes with MGP gene deletion, proved that elevated glucose levels lead to increase in morphogenetic protein signaling, endothelial-mesenchymal transitioning and osteoinduction. Enhancement of MGP expression through a transgene limited this process. Similarly, Doyon et al., in their mouse model of diabetes showed that the levels of active MGP were reduced and correlated with calcifications in the aortic wall. We found a substantial independent correlation of dp-ucMGP with CF-PWV. This suggests that MGP might have an intrinsic role in inhibiting large artery calcifications and stiffness in type 2 diabetes.

Multiple prospective observational studies have proven the association of dp-ucMGP with cardiovascular morbidity and mortality. Dalmeijer et al., in a subgroup analysis of the 518 participants with type 2 diabetes enrolled in the EPIC-NL prospective cohort study, demonstrated an association of elevated dp-ucMGP with a higher risk of cardiovascular disease (hazard ratio: 1.21), heart failure (hazard ratio: 1.75), and peripheral arterial disease (hazard ratio: 1.32). Concordant with prior studies, we found higher circulating dp-ucMGP among participants taking warfarin. This supports the crucial role of vitamin K in activating MGP and raises the issue of whether warfarin administration causes deleterious long-term effects on the arterial wall. Multiple studies have associated dietary intake of vitamin K with arterial stiffness. Whereas the trials of vitamin K1 supplementation have yielded inconsistent results, a recent trial with vitamin K2 supplementation has shown promising results. Participants with diabetes, however, were neither targeted in the prior trials, nor are being targeted in any of 6 ongoing vitamin K supplementation trials (May 2016; clinicaltrials.gov search). Our findings support the notion that MGP might have a critical role in inhibiting large artery stiffness in type 2 diabetes, and targeting MGP might be effective in the patients with diabetes. This needs to be tested in future studies with experimental designs.

An important novel finding of our study is that African American participants with type 2 diabetes demonstrated lower levels of dp-ucMGP. Multiple cross-sectional studies have found significant independent impact of race and ethnicity on large artery stiffness. Shah et al., in a study of adolescents and young adults with type 2 diabetes, reported higher large artery stiffness (as determined by CF-PWV) in participants with African American ethnicity compared to Caucasian participants. Mechanisms underlying the effect of race and ethnicity on large artery stiffness have not been explored previously. Wei et al., in a recent cross-sectional population-based analyses, found significantly lower dp-ucMGP levels in Black South African participants compared to White South African participants. In our study, we found inverse association of dp-ucMGP with African American ethnicity. This novel finding could reflect either (i) decreased expression of MGP in this subgroup leading to reduced circulating dp-ucMGP or (ii) a compensatory increase in maturation (posttranslational modification) of MGP in response to a primary stiffening process leading to lower circulating dp-ucMGP. Future studies will be required to assess the biologic basis of the lower levels of dp-ucMGP in African Americans with diabetes, and to explore the role of this pathway in mediating the influence of race and ethnicity on large artery stiffness.

Our study should be interpreted in the context of its strengths and limitations. Our study is the first to assess the association of circulating dp-ucMGP with large artery stiffness in type 2 diabetes. Enrollment of a multiethnic cohort allowed us to identify the differential association of ethnicity with dp-ucMGP.
with dp-ucMGP. We assessed CF-PWV, the gold standard index of large artery stiffness, and performed appropriate adjustments for distending pressure (i.e., mean arterial pressure) as recommended by current guidelines.29 Our study also has limitations. We enrolled our participants through convenience sampling and the majority of the participants were males, given the characteristics of the VA Medical Center patient population. Further studies will be required in larger populations with diabetes mellitus that also include other ethnic/race minorities.

In conclusion, in participants with type 2 diabetes, we found an independent correlation of dp-ucMGP with large artery stiffness after adjusting for potential confounders. dp-ucMGP showed positive correlation with warfarin use, supporting the role of vitamin K in MGP activation in this population. Our findings form the basis of further investigation into the role of vitamin K supplementation in reducing arterial stiffness in type 2 diabetes.

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J.A.C. contributed to study design, data collection, analysis, draft of manuscript, and obtaining funding. M.S. contributed to data analysis and draft of manuscript. I.V., S.V., U.K., A.T., N.E.A.D., C.V. contributed towards obtaining data. A.A.S., M.R.K., M.B. contributed towards quantifying data. S.R.A. contributed towards study design, data collection, and obtaining funding. All authors were involved in the acquisition and/or interpretation of the data, made critical revision of the manuscript for important intellectual content, and provided final approval of the version to be published.

DISCLOSURE

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