Angiotensin-converting enzyme inhibitors increase anti-fibrotic biomarkers in African Americans with left ventricular hypertrophy

Cesar A. Romero MD, PhD1 | Shobi Mathew MS2 | Benjamin Wasinski MS2 | Brian Reed BS2 | Aaron Brody MD2 | Rachelle Dawood BS2 | Michael J. Twiner MD, PhD2 | Candace D. McNaughton MD, PhD3 | Rafael Fridman MD4 | John M. Flack MD5 | Oscar A. Carretero MD1 | Phillip D. Levy MD, MPH2

1Hypertension and Vascular Research Division, Internal Medicine Department, Henry Ford Hospital, Detroit, MI, USA
2Department of Emergency Medicine and Integrative Biosciences Center, Wayne State University, Detroit, MI, USA
3Department of Emergency Medicine, Vanderbilt University Medical Center and Geriatric Research Education Clinical Center VA Medical Center, Nashville, TN, USA
4Department of Pathology and Oncology, Wayne State University, Detroit, MI, USA
5School of Medicine Department of Internal Medicine, Southern Illinois University, Springfield, IL, USA

Abstract

Angiotensin-converting enzyme inhibitors (ACEi) are part of the indicated treatment in hypertensive African Americans. ACEi have blood pressure-independent effects that may make them preferred for certain patients. We aimed to evaluate the impact of ACEi on anti-fibrotic biomarkers in African American hypertensive patients with left ventricular hypertrophy (LVH). We conducted a post hoc analysis of a randomized controlled trial in which hypertensive African American patients with LVH and vitamin D deficiency were randomized to receive intensive antihypertensive therapy plus vitamin D supplementation or placebo. We selected patients who had detectable lisinopril (lisinopril group) in plasma using liquid chromatography/mass spectrometry analysis and compared them to subjects who did not (comparison group) at the one-year follow-up. The pro-fibrotic marker type 1 procollagen C-terminal propeptide (PICP) and the anti-fibrotic markers matrix metalloproteinase-1 (MMP-1), tissue inhibitor of metalloproteinases 1 (TIMP-1), telopeptide of collagen type I (CITP), and N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) peptide were measured. Sixty-six patients were included, and the mean age was 46.2 ± 8 years. No difference was observed in the number and intensity of antihypertensive medications prescribed in each group. Patients with detectable lisinopril had lower blood pressure than those in the comparison group. The anti-fibrotic markers Ac-SDKP, MMP-1, and MMP-1/TIMP-1 ratio were higher in patients with detectable ACEi (all \( p < .05 \)). In a model adjusted for systolic blood pressure, MMP-1/TIMP-1 (\( p = .02 \)) and Ac-SDKP (\( p < .001 \)) levels were associated with lisinopril. We conclude that ACEi increase anti-fibrotic biomarkers in hypertensive African Americans with LVH, suggesting that they may offer added benefit over other agents in such patients.

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1 | INTRODUCTION

Hypertension is the leading risk factor for cardiovascular mortality and has a particularly early incidence and high prevalence in the African American population. Elevated blood pressure (BP) leads to vascular, myocardial, and renal tissue damage, which are responsible for the high burden of disease in this population. With the progression of hypertension, adaptive and pathologic changes are observed in the heart and vascular beds. Some of these changes are macroscopic, such as left ventricular hypertrophy (LVH) and arterial stiffness, while other alterations are microscopic, including endothelial dysfunction, vascular rarefaction, inflammation, and fibrosis. Myocardial and vascular fibrosis are of particular importance because they can lead to early cardiac dysfunction, accelerate the vascular damage by altering physiological vascular mechanics, and decrease tissue oxygenation.

Fibrosis is a dynamic process characterized by the formation, deposition, and late consolidation (ie, crosslinking) of fibrotic fiber, specifically collagen. Simultaneous with the profibrotic process, a series of anti-fibrotic mechanisms promote collagen release after the injury is healed and before extensive scarring (crosslinking) occurs. Cardiac fibroblasts synthesize type I and III collagen fibers in the interstitium of the heart when a stressor or injury occurs, as in hypertension. During the extracellular conversion of procollagen type I into mature collagen type I, type 1 procollagen C-terminal propeptide (PICP) is generated and released from the heart into the circulation. Serum PICP levels correlate with myocardial collagen and collagen type I deposition. On the other hand, matrix metalloproteinase 1 (MMP-1), or cardiac collagenase, is the key enzyme for interstitial collagen degradation. MMP-1 releases the carboxy-terminal telopeptide of collagen type I (CITP) into the circulation when collagen degradation occurs. MMP-1 is regulated by the tissue inhibitor of matrix metalloproteinase 1 (TIMP-1); thus, net MMP-1 activity can be estimated from the MMP-1/TIMP-1 ratio, with a higher ratio suggesting more collagen degradation or an anti-fibrotic effect. Unfortunately, after crosslinking, collagen is resistant to the MMP-1 degradative process. The PICP/CITP ratio reflects the balance between type I collagen synthesis and degradation. It is possible to predict the intensity of collagen crosslinking with the CITP/MMP-1 ratio, where a higher value is associated with less crosslinked cardiac collagen.

Part of the adverse cardiac remodeling and fibrosis are explained by the renin-angiotensin-aldosterone system (RAAS) which includes angiotensin II (Ang II) via angiotensin receptor 1 (AT1). Ang II production has untoward effects leading to remodeling of the cardiovascular tissues, including stimulation and proliferation of cardiac fibroblasts. RAAS activation stimulates collagen deposition and is involved in cardiac fibrosis. Matrix metalloproteinases (MMPs) and the tissue inhibitors of matrix metalloproteinases (TIMPs) are fundamental mediators and regulators of the cardiac fibrotic process and sensitive to the RAAS modulation. Specifically, MMP-9 and TIMP-1 are known to be involved in models of cardiac disease. Previous studies have indicated that Ang II could stimulate vascular smooth muscle cells, endothelial cells, and extracellular matrix (ECM) of cerebral vessels to secrete MMP-9, an effect that was prevented by using captopril. Cardiac myocytes are known to release large amounts of MMP-1. In addition, MMP-1 and TIMP-1 play a role in maintaining the ECM architecture. Imbalances in either of these proteinases reflect a disruption in the architecture of myocardial tissue.

Treatment with RAAS inhibitors may provide benefit independent of BP reduction and are an important component of combination therapy for African American patients. An important meta-analysis indicated that angiotensin-converting enzyme inhibitors (ACEi) have an additional protective effect over angiotensin receptor blockers (ARB) in myocardial infarction incidence, independent of the BP-reducing effects. This may be because ACEi specifically induce the release of an anti-fibrotic and anti-inflammatory tetrapeptide called N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP). AcSDKP is degraded by the N-terminal domain of the ACE enzyme and has been proposed as a marker of medication adherence in patients prescribed ACEi. AcSDKP has been shown to have strong anti-fibrotic effects in heart, kidney, and vascular tissues in vitro and in vivo. It has also been suggested that many of the protective effects of ACEi can be ascribed to this peptide. However, it has also been shown that ARBs and calcium channel blockers may have direct and indirect anti-fibrotic effects. No comparative study has yet evaluated differences in anti-fibrotic markers in hypertensive African American patients with adaptive remodeling taking ACEi vs. antihypertensive medications from other classes. Thus, we sought to evaluate anti-fibrotic markers in hypertensive African American patients with LVH after one year of standard intensive treatment, comparing levels in patients who were treated with the ACEi lisinopril with those who received other classes of antihypertensive medications.

2 | METHODS

We performed a post hoc analysis of a vitamin D randomized controlled trial (NCT01360476) that recruited African American men and women aged 30 to 74 who presented at the emergency departments affiliated with Wayne State University in Detroit, MI, USA, with elevated BP. Eligible individuals required a systolic BP (SBP) ≥ 160 mmHg at first measurement and at one-hour post-triage with evidence of LVH on cardiac magnetic resonance (CMR) imaging at outpatient follow-up 1-2 weeks later. Inclusion and exclusion criteria are shown in Table 1. Participants received standard antihypertensive therapy according to current guidelines for hypertensive African Americans, which included initial combination therapy (diuretic plus ACEi or calcium channel antagonist) for most. Lisinopril was the ACEi prescribed in all patients receiving ACEi. All patients received a prescription for antihypertensive medications aimed at achieving intensive BP control (uniform goal SBP < 130 mmHg), utilizing an evidence-based, standardized algorithm, for the duration of the study. The number of prescribed antihypertensive...
medications, as well as the therapeutic intensity score (TIS), was calculated for each antihypertensive medication prescribed in each patient. Additionally, a total TIS score (all hypertensive medications combined) was also calculated. TIS is a summary measure that accounts for the number of medications and the relative doses a patient received. Hypertension treatment was supplemented with vitamin D (50 000 IU) or placebo administered every other week for 52 weeks (26 total doses). Participants were followed for one year, with six antihypertensive medication titrations occurring during weeks 2, 8, 16, 28, 40, and 52.

BP measurements were obtained using the BpTru™ device. BpTru™ measurements are equivalent to the daytime average of the ambulatory BP monitoring when 5 measurements without a health professional present are performed. BP was measured with an appropriately sized cuff. Five seated BP measurements were taken over 5 minutes. The average was used in the analysis.

CMR imaging was performed using standard protocols. Males required a left ventricular mass index (LVMI) > 89 g/m² and females required a LVMI > 73 g/m² to diagnose LVH and to be included into the study.

Carotid-femoral pulse wave velocity (PWV) was measured at screening and at 16 weeks and one year (52 weeks) post-randomization using a commercially available, non-invasive applanation tonometry device (SpyghmoCor; AtCor Medical, West Ryde, Australia).

For this post hoc analysis, participants receiving antihypertensive therapy were divided into two groups: one group with detectable lisinopril in serum (lisinopril group) at 16 and 52 weeks and those in whom no lisinopril was detectable (control group). We used liquid chromatography-mass spectrometry (LC–MS) to measure the presence and levels of lisinopril in banked serum samples obtained at the 16-week and 52-week (one-year) follow-up appointments for comparison. Patients in the lisinopril group had to have detectable lisinopril at both 16- and 52-week follow-up appointments, while those in the control group had no detectable lisinopril at those time points. Patients with detectible lisinopril may not have been originally prescribed lisinopril at baseline; however, lisinopril may be added during the subsequent medication titration phase. As a result, in Table 1, there are some patients with detectible lisinopril who were not prescribed lisinopril at baseline. From the 52-week samples, the pro-fibrotic marker type 1 procollagen C-terminal propeptide (PICP) and the anti-fibrotic markers matrix metalloproteinase-1 (MMP-1), tissue inhibitor of MMP-1 (TIMP-1), the MMP-1/TIMP-1 ratio, telopeptide of collagen type I (CITP), and Ac-SDKP were measured. PICP (Cat # 8003; Quidel, USA), CITP (Cat # HC0761; Neo Scientific, USA), MMP-1 (Cat # DMP100; R&D Systems, USA), TIMP-1 (Cat # DTM100; R&D Systems, USA), and Ac-SDKP (Cat # A05881; SPI-BIO, France) were measured using commercial enzyme-linked immunosorbent assay kits according to the manufacturers’ instructions.

The study was approved by the local Institutional Review Board at Wayne State University and was in accordance with the ethical standards set forth by the Institutional Review Board for utilization of humans in research.

### 2.1 Statistics

Data were presented as frequencies (%) or mean ± standard deviation. Where normal distributions were observed, t tests and ANOVA were used to compare continuous variables between two or more categories, respectively. The Wilcoxon signed-rank test was used when non-normal distributions were observed. Ordinary least squares linear regression was used to determine the association between lisinopril (independent) and individual markers (dependents), controlling for week 52 SBP. Two separate sets of models were used. In one set, detectable presence of lisinopril at the week 52 visit was treated as a dichotomous indicator. In the second set, the observed lisinopril concentration at week 52 was used as a continuous predictor, to account for observed individual variation in dosage and/or adherence. Because of the order-of-magnitude variation observed in lisinopril levels, the reported week 52 concentrations were log-transformed prior to inclusion in the regression models. An alpha level of 0.05 was used for all comparisons. SAS (SAS Institute, Cary, NC, USA) v9.4 was used for analyses.
Three hundred fifty-three patients were screened, and 113 patients with CMR-confirmed LVH were randomized. This sub-analysis included 66 patients that had detectible antihypertensive medication serum levels at 16 and 52 weeks. The clinical characteristics of control and lisinopril groups are shown in Table 2. The average age of this cohort of hypertensive patients was 46.2 ± 8 years. The average age of patients with detectible lisinopril was 5 years older than that of patients without detectible lisinopril, with no difference in gender and body weight distribution. The number of prescribed antihypertensive medications was similar between the groups, and no difference was observed in vitamin D supplementation. TIS for each individual medication (data not shown) and the total combined TIS for all the antihypertensive medication were similar between those with detectible lisinopril and the comparison group (Table 2). The classes of antihypertensive drugs prescribed were equally distributed, except for ACEi and ARBs, which were the focus of these analyses. One patient in the lisinopril group was prescribed an ARB and an ACEi simultaneously.

There were four patients who were initially prescribed ACEi in whom the drug was not detected in two consecutive screenings at 16 and 52 weeks. Because the main criterion of this analysis was the presence of detectible lisinopril, these four patients were included in the comparison group. Despite the same number of prescribed medication and TIS score, BP was lower in the lisinopril group after one year of treatment. LVMI assessed with CMR imaging was similar between the groups at the beginning of the study and numerically lower in patients with detectible lisinopril concentrations at one year of treatment (84 ± 13.6 vs 80.8 ± 12.7 g/m²); however, we did not detect a statistically significant difference in LVMI change at one year. Both groups experienced similar regression of structural heart damage after one year of intensive therapy, and arterial stiffness was similar between the groups (Table 2).

The pro-fibrotic and anti-fibrotic markers were compared between the groups after one year of antihypertensive therapy. No difference was observed in the pro-fibrotic marker PICP between those receiving lisinopril and the comparison group (Table 3). However, the anti-fibrotic biomarkers Ac-SDKP, MMP-1, and the MMP-1/TIMP-1 ratio significantly increased in the group with detectible lisinopril (Table 3). In a linear regression model adjusted for SBP, the anti-fibrotic markers Ac-SDKP and MMP-1/TIMP-1 ratio were independently associated with the presence of lisinopril (Table 4) and with lisinopril concentrations (Table S1). SBP was associated with LVMI and with the presence of the pro-fibrotic marker PICP (Table 4).

We observed that the majority of the patients with detectible lisinopril had a high Ac-SDKP level, in agreement with previous reports.21 However, six patients demonstrated low Ac-SDKP levels despite detectible lisinopril concentrations, suggesting that these patients may not have benefited from the anti-fibrotic effects of ACEi (Figure 1). Six (16.6%) lisinopril patients had Ac-SDKP levels lower than 4.5 nM, 5 (13.9%) had levels between 4.5 and 5 nM, and 25 (69.4%) had levels >5 nM. We arbitrarily chose the 5 nM level based on our previous experience to separate those who are responsive (>5 nM) and non-responsive (<5 nM). Table 5 shows the comparisons of clinical and biochemical characteristics between the respondent and non-respondent groups. There was no difference in

### TABLE 2 General characteristics of patients treated or not treated with ACE inhibitors

|                         | Comparison Group | Lisinopril Group | p-value |
|-------------------------|------------------|------------------|---------|
| N                       | 30               | 36               |         |
| Age, years (sd)         | 43.7 ± 7.9       | 48.7 ± 7.5       | .01     |
| Female (%)              | 60.0             | 44.0             | .21     |
| BMI (kg/m²)             | 35.9 ± 10.8      | 35.1 ± 7.1       | .98     |
| Number of prescribed antihypertensive drugs | 2.3 ± 0.7 | 2.7 ± 0.8 | .07 |
| ACEi (%)                | 13.0             | 94.4             | <.001   |
| ARB (%)                 | 56.7             | 2.8              | <.001   |
| Amlodipine (%)          | 53.3             | 66.7             | .27     |
| Diuretics (%)           | 86.7             | 77.8             | .35     |
| Therapeutic intensity score (sd) | 1.5(0.7) | 1.5(0.6) | .62 |
| Supp. Vitamin D (%)     | 56.7             | 41.7             | .22     |
| SBP basal (mmHg)        | 161.0 ± 29.3     | 162.2 ± 24.0     | .85     |
| DBP basal (mmHg)        | 100.9 ± 15.3     | 102.2 ± 12.1     | .71     |
| SBP at one year (mmHg)  | 139.5 ± 20.7     | 129.6 ± 13.8     | .02     |
| DBP at one year (mmHg)  | 94.3 ± 12.8      | 86.3 ± 9         | <.01    |
| LVMI at one year (g/m²) | 84.0 ± 13.6      | 80.8 ± 12.7      | .33     |
| Changes in LVMI at one year—basal (g/m²) | -12.9 ± 15.5 | -17.9 ± 11.2 | .10 |
| Pulse Wave Velocity (m/s) | 6.6 ± 3.4 | 6.6 ± 3.2 | .94 |

Abbreviations: ACEi, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin II receptor blocker; BMI, body mass index; DBP, diastolic blood pressure; LVMI, left ventricular mass index; SBP, systolic blood pressure; sd, standard deviation.

### TABLE 3 Fibrotic and anti-fibrotic biomarkers in African Americans treated or not treated with angiotensin-converting enzyme inhibitors

|                         | Comparison Group | Lisinopril Group | p-value |
|-------------------------|------------------|------------------|---------|
| PICP/CITP ratio         | 41.1 ± 32.7      | 46.6 ± 44.2      | .59     |
| PICP (nM)               | 84.7 ± 31.9      | 85.2 ± 28.4      | .83     |
| CITP (nM)               | 3.6 ± 2.8        | 3.3 ± 2.9        | .63     |
| MMP-1 (nM)              | 3.4 ± 2.6        | 5.5 ± 4.0        | .04     |
| TIMP-1 (nM)             | 166.8 ± 36.6     | 155 ± 34.6       | .25     |
| MMP-1/TIMP-1 ratio      | 0.02 ± 0.02      | 0.04 ± 0.03      | .04     |
| Log CITP/MMP-1 ratio    | 1.03 ± 4.10      | 0.71 ± 4.16      | .26     |
| Ac-SDKP (nM)            | 3.9 ± 2.6        | 6.3 ± 2.8        | <.001   |

Abbreviations: Ac-SDKP, N-acetyl-seryl-aspartyl-lysyl-proline; CITP, telopeptide of collagen type I; MMP-1, matrix metalloproteinase-1; PICP, type 1 procollagen C-terminal propeptide; TIMP-1, tissue inhibitor of metalloproteinases 1.
No differences were observed in LVMI and PWV. CITP and TIMP-1 levels were higher in patients with high Ac-SDKP levels; however, the difference did not reach statistical significance due to the low number of cases.

### DISCUSSION

In this study, we found that detectable lisinopril in the serum of hypertensive African American patients with LVH on CMR was associated with an increase in the anti-fibrotic markers MMP-1 and Ac-SDKP. In all patients treated with antihypertensive medication, with or without ACEi, BP decreased and LVH improved after one year of treatment with goal SBP < 130 mmHg. However, in this post hoc analysis, patients with detectable lisinopril had greater decrease in BP and improvement in LVH despite being prescribed a similar number and intensity of antihypertensive medications. We also found that the expected increase in Ac-SDKP levels was not observed in approximately one-third of patients with detectable levels of lisinopril, suggesting that a differential anti-fibrotic response to ACEi might exist. This finding has potential treatment implications.

MMP-1, secreted by fibroblasts and cardiomyocytes, is a key enzyme that participates in collagen type I degradation and the release of CITP, telopeptide of collagen type I; LVMI, Left ventricular mass-Indexed; MMP-1, matrix metalloproteinase-1; PICP, type 1 procollagen C-terminal propeptide; TIMP-1, tissue inhibitor of metalloproteinases 1.

### TABLE 4 Regression model controlling for systolic blood pressure and detectible serum lisinopril at week 52

|                | Week 52 SBP | Liisinopril |
|----------------|-------------|-------------|
|                | $R^2$       | Estimate    | 95% Conf. Limits | p-Value | Estimate    | 95% Conf. Limits | p-Value |
| LVMI (g/m$^2$) | .1342       | 0.2636      | [0.085, 0.442]  | .004    | -0.5463    | [-6.911, 5.818]  | .864    |
| PICP/CITP      | .0237       | 0.3126      | [-0.255, 0.88]  | .275    | 8.603      | [-11.628, 28.834] | .399    |
| PICP           | .0704       | 0.4603      | [0.039, 0.881]  | .032    | 4.9843     | [-10.029, 19.998] | .509    |
| CITP           | .0057       | 0.0095      | [-0.032, 0.051] | .653    | -0.1895    | [-1.683, 1.304]  | .801    |
| MMP-1/TIMP-1   | .1191       | -0.0002     | [-0.001, 0]     | .186    | 0.0139     | [0, 0.027]       | .043    |
| MMP-1          | .0893       | -0.0371     | [-0.089, 0.015] | .156    | 1.4337     | [-0.411, 3.278]  | .125    |
| TIMP-1         | .0274       | -0.0235     | [-0.54, 0.493]  | .927    | -11.9787   | [-30.375, 6.417] | .198    |
| Ac-SDKP        | .1666       | 0.0148      | [-0.025, 0.054] | .456    | 2.4915     | [1.085, 3.898]   | .001    |

Abbreviations: Ac-SDKP, N-acetyl-seryl-aspartyl-lysyl-proline; CITP, telopeptide of collagen type I; LVMI, Left ventricular mass-Indexed; MMP-1, matrix metalloproteinase-1; PICP, type 1 procollagen C-terminal propeptide; TIMP-1, tissue inhibitor of metalloproteinases 1.

**FIGURE 1** Scatter plot displaying the relationship between serum lisinopril and Ac-SDKP. Ac-SDKP has a moderate correlation ($R^2 = .2$) with lisinopril levels; however, some patients showed low Ac-SDKP level despite the presence of lisinopril, suggesting variable Ac-SDKP response (square area).
decreases with hypertension and in hypertensive patients, while TIMP-1 does not increase or display any changes.29 This is particularly true for patients with LVH, which collectively suggests that in hypertension, there is a pro-fibrotic state favoring collagen deposition in the heart.27 The use of a RAAS antagonist has been shown to modulate MMPs and TIMP-1 in vitro and in animal models.14 In our study, SBP was associated with collagen deposition (PICP levels), in agreement with this concept. Laviades et al studied a small cohort of patients who received other antihypertensive medications, but observed no difference in total TIMP-1 levels after lisinopril treatment. In our study, in addition to confirming those changes in the heart, arteries, and kidney. In the past, the presence of Ac-SDKP has been proposed as a marker of medication adherence in patients prescribed ACEi.21 In our study, we measured the presence of lisinopril using LC–MS and observed that approximately one-third of the patients with detectible lisinopril did not display an increase in Ac-SDKP levels as we had expected. Thus, there may be variability in the response to ACEi specifically related to the pleiotropic effects of these drugs, since the degree of BP reduction is similar in those with high and low levels of Ac-SDKP. Several ACE polymorphisms have been described, including insertion/deletion (I/D) polymorphisms. The exploration of the effects of the I/D polymorphism of the gene encoding angiotensin I converting enzyme on Ac-SDKP is limited to a single study.35 This study indicated that the DD polymorphism only affects the timing of Ac-SDKP metabolism, with no effects on the basal Ac-SDKP level even in patients who received ACEi along with an exogenous Ac-SDKP infusion. However, other polymorphisms have not been explored. Interestingly, in patients with high Ac-SDKP, we observed a decrease in CITP and an increase in TIMP-1, albeit without statistical power to determine whether this was simply a fortuitous finding. The decrease in CITP is in line with the anti-crosslinking effects of Ac-SDKP, as previously described.34 We previously reported that Ac-SDKP increases TIMP-1 in myocardial tissue, preventing cardiac rupture due to exaggerated anti-fibrotic effects.36 Currently, it is not known exactly how TIMP-1 is regulated in the

### Table 5: Clinical and biochemical characteristics based on N-acetyl-seryl-aspartyl-lysyl-proline level in lisinopril-treated patients

|                      | Ac-SDKP < 5nM | Ac-SDKP > 5 nM | p-value |
|----------------------|--------------|---------------|---------|
| N                    | 11           | 25            | .60     |
| Age (years)          | 47.2 ± 2.8   | 49.3 ± 7.2    | .28     |
| Female (%)           | 27.3         | 52.0          | .28     |
| BMI (kg/m²)          | 34.6 ± 5.2   | 35.3 ± 7.8    | .77     |
| SBP basal (mmHg)     | 131.0 ± 9.2  | 129 ± 15.7    | .26     |
| DBP basal (mmHg)     | 84.4 ± 7     | 87.1 ± 9.8    | .35     |
| SBP at one year (mmHg)| -32.1 ± 25.7 | -37.5 ± 23.2  | .56     |
| DBP at one year (mmHg)| -17.4 ± 14.5 | -17.3 ± 11.82 | 1.00    |
| LVM at one year (g/m²)| 83.3 ± 9.9   | 79.7 ± 13.8   | .38     |
| Pulse Wave Velocity (m/s)| 7.34 ± 2.9 | 6.19 ± 3.3    | .34     |
| PICP/CITP ratio      | 52.6 ± 38    | 43.9 ± 47.2   | .31     |
| PICP (nM)            | 75.9 ± 17    | 89.3 ± 31.6   | .25     |
| CITP (nM)            | 2.5 ± 2.6    | 3.6 ± 3.1     | .07     |
| MMP1/TIMP1 ratio     | 0.038 ± 0.032| 0.038 ± 0.031 | 1.00    |
| MMP-1 (nM)           | 5.4 ± 4.6    | 5.5 ± 3.8     | .90     |
| TIMP-1 (nM)          | 139.9 ± 27.1 | 161.7 ± 35.9  | .06     |
| Log CITP/MMP-1 ratio | 0.61 ± 4.76  | 0.76 ± 4.01   | .57     |
| Ac-SDKP (nM)         | 4.05 ± 0.89  | 7.27 ± 2.79   | <.001   |

Abbreviations: Ac-SDKP, N-acetyl-seryl-aspartyl-lysyl-proline; CITP, telopeptide of collagen type I; MMP-1, matrix metalloproteinase-1; PICP, type 1 procollagen C-terminal propeptide; TIMP-1, tissue inhibitor of metalloproteinases 1.
context of ACE inhibition, but we speculate TIMP-1 may be playing a role in preventing exaggerated extracellular matrix degradation in some patients with an extensive anti-fibrotic response. The variable response in TIMP-1 according to Ac-SDKP levels may explain why some authors report no change while others report an increase in TIMP-1 under ACEI. Pharmacogenomic and metabolomic profiles are an important aspect of precision medicine, a personalized therapeutic approach to attain the best results for an individual patient. The RAAS system, particularly ACE, produces a variety of BP responses, side effects, and pharmacokinetic properties related to ACE polymorphisms and differences in drug metabolism enzymes. Thus, these variable responses may help to identify patients more susceptible to the anti-fibrotic effects of ACE inhibition. However, more studies are needed to shed light on the mechanism behind these distinct responses.

Similar regression of structural heart damage by CMR after one year between the two groups confirm the importance of BP reduction over antihypertensive class. However, anti-fibrotic and fibrotic biomarkers have been associated with histological myocardial changes in many studies and is expected to have more sensitivity to detect pre-clinical or incipient changes than CMR. Other studies have shown this dissociation between biomarkers and CMR.

One important aspect of this study, other than the increase of the anti-fibrotic markers, is that we did not observe a difference in collagen metabolism biomarkers between the groups at the one-year follow-up, confirming that BP control is the most important factor for improving organ fibrosis. Other drug classes, such as ARBs and calcium channel blockers, also have anti-fibrotic effects that may explain the similar collagen metabolite results. Additionally, the variability of the PICP level and the relative low number of patients may contribute to explain the lack of statistical differences between the groups despite the BP differences. Longer follow-up would potentially provide more robust data for determining any differences in the fibrosis rate, crosslinking state, or target organ damage.

Despite the similar number and intensity of medications prescribed, patients with detectible lisinopril serum concentrations had the largest decrease in BP than patients not prescribed lisinopril or with undetectable serum lisinopril concentration. The explanation for this finding is unclear, but may include medication adherence or other factors, such as diet and physical exercise. MMP-1 and Ac-SDKP are associated with the presence of lisinopril (adjusted for BP), indicating a direct effect of ACEi on these variables. Although this study was not designed to compare ARB and ACEi, based on our findings and the current guidelines, our study supports the use of ACEi in African American hypertensive patients in addition to diuretics or calcium antagonists, reserving ARB as an alternative for those who suffer from adverse reactions to ACEi.

There are some aspects of this study that are important to consider. This is a post hoc analysis of a clinical trial evaluating the effect of vitamin D supplementation in hypertension; thus, it was not originally conceived to evaluate the effect of ACEi on cardiac fibrosis and its biomarkers. However, the number of patients receiving vitamin D supplementation was similar in both groups, decreasing the chance of any vitamin D-related effect. Additionally, any non-significance difference in biomarkers can be underpowered. The number of patients, the follow-up period, and the age of the participants made it difficult to establish hard end points or surrogates of end organ damage. Thus, the findings cannot be extrapolated to generate any conclusion on the benefits of ACEi in this population. Moreover, because we measured lisinopril serum concentration at two discrete time points, it is possible that corresponding values represent recent intake rather than long-term medication adherence. However, we did see relationships between increasing lisinopril serum concentrations and changes in fibrosis, BP, and LV mass, suggesting that detectable ACEi by LC-MS reflected protracted medication use.

Our study contributes to the knowledge of heart damage associated with hypertension. The cutting-edge technology used in our study to evaluate a very specific hypertensive population with LVH under intensive BP therapy provides unique and important insights to help further our understanding of the fibrotic process in hypertension. In addition, our study generates new hypotheses to explore the contribution of ACEi to the treatment of hypertension beyond their BP-reducing effects.

We conclude that the use of lisinopril in hypertensive African Americans with LVH is associated with an increase in anti-fibrotic markers and a reduction in LV mass after one year of intensive antihypertensive therapy.

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CONFLICT OF INTEREST
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Cesar A. Romero, MD, PhD, Benjamin Wasinski, MS, Aaron Brody, MD, Candace D. McNaughton, MD, PhD, Rafael Fridman, MD, Oscar A. Carretero, MD, and Phillip D. Levy, MD, MPH involved in study conception and design, and data collection, interpretation of data, drafting of the manuscript and revising it critically for important intellectual content, and final approval of the manuscript submitted. Shobi Mathew, MS, Michael J. Twiner, MD, PhD, and John M. Flack, MD involved in study conception and design, and interpretation of data, drafting of the manuscript and revising it critically for important intellectual content, and final approval of the manuscript submitted. Brian Reed, BS involved in analysis and interpretation of data and final approval of the manuscript submitted. Rachelle Dawood, BS involved in data collection, interpretation of data, drafting of the manuscript and revising it critically for important intellectual content, and final approval of the manuscript submitted.
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**Supporting Information**

Additional supporting information may be found online in the Supporting Information section.