High rate of left ventricular hypertrophy on screening echocardiography among adults living with HIV in Malawi

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ABSTRACT

Background There are limited data on structural heart disease among people living with HIV in southern Africa, where the success of antiretroviral therapy (ART) has drastically improved life expectancy and where risk factors for cardiovascular disease are prevalent.

Methods We performed a cross-sectional study of screening echocardiography among adults (≥18 years) with HIV in Malawi presenting for routine ART care. We used univariable and multivariable logistic regression to evaluate correlates of abnormal echocardiogram.

Results A total of 202 individuals were enrolled with a median age of 45 years (IQR 39–52); 52% were female, and 27.7% were on antihypertensive medication. The most common clinically significant abnormality was left ventricular hypertrophy (LVH) (12.9%, n=26), and other serious structural heart lesions were rare (<2% with ejection fraction less than 40%, moderate-severe valve lesions or moderate-severe pericardial effusion). Characteristics associated with abnormal echocardiogram included older age (OR 1.04, 95% CI 1.01 to 1.08), higher body mass index (OR 1.09, 95% CI 1.02 to 1.17), higher mean systolic blood pressure (OR 1.03, 95% CI 1.02 to 1.05) and higher mean diastolic blood pressure (OR 1.03, 95% CI 1.01 to 1.05). In a multivariable model including age, duration on ART, body mass index, and systolic and diastolic blood pressure, only mean body mass index (adjusted OR 1.10, 95% CI 1.02 to 1.19), systolic blood pressure (aOR 1.05, 95% CI 1.03 to 1.08) and diastolic blood pressure (aOR 0.96, 95% CI 0.92 to 1.00) remained associated with abnormal echocardiogram.

Conclusions LVH was common in this population of adults on ART presenting for routine care and was associated with elevated blood pressure. Further research is needed to characterise the relationship between chronic hypertension, LVH and downstream consequences, such as diastolic dysfunction and heart failure in people living with HIV.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ While HIV is known to be associated with cardiovascular disease, there are little data from southern Africa on the prevalence of and risk factors for structural heart disease among people living with HIV, due in part to poor availability of echocardiography.

WHAT THIS STUDY ADDS

⇒ Left ventricular hypertrophy was common in a population of adults on antiretroviral therapy (ART) in Malawi and was associated with known hypertension and elevated blood pressure. This is among the first studies from southern Africa to suggest the clinical consequences of uncontrolled hypertension among people living with HIV on ART.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ Future research should involve longitudinal follow-up among larger cohorts in Africa to understand the association between hypertension and cardiac disease, and policy and practice should focus on identifying and aggressively treating hypertension among adults with HIV.

INTRODUCTION

HIV is associated with an excess burden of non-communicable diseases (NCDs), including cardiovascular disease, which occurs at a higher frequency compared with age-matched individuals without HIV, even in the presence of antiretroviral therapy (ART) and viral suppression. While there is rapidly expanding knowledge of cardiac conditions associated with HIV in high-income countries, there are limited data from sub-Saharan Africa, where the success of ART programmes has drastically improved life expectancy and risk factors for cardiovascular disease are prevalent. HIV is associated with an increased risk for structural heart disease, including altered cardiac chamber size and volume and diastolic dysfunction. Changes in left ventricular (LV) mass and diastolic dysfunction have been described in studies of people living with HIV even when controlling for...
age, sex, ART and cardiovascular risk factors.\textsuperscript{8-10} Data on structural heart disease and associated risk factors in people living with HIV in sub-Saharan Africa remain limited as a result of poor availability of echocardiography, which has been challenging to provide as part of routine care due to lack of equipment and limited expertise to perform echocardiograms and interpret findings.

Malawi is a small country in southern Africa with an adult HIV prevalence of 8.1\%\textsuperscript{11} and limited screening and treatment for most NCDs. Partners in Hope (PIH) Medical Center is a faith-based, non-governmental hospital that provides free HIV care in the urban setting of Lilongwe, Malawi to an active cohort of over 5000 patients on ART. PIH was among the earliest facilities in Malawi to adopt an integrated approach to screening and treating hypertension as part of HIV clinical care, prior to the formal introduction of blood pressure screening as part of the Malawi HIV guidelines.\textsuperscript{12} In 2016, in collaboration with the University of California, Los Angeles (UCLA), PIH introduced echocardiography as part of their integrated care services for people living with HIV. To launch this programme, UCLA cardiologists provided echocardiogram training to clinicians and radiology technicians at PIH. We embedded a cross-sectional study within this echocardiography training programme to explore the prevalence of structural heart disease and to evaluate risk factors associated with abnormal echocardiogram in adults on ART.

METHODS

Recruitment, screening and enrolment

Individuals in the ART clinic were recruited while waiting to receive routine HIV care at PIH. Those who expressed interest in the study were taken to a private room for written informed consent and further screening procedures. Individuals were eligible if they were \geq 18 years of age and had been receiving ART from PIH for at least 1 year at the time of study entry. Pregnant women and individuals with any clinical condition that required urgent medical attention on the day of recruitment were excluded. All recruitment, enrolment and study procedures took place between 1 June 2016 and 31 October 2017. This was a cross-sectional substudy nested within a larger observational cohort study of hypertension incidence and prevalence, which has been previously published.\textsuperscript{13}

Study procedures

After providing written informed consent, participants completed an interviewer-administered paper survey about HIV clinical history (duration on ART, adherence to ART), lifestyle (diet, exercise, substance use), and clinical comorbidities (diabetes, hypertension, known heart disease). Participant charts were reviewed for HIV disease characteristics (HIV diagnosis, duration on ART, ART regimen and viral load within 12 months prior to study entry, if available) and medications taken for hypertension, if any.

All participants had blood pressure measured and recorded at least twice during the study visit: at the patient registration area (part of routine care at the HIV clinic) and by the study nurse at the beginning of the study visit. For those with an elevated blood pressure on either of those measurements (\geq 140 mm Hg systolic and/or \geq 90 mm Hg diastolic), a third blood pressure was obtained at the end of the study visit by the study nurse.

Participants were selected based on convenience (individuals willing to participate at the time of a routine ART clinic visit) with the goal of enrolling 200 clients, to allow enough data to explore structural heart disease. Participants were provided the equivalent of US$1.50 for the study visit.

Patient and public involvement

Patients were not directly involved in the design of this study but were informed about the study and possible benefits through health talks in the clinic waiting area. Dissemination of study results includes sharing findings with patients and key stakeholders, including the Malawi Ministry of Health.

Echocardiography

Cardiologists from UCLA performed an in-person training in Malawi with physicians, clinical officers and a radiology technician using a ClearVue 65 ultrasound machine with an S4-1 MHZ probe (Philips, Amsterdam, Netherlands). Training occurred over a period of 1 week and focused on a limited set of findings, including left ventricle size; end diastolic diameter; presence of left ventricular hypertrophy (LVH) defined by septum or wall thickness \geq 1.2 cm; LV systolic function; left atrial size (enlargement); aortic, mitral and tricuspid valve abnormalities; presence and size of pericardial effusion; and inferior vena cava size and presence/absence of collapse with inspiration (see online supplemental appendix for echocardiogram report form). The echocardiogram protocol was informed by similar training programmes in resource-limited settings with inclusion of specific parameters based on available resources in Malawi for treatment of cardiac disease identified, level of clinical providers being trained and type of ultrasound equipment available.\textsuperscript{14-16} After the initial in-person training, remote support was provided by UCLA cardiologists through file-sharing via a secure server. For this process, the individual in Malawi performing the examination completed an echocardiogram report, and the report findings and images were verified by an UCLA cardiologist. Any errors in the interpretation were discussed with the sonographer in Malawi via email or teleconference, and the final echocardiogram reading was reconciled and uploaded to the study record.
Statistical analysis

Summary statistics were generated to describe characteristics of participants, and χ² and rank-sum tests were used to compare characteristics of participants by gender and by presence of LVH on echocardiogram. Univariate and multivariable logistic regression were used to evaluate correlates of abnormal echocardiogram. Variables for the multivariate regression were selected based on literature showing associations with the outcome of interest. For blood pressure, the average of all readings performed at the study visit was used for the analysis. Associations with elevated blood pressure and LVH on echocardiogram were explored using blood pressure as a continuous variable and by categories of elevation defined based on Malawi’s national clinical guidelines as follows: mild elevation as systolic 140–159 mm Hg and/or diastolic 90–99 mm Hg; moderate elevation as systolic 160–179 mm Hg and/or diastolic 100–109 mm Hg and severe elevation as systolic ≥180 mm Hg and/or diastolic ≥110 mm Hg. For the analyses, moderate and severe were combined into one category as systolic ≥160 mm Hg and/or diastolic ≥100 mm Hg. A p-value of <0.05 was considered significant for all analyses.

RESULTS

Table 1 describes sociodemographic and clinical characteristics of the cohort at baseline overall and by sex. A total of 202 individuals were enrolled with a median age of 45 years (IQR 39–52); 52% were females, and the median duration on ART was 6.8 years (IQR 4.6–8.8). The majority of participants (79%, n=160) were on ART with efavirenz/lamivudine/tenofovir disoproxil fumarate, the standard first-line regimen at the time under study. Viral load values within the year prior to enrolment were available in 32.7% (66/202) of the participants and the majority (97.0%) had virological suppression (<1000 copies/mL). Diabetes was present in 4.0% of the population (n=8) and rates of current tobacco and alcohol use were low (2.0% (n=4) and 16.8% (n=34), respectively). Fifty-six participants (27.7%) were on antihypertensive medication. In sex-stratified analyses, women were slightly younger than men (43 vs 46 years, p=0.03),
were less likely to report smoking (no women vs 4.1% of men, p=0.04) and alcohol use (5.7% vs 28.9%, p<0.001) and had higher body mass index (BMI) (25.2 vs 22.6, p=0.005).

Fifty-four individuals (26.7% of the total population) had one or more abnormalities on echocardiogram. Twenty-four (11.9%) had mild aortic or mitral valvular regurgitation (8 aortic valve and 16 mitral valve), and 10 (5.0%) had mild thickening or calcification of the aortic or mitral valve (4 aortic valve and 6 mitral valve). Twelve individuals (5.9%) had tricuspid regurgitation, with 11 of these described as mild and only 1 as moderate/severe tricuspid regurgitation. Twenty-six participants (12.9%) had LVH (mean overall septal thickness of 1.35 cm and SD 0.27 cm; mean for men 1.41 cm and SD 0.32 cm; mean for women 1.28 cm and SD 0.16). Eight participants (4.0%) had reduced LV systolic function (one individual with ejection fraction of 35% and the remainder 40%–50%) and six (3.0%) had pericardial effusion, with one characterised as moderate and the remainder as small. Of the individuals with reduced LV function, median age was 53.5 years (IQR 46–59), 37.5% were women, and 5 individuals were on antihypertensive medications.

Characteristics associated with abnormal echocardiogram included older age (OR 1.04, 95% CI 1.01 to 1.08), higher BMI (OR 1.09, 95% CI 1.02 to 1.17), higher mean systolic blood pressure (OR 1.03, 95% CI 1.02 to 1.05) and higher mean diastolic blood pressure (OR 1.03, 95% CI 1.01 to 1.05) (table 2). In a multivariable model including age, duration on ART, BMI, and systolic and diastolic blood pressure, mean BMI (adjusted OR (aOR) 1.10, 95% CI 1.02 to 1.19), systolic blood pressure (aOR 1.05, 95% CI 1.03 to 1.08) and diastolic blood pressure (aOR 0.96, 95% CI 0.92 to 1.00) remained associated with abnormal echocardiogram (table 2).

Those with LVH on echocardiogram (n=26) were more likely to be older (51.5 vs 45, p<0.001), have a higher BMI (28.3 vs 23.5, p=0.002) and have higher mean systolic blood pressure (169 vs 134 mm Hg, p<0.001) and diastolic blood pressures (94 vs 82 mm Hg, p<0.001) (table 3). In an analysis of blood pressure by category, those with LVH were less likely to have blood pressure <140/90 mm Hg (7.7% vs 52.0%, p<0.001).

### Table 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal echocardiogram n=148</th>
<th>Abnormal echocardiogram n=54</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (IQR)</td>
<td>45 (38.5–52)</td>
<td>49.5 (40–56)</td>
<td>1.04 (1.01 to 1.08)</td>
<td>0.01</td>
<td>1.00 (0.96 to 1.04)</td>
<td>0.95</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>77 (52.0)</td>
<td>28 (51.9)</td>
<td>0.99 (0.53 to 1.85)</td>
<td>0.98</td>
<td>1.03 (0.51 to 2.1)</td>
<td>0.92</td>
</tr>
<tr>
<td>Median BMI (IQR)*</td>
<td>23.4 (20.6–26.5)</td>
<td>26.2 (22.5–29.0)</td>
<td>1.09 (1.02 to 1.17)</td>
<td>0.01</td>
<td>1.10 (1.02 to 1.19)</td>
<td>0.01</td>
</tr>
<tr>
<td>Median duration on ART (IQR)*</td>
<td>6.8 (4.1–8.6)</td>
<td>6.7 (5.4–9.7)</td>
<td>1.08 (0.98 to 1.19)</td>
<td>0.13</td>
<td>1.04 (0.92 to 1.17)</td>
<td>0.58</td>
</tr>
<tr>
<td>Mean systolic blood pressure (SD)</td>
<td>132.8 (23.1)</td>
<td>154.1 (28.5)</td>
<td>1.03 (1.02 to 1.05)</td>
<td>&lt;0.001</td>
<td>1.05 (1.03 to 1.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean diastolic blood pressure (SD)</td>
<td>81.7 (14.1)</td>
<td>88.2 (16.3)</td>
<td>1.03 (1.01 to 1.05)</td>
<td>0.01</td>
<td>0.96 (0.92 to 1.00)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*BMI missing for one participant with abnormal echo; duration on ART missing for one participant with normal echo.

ART, antiretroviral therapy; BMI, body mass index.

### Table 3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No LVH on echocardiogram n=176</th>
<th>LVH on echocardiogram n=26</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (IQR)</td>
<td>45 (39–52)</td>
<td>51.5 (43–63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>93 (52.8)</td>
<td>12 (46.2)</td>
<td>0.52</td>
</tr>
<tr>
<td>Median BMI (IQR)†</td>
<td>23.5 (20.9–26.8)</td>
<td>28.3 (23.2–30.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Median duration on ART (IQR)†</td>
<td>6.8 (4.2–8.6)</td>
<td>7.0 (5.6–10.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean systolic blood pressure, mm Hg (SD)</td>
<td>134 (23.4)</td>
<td>169 (25.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean diastolic blood pressure, mm Hg (SD)</td>
<td>82 (14.2)</td>
<td>94 (15.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood pressure &lt;140/90 mm Hg, n (%)</td>
<td>100 (56.8)</td>
<td>2 (7.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood pressure 140–159 and/or 90–99 mm Hg, n (%)</td>
<td>46 (26.1)</td>
<td>8 (30.8)</td>
<td></td>
</tr>
<tr>
<td>Blood pressure ≥160 and/or ≥100 mm Hg, n (%)</td>
<td>30 (17.1)</td>
<td>16 (61.5)</td>
<td></td>
</tr>
</tbody>
</table>

*P values determined by χ² and t-tests or rank-sum tests.
†BMI missing for one participant with LVH; duration on ART missing for one participant with no LVH.

ART, antiretroviral therapy; BMI, body mass index.
and/or diastolic significance (table 4). Those with LVH had higher average systolic blood pressure 140–159 mm Hg (72.2% vs 44.7%), although differences by blood pressure category did not reach statistical significance (table 4).

Eight participants with LVH were not on antihypertensive medications, and of these, two had blood pressure <140/90 mm Hg at the study visit, three had systolic blood pressure 140–159 mm Hg and/or diastolic 90–99 mm Hg, and three had systolic blood pressure ≥160 and/or diastolic ≥100 mm Hg.

**DISCUSSION**

We found a low prevalence of serious cardiac abnormalities on echocardiogram in this adult population presenting for routine ART care in Malawi. We did find a high proportion of individuals with LVH on echocardiogram—most of whom were on antihypertensive medications (18 out of 26) and had significantly elevated blood pressure at the study visit. Hypertension is common in people living with HIV in Africa, with studies showing a prevalence ranging from 11% to 46% in the region, depending on the cohort evaluated. However, there are few studies that evaluate associations between blood pressure control and LVH or LV dysfunction. Cross-sectional studies from Africa have found that people living with HIV have higher LV mass and higher rates of diastolic dysfunction even in the absence of hypertension and other cardiovascular risk factors, suggesting that HIV itself may independently contribute to increased LV mass and LV dysfunction. Proposed mechanisms for this include chronic inflammation and immune activation, even in the setting of viral suppression and increased pericardial fat density, which is common in people living with HIV and has been associated with coronary artery calcification and myocardial infarction. A recent cross-sectional study from Kenya found a low rate of diastolic dysfunction in people living with HIV and a similar rate as compared with age and sex-matched individuals without HIV; however, individuals with HIV did have significantly higher LV mass index and left atrial volume. None of the participants in this study had hypertension, limiting comparisons with our study population.

LVH is a marker of hypertension-related organ damage and is associated with congestive heart failure, coronary heart disease and stroke in the general population, with the majority of data on clinical outcomes from high-income countries. However, there is an emerging body of evidence for an association between hypertension and adverse clinical outcomes from studies of people living with HIV in resource-constrained settings. In a cohort from Haiti with an average age of 39 years and median follow-up time of approximately 7 years, hypertension was independently associated with increased mortality (HR 2.47, 95% CI 1.10 to 5.57) after adjustment for age, sex, HIV clinical stage and CD4 count. In a retrospective analysis of almost 50 000 medical records of people living with HIV in Kenya, men with systolic hypertension (≥140 mm Hg) had a higher

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**Table 4** Characteristics of participants on antihypertensive medications with versus without left ventricular hypertrophy (LVH) on echocardiogram (n=56)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No LVH on echocardiogram n=38</th>
<th>LVH on echocardiogram n=18</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (IQR)</td>
<td>50.5 (42–57)</td>
<td>54.5 (51–66)</td>
<td>0.02</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>28 (73.7)</td>
<td>7 (38.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Median BMI (IQR)†</td>
<td>24.0 (22.0–27.7)</td>
<td>27.6 (22.5–30.4)</td>
<td>0.30</td>
</tr>
<tr>
<td>Median duration on ART (IQR)</td>
<td>7.3 (5.0–9.8)</td>
<td>8.1 (6.1–11.4)</td>
<td>0.25</td>
</tr>
<tr>
<td>Mean systolic blood pressure, mm Hg (SD)</td>
<td>156 (19.4)</td>
<td>174 (20.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean diastolic blood pressure, mm Hg (SD)</td>
<td>93 (11.3)</td>
<td>96 (15.7)</td>
<td>0.47</td>
</tr>
<tr>
<td>Blood pressure ≤140/90 mm Hg, n (%)</td>
<td>5 (13.2)</td>
<td>0 (0.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Blood pressure 140–159 and/or 90–99 mm Hg, n (%)</td>
<td>16 (42.1)</td>
<td>5 (27.8)</td>
<td></td>
</tr>
<tr>
<td>Blood pressure ≥160 and/or ≥100 mm Hg, n (%)</td>
<td>17 (44.7)</td>
<td>13 (72.2)</td>
<td></td>
</tr>
</tbody>
</table>

*P values determined by χ² and t-tests or rank-sum tests.
†BMI missing for one participant with LVH.

ART, antiretroviral therapy; BMI, body mass index.
mortality risk compared with men who were normotensive. The analysis excluded men with advanced HIV disease (defined by CD4 count <350 cells/mm² or WHO stage 2 or higher), thus making mortality from opportunistic infections unlikely. While these studies are limited in their ability to evaluate whether mortality was from a cardiovascular cause, they provide important data from resource-limited settings that signal interventions aimed at blood pressure control may have significant benefit at a population level. Currently, there are significant health system barriers to hypertension care in countries with the highest prevalence of HIV, including frequent stockouts of blood pressure medications, a lack of providers trained in hypertension management and a lack of public health messaging and programming geared towards blood pressure control (relative to campaigns around communicable infections, such as HIV, malaria and tuberculosis (TB)).

In the absence of addressing these barriers, the benefits of HIV programmes may be partly offset by morbidity and mortality due to NCDs, particularly cardiovascular disease; however, the infrastructure developed for HIV can be leveraged to improve access to NCD care (including free or affordable medications), should the resources be made available for integration.

Apart from LVH, we found low rates of other serious echocardiographic abnormalities, including valvular disease and cardiomyopathy. We did not find evidence of rheumatic heart disease in our population, and this is consistent with the literature, including several studies in children with HIV that have found low rates of rheumatic heart disease relative to the general population, and raise whether HIV may be protective against this condition. A low rate of echocardiographic valvular disease was also found in a recent South African study in adults with HIV in which the majority of findings were characterised as trivial (<1% with any moderate valvular dysfunction among 394 individuals with HIV). It will be important to perform longitudinal studies of individuals living with HIV to determine whether mild valvular changes and mild reductions in ejection fraction progress to clinically significant disease.

Pericardial effusions were detected on echocardiogram in only six study subjects, and these were predominately small and were not associated with evidence of tamponade physiology either clinically or based on echocardiogram. A recent study of adults with HIV in South Africa found low rates of pericardial effusion and no difference by ART status and regimen. TB is endemic in southern Africa and can present with pericardial disease, and the presence of pericardial effusion should prompt consideration of this diagnosis. In our study population, individuals were presenting for routine care and we do not have data on whether they reported signs or symptoms of TB. However, individuals were only included in the study if they were stable at the time of the visit. Any individual with an acute illness was excluded, reducing the probability of active TB in the cohort included in the study.

Study limitations

This study has several limitations. Echocardiograms were performed on individuals on ART who were presenting for routine HIV care. Screening asymptomatic individuals increases the likelihood of incidental findings that are not likely to be clinically significant. Our study was cross-sectional and therefore we cannot determine whether findings such as mild valvular disease and effusions improve or worsen over time, and we also cannot characterise historical blood pressure control to evaluate the association with blood pressure and LVH longitudinally. The echocardiography was a limited examination designed to be feasible in resource-limited settings for individuals completing a short training course; therefore, it did not include detailed descriptions of cardiac valve disease, precise characterisation of ejection fraction and determinations of LV diastolic dysfunction, such as the E-to-A wave ratio. Our study population had low rates of clinical comorbidities such as diabetes, and low rates of substance use, limiting our ability to evaluate associations between heart disease and these other risk factors. Additionally, CD4 counts and viral load tests were not routinely performed at the time these data were collected, and therefore we could not evaluate associations with HIV disease status and echocardiographic findings.

Conclusion

We found high rates of LVH in this population of adults on ART in association with known hypertension and elevated blood pressure. Further research is needed in larger African cohorts and with longitudinal follow-up to understand the risk of disease progression in those with structural cardiac abnormalities and to better characterise the relationship between chronic hypertension, LVH and downstream consequences, such as diastolic dysfunction and heart failure.

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Contributors RH conceived of the study idea and developed the protocol with FC, TC, ZPB, JWC and BAB. FC, BAB, DK, KG, ZPB, MC, CK, DN and HS supported study implementation. RH, CM and TC performed data analyses with input from all study team members. CM, JvD, AM, SP, JSC and JWC helped to write and revise the manuscript. All authors provided manuscript edits and final review. RH is responsible for the overall content of the finished work as guarantor.

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