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ORIGINAL RESEARCH

Transmethylamine-N-Oxide Is Associated With Diffuse Cardiac Fibrosis in People Living With HIV

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BACKGROUND: People living with HIV are at increased risk of developing diastolic dysfunction, heart failure, and sudden cardiac death, all of which have been characterized by higher levels of myocardial fibrosis. Transmethylamine-N-oxide (TMAO), a dietary gut metabolite, is linked to the development of myocardial fibrosis in animal models. However, it is unclear whether TMAO plays a role in the development of myocardial fibrosis in people living with HIV.

METHODS AND RESULTS: The study population consisted of participants enrolled in the multisite cross-sectional study called CHART-HIV (Characterizing Heart Function on Anti-Retroviral Therapy). Participants underwent echocardiography, cardiac magnetic resonance imaging, biomarker analysis, and targeted assessment of gut-related circulating metabolites; diastolic dysfunction was determined by study-specific criteria. Multivariable linear regression models were performed to examine the relationship of gut-related metabolites with serum and imaging measures of myocardial fibrosis. Models were adjusted for traditional cardiovascular, inflammatory, and HIV-related risk factors. Diastolic dysfunction was present in 94 of 195 individuals (48%) in CHART-HIV; this cohort demonstrated higher prevalence of hypertension, hyperlipidemia, and chronic kidney disease as well as higher plasma levels of both TMAO and choline. TMAO levels were associated with parameters reflecting increased left ventricular filling pressures and with a marker of the innate immune system. TMAO levels correlated with diffuse myocardial fibrosis (R=0.35; P<0.05) as characterized by myocardial extracellular volume fraction as well as biomarkers reflective of myocardial fibrosis.

CONCLUSIONS: In this study of people living with HIV, the gut metabolite TMAO was associated with underlying diffuse myocardial fibrosis and found to be a potential marker of early structural heart disease. The mechanistic role of the gut microbiome in HIV-associated cardiovascular disease warrants further investigation.

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Key Words: diastolic dysfunction ■ HIV ■ myocardial fibrosis ■ transmethylamine-N-oxide

HIV continues to be a global epidemic. At the end of 2018, there were 37.9 million individuals living with HIV worldwide and 1.2 million living with HIV in the United States.[1] The widespread use of antiretroviral therapy (ART) has substantially improved the life expectancy of people living with HIV (PLWH) such that their lifespan now approaches that of the general population. However, as this population ages, their risk of developing chronic cardiovascular conditions increases as well. By the year 2030, it is predicted that >70% of PLWH will be aged ≥50 years and 78% of PLWH will be diagnosed with cardiovascular disease.[2]

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CLINICAL PERSPECTIVE

What Is New?

- We found that plasma transmethylamine-N-oxide levels were associated with biomarkers and imaging correlates of diffuse myocardial fibrosis in a cohort of individuals with well-controlled chronic HIV infection.
- In this cohort, transmethylamine-N-oxide levels also correlated with a marker of innate immune system activation, sCD14 (soluble CD14), and adjustment for sCD14 partially attenuated the relationship between transmethylamine-N-oxide and diffuse myocardial fibrosis.
- Finally, transmethylamine-N-oxide levels demonstrated a relationship with echocardiographic measures of early structural heart disease such as elevated left ventricular filling pressures.

What Are the Clinical Implications?

- As individuals with HIV are living longer, understanding the biological pathways by which chronic HIV infection increases the susceptibility to developing structural heart disease will be essential for decreasing morbidity and mortality in this population.
- Our study raises the possibility that abnormalities in gut function, namely, gut metabolite generation and absorption, may contribute to the increased prevalence of structural heart disease seen in individuals with chronic HIV infection and warrants further investigation.

Nonstandard Abbreviations and Acronyms

DD	diastolic dysfunction
ECV	extracellular volume
PLWH	people living with HIV
TMAO	transmethylamine-N-oxide

Compared with the general population, PLWH are more susceptible to developing heart failure (HF). With the use of ART, the phenotype of HIV-associated HF has evolved from primarily reduced ejection fraction into predominantly diastolic dysfunction (DD) and preserved ejection fraction.[3] Epidemiological studies show that PLWH now have a 15% to 30% prevalence of HF with preserved ejection fraction, and almost 50% prevalence of subclinical cardiomyopathy with echocardiographic features of DD.[4] Little is known about the pathogenesis and mechanisms of DD in PLWH, but there have been early studies highlighting the role of myocardial fibrosis as a mediator of HIV-related DD and subsequent HF with preserved ejection fraction.[5–7] Given the growing prevalence of HIV-related DD, there is a substantial need to better delineate the mechanisms by which chronic HIV infection leads to structural heart disease.

Abnormal intestinal function and gut microbiota have been previously implicated in the pathogenesis, severity, and progression of cardiometabolic disease and HF.[8] Mechanisms of action include splanchnic circulation congestion, heightened inflammatory responses, and bacterial translocation.[9,10] Recently, it has been shown that levels of the gut microbe-derived dietary metabolite, transmethylamine-N-oxide (TMAO), are elevated in the setting of HF; TMAO levels also correlate with echocardiographic indexes of DD and are independently prognostic of increased mortality in individuals with systolic HF.[9,11] In mouse models, diets with increased TMAO lead to adverse ventricular remodeling via fibrosis[12–14]; conversely, removal of dietary TMAO attenuates cardiac dysfunction in pressure-overloaded states.[15] Plasma TMAO levels are largely determined by temporal dietary intake of precursors (choline, phosphatidylcholine, L-carnitine), the presence of gut microbial species capable of converting precursors to transmethylamine (TMA), and the intrahepatic conversion of transmethylamine to TMAO by FMO3 (flavin monooxygenases 3). Age, body mass index, and renal dysfunction are additional factors known to influence TMAO levels.[14,16]

The gut also plays a key role in HIV disease pathogenesis as HIV preferentially infects $CD4^+$ T cells in the gut, resulting in increased gut permeability and microbial translocation. Microbial translocation is responsible in part for the chronic inflammatory state seen in PLWH.[17] Individuals with longstanding HIV have distinct alterations in the composition of their gut microbiome,[18] which has a critical downstream effect on the production of specific dietary metabolites. Interestingly, animal studies have mechanistically linked TMAO to inflammation,[19] and TMAO levels correlate with markers of monocyte activation in PLWH.[20]

We propose a role for gut metabolites in the development of structural heart disease in the setting of treated HIV infection. Specifically, we sought to identify a plausible biological pathway leading to structural heart disease in the population with HIV by investigating cross-sectional associations between gut metabolites, diastolic indexes, and myocardial fibrosis in PLWH.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CHART-HIV Study

The CHART-HIV (Characterizing Heart Function on Anti-Retroviral Therapy [principal investigator, Dr Javed Butler, National Heart, Lung, and Blood Institute PO515992]) study is a multisite, cross-sectional study conducted by the Heart Failure Network and has been described in detail previously.[21] Briefly, the CHART-HIV study enrolled ART-treated participants who were HIV positive, virally suppressed, and aged >40 years divided into those with (1) normal diastolic function (HIV+/DD– cohort) and (2) DD (HIV+/DD+ cohort), as defined in the next section.[22] Inclusion criteria were individuals infected with HIV on ART for >6 months with HIV RNA levels <200 copies/mL. Notable exclusion criteria include history of AIDS (defined as CD4 <200 or AIDS-defining opportunistic infections) or past left ventricular (LV) ejection fraction <50%.

A total of 282 individuals across 11 sites were screened, and 195 individuals with well-controlled HIV were enrolled (Figure S1). All individuals provided written informed consent; the institutional review board approval was obtained at each study site. All enrolled individuals underwent phenotypic characterization with blood sampling (HIV characteristics, inflammatory panel, fibrosis markers, proteomics, and metabolomics), echocardiography, and cardiac magnetic resonance imaging.

Echocardiography

All study participants underwent comprehensive 2-dimensional, M-mode, Doppler, and tissue Doppler echocardiography. All studies were acquired using a standardized protocol and were interpreted by an echocardiography core laboratory at Northwestern University blinded to all other data. DD was defined according to the following prespecified criteria: (1) septal e' velocity <7 cm/s or lateral e' velocity <10 cm/s and (2) left atrial volume index >28 mL/m^2 or LV hypertrophy (LV mass index >95 g/m^2 in women or >115 g/m^2 in men, or relative LV wall thickness >0.42). The rationale for the DD criteria used in the CHART-HIV study has been previously described.[21]

Magnetic Resonance Imaging

All study participants underwent a standardized cardiac magnetic resonance (CMR) imaging protocol, including a steady-state free procession acquisition, retrospective ECG gating and breath-hold imaging sequence, and delayed enhanced imaging after contrast administration as specified by a CMR core laboratory at Duke University. T1 mapping for extracellular volume (ECV) measurement was performed at sites capable of receiving and integrating a standardized T1-mapping sequence developed by the CMR core laboratory; this was performed in a subset of the cohort (n=81 [42%]; DD+, n=29; DD–, n=52). All CMR data were analyzed blinded to all other data.

Gut Metabolites

Serum samples were collected from all patients in the CHART-HIV study cohort (within 3 months of enrollment) and stored at −80 °C. Levels of 4 gut metabolites (TMAO, choline, betaine, L-carnitine) were measured by stable isotope dilution liquid chromatography tandem mass spectroscopy at the Duke University core laboratory. The accuracy of the TMAO assay has been described previously.[23] Cardiac biomarkers (NT-proBNP [N-terminal pro–brain natriuretic peptide], troponin-I, galectin-3, GDF-15 [growth differentiating factor 15], and ST-2) were also assessed as part of the CHART-HIV study.[21]

Statistical Analysis

Comparisons between individuals with and without DD were performed. Continuous variables were summarized using mean±SD or median (interquartile range), and differences between groups were analyzed using *t* tests or Wilcoxon rank-sum test where appropriate. Discrete variables were summarized using number (percentage) and compared using the χ^2 test or Fisher's exact test if the sample size in any cell was <5. Robust linear regression models were performed to examine the associations of gut metabolites with levels of echocardiographic parameters, CMR variables, and biomarkers of fibrosis. Given the skewed distribution of gut metabolites and some biomarkers, the data were $\log_2$-transformed. When included in the model as independent variables, the coefficients reflect the effect of per doubling; when included as dependent variables, the coefficient represents the percentage differences per unit increment of corresponding independent variable. All models were performed as unadjusted and adjusted for traditional cardiovascular, inflammatory, and HIV-related risk factors (diabetes mellitus, systolic blood pressure, CD4 count, HIV RNA, illicit drug use, and hepatitis C virus coinfection) in addition to factors known to independently affect plasma TMAO levels (age, body mass index, glomerular filtration rate). Spearman rank correlation coefficients were calculated between gut metabolites and magnetic resonance imaging–assessed cardiac fibrosis and among cardiac fibrosis markers. All analyses were done using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

Demographic and clinical characteristics of the CHART-HIV study cohort have been described previously

(Table 1).[22] A total of 94 (48%) participants met the predefined criteria for DD. The average age of those with DD (DD+) was 58.0 years compared with 52.5 years for those without DD (DD−; $P<0.01$). The DD+ group had a higher prevalence of medication-treated hypertension, hyperlipidemia, and chronic kidney disease and a trend toward increased prevalence of diabetes mellitus; 8.5% of DD+ individuals had a prior diagnosis of HF compared with 2% in the DD− group. There was no difference in duration of HIV diagnosis, average CD4 count, or HIV viral load; the DD+ group had increased prior exposure to nucleoside reverse transcriptase inhibitor–based ART ($P<0.01$).

As expected, echocardiographic parameters reflecting ventricular stiffness and elevated filling pressures were significantly higher in the DD+ group (Table 1). Although focal myocardial fibrosis (reported as percentage of LV mass) was significantly increased in the DD+ group, the degree of diffuse myocardial fibrosis (assessed by ECV) was similar across both groups as has been previously reported in the CHART-HIV study.[22] Analysis of individual echocardiographic parameter associations with ECV was also performed; there were no significant associations identified (Table S1).

We then compared levels of the gut metabolites, initially stratified by presence of DD (Table 1). Plasma levels of both TMAO and choline were higher in the DD+ group, whereas levels of betaine and L-carnitine were similar in those with and without DD. Next, we determined the association of TMAO levels with individual echocardiographic parameters of DD (Table 2) given the limitations of the binary classification of DD. Multivariable analysis revealed a statistically significant association between TMAO and *E/e′* (septal and average of septal and lateral). There were minimal associations in multivariable analysis between the remaining gut metabolites (choline, betaine, L-carnitine) with echocardiographic indexes of DD (Table S2).

Next, we assessed for correlation of gut metabolites with subtypes of myocardial fibrosis. There was a modest correlation of betaine with percentage of scar, a marker of focal myocardial fibrosis on CMR ($R=0.21$; $P<0.01$). There was moderate correlation between levels of TMAO and CMR-based ECV, a marker of diffuse myocardial fibrosis ($R=0.35$; $P<0.01$; Figure 1, blue box); this association remained significant in multivariate analysis (Table 3). There were no significant differences in baseline characteristics between individuals who did and did not undergo T1 mapping (Table S3), with the exception of estimated glomerular filtration rate (although both groups remained in the normal range) and body mass index (both groups >25).

CHART-HIV study participants also underwent testing of plasma levels of well-characterized biomarkers that have previously been implicated in myocardial injury, stress, inflammatory, and fibrotic pathways. In unadjusted analysis, TMAO was positively correlated with all relevant biomarkers (NT-proBNP, troponin-I, galectin-3, GDF-15, IL-6 [interleukin 6]) except ST-2 (Table 3). In multivariable analysis, the associations between TMAO and NT-proBNP, troponin-I, and galectin-3 remained statistically significant, matching the correlations seen in Table 3. TMAO, NT-proBNP, and galectin-3 levels were correlated with ECV on CMR (TMAO and galectin-3, $R=0.35$; NT-proBNP, $R=0.25$); NT-proBNP and troponin-I also showed modest correlation with CMR scar percentage (Figure 1, orange box). Of note, mineralocorticoid receptor use (antifibrotic) and history of prior myocardial infarction were considered for inclusion in the multivariable models but had too few observations within the cohort.

Finally, we examined the relationship between TMAO, ECV, and markers of innate immune system activation. TMAO was significantly associated with the monocyte activation marker sCD14 (soluble CD14), but not sCD163 (soluble CD163); this association remained significant after adjusting for confounders (Table 3). Soluble CD14 also demonstrated a significant relationship with ECV, whereas soluble CD163 did not. Adjusting for sCD14 but not sCD163 partially attenuated the association between TMAO and ECV (Table S4).

DISCUSSION

Our study demonstrates a relationship between TMAO and diffuse myocardial fibrosis among individuals with well-controlled HIV infection. We found that TMAO was associated with 2 distinct measures of myocardial fibrosis: ECV on CMR (marker of diffuse myocardial fibrosis) and plasma biomarkers reflective of known fibrotic pathways. TMAO was also associated with echocardiographic indexes of increased LV filling pressures. Notably, the relationship between TMAO and diffuse myocardial fibrosis was found in a participant population that was largely free of clinical HF, suggesting that diffuse myocardial fibrosis in HIV underlies the earliest stages of structural heart disease (Figure 2).

People infected with HIV have a 1.5-fold to 2-fold increased risk of HF compared with people not infected with HIV, and much of this risk can be accounted for by the increased incidence and prevalence of DD in PLWH.[4,24,25] The development of atrial fibrillation, secondary pulmonary hypertension, and HF with preserved ejection fraction as a result of abnormal diastolic function all lead to substantial morbidity and mortality,[26] highlighting the need to identify pathways by which chronic HIV infection results in these critical structural changes. In the general population, myocardial fibrosis has been correlated with degree of DD,

Table 1. Summary of CHART-HIV Characteristics Stratified by Presence of Diastolic Dysfunction

Clinical characteristics	Diastolic dysfunction, n=94	No diastolic dysfunction, n=101	*P* value
Age, y	58.0±8.1	52.5±5.7	<0.01
Birth sex, male	67 (71)	72 (71)	0.99
Race, Black	50 (53)	59 (58)	0.46
Body mass index, kg/m^2	30.6±7.3	28.1±6.3	0.02
Heart failure diagnosis	8.5 (9)	2.0 (2)	0.05
Hypertension	59 (63)	38 (38)	<0.01
Systolic blood pressure, mm Hg	132.3±14.2	125.0±14.7	<0.01
Hyperlipidemia	41 (44)	21 (21)	<0.01
Low-density lipoprotein, mg/dL	105.1±34.4	101.5±26.6	0.45
Diabetes mellitus	19 (20)	11 (11)	0.07
Hemoglobin A1C, %	5.7 (5.4–6.3)	5.5 (5.1–6.7)	0.22
Chronic kidney disease	14 (15)	2 (2)	<0.01
eGFR, mL/min	77.9 (65.3–93.4)	90.0 (72.9–104.9)	<0.01
HCV coinfection	14 (13)	14 (14)	0.98
Illicit drug use	31 (34)	39 (39)	0.71
Duration of HIV, y	16.5 (9.4–22.4)	15.2 (9.9–22.2)	0.44
CD4 count, cells/mm^3	670 (416–849)	680 (469–838)	0.93
HIV RNA, copies/mL	20 (20–40)	20 (20–40)	0.82
Ever use of NRTI or combination NRTI	64 (70)	46 (46)	0.01
Current use of NRTI or combination NRTI	34 (37)	42 (42)	0.47
Echocardiography	**Diastolic dysfunction, n=94**	**No diastolic dysfunction, n=101**	***P* value**
Left ventricular mass index, g/m^2	97.2±21.5	86.9±19.1	<0.01
Biplane left ventricular ejection fraction, %	60.1±4.9	60.1±4.4	0.86
Biplane left atrial volume index, mL/m^2	27.6±7.4	26.9±7.0	0.4
Mitral E/A ratio	0.9±0.2	1.2±0.3	<0.01
E/*e*' ratio	9.6±2.5	7.1±1.7	<0.01
Estimated pulmonary capillary wedge pressure, mm Hg	17.1±1.6	15.6±0.9	<0.01
Left ventricular global longitudinal strain, %	17.9±3.9	19.4±3.3	<0.01
Left ventricular early diastolic strain rate, 1/s	0.8±0.3	1.0±0.3	<0.01
Cardiac magnetic resonance	**Diastolic dysfunction, n=85**	**No Diastolic dysfunction, n=95**	***P* value**
Scar as % of left ventricular myocardial mass	0.5±1.8	0.1±0.4	<0.01
ECV, %	26.4±3.6 (n=29)	27.9±3.5 (n=52)	0.07
Biomarkers	**Diastolic dysfunction, n=94**	**No diastolic dysfunction, n=101**	***P* value**
N-terminal pro–brain natriuretic peptide, pg/mL	36.1 (22.7–85.3)	26.2 (12.0–46.8)	<0.01
Troponin-I, pg/mL	3.8 (3.1–4.9)	3.1 (2.4–3.7)	<0.01
ST-2, pg/mL	24.9±9.9	24.0±7.9	0.48
Galectin-3, pg/mL	11.6±4.9	10.8±4.4	0.25
GDF-15, pg/mL	867 (628–1385)	735 (570–1078)	0.07
IL-6, pg/mL	1.0 (0.7–1.6)	0.9 (0.5–1.3)	0.08
Gut metabolites, median	**Diastolic dysfunction, n=94**	**No diastolic dysfunction, n=101**	***P* value**
TMAO, µmol/L	4.2 (2.8–6.4)	3.5 (2.4–4.7)	<0.01
Choline, µmol/L	9.5 (8.0–12.0)	8.9 (6.8–10.8)	0.03
Betaine, µmol/L	37.2 (29.9–45.3)	37.8 (31.0–48.9)	0.67
L-carnitine, µmol/L	38.3 (31.4–46.3)	37.9 (29.3–44.9)	0.29

Categorical data are summarized as number (percentage) and continuous data are summarized as mean±SD or median (interquartile range). CHART-HIV indicates Characterizing Heart Function on Anti-Retroviral Therapy; ECV, extracellular volume; E/e′, early mitral inflow velocity/mitral annular early diastolic velocity; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiating factor 15; HCV, hepatitis C virus; IL-6, interleukin 6; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; and TMAO, transmethylamine-N-oxide.

Table 2. Association of TMAO Levels With Echocardiographic Correlates of Diastolic Dysfunction

Parameter	Effect of (doubling of) TMAO			
	Unadjusted		Adjusted*	
	Estimate (95% CI)	*P* value	Estimate (95% CI)	*P* value
Septal *e′* velocity, cm/s	−0.38 (−0.67 to −0.10)	0.01	−0.23 (−0.53 to 0.08)	0.15
Lateral *e′* velocity, cm/s	−0.33 (−0.74 to 0.07)	0.11	−0.14 (−0.52 to 0.23)	0.46
Septal *E/e′* ratio	0.48 (0.14 to 0.81)	0.01	0.41 (0.04 to 0.77)	0.03
Lateral *E/e′* ratio	0.36 (0.06 to 0.66)	0.02	0.21 (−0.11 to 0.53)	0.20
Average *E/e′* ratio	0.42 (0.12 to 0.73)	0.01	0.35 (0.04 to 0.66)	0.03
Global longitudinal strain, %	−0.01 (−0.49 to 0.47)	0.96	−0.05 (−0.58 to 0.48)	0.86
Left atrial end systolic volume index, mL/m^2	−0.37 (−1.30 to 0.57)	0.44	−0.33 (−1.38 to 0.72)	0.54
Tricuspid regurgitation peak velocity, m/s	0.01 (−0.04 to 0.06)	0.71	−0.01 (−0.06 to 0.05)	0.84

E/e′ indicates early mitral inflow velocity/mitral annular early diastolic velocity; TMAO, transmethylamine-N-oxide.
*Multivariable analysis adjusted for age, body mass index, diabetes mellitus, estimated glomerular filtration rate, systolic blood pressure, CD4 count, HIV RNA, antiretroviral therapy, illicit drug use, and hepatitis C virus coinfection.

and diffuse myocardial fibrosis (as assessed by ECV on CMR) has specifically been implicated in the pathogenesis of HF with preserved ejection fraction.[27] This established relationship is important given that previous findings have demonstrated an association of HIV with myocardial fibrosis. Specifically, individuals with suppressed viremia have increased levels of diffuse myocardial fibrosis compared with age-matched controls,[6,7] which has been confirmed in a cohort of women[28] and in histological studies.[29] However, we still do not clearly understand the mechanism by which HIV leads to myocardial fibrosis nor the impact of myocardial fibrosis on clinical cardiovascular sequelae.

There is a growing body of literature that the intestinal microbiome plays a key role in the pathogenesis of disease states in PLWH. In early infection, HIV-1 targets and replicates in gut-associated lymphoid tissue resulting in CD4$^+$ T cell depletion with subsequent intestinal epithelial cell damage, mucosal inflammation, and chronic immune activation. Investigation into

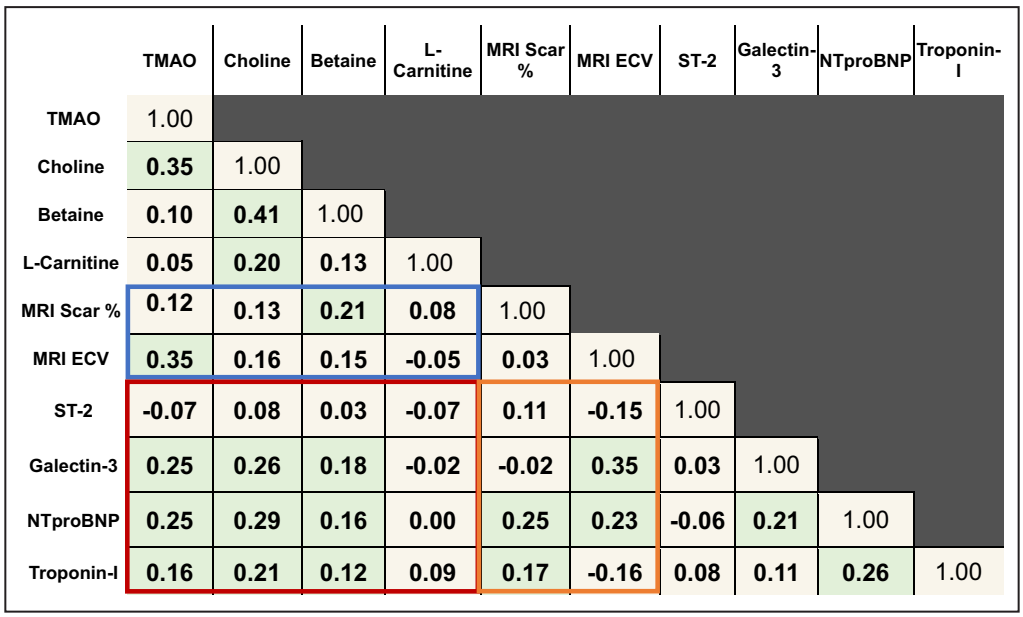

Figure 1. Spearman rank correlation values of gut metabolites, cardiac magnetic resonance measures, and biomarkers.
Correlation values of gut metabolites with MRI measures are shown in the blue box, gut metabolites with biomarkers in the red box, and MRI measures with biomarkers in the orange box. Statistically significant correlation values (P<0.05) are shaded green. ECV indicates extracellular volume; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro–brain natriuretic peptide; and TMAO, transmethylamine-N-oxide.

Table 3. Association of TMAO Levels With MRI Measures and Biomarkers

	Effect of (doubling of) TMAO			
	Unadjusted		Multivariable adjusted*	
	Estimate (95% CI)	*P* value	Estimate (95% CI)	*P* value
Cardiac MRI measures				
MRI scar % >0	1.52 (0.97 to 2.39)	0.07	1.37 (0.7 to 2.67)	0.36
MRI ECV	1.32 (0.58 to 2.07)	<0.01	1.47 (0.64 to 2.30)	<0.01
Biomarkers				
NT-proBNP %, pg/mL†	32.8 (15.5 to 52.8)	<0.01	24.7 (9.6 to 41.9)	<0.01
Troponin-I %, pg/mL†	9.4 (0.2 to 19.5)	0.05	9.7 (0.8 to 19.3)	0.03
ST-2 %, ng/mL†	−2.9 (−6.8 to 1.1)	0.15	−2.5 (−6.8 to 2.1)	0.29
Galectin-3 %, ng/mL†	8.4 (3.9 to 13.1)	<0.01	4.9 (0.2 to 9.89)	0.04
GDF-15 %, pg/mL†	11.7 (4.5 to 19.5)	<0.01	4.7 (−2.2 to 12.1)	0.27
IL-6 %, pg/mL†	17.4 (6.72 to 29.2)	<0.01	7.2 (−2.5 to 17.9)	0.12
sCD14, ng/mL	66.12 (5.79 to 126.45)	0.03	88.43 (20.79 to 156.07)	0.01
sCD163, ng/mL	17.27 (−16.67 to 51.20)	0.32	0.82 (−33.26 to 34.90)	0.96

ECV indicates extracellular volume; GDF-15, growth differentiating factor 15; IL-6, interleukin 6; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro–brain natriuretic peptide; sCD14, soluble CD14; sCD163, soluble CD163; and TMAO, transmethylamine-N-oxide.

* Multivariable analysis adjusted for age, body mass index, diabetes mellitus, estimated glomerular filtration rate, systolic blood pressure, CD4 count, HIV RNA, illicit drug use, and hepatitis C virus coinfection.

† Log_2-transformed variables because of skewness. Coefficients represent the percentage difference per doubling of TMAO.

alterations in gut metabolites, microbial diversity, and bacterial community function in the setting of HIV infection are ongoing with previous reports linking TMAO to atherosclerosis in HIV, with associations in carotid artery disease,[30] plaque burden and calcium score,[31] and coronary stenosis.[32] As a downstream metabolite of choline, L-carnitine, and betaine, there may be TMAO-dependent pathophysiological mechanisms for HIV-associated cardiovascular disease. Our finding of an association between TMAO and ECV adds to the emerging role of the gut in HIV-associated comorbidities. This is crucial given that even effectively treated and suppressed PLWH manifest a chronic inflammatory milieu with apparent myocardial edema and fibrosis, all leading to increased ECV.[33]

Furthermore, our study shows distinct associations between TMAO and the following 3 biomarkers associated with myocardial fibrosis: troponin-I, galectin-3,

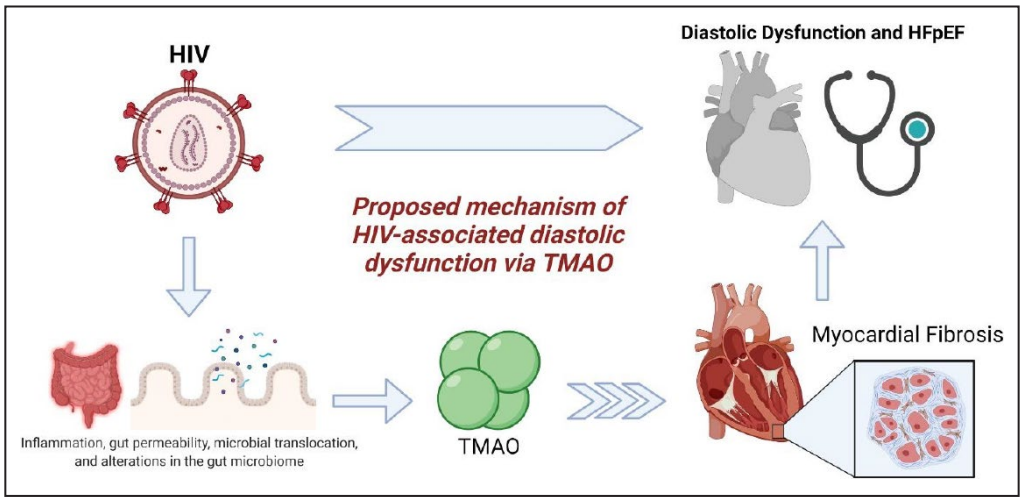

Figure 2. Proposed mechanism of HIV leading to diastolic dysfunction via TMAO and the gut. HIV and the intestinal microbiome interact substantially, leading to gut permeability, inflammation, and immune activation and microbial translocation. TMAO is upregulated in this pathway and is associated with diffuse myocardial fibrosis, which may be a precursor to structural heart disease and diastolic dysfunction. Figure created with Biorender.com. HFpEF indicates heart failure with preserved ejection fraction; and TMAO, transmethylamine-N-oxide.

and NT-proBNP. Historically, serum troponin-I has been considered a reflection of myocardial injury, but more recently, has been shown to correlate with imaging markers of fibrosis including late gadolinium enhancement and ECV.[34] Galectin-3 is well known as a marker of inflammation and fibrosis, driving fibroblast proliferation and collagen deposition to cause cardiac dysfunction and incident HF.[35,36] NT-proBNP, normally reflective of myocardial strain, has been shown in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort to be strongly associated with diffuse myocardial fibrosis, as assessed by ECV.[37]

PLWH have a persistent chronic inflammatory state leading to subtle structural changes (increases in myocardial edema and fibrosis); we expect that a subset of these individuals will manifest changes in diastolic function (via increased filling pressures and abnormal relaxation). In our population, TMAO levels were associated with a subset of abnormal diastolic indexes such as increased LV filling pressures. Taken together, our findings suggest that in the setting of HIV, TMAO may reflect underlying diffuse myocardial fibrosis and may be an early marker of remodeling. Of note, despite the aforementioned relationship, the CHART-HIV study participants who met the study criteria for DD exhibited higher levels of focal but not diffuse myocardial fibrosis. One potential explanation for this is that the prespecified criteria used to define DD in the CHART-HIV study led to the inclusion of a population with early and/or mild disease.

Whether TMAO is a marker reflective of the fibrosis pathway or is involved in the mechanistic process itself (initiated at the level of the gut microbiome) remains unknown. Independent of HIV, a direct role for TMAO in a biological pathway leading to fibrosis has been demonstrated in animal and in vitro models.[12–14,38] Namely, the direct addition of TMAO to an already profibrotic milieu (either doxorubicin toxicity or pressure-overload state) resulted in excess amounts of cardiac fibrosis through fibroblast proliferation and collagen.[15,39] A growing body of work supports a specific role for TMAO in the activation of inflammatory pathways, namely, the innate immune system, in individuals with and without HIV.[20,40] Here we show that TMAO levels are associated with the classical monocyte marker sCD14, but not with sCD163. The specific correlation with sCD14 may reflect its more defined role as a marker of innate immune system activation driven by gut microbial translocation in PLWH.[41]

A preclinical role for TMAO has not yet been investigated in the general population, but interventions targeting various steps of the TMAO pathway are an area of active research, ranging from dietary modifications, transformation of the gut microbiome, to drugs targeting the microbiome-specific machinery.[42,43] Unfortunately, interventions targeting the gut in HIV have not significantly impacted inflammatory biomarkers,[44] although TMAO has not been specifically studied in these interventions. Early ART initiation or HIV curative strategies may reduce the eventual development of cardiac fibrosis; in addition, therapeutic strategies targeting TMAO[42] or use of antifibrotic agents such as mineralocorticoid receptor antagonists could also be considered.

Finally, it is important to note that our study population consists of well-treated participants who were virally suppressed and HIV positive. This is notable in that the profibrotic role of TMAO may be potentiated in the presence of uncontrolled viremia, HF, or coronary artery disease. Conversely, HIV infection may singlehandedly predispose these individuals to elevated TMAO levels at baseline, even in the absence of an ongoing insult; this may in part be attributed to its unique microbiome signature.[18] Further research is needed to understand the relationship of TMAO and myocardial fibrosis in the population not infected with HIV.

There are several limitations to note in this study. First, this study was a cross-sectional study and thus we cannot infer causality. Second, sample size was modest, limiting the ability to detect significant relationships. Moreover, standard T1 mapping software for CMR was only available at certain sites and thus only able to be performed in a subset of the cohort. However, even with these limitations, the CHART-HIV study remains one of the largest evaluations of individuals infected with HIV with CMR and novel biomarkers, with a specific focus on well-treated individuals with and without structural heart disease who were virally suppressed. Third, gut metabolite panels were not performed in the fasted state across the CHART-HIV study, and we do not have information on CHART-HIV study participant diets before the blood draw, which may have an impact on gut metabolite levels. Fourth, although calculated ECV is largely regarded as a measure of diffuse myocardial fibrosis, it can also represent changes attributed to inflammation, edema, and infiltration; thus, ECV is not categorically specific for myocardial fibrosis. Finally, we did not include formal adjustments for multiple comparisons as we hypothesized that associations among biomarkers would show a biologically coherent pattern. This dictates that results should be mutually reinforcing rather than a series of independent tests and so formal multiple comparisons adjustments would not be appropriate.

Overall, we believe these findings are hypothesis generating and provide important signals toward understanding the underlying mechanism of structural heart disease in PLWH. Future studies with larger cohorts, longitudinal imaging, and a dedicated non-HIV control group will be important in understanding the implications for this growing population.

CONCLUSIONS

In conclusion, we demonstrate that among treated PLWH who are virally suppressed, levels of TMAO are independently associated with measures of diffuse myocardial fibrosis, as quantified by CMR, echocardiographic measures of DD, and established biomarkers of cardiac fibrosis. Given the role of the gut in both HF and HIV disease pathogenesis, our findings suggest that HIV may lead to CVD via its impact on the gut microbiome. Additional studies will be needed to further delineate the role of TMAO in structural heart disease and clinical sequelae in HIV and whether TMAO may represent a targetable pathway to prevent or treat individuals with HIV.

ARTICLE INFORMATION

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Disclosures

Dr Hsue has received honoraria from Gilead and Merck, outside of the submitted work. The remaining authors have no disclosures to report.

Supplementary Material

Tables S1–S4
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Association of echocardiogram parameters with cardiac MRI extracellular volume.

	Effect on ECV (per doubling of parameter)			
Parameter	**Unadjusted**		**Adjusted†**	
	Estimate (95% CI)	**p-value**	**Estimate (95% CI)**	**p-value**
Septal e' velocity (cm/s)	0.30 (-0.01, 0.62)	0.06	0.21 (-0.17, 0.59)	0.28
Lateral e' velocity (cm/s)	0.15 (-0.12, 0.42)	0.27	0.09 (-0.21, 0.39)	0.56
Septal a' velocity (cm/s)	-0.28 (-0.64, 0.08)	0.12	-0.13 (-0.57, 0.32)	0.58
Lateral a' velocity (cm/s)	-0.11 (-0.43, 0.20)	0.49	0.12 (-0.22, 0.46)	0.47
Septal E/e' ratio	-0.0002 (-0.34, 0.34)	1.0	-0.05 (-0.45, 0.35)	0.80
Lateral E/e' ratio	0.06 (-0.26, 0.39)	0.70	0.01 (-0.39, 0.41)	0.97
Average E/e' ratio	0.05 (-0.32, 0.41)	0.80	-0.03 (-0.49, 0.44)	0.91
Global longitudinal strain (%)	-0.09 (-0.32, 0.14)	0.45	0.01 (-0.28, 0.29)	0.95

Left atrial end systolic volume index (mL/m^2)	-0.09 (-0.21, 0.02)	0.12	-0.08 (-0.21, 0.05)	0.22
Tricuspid regurgitation peak velocity (m/s)	1.20 (-1.79, 4.19)	0.43	0.65 (-2.25, 3.56)	0.66

Table S2. Association of choline, betaine, and L-carnitine levels with echocardiographic correlates of diastolic dysfunction.

Parameter	Effect of (doubling of) choline				Effect of (doubling of) betaine				Effect of (doubling of) L-carnitine			
	Unadjusted		Adjusted†		Unadjusted		Adjusted†		Unadjusted		Adjusted†	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Septal e' velocity (cm/s)	-0.40 (-1.10, 0.31)	0.27	0.36 (-0.41, 1.13)	0.36	0.25 (-0.49, 0.98)	0.51	0.49 (-0.25, 1.20)	0.31	-0.37 (-1.08, 0.34)	0.31	0.08 (-0.74, 0.90)	0.85
Lateral e' velocity (cm/s)	-1.11 (-2.10, -0.12)	0.03	0.23 (-0.71, 1.17)	0.63	-0.52 (-1.54, 0.49)	0.31	-0.29 (-1.20, 0.62)	0.53	-0.44 (-1.38, 0.50)	0.35	0.06 (-0.90, 1.03)	0.90
Septal E/e' ratio	0.48 (-0.33, 1.30)	0.24	-0.02 (-0.90, 0.87)	0.97	-0.18 (-1.01, 0.65)	0.67	0.07 (-0.8, 0.94)	0.87	0.31 (-0.50, 1.12)	0.50	-0.38 (-1.31, 0.55)	0.43
Lateral E/e' ratio	0.73 (-0.01, 1.47)	0.05	-0.09 (-0.87, 0.68)	0.81	0.42 (-0.35, 1.18)	0.29	0.48 (-0.29, 1.25)	0.22	0.29 (-0.45, 1.03)	0.45	-0.30 (-1.12, 0.51)	0.47
Average E/e' ratio	0.58 (-0.17, 1.33)	0.13	-0.03 (-0.82, 0.76)	0.94	0.06 (-0.71, 0.82)	0.88	0.24 (-0.52, 1.00)	0.53	0.35 (-0.39, 1.10)	0.36	-0.34 (-1.16, 0.48)	0.42
Global longitudinal strain (%)	-0.47 (-1.65, 0.70)	0.43	-0.26 (-1.55, 1.03)	0.69	-0.45 (-1.63, 0.73)	0.46	-1.07 (-2.32, 0.19)	0.10	0.61 (-0.52, 1.74)	0.29	0.49 (-0.86, 1.84)	0.48
Left atrial end systolic volume index (mL/m^2)	-0.17 (-2.40, 2.06)	0.88	-0.91 (-3.50, 1.69)	0.49	2.74 (0.53, 4.95)	0.02	2.58 (0.09, 5.06)	0.04	0.74 (-1.46, 2.93)	0.51	0.46 (-2.22, 3.14)	0.74

Tricuspid regurgitation peak velocity (m/s)	0.07 (-0.05, 0.18)	0.24	0.02 (-0.10, 0.15)	0.70	0.07 (-0.05, 0.19)	0.27	0.04 (-0.08, 0.16)	0.49	0.02 (-0.11, 0.15)	0.75	-0.002 (-0.14, 0.14)	0.97

†Multivariable analysis adjusted for age, body mass index, diabetes, eGFR, systolic blood pressure, CD4 count, HIV RNA, antiretroviral therapy, illicit drug use, and hepatitis C coinfection.

Table S3. Summary of baseline characteristics stratified by participants who received cardiac MRI T1 mapping.

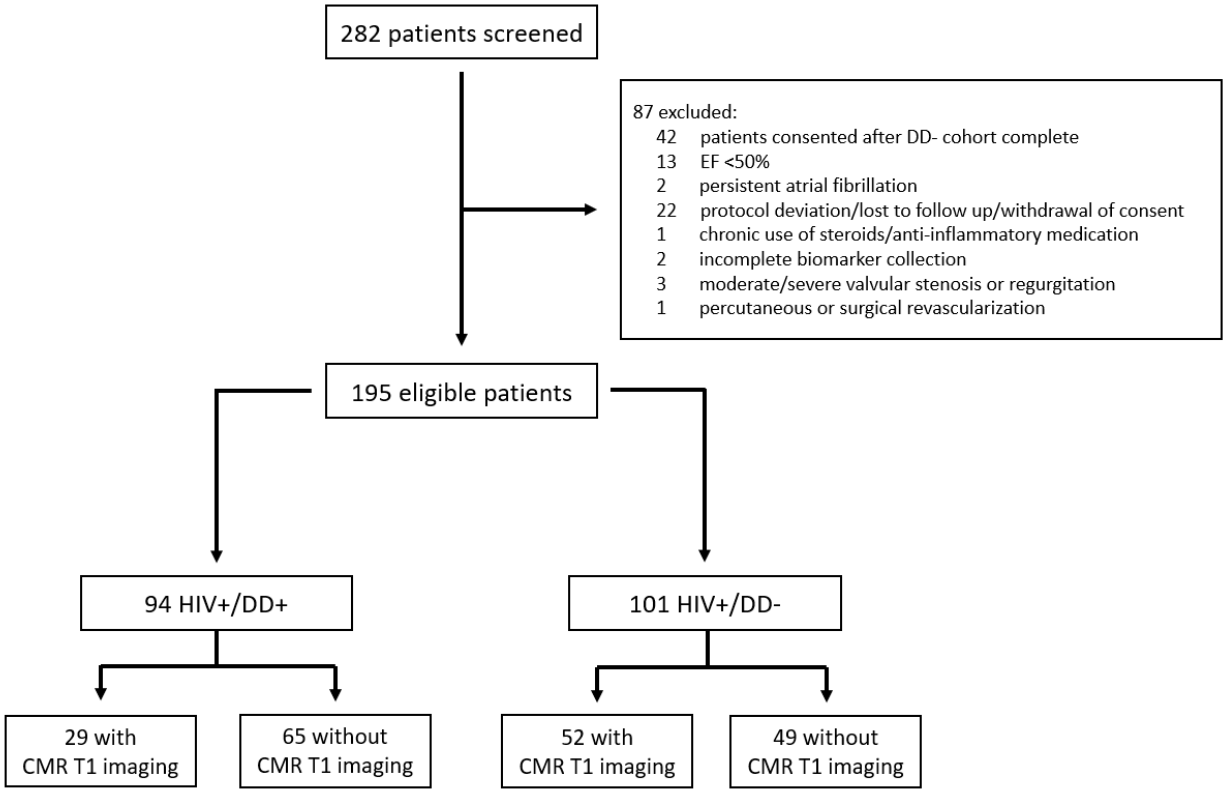
Clinical Characteristics	T1 Mapping (n = 81)	No T1 Mapping (n = 112)	*P* value
Age (years)	54.4 ± 6.6	55.6 ± 8.1	0.26
Birth gender male	60 (72)	79 (71)	0.79
Race, Black	55 (66)	54 (48)	0.01
CD4 count (cells/mm^3)	680 (510, 830)	661 (392, 847)	0.85
HIV RNA (copies/mL)	22 (20, 44)	31 (21, 68)	0.21
eGFR (ml/min)	88.2 (71.3, 106.3)	79.2 (67.6, 95.4)	<0.01
Hypertension	43 (52)	54 (49)	0.66
Hyperlipidemia	26 (32)	36 (32)	0.99
Chronic kidney disease	4 (5)	12 (11)	0.18
Body mass index, kg/m^2	27.5 ± 5.8	30.5 ± 8.1	0.03
Diabetes	12 (15)	18 (16)	0.76
HCV coinfection	12 (15)	15 (14)	0.85
Illicit drug use	32 (40)	38 (35)	0.48
Ever use of NRTI or combination NRTI	43 (54)	67 (60)	0.36
Current use of NRTI or combination NRTI	36 (44)	40 (36)	0.25
Left ventricular mass index (g/m^2)	89.8 (73.2, 101.8)	88.6 (74.4, 104.2)	0.91
Biplane left ventricular ejection fraction (%)	59.4 ± 4.2	60.6 ± 4.9	0.07
Mitral E/A ratio	1.0 ± 0.3	1.0 ± 0.3	0.49

Table S4. Relationship between monocyte activation markers, TMAO, and ECV.

Parameter	Effect on ECV (per doubling of parameter)					
	Unadjusted		Adjusted†			
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value		
sCD14	0.002 (0.001, 0.004)	0.001	0.002 (0.001, 0.003)	0.01		
sCD163	0.002 (-0.002, 0.005)	0.32	0.001 (-0.003, 0.005)	0.64		
	Unadjusted		Adjusted for sCD14		Adjusted for sCD163	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
TMAO (per doubling)	1.32 (0.58, 2.07)	<0.0001	1.18 (0.45, 1.89)	0.001	1.29 (0.52, 2.06)	0.001

†Multivariable analysis adjusted for age, body mass index, diabetes, eGFR, systolic blood pressure, CD4 count, HIV RNA, antiretroviral therapy, illicit drug use, and hepatitis C coinfection

Figure S1. Flow diagram of The Characterizing Heart Function on Anti-Retroviral Therapy (CHART-HIV) study.



Adapted from original publication of CHART-HIV study[21].