Comprehensive Cardiac Magnetic Resonance Imaging and Spectroscopy

Reveals a High Burden of Myocardial Disease in HIV Patients

Running title: Holloway et al.; HIV-related heart disease

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Abstract:

Background—To characterise myocardial abnormalities in a contemporary group of human immunodeficiency virus (HIV)-infected individuals on combination antiretroviral therapy (cART). HIV infection continues to be endemic worldwide. Whilst treatments are successful, it remains controversial whether patients receiving optimal therapy have structural, functional or biochemical cardiac abnormalities which may underlie the increased cardiac morbidity and mortality.

Methods and Results—Volunteers with HIV on cART and age-matched controls, without a history of cardiovascular disease, underwent cardiac Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) for the determination of cardiac function, myocardial fibrosis and myocardial lipid content. A total of 129 participants were included in this analysis. Compared to age-matched controls (n = 39, 30.23%), HIV-infected subjects on cART (n = 90, 69.77%) had 47% higher median myocardial lipids and 74% higher median plasma triglycerides levels (both p < 0.001). Myocardial fibrosis, predominantly in the basal infero-lateral wall of the left ventricle, was observed in 76% of HIV-infected subjects compared to 13% of controls (p < 0.001). Peak myocardial systolic and diastolic longitudinal strain were also lower in HIV-infected individuals compared to controls and remained statistically significant after adjusting for available confounders.

Conclusions Comprehensive cardiac imaging revealed cardiac steatosis, alterations in cardiac function and a high prevalence of myocardial fibrosis in a contemporary group of asymptomatic HIV-infected subjects on cART. Cardiac steatosis and fibrosis may underlie cardiac dysfunction and increased cardiovascular morbidity and mortality in subjects with HIV.

Key words: HIV, cardiac function, cardiac metabolism, myocardial fibrosis
Introduction

Human immunodeficiency virus (HIV) infection continues to present a major public health concern in both the developed and developing world, with a global prevalence of ~34 million people.\textsuperscript{1} HIV infection has been associated with a broad spectrum of cardiac diseases, including pericardial, myocardial and coronary artery disease. Combination antiretroviral therapy (cART) has had a profound impact on HIV-related morbidity and mortality, though long-term treatment is associated with metabolic sequelae and cardiovascular disease (CVD).\textsuperscript{2,3} While it is widely accepted that traditional risk factors play a major role in HIV-related CVD,\textsuperscript{4} the extent to which metabolic complications of therapy pose additional risk in the contemporary cART treated patient remains controversial. Furthermore, myocardial disease was reported widely in the pre-cART era, where a high incidence of cardiomyopathy with cardiac systolic and diastolic dysfunction was found, but comprehensive analysis of a contemporary cohort of patients receiving effective therapy is lacking\textsuperscript{5}. Recent echocardiographic studies have reported a low prevalence of HIV-related myocardial disease is in settings with unrestricted access to cART and / or a low incidence of tuberculosis; however, isolated ventricular dilatation, systolic dysfunction without dilatation and diastolic dysfunction have been described using echocardiography.\textsuperscript{6,7} The pathogenesis of myocardial disease in HIV infection remains ill defined, but is likely to be multifactorial: myocarditis from infectious and non-infectious causes, the direct effects of HIV infection, including increased circulating pro-inflammatory cytokines, and complications of cART, such as abnormal fat metabolism.

Magnetic resonance (MR) imaging and spectroscopy techniques allow the concurrent determination of cardiac contractile function, fibrosis and fat metabolism and thus offer the potential to obtain a highly accurate and sensitive assessment of multiple structural, functional...
and biochemical cardiac parameters. We report on the first comprehensive study to employ cardiac MR imaging and spectroscopy in asymptomatic HIV patients receiving effective cART, to determine the prevalence and extent of myocardial abnormalities.

Methods

Study subjects

Subjects with HIV infection on cART (n = 90) were recruited during 2011 and 2012 to an observational study from four HIV centres in Oxfordshire, UK. Inclusion criteria were age at least 18 years with no history of cardiovascular disease, Hepatitis C or injection drug use or contraindications to cardiac MR assessment. All subjects fulfilling these criteria from the outpatient centres were invited to participate and the first 90 responders were included. The study was approved by Oxfordshire Research Ethics Committee (IRB ref. 10/H0604/95) and all subjects gave written informed consent prior to enrolment. All HIV-seropositive subjects had initiated cART in accordance with national treatment guidelines. A control group that comprised healthy subjects matched for age and ethnicity (self-defined) and with low risk of HIV infection (n = 39) was studied contemporaneously. Early morning assessments took place at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR) after an overnight fast. Both Magnetic Resonance (MR) imaging and spectroscopy scans and bloods were performed during a 3-hour fasting visit. All analyses were performed in a blinded manner, with sequential numbering of subjects, with HIV status revealed only after analysis was complete.

Body mass and metabolic marker assessments

Height, weight, hip and waist circumference were recorded. Body fat percentages, water percentages and lean body weights were measured using a bioimpedance analyzer (Bodystat,
Douglas, Isle of Man). For measurement of subcutaneous and visceral fat, transverse abdominal images were acquired using a single breath-hold, 1-slice, water suppressed T1-weighted turbo spin echo sequence centred on L4 at 3T MRI, using a modified from previous similar acquisition. Images were manually contoured for subcutaneous and visceral fat volume using standard Siemen workstation analysis. A fasting blood sample was obtained for measurement of plasma glucose, free fatty acids and cholesterol. Insulin was measured using ELISA (Mercodia AB, Uppsala, Sweden). To calculate the homeostasis model assessment for insulin resistance (HOMA-IR) the following formula was used: fasting insulin (pmol/l) x fasting glucose (mM/l)/135.9

1H Magnetic Resonance Spectroscopy

All 1H MRS studies were performed on a 3 Tesla MR scanner (Tim Trio, Siemens Healthcare, Germany). The 1H MRS sequence was based on a conventional STEAM sequence that was modified to achieve a short TE of 10 ms, as previously described. A voxel, or area of interest, was placed in a mid-ventricular slice in the interventricular septum of a short axis image (Figure 1). Cardiac gated spectra were acquired as previously described. Acquisition consisted of six breath-holds of about 16 s each, five breath-holds which allowed for the acquisition of 35 nonaveraged water-suppressed spectra. Four nonaveraged water spectra were acquired with a minimum TR of 4 s in a separate breath-hold setting the water suppression RF pulse power to zero. Spectra were analysed using Matlab and the AMARES algorithm included in the jMRUI package 4, as previously described. An example of a proton MRS cardiac spectrum is in Figure 1C. Previous studies in our department have demonstrated good reproducibility with this single breath hold method, with a 19% coefficient of variance.
Left and right ventricular cardiac volumes, mass, and function were assessed using cardiac MRI in a 1.5 Tesla Avanto MR system (Erlangen, Germany) as previously described.11 A stack of steady state free precession (SSFP), short axis cine images were obtained using breath hold and cardiac gating with subsequently analysis of cardiac volumes and function using Argus processing (Siemens Medical Solutions, Erlangen, Germany). Cardiac MR is considered the gold standard for assessment of cardiac function with excellent reproducibility.

**Myocardial Tagging**

Semi-automated analysis was performed using CIM software (CIMTag2D v.7, Auckland MRI Research Group, New Zealand) by aligning a grid to the myocardial tagging planes in end diastole. End systole was determined visually, and tags are adjusted at each frame through the cardiac cycle. A four chamber, horizontal long axis, and a mid-short axis image were acquired for each subject. From the mid short axis peak systolic and diastolic circumferential strain rates was derived. From the horizontal long axis view, peak systolic and diastolic longitudinal strain rates were determined.

**Assessment of Myocardial Fibrosis using Late gadolinium enhancement and T1 mapping**

Late gadolinium enhancement CMR was performed with a T1-weighted segmented inversion-recovery turbo fast low-angle shot (FLASH) sequence (echo time 4.8 ms, voxel size 1.4 × 2.4 × 7 mm, flip angle 20°) following a 6 minute time delay after the administration of 0.1 mmol/kg gadolinium contrast agent (Dotarem: gadoterate meglumine). The inversion time was adjusted for optimal nulling of normal myocardium. All images were analysed using in-house software written in IDL (Interactive Data Language, Ver. 6.1, Exelis Visual Information Solutions, Inc., Boulder, USA). Myocardial contours were drawn semi-automatically by CJH and NN and then two forms of analysis were performed. Firstly, visual analysis was performed where two
experienced operators scored the presence or absence of fibrosis on blinded data. In addition, quantitative analysis for scar % was performed using IDL software (Boulder, USA). Whilst myocardial contours were derived by the program, some contours required adjustment to ensure only myocardium was included in the analysis. The program then produced values for scar size (%) and T1 values. T1 mapping was performed using a Short Modified Look Locker Inversion recovery (ShMOLLI) technique, where adjustment for heart rate is not required. Heart rates were adjusted for during acquisition. Quality assessment of T1-maps and late gadolinium images were performed as previously described.

**Statistical analysis**

No formal sample size calculation was performed as the study was conceived as exploratory. Data were summarised as medians and Interquartile range (IQR) for continuous variables and numbers and percentages for discrete variables. Wilcoxon-Mann Whitney test (for continuous variables) and chi square (for categorical variables) tests were used to explore whether there were differences between the HIV group and control group with respect to cardiac function variables and in cardiovascular risk factors.

To further explore the association between HIV status as independent variable and the cardiac function variables, Myocardial fat, Ejection Fraction (EF), Cardiac relaxation (EA), Circumferential systolic strain (CS), peak circumferential diastolic strain rate (CD), Longitudinal systolic strain (LS) and peak longitudinal diastolic strain rate (LD), separate median regression models were used, with the controls group included as a reference category. Median regression were conducted using stata command qreg, qreg fits quantile (including median) regression models, also known as least-absolute-value models (LAV ). The quantile regression models fit by qreg in stata express the quantiles (in our case the median) of the conditional distribution as
linear functions of the independent variables. This is a similar approach to multiple linear regression which analyses the mean. Median regression relaxes the normality assumption of the residuals required in multiple linear regression. All regression models were adjusted for available known confounders, i.e. HDL cholesterol, glucose, smoking, visceral fat, age, sex and ethnicity. Results from median regression models were presented as $\beta$ coefficients which represent the average difference in an outcome and 95% confidence intervals (CI). Statistical analyses were performed using stata version 12.1 (StataCorp, College Station, TX, USA).

**Results**

All subjects, regardless of HIV status, were asymptomatic without a history of chest pain or dyspnoea (all were NYHA class 1). Two patients with HIV were unexpectedly found to have subendocardial myocardial infarction on cardiac MR, so all their data were excluded from subsequent analysis.

**Participant characteristics**

HIV-seropositive subjects had diagnosed infection for a mean (SD) of 7.44 (6.01) years. Of all HIV subjects, 84% had plasma viremia consistently suppressed to $<50$ copies/ml. Of those who were not virologically suppressed, 27% had initiated cART within the preceding 6 months. cART regimens included a non-nucleoside reverse transcriptase inhibitor (NNRTI) or ritonavir-boosted protease inhibitor (PI) in 68% and 32% subjects, respectively. Mean CD4+ cell counts at enrolment were 546 (179) cells/$\mu$L. Pre-ART CD4+ cell nadir was $<$200 cells/$\mu$L in 55% of patients. Patients and control subjects had, on average, similar age and traditional cardiovascular risk factors. No participant had a history of diabetes. 14 (15%) subjects with HIV and 6 (15%) of controls had a history of treated hypercholesterolaemia. 14% of HIV subjects and 15% of controls had a history of treated hypercholesterolaemia.
controls were taking medications for cholesterol: 8 (9%) of subjects with HIV were taking statins and 4 (5%) were taking fibrates. This is compared to controls, where 6 (15%) were taking statins, but none were on fibrates. Hypertension was reported in 14 (15%) subjects with HIV and 4 (10%) of control subjects. A family history of coronary artery disease reported in 13 (14%) subjects with HIV and 7 (18%) control subjects (all p values were above 0.05). Controls subjects were recruited by local advertising. Due to local ethics requirements, control subjects’ HIV status was not confirmed by testing and their negative status was self-declared only.

Characteristics of the study subjects by HIV status (HIV and controls groups) are shown in Table 1.

**Weight and metabolic markers**

There were no significant differences in total body weight, waist circumference, body mass index, lean body weight and body fat composition or hip measurements between HIV-infected and control subjects, nor was there any significant difference in subcutaneous and visceral fat as determined by MR at L4. (Table 1). Rates of metabolic syndrome, using the 2006 International Diabetes Federation consensus worldwide definition of the metabolic syndrome, were not statistically different between the two groups (22.73 in HIV subjects versus 15.38% in controls, p = 0.34).

HIV-infected subjects had lower median HDL cholesterol (1.10 (0.90, 1.30) vs. 1.30 (0.90, 1.70) mM, p = 0.03), higher total cholesterol/HDL ratios (4.20 (3.08, 5.05) vs. 3.55 (2.7, 4.00), p = 0.02) and 74% increase in the median plasma triglycerides (1.22 (0.87, 1.77) mM vs. 0.70 (0.56, 1.05) mM, p <0.001) compared to controls, whereas total and LDL cholesterol were not statistically different in the two groups (Table 1). Median plasma glucose was marginally higher in the HIV cohort (5.30 (4.50, 5.80) versus 4.80 (4.50, 5.30) mM, p = 0.04), but insulin
and free fatty acids mean levels in the two groups were not statistically different (Table 1). 6
(7%) patients in the HIV group had impaired glucose tolerance, defined as a fasting glucose of
equal or greater than 6.1 to 6.9mM (110mg/dl to 125mg/dl) and 4 (5%) had fasting glucose
above 7.0mmol/l (126mg/dl, all less than 8.0 mM). This is compared to 2 (5%) subjects with
fasting glucose in the 6.1 to 6.9mM range and no control subject with a fasting glucose in the
diabetic range.

Myocardial Lipids

The median myocardial lipids were elevated by 47% in the HIV group, compared to the controls
(0.53 (0.30, 0.85) versus 0.36 (0.21, 0.55 ) p<0.001, Table 1) and this was independent to serum
triglycerides. Median regression modelling confirmed the significant association between HIV
and myocardial steatosis; subjects with HIV had significantly higher mean levels of myocardial
fat: adjusted beta coefficients (95%CIs) was β= 0.27 (0.04, 0.51 ) Table 2.

Association between cardiac function and HIV status

In multiple linear regression models (adjusted for HDL cholesterol, glucose, smoking, visceral
fat, age, sex and ethnicity) the association between HIV and alterations in myocardial tagging
parameters remained statistically significant (Table 2).

Patchy and diffuse myocardial fibrosis in HIV

Late gadolinium imaging MR techniques demonstrated that 76% of HIV-infected subjects had
evidence of patchy myocardial fibrosis, on visual assessment. All but one of these had patchy
fibrosis in a mid-wall and subepicardial distribution in the basal infero-lateral wall (Figure 2).
This is a typical pattern suggesting previous myocarditis. In addition, 15% of HIV-infected
subjects had scar in the basal septum. For comparison, only 13% of control subjects had
evidence of any myocardial fibrosis. As mentioned above, two HIV-seropositive subjects had
subendocardial myocardial infarction and subsequently epicardial coronary disease confirmed at angiography, so these subjects were excluded from the analyses. No subjects in the control group had evidence of infarction. Using volumetric analysis methods, the burden of left ventricular patchy fibrosis was 3.97 (2.27, 6.20) % in the HIV-infected cohort compared to 1.87 (0.99, 3.49)% in controls (p < 0.001, Figure 3).

There was no association between duration of diagnosed HIV infection, recent CD4 count or viral load, or pre-therapy CD4 nadir with the alterations in cardiac function parameters, cardiac steatosis or measures of myocardial fibrosis.

Discussion
This is the first study to provide an extensive assessment of cardiac structure, function and metabolism using MR in asymptomatic HIV-seropositive patients. In this cross-sectional analysis, our major findings are cardiac steatosis, in association with deranged plasma lipids, altered myocardial function and a high rate of myocardial fibrosis in nearly all patients with treated HIV infection. These metabolic and structural cardiac abnormalities have not been previously reported and may in part explain the cardiac functional abnormalities and increased mortality reported in subjects receiving cART.13

Whilst plasma metabolic derangements have been well described in the era of cART,14 this study is the first to demonstrate cardiac steatosis in HIV subjects. We hypothesize that cardiac steatosis is a sequela of cART-induced hyperlipidemia and dysglycaemia, though prospective studies before and after cART are needed to test this theory. The association between treated HIV infection and myocardial fat remained after adjustment for potential known confounders. The extent of cardiac steatosis we observed in subjects with HIV did not quite
reach cardiac fat levels reported in obese and diabetic individuals, though was considerably higher than observed in controls within our centre. The heart does not store lipid, in contrast to other organs such as the liver, and cardiac fat rarely exceeds 2% even in significant disease. The extent of cardiac steatosis found in this study is of uncertain clinical significance but given its possible association with antiretroviral drugs to which patients may be continuously exposed for several decades, this warrants further investigation. The elevated myocardial lipid in subjects with HIV was accompanied by plasma lipid abnormalities that are of similar magnitude to those reported in previous studies in HIV cohorts, and a causal relationship is certainly plausible given the link between plasma and cardiac lipid abnormalities in other disease, such as diabetes and obesity. We did not find any significant association between classes of antiretroviral drugs and alterations in cardiac steatosis, though this study was not adequately powered to show this. Larger prospective studies are needed to determine the role of individual medications and combinations of therapies. Our study was not designed to assess extra-cardiac fat, so further studies are needed to see if paracardiac fat in these subjects correlates with myocardial steatosis.

Positron Emission Tomography has demonstrated no changes in myocardial fatty acid uptake, esterification and utilization in HIV-positive subjects compared to controls. We have extended these observations, with our data showing increased storage of fat in the myocardium in patients with HIV on cART. Long term exposure to antiretroviral medications can confer a substantial metabolic risk for a large proportion of patients with HIV, with up to half having dyslipidaemias and a third with impaired glucose tolerance, further compounding cardiovascular risk. Peripheral metabolic complications in other diseases such as diabetes and obesity are associated with increased cardiac functional derangements, morbidity and mortality, which are independent of blood pressure, body mass index and coronary disease. Whether lipid and
glucose abnormalities predispose to cardiac functional alterations in HIV is unknown.

The high prevalence of myocardial fibrosis in the HIV group provides a second potential mechanism for altered cardiac function in the HIV population. The vast majority of patients with HIV had evidence of patchy myocardial fibrosis in the basal infero-lateral wall of the left ventricle. Whilst this pattern of myocardial fibrosis is not specific for a single aetiology, it likely represents previous myocarditis.\textsuperscript{21} In studies before the advent of cART, myocarditis was revealed on biopsy in the vast majority of HIV patients with dilated cardiomyopathy.\textsuperscript{22} However, sub-clinical myocarditis has not, to our knowledge, been widely reported in the cART-era. Our data may represent sub-clinical myocarditis in most people with HIV despite effective cART. It is important to appreciate that whilst there is a six-fold difference in fibrosis prevalence between groups (13\% vs 76\%, control vs HIV) there is only a 2.1\% difference in scar burden between groups. We believe this highlights that whilst scar prevalence is high, the volume of fibrosis is low. In addition, residual scar burden from myocarditis of all aetiologies is usually low. The cause of myocarditis in this group is unknown, but given that fibrosis was observed in nearly all HIV patients, the findings may either represent myocardial inflammation during primary HIV infection, a chronic inflammatory response or a healed scar. Increased overall T1 mapping values compared to controls were also observed which may also reflect myocardial fibrosis, though this association requires further confirmation. Of note, myocardial T1 values were elevated despite the increased myocardial fat demonstrated on proton spectroscopy, which would be expected to decrease T1 values, further supporting the concept of diffuse myocardial fibrosis in these patients. Regardless of the cause, given the well-established association of myocardial scarring with morbidity and mortality in cardiomyopathy, measured using cardiac magnetic resonance,\textsuperscript{23} HIV-related cardiac fibrosis could indicate a potential increased risk of cardiac morbidity.\textsuperscript{24}
This hypothesis warrants further investigation in longitudinal studies. We did not acquire post contrast T1 images in this study, though this would be of interest in further work.

Our findings of altered myocardial function in patients with HIV are consistent with previous echocardiography studies demonstrating systolic and diastolic dysfunction\(^7\) in asymptomatic subjects on cART.\(^6\) We have confirmed and extended these findings using the gold standard for cardiac functional assessment, cardiac MRI. We propose that cardiac steatosis, secondary to cART, and myocardial fibrosis may underlie the dysfunction observed. It is conceivable that, in addition to the known increased incidence of HIV-related coronary disease\(^5\), cardiac steatosis and fibrosis may partially underlie the increased cardiac morbidity and mortality seen in this group.

As the clinical implications of our results have yet to be determined and MRS is not yet a clinical tool, we do not advocate cardiac MR scans for the routine assessment of asymptomatic subjects with HIV. We believe the findings are hypothesis generating and may help develop further studies to determine the pathogenesis of early cardiac disease in patients with treated HIV. Recently, the concept of HIV-induced premature aging has been proposed as a mechanism for changes in cardiac pathological findings.\(^25\) This hypothesis is not supported by our data as the extent of cardiac steatosis,\(^26\) the near universal finding of myocardial fibrosis and the reduction in systolic strain are not characteristic features of the aging heart.\(^27\)

**Limitations**

This study was cross-sectional and exploratory, so it is not possible to assign causal links between cART, cardiac steatosis, fibrosis and the altered function seen in the HIV group. Whilst cART has been associated with lipid abnormalities and is a plausible explanation for cardiac steatosis in this study, HIV itself has been modestly associated with deranged lipid metabolism.
and diet and genetic factors have not been accounted for as potential confounders in this study. Dysglycemia or diabetes may be expected to affect the MRI results including higher rate of LGE, abnormal t1 mapping, and steatosis. Though the statistics accounted for glucose measurements, glucose as a single point in time glucose may incompletely control for the effects of chronic dysglycemia, which is more prevalent in the patients with HIV. In addition, we did not measure perfusion abnormalities in the HIV group; a greater burden of coronary disease in this group may account for some of the functional changes. Larger prospective studies in subjects on cART and those who are cART-naive are required to determine at what stage cardiac steatosis, fibrosis or dysfunction occur, and to determine any direct effect of HIV itself. In addition, given the diverse combinations of antiretroviral drugs used by patients in this study, we were not able to identify drug- or drug class-specific effects; this would necessitate a suitably powered prospective study. Self-reporting of negative HIV status in controls was an additional limitation of this study, though any false status may have demonstrated a greater difference between the two groups. Because of multiple testing concerns in this study, all findings are interpreted as hypothesis generating and require confirmation in larger cohorts.

**Conclusion**

In this study we have demonstrated a previously unappreciated high burden of metabolic, structural and functional abnormalities in HIV subjects on cART using cardiac MRI and MRS. Whilst the clinical implications of these findings are uncertain, our study generates the hypothesis that these abnormalities, in addition to coronary disease, account for the increased cardiac morbidity and mortality observed in people living with chronic HIV infection, and larger prospective studies are needed to address this further.
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Conflict of Interest Disclosures: None.

References:


Table 1. Subject characteristics by HIV status

<table>
<thead>
<tr>
<th></th>
<th>HIV-infected 90 (69.77%)</th>
<th>Controls 39 (30.23%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic and blood pressure measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>68 (75.56)</td>
<td>26 (66.67)</td>
<td>0.30</td>
</tr>
<tr>
<td>White or European ancestry</td>
<td>58 (68.24)</td>
<td>34 (87.18)</td>
<td>0.03</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>20 (22.73)</td>
<td>6 (15.38)</td>
<td>0.34</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>40.00 (35.00, 53.00)</td>
<td>43.00 (37.00, 52.00)</td>
<td>0.70</td>
</tr>
<tr>
<td>Smoking (pack years)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.00 (0.00, 13.00)</td>
<td>0.14</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>131.00 (122.00, 143.00)</td>
<td>127.00 (119.00, 137.00)</td>
<td>0.25</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78.00 (70.00, 86.00)</td>
<td>78.00 (71.00, 84.00)</td>
<td>0.61</td>
</tr>
<tr>
<td>Anthropometric Measurement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.40 (69.00, 87.00)</td>
<td>74.30 (69.00, 86.70)</td>
<td>0.94</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 (1.68, 1.83)</td>
<td>1.75 (1.67, 1.80)</td>
<td>0.51</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>24.49 (23.14, 26.44)</td>
<td>25.10 (22.46, 28.13)</td>
<td>0.57</td>
</tr>
<tr>
<td>Lean body weight (kg)</td>
<td>57.30 (47.80, 69.10)</td>
<td>57.50 (51.60, 63.90)</td>
<td>0.92</td>
</tr>
<tr>
<td>% body fat</td>
<td>22.80 (17.00, 29.50)</td>
<td>23.60 (19.90, 28.90)</td>
<td>0.41</td>
</tr>
<tr>
<td>Subcutaneous fat in (cm²)</td>
<td>192.10 (144.60, 257.20)</td>
<td>188.80 (139.30, 264.80)</td>
<td>0.66</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>86.00 (80.00, 93.00)</td>
<td>91.00 (83.50, 98.00)</td>
<td>0.06</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>97.00 (93.00, 101.00)</td>
<td>95.00 (89.00, 102.00)</td>
<td>0.41</td>
</tr>
<tr>
<td>Visceral fat in (cm²)</td>
<td>73.20 (44.40, 116.70)</td>
<td>85.75 (50.40, 119.70)</td>
<td>0.31</td>
</tr>
<tr>
<td>Plasma Metabolites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
<td>4.60 (3.40, 5.10)</td>
<td>4.45 (3.40, 5.10)</td>
<td>0.86</td>
</tr>
<tr>
<td>HDL cholesterol (mM)</td>
<td>1.30 (0.90, 1.70)</td>
<td>1.10 (0.90, 1.30)</td>
<td>0.03</td>
</tr>
<tr>
<td>LDL cholesterol (mM)</td>
<td>2.60 (1.90, 3.30)</td>
<td>2.50 (1.85, 3.20)</td>
<td>0.62</td>
</tr>
<tr>
<td>Cholesterol / HDL ratio</td>
<td>3.55 (2.72, 4.00)</td>
<td>4.20 (3.08, 5.05)</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>0.70 (0.56, 1.05)</td>
<td>1.22 (0.87, 1.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>4.80 (4.50, 5.30)</td>
<td>5.30 (4.50, 5.80)</td>
<td>0.04</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>34.00 (25.00, 56.00)</td>
<td>38.00 (26.00, 54.50)</td>
<td>0.70</td>
</tr>
<tr>
<td>Free fatty acids (mM)</td>
<td>0.45 (0.27, 0.71)</td>
<td>0.40 (0.29, 0.53)</td>
<td>0.28</td>
</tr>
<tr>
<td>Cardiac parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial lipids (%)</td>
<td>0.36 (0.21, 0.55)</td>
<td>0.53 (0.30, 0.85)</td>
<td>0.003</td>
</tr>
<tr>
<td>Left ventricular ejection Fraction (%)</td>
<td>69.65 (66.00, 73.50)</td>
<td>69.40 (64.40, 73.10)</td>
<td>0.202</td>
</tr>
<tr>
<td>Adjusted LV End Diastolic Volume (ml/BSA)</td>
<td>75.89 (70.06, 88.35)</td>
<td>74.31 (65.70, 83.38)</td>
<td>0.138</td>
</tr>
<tr>
<td>Adjusted LV End Systolic Volume (ml/BSA)</td>
<td>23.07 (18.41, 27.61)</td>
<td>23.12 (19.43, 27.33)</td>
<td>0.054</td>
</tr>
<tr>
<td>Adjusted LV Stroke Volume (ml/BSA)</td>
<td>54.69 (48.41, 61.71)</td>
<td>51.20 (45.40, 55.82)</td>
<td>0.0323</td>
</tr>
<tr>
<td>Circumferential systolic strain (%)</td>
<td>-18.71 (-19.77, -17.98)</td>
<td>-17.23 (-19.27, -16.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak circumferential diastolic strain rate (%/sec)</td>
<td>78.07 (65.52, 97.28)</td>
<td>70.31 (57.19, 81.17)</td>
<td>0.0225</td>
</tr>
<tr>
<td>Longitudinal systolic strain (%)</td>
<td>-14.46 (-15.07, -13.03)</td>
<td>-12.91 (-14.75, -11.48)</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak longitudinal diastolic strain rate (%/sec)</td>
<td>42.47 (30.00, 57.11)</td>
<td>33.48 (23.59, 47.14)</td>
<td>0.01</td>
</tr>
<tr>
<td>T1 mapping shMOLLI value (ms)</td>
<td>950.51 (934.41, 973.73)</td>
<td>962.33 (942.8, 986.13)</td>
<td>0.042</td>
</tr>
<tr>
<td>Late Gadolinium Enhancement (3sd)</td>
<td>3.97 (2.27, 6.20)</td>
<td>1.87 (0.99, 3.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of Late gadolinium enhancement</td>
<td>68 (75.56%)</td>
<td>5 (12.82%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are mean (SD) for continuous variables or count (%) for categorical variables. P value: Wilcoxon-Mann Whitney test for (ranksum command in stata) continuous variables and chi-square test for categorical variables. The International Diabetes Federation consensus worldwide definition of the metabolic syndrome (2006).
Table 2. Association between cardiac function and presence of HIV: linear regression models to demonstrate the association between outcome variables with HIV status as an independent predictor after adjustment for risk factors (listed below)

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>95% (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial fat</td>
<td>0.27</td>
<td>(0.04 , 0.510 )*</td>
</tr>
<tr>
<td>Left Ventricular ejection fraction (LVEF)</td>
<td>-2.38</td>
<td>(-5.61 , 0.860 )</td>
</tr>
<tr>
<td>Circumferential systolic strain (CS)</td>
<td>1.46</td>
<td>(0.30 , 2.618 )*</td>
</tr>
<tr>
<td>Peak circumferential diastolic strain rate (CD)</td>
<td>-16.14</td>
<td>(-26.09 , -6.197 )**</td>
</tr>
<tr>
<td>Longitudinal systolic strain (LS)</td>
<td>1.27</td>
<td>(0.10 , 2.440 )*</td>
</tr>
<tr>
<td>Peak longitudinal diastolic strain rate (LD)</td>
<td>-13.72</td>
<td>(-26.54 , -0.893 )*</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, *** p<0.001. Adjusted for HDL cholesterol, glucose, smoking, visceral fat, age, sex and ethnicity. β beta coefficient, CI: Confidence intervals. Average difference between HIV patients and controls (reference controls).

Figure Legends:

Figure 1. Voxel selection for $^1$H magnetic resonance spectroscopy. A. Short axis image with a voxel in the interventricular septum of a midventricular slice (white box). The green box shown in Figure 1 is the adjustment volume (for frequency calibration). B. Four chamber image showing slice selection. C. An example of $^1$H MR spectrum from a subject with HIV.

Figure 2. Two examples of cardiac magnetic resonance short axis image of the heart.

Demonstrating diffuse left ventricular mid-wall enhancement, particularly prominent in the basal infero-lateral wall.

Figure 3. Box plots showing differences in cardiac parameters between subjects with HIV and controls (Symbol represents median). (A): Myocardial lipids (%) (p =0.003), (B): Left Ventricular ejection fraction (%) (EF) (p=0.202), (C): End Diastolic volumes/Body surface area
(ml/BSA) (p=0.138). (D): End systolic volumes/Body surface area (ml/BSA) (p=0.054), (E): Stroke volumes /Body surface area (ml/BSA) (p=0.0323), (F): Circumferential systolic strain (%) (p<0.001), (G): Peak circumferential diastolic strain rate (%/sec) (p=0.0225), (H): Longitudinal systolic strain(%) (p=0.01), (I): Peak longitudinal diastolic strain rate (%/sec) (p=0.01), (J): Average T1 mapping-shMOLLI cardiac values(ms) (p=0.042), (K): Average volume of late gadolinium enhancement(%) (p<0.001).
Figure 1

Suppressed water peak

Myocellular lipid peak
Figure 3
Comprehensive Cardiac Magnetic Resonance Imaging and Spectroscopy Reveals a High Burden of Myocardial Disease in HIV Patients

Cameron Holloway, Ntobeko Ntusi, Joseph Suttie, Masliza Mahmod, Emma Wainwright, Genevieve Clutton, Gemma Hancock, Philip Beak, Abdelouahid Tajar, Stefan K. Piechnik, Jürgen E. Schneider, Brian Angus, Kieran Clarke, Lucy Dorrell and Stefan Neubauer

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