Multisite prospective Liver Disease and Reproductive Ageing (LIVRA) study in US women living with and without HIV

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ABSTRACT

Purpose The Liver Disease and Reproductive Ageing (LIVRA) study leverages the infrastructure of the decades-long multicentre prospective Women’s Interagency HIV Study (WHIS) to examine the contributions of HIV, hepatitis C virus (HCV) and ageing to liver disease progression in women.

Participants From 2013 to 2018, LIVRA enrolled 1576 participants (77 HIV-seropositive only, 248 HIV/HCV-seropositive, 868 HIV-seropositive only and 383 HIV/HCV-seronegative) who underwent vibration controlled transient elastography (VCTE). A VCTE quality assurance programme was established to ensure consistency and accuracy for longitudinal assessment of steatosis (fatty liver) via the controlled attenuation parameter (CAP) and fibrosis via liver stiffness (LS). Demographic, lifestyle factors, anthropometry, clinical and medication history, host genetics, immune markers and hormone levels were collected as part of the WHIS.

Findings to date At baseline, 737 of 1543 women with CAP measurements had steatosis (CAP ≥248 dB/m) and 375 of 1576 women with LS measurements had significant fibrosis (LS ≥7.1 kPa), yielding a prevalence of 48% and 24%, respectively. On multivariable analysis, waist circumference (WC) and insulin resistance were independently associated with higher CAP (17.8 dB/m per 10 cm (95% CI: 16.2 to 19.5) and 1.2 dB/m per doubling (95% CI: 0.8 to 1.6), respectively). By contrast, HIV/HCV seropositivity and HCV seropositivity alone were associated with less steatosis compared with HIV/HCV-seronegative women, although the latter did not reach statistical significance (−0.2 dB/m (95% CI: −18.2 to −0.3) and −0.1 dB/m (95% CI: −23.8 to 3.1), respectively). Factors independently associated with higher LS were age (4.4% per 10 years (95% CI: 0.4% to 8.4%)), WC (5.0% per 10 cm (95% CI: 3.3% to 6.6%)), CAP steatosis (0.6% per 10 dB/m (95% CI: 0.1% to 1.0%)), HIV/HCV seropositivity (33% (95% CI: 24% to 44%)) and HCV seropositivity alone (43% (95% CI: 28% to 60%)). Excluding scans that were invalid based on traditional criteria for unreliability did not affect the results.

Future plans Enrolled women undergo VCTE at 3-year intervals unless LS is ≥9.5 kPa, indicating advanced fibrosis, in which case VCTE is performed annually. Participants also undergo VCTE every 6 months until 18 months after HCV treatment initiation. Analysis of the data collected will provide insights into the impact of ageing/ovarian function, host genetics, immune function and contemporary HIV and HCV treatments on liver disease progression.

Strengths and limitations of this study

The Liver Disease and Reproductive Ageing (LIVRA) study is designed to examine the contributions of HIV, hepatitis C virus, ageing and ovarian function, immune function, host genomics and contemporary antiviral treatments on hepatic steatosis (fatty liver) and fibrosis progression measured via longitudinal vibration controlled transient elastography (VCTE).

The study leverages the infrastructure of the decades-long multicentre prospective Women’s Interagency HIV Study (WHIS), which collects biological specimens, sociodemographic data, lifestyle (substance use, food insecurity and physical activity) factors, anthropometry, clinical and medication history, host genetics, immune markers and hormone levels.

A rigorous VCTE quality assurance programme was developed to ensure consistency and accuracy of hepatic steatosis and fibrosis assessments.

Although limited to women, the LIVRA VCTE protocol is currently being implemented in men enrolled into the newly integrated Multicentre AIDS Cohort Study (MACS)-WHIS Combined Cohort Study.

The LIVRA study will allow investigation into the natural history of and potentially modifiable risk factors for hepatic steatosis and fibrosis in women with and without HIV.

INTRODUCTION

Liver disease is a leading cause of morbidity and mortality among persons living with HIV (PLWH). However, the determinants of hepatic steatosis (fatty liver) and fibrosis progression among women living with HIV in the modern era of HIV and hepatitis C virus (HCV) treatment are not well known. Seminal fatty liver disease cohort studies have excluded PLWH and those with cleared
HCV,\(^2\) and studies of hepatic steatosis in PLWH are often small in sample size. Moreover, the vast majority of studies of hepatic steatosis among PLWH have been conducted in men even though women represent over 50% of all PLWH worldwide\(^4\) and close to 25% of all PLWH in the USA.\(^5\)

In HIV-seronegative populations, women have a lower overall risk of steatosis than men but a higher risk of advanced fibrosis once steatosis is established, particularly after age 50 years.\(^6\) Understanding the factors associated with steatosis and its impact on fibrosis among women in general, and especially in those living with HIV, is of essential importance. The Liver Disease and Reproductive Ageing (LIVRA) study was designed as an ancillary study of the Women’s Interagency HIV Study (WIHS) to evaluate the contributions of HIV, HCV and reproductive ageing to steatosis and fibrosis progression as measured by longitudinal vibration controlled transient elastography (VCTE) among a large cohort of women living with or without HIV.

**COHORT DESCRIPTION**

**Study population and design**

The WIHS (now part of the MACS-WIHS Combined Cohort Study\(^7\) (MWCCS)) was a multicentre prospective cohort study established to investigate the course of HIV and associated conditions among women living with and without HIV. The WIHS enrolled 4982 women (3678 living with HIV infection and 1304 living without HIV infection) during four recruitment waves: 1994–1995, 2001–2002, 2011–2012 and 2013–2015 from 10 US cities (Bronx and Brooklyn, New York; Chicago, Illinois; San Francisco, California; Los Angeles, California; Washington District of Columbia; Atlanta, Georgia; Chapel Hill, North Carolina; Miami, Florida; Jackson, Mississippi and Birmingham, Alabama). Full details of recruitment, retention and demographics have been published previously.\(^8\) Eligibility criteria varied with each wave of enrolment mainly to recruit women with earlier HIV disease stage and who had never taken older antiretroviral drugs associated with increased metabolic perturbations during later waves (ie, didanosine and stavudine). Eligible women had documented seroconversion to reactive anti-HIV serology and if positive, a confirmatory test or if they were HIV-seronegative had risk factor(s) for HIV exposure. Baseline sociodemographic characteristics and HIV risk factors were similar between HIV-seropositive and HIV-seronegative women.

At semi-annual research visits, participants completed a physical examination, provided biological specimens (eg, serum, plasma, peripheral blood mononuclear cells, urine) and completed interviewer-administered questionnaires to obtain information regarding sociodemographics, lifestyle data (eg, substance use, food security, physical activity) and clinical, psychosocial and medication history. Host genetic data and genome-wide association studies are also available in the WIHS, as are markers of immune activation and inflammation and hormone levels.

From December 2013 to December 2018, 1576 of the approximately 2100 active WIHS women were enrolled into the LIVRA ancillary study. Eligibility included age 35–60 years old for women without chronic HCV. All women with chronic HCV infection were eligible for enrolment, regardless of age, as long as they were not currently receiving anti-HCV therapy. Women with positive hepatitis B surface antigen, haemochromatosis, autoimmune hepatitis or primary biliary cholangitis were excluded, as were women who reported using medications associated with steatosis (ie, systemic corticosteroids, amiodarone, methotrexate), signs of decompensated cirrhosis, current cancer or severe renal insufficiency. Women who were pregnant or had an implantable cardiac device were excluded per the VCTE manufacturer (Fibroscan; Echosens, Paris, France).

As part of the study protocol, longitudinal VCTE examinations were performed at 3-year intervals after their baseline measurement unless liver stiffness (LS) values are ≥9.5 kPa, consistent with advanced fibrosis, in which case VCTE are performed annually. Additionally, participants who report HCV treatment after enrolment undergo VCTE every 6 months until 18 months after HCV treatment initiation.

**VCTE performance and quality assurance**

VCTE reliability is operator-dependent,\(^9\) and therefore proper operator training and experience is important, especially in multicentre studies involving multiple operators. VCTE operators at each site were first trained by Echosens to: (1) operate the device, (2) obtain at least 10 measurements that the device deemed as valid per scan and (3) recognise images that the device deemed valid but required manual adjustment of probe placement by the operator due to interference from vessel, breathing or lung, and not being completely in the liver (eg, probe placed too low or needing to switch to the XL probe due to subcutaneous fat interference). Operators at each WIHS site were then certified by Echosens after demonstrating proficiency in scanning three to five volunteers.

A quality assurance (QA) programme was implemented which required operators to complete at least 50 additional training examinations on subjects with a wide range of body types and within the age range of eligible participants. This benchmark was selected because a large multicentre study indicated that at least 50 examinations were needed before an operator was considered proficient to consistently acquire valid scans.\(^10\) Each of the first 25 examinations was reviewed by the QA team, which comprised two clinicians and an experienced VCTE operator (table 1). On completion of the review, a written report was provided with the additional option of individualised verbal feedback or an in-person hands-on training and observation. A similar procedure was followed for the next 25 examinations. After completion of 50 training examinations, the final report indicated whether: (1) the
operator was certified; (2) additional examinations were needed and/or (3) a site visit for in-person training was warranted. Twenty-nine operators submitted a median of 55 scans (IQR 51, 62) for review before being certified. To maintain certification, operators are required to perform at least 20 examinations per year.

Assessment of hepatic steatosis and fibrosis

Hepatic steatosis was estimated in dB/m using the VCTE-controlled attenuation parameter (CAP) software and fibrosis was estimated using LS measurements in kPa. Participants were instructed to fast for at least 3 hours prior to VCTE. Operators were instructed to manually switch from the M probe to the XL probe if images suggested subcutaneous fat interference in the measurement range of the probe, that is, >2.5 cm distance from skin to liver capsule. All examinations had at least 10 successful measurements. At the time of analyses, examinations were flagged as having abnormal validity if they had an IQR/median LS ratio >30% and/or success rate <60% as per prior published criteria, or poor image quality. The QA team manually reviewed scan images and data on all VCTE examinations with LS ≥9.3 kPa, a random selection of 10% of examinations with LS <9.3 kPa and those with CAP <150 dB/m. This latter criterion was established after image review suggested that some scans with very low CAP values, including those with the lower bound CAP value of 100 dB/m, may partly capture data from outside of the liver.

Of the 1576 VCTEs performed, 56 had an IQR/median >30% (3.6%), 129 had a success rate <60% (8.2%) and 49 had poor image quality on review (3.1%). These categories were not mutually exclusive; 193 had at least one of these indices (12%). Due to an early technical error with the VCTE device, CAP values were missing for 33 women. Steatosis was defined by CAP ≥248 dB/m, which was the optimal cut-off for selecting mild or greater steatosis in a meta-analysis including data from 2735 patients with liver biopsies and was 83% sensitive and 72% specific in detecting mild or greater steatosis in a subset of our cohort when compared with magnetic resonance spectroscopy-measured liver fat fraction. The following fibrosis categories were used: significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa) and cirrhosis (LS ≥12.5 kPa).

Covariates

Race/Ethnicity was self-reported as white, black or Hispanic (including white and black Hispanic). Menopause stage was determined based on the Stages of Reproductive Ageing Workshop criteria. Self-reported alcohol consumption was categorised as: none; light (>0–7 drinks/week); moderate (7–12 drinks/week) or heavy (>12 drinks/week). Additional demographic and behavioural covariates were obtained through self-report. Body mass index was calculated in kg/m², waist circumference was measured in cm and the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using 8-hour fasting insulin and glucose values.

HIV and HCV seropositivity were defined by documentation of a reactive HIV enzyme immunoassay and reactive second-generation or third-generation HCV enzyme immunoassay, respectively. At WIHS study entry, women underwent testing for HCV antibody (Ab) with HCV RNA if positive. If negative, HCV Ab testing was repeated at last WIHS study visit. For those who became HCV Ab positive, additional retrospective HCV Ab testing was performed to determine when HCV incidence occurred, and then HCV RNA testing was performed at least 2 years later to determine whether they had spontaneously cleared or had chronic infection. For all chronic infections, HCV RNA testing was performed at later visits to determine
### Table 2  Characteristics of the study population by HIV and HCV antibody status

<table>
<thead>
<tr>
<th>Median (IQR) or %</th>
<th>HIV−/HCV+ (n=77)</th>
<th>HIV+/HCV+ (n=248)</th>
<th>HIV+/HCV− (n=868)</th>
<th>HIV−/HCV− (n=383)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sociodemographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 (49, 60)</td>
<td>56 (53, 60)</td>
<td>49 (43, 54)</td>
<td>47 (41, 54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Black</td>
<td>64%</td>
<td>67%</td>
<td>76%</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>7%</td>
<td>15%</td>
<td>9%</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>22%</td>
<td>17%</td>
<td>11%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7%</td>
<td>2%</td>
<td>4%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Menopause stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Premenopause</td>
<td>16%</td>
<td>7%</td>
<td>33%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Perimenopause</td>
<td>13%</td>
<td>8%</td>
<td>14%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>Postmenopause</td>
<td>71%</td>
<td>85%</td>
<td>53%</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>Household income (US$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;6000</td>
<td>29%</td>
<td>16%</td>
<td>12%</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>6000–12000</td>
<td>44%</td>
<td>49%</td>
<td>35%</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td>12 001–36000</td>
<td>7%</td>
<td>13%</td>
<td>15%</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>&gt;36000</td>
<td>9%</td>
<td>9%</td>
<td>17%</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Less than high school</td>
<td>43%</td>
<td>39%</td>
<td>31%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>35%</td>
<td>29%</td>
<td>34%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>More than high school</td>
<td>22%</td>
<td>32%</td>
<td>35%</td>
<td>41%</td>
<td></td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>None</td>
<td>51%</td>
<td>63%</td>
<td>51%</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>27%</td>
<td>25%</td>
<td>40%</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>4%</td>
<td>3%</td>
<td>3%</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Heavy</td>
<td>18%</td>
<td>8%</td>
<td>6%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>71%</td>
<td>54%</td>
<td>35%</td>
<td>42%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current marijuana use</td>
<td>30%</td>
<td>26%</td>
<td>20%</td>
<td>27%</td>
<td>0.05</td>
</tr>
<tr>
<td>Ever injection drug use</td>
<td>36%</td>
<td>23%</td>
<td>1%</td>
<td>4%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30 (25, 36)</td>
<td>28 (23, 32)</td>
<td>31 (26, 36)</td>
<td>32 (27, 38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99 (88, 115)</td>
<td>97 (86, 108)</td>
<td>99 (89, 111)</td>
<td>100 (88, 113)</td>
<td>0.12</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.2 (1.6, 6.8)</td>
<td>2.48 (1.3, 4.9)</td>
<td>2.1 (1.3, 3.8)</td>
<td>1.8 (1.1, 3.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Liver-related</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>26 (19, 37)</td>
<td>24 (18, 35)</td>
<td>19 (15, 23)</td>
<td>17 (14, 20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>23 (15, 34)</td>
<td>18 (12, 30)</td>
<td>15 (12, 21)</td>
<td>14 (11, 18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HIV-related</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable HIV RNA</td>
<td>69%</td>
<td>72%</td>
<td>69%</td>
<td>69%</td>
<td>0.60</td>
</tr>
<tr>
<td>CD4 current (cells/mm³)</td>
<td>616 (428, 869)</td>
<td>656 (435, 870)</td>
<td>215 (107, 320)</td>
<td>222 (103, 361)</td>
<td>0.35</td>
</tr>
<tr>
<td>CD4 nadir (cells/mm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>History of clinical AIDS</td>
<td>42%</td>
<td>28%</td>
<td>42%</td>
<td>28%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current ART</td>
<td>89%</td>
<td>89%</td>
<td>89%</td>
<td>89%</td>
<td>0.81</td>
</tr>
<tr>
<td>NNRTI</td>
<td>30%</td>
<td>29%</td>
<td>30%</td>
<td>29%</td>
<td>0.84</td>
</tr>
<tr>
<td>PI</td>
<td>26%</td>
<td>25%</td>
<td>26%</td>
<td>25%</td>
<td>0.80</td>
</tr>
<tr>
<td>INSTI</td>
<td>44%</td>
<td>48%</td>
<td>44%</td>
<td>48%</td>
<td>0.22</td>
</tr>
<tr>
<td>EI</td>
<td>1.6%</td>
<td>0.8%</td>
<td>1.6%</td>
<td>0.8%</td>
<td>0.26</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; EI, entry inhibitor; HCV, hepatitis C virus; HOMA-IR, homeostatic model assessment of insulin resistance; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
which had cleared following treatment. HCV RNA was performed using either the COBAS Amplicor Monitor V.2.0 or the COBAS Taqman assay, as previously described (both from Roche Diagnostics, Branchburg, New Jersey, USA). HCV genotyping was conducted on a subset of HCV viraemic women using the NC TRUGENE HCV 5 NC Genotyping Kit (Bayer HealthCare, Tarrytown, New York, USA), as previously described.

**Statistical analysis**

For the purposes of this analysis, HIV and HCV infection categories were defined by serostatus. We compared participant characteristics by infection category using analysis of variance or Kruskal-Wallis tests for continuous variables and \( \chi^2 \) or Fisher’s exact tests for categorical variables, as appropriate. We used unadjusted and multivariable adjusted linear regression models to examine associations with CAP and LS. LS had a right-skewed distribution and therefore was log-transformed to normalise its distribution, and the regression coefficients and their CIs were exponentiated to calculate percentage differences attributable to each covariate. These analyses were performed for all women with successful VCTEs, and sensitivity analyses were performed excluding the scans with IQR/median >30%, success rate <60%, poor image quality on QA review or any one of these criteria. In linear regression models where there were missing covariate information, we used the full information maximum likelihood approach in the setting of path analysis instead of the multiple imputation approach for its efficiency. All analyses were performed using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA).

**Baseline characteristics**

Among the 1576 WIHS women enrolled in LIVRA, 77 had HCV monoinfection, 248 had HIV/HCV coinfection, 868 had HIV infection alone and 383 had neither infection (table 2). About three-quarters were black, and median

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**Figure 1** (A) Prevalence of VCTE-estimated hepatic steatosis (CAP ≥248 dB/m) by HIV and HCV antibody status. (B) Prevalence of VCTE-estimated significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa) and cirrhosis (LS ≥12.5 kPa), as estimated by VCTE-measured liver stiffness, by HIV and HCV antibody status. Ab, antibody; CAP, controlled attenuation parameter; HCV, hepatitis C virus; LS, liver stiffness; VCTE, vibration controlled transient elastography.
age was 51 years (IQR 44, 56), with 31% premenopausal, 13% perimenopausal and 56% postmenopausal. Nearly half reported an annual household income of ≤ US$12,000, and one-third of the cohort completed less than a high school education. Most women with HIV were taking antiretroviral therapy (ART) and had undetectable HIV RNA. Among the 325 women with HCV seropositivity, 136 were HCV viraemic at the baseline LIVRA visit (42%), 98 had cleared HCV with treatment (30%) and 91 had cleared HCV spontaneously (28%).

**PATIENT AND PUBLIC INVOLVEMENT**

Participants of the LIVRA study were not involved in the development of the study question or the liver disease outcomes. However, the national community advisory board of the WIHS (now MWCCS) approved the study of liver disease progression using VCTE as a high scientific priority.

**FINDINGS TO DATE**

At baseline, women with neither HIV nor HCV had higher median CAP values than women with HCV monoinfection (247 dB/m (IQR 204, 281) vs 232 dB/m (IQR 191, 285)), and women with HIV monoinfection had higher values than women with HIV/HCV coinfection (246 dB/m (IQR 208, 290) vs 237 dB/m (IQR 200, 272)). Similarly, the prevalence of hepatic steatosis based on CAP ≥248 dB/m was highest in women with neither infection (50%), followed by those with HIV monoinfection (49%), HIV/HCV coinfection (43%) and HCV monoinfection (38%) (figure 1A). The overall prevalence of steatosis was 48%, which is slightly higher than the 43% prevalence observed among women in the 2017–2018 National Health and Nutrition Examination Survey study, which used a CAP cut-off of 263 dB/m, and higher than the 39% reported in a retrospective study of 1511 PLWH from Canada and Italy, which used the same 248 dB/m CAP cut-off as our study. The latter study included mostly white men, whereas our study included mostly black women, a group that has traditionally been found to have lower risk of hepatic steatosis.

On multivariable analysis, greater waist circumference (a marker of visceral obesity) and greater HOMA-IR were significantly associated with higher CAP values (table 3). Our findings are consistent with other studies that have demonstrated visceral adiposity and insulin resistance to be key mediators in the pathogenesis of fatty liver disease. However, HIV infection was not associated with CAP, suggesting that the clinical phenotype of fatty liver disease in PLWH is predominantly caused by metabolic dysregulation that appears to be independent of HIV infection. By contrast, HCV seropositivity was associated with significantly lower CAP values in analyses adjusted for alcohol use, smoking status and menopause stage. This is consistent with our prior finding that non-genotype 3 HCV infection was associated with significantly lower magnetic resonance spectroscopy-measured liver fat fraction among 356 men and women with or without HIV and HCV. Further research on the potential mechanisms underlying this observation as well as the impact of HCV clearance on fatty liver disease are warranted.

As expected, women with HCV had higher median LS values compared with women without HCV, with the...
highest LS observed among those with HCV monoinfection (6.9 kPa (IQR 5.0, 10.7)), followed by those with HIV/HCV coinfection (6.2 kPa (IQR 4.6, 9.1)), HCV monoinfection (5.0 kPa (IQR 3.9, 6.7)) and those with neither infection (4.9 kPa (IQR 3.9, 6.2)). Prevalence of significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa) and cirrhosis (LS ≥12.5 kPa) was also higher among women with HCV compared with those without HCV (figure 1B). In multivariable analysis, HIV/HCV coinfection, HCV monoinfection, older age, greater waist circumference and greater CAP values were each independently associated with higher LS (table 3). These findings have important implications for women as visceral adiposity and metabolic abnormalities increase during the menopausal transition, independent of chronologic age.25 Oestrogen depletion occurs during the late phase of perimenopause and is associated with increased levels of inflammatory cytokines and mucosal barrier dysfunctions.27 These changes may result in increased gut microbial translocation and inflammation,28 factors that have also been linked with steatosis and fibrosis.29 30 Studies are underway evaluating the impact of ovarian follicular reserve on steatosis and fibrosis among LIVRA participants.

In analysis restricted to women living with HIV, HIV-related factors such as CD4 count, HIV RNA and ART regimen, were not significantly associated with CAP or LS on cross-sectional analysis. However, the potential impact of ART class, including integrase strand transfer inhibitors, on incident steatosis and fibrosis will be an important area of research within the cohort prospectively. Finally, a notable finding was that the factors associated with steatosis and fibrosis were similar after excluding VCTE examinations with IQR/median >30%, success rate <60%, poor image quality or any of these three criteria (online supplemental tables 1 and 2). In the setting of a large multicentre epidemiological study, these findings provide assurance about the rigour of our data collection approach. In clinical practice, currently accepted VCTE validity criteria should be adhered to.

Strengths and limitations
To our knowledge, this is the most comprehensive cohort study evaluating liver disease among US women using VCTE and the largest study of VCTE in women living with HIV. However, our cohort has limitations. First, there are demographic differences by HIV and HCV serostatus, and thus unmeasured confounders may remain despite adjustment for multiple variables. Second, we rely on VCTE to assess liver disease, rather than liver biopsy. However, liver biopsy is infeasible in large cohorts such as ours. Finally, our findings may not be generalisable to men, but our VCTE protocol is currently being implemented in men enrolled in the newly integrated MWCCS allowing us to address sex differences.

A major strength of the LIVRA study is that it is conducted in a large, nationally representative cohort of women living with HIV in the USA,31 where racial and ethnic minorities, people living below the poverty line and those with less than a high school education are at higher risk for liver disease.32 Second, LIVRA has detailed longitudinal HIV and HCV data allowing the study of liver steatosis and fibrosis trajectories in women with active HCV viraemia, spontaneous HCV clearance and treated HCV clearance. Third, the inclusion of women with and without HIV and HCV infections will allow us to evaluate the association of viral factors with liver disease using seronegative controls. Finally, due to our rigorous QA programme, a low proportion of VTCE scans were invalid based on traditional criteria for unreliability and excluding these scans did not impact our results. With longitudinal assessments, the LIVRA study will enable evaluation of hepatic steatosis and fibrosis progression in women and support investigation of mechanistic pathways and potentially modifiable risk factors for liver disease in high-risk women.

COLLABORATION
The datasets generated and analysed during the current study are not publicly available but are available from the principal investigator of the LIVRA study on reasonable request and on approval by the Executive Committee of the MACS-WIHS Combined Cohort Study. Please see https://statepi.jhsph.edu/mwccs/work-with-us/ for further information on how to work with our cohort.

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Disclaimer

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Competing interests

AA has received consulting fees from Merck, Viiv Healthcare and Gilead Sciences; Merck and Gilead Sciences have provided her institution with funding for her research. JCP has received consulting fees from Theratechnologies; Gilead Sciences and Merck have provided her institution with funding for her research. PCT, Merck has provided her institution with funding for her research; Gilead and Lilly have also provided her institution with funding for her to conduct industry-sponsored clinical trials. MKH reports consulting for Sanofi. All other authors have no conflicts of interest to report.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Consent obtained directly from patient(s)

Ethics approval

The study was approved by the Institutional Review Boards (IRB) of all participating sites and all participants provided written informed consent to undergo longitudinal VCTE exam during the WHS study visit. Each participating institution’s IRB has been formally designated to review and monitor biomedical research involving human subjects, with the primary responsibility being the protection of subjects from undue risk and from deprivation of personal rights and dignity, which are the touchstones of ethical research.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Data may be obtained from a third party and are not publicly available. The MACS or WHS Public Use Data Set (PDS) is available to investigators. To submit a request form to gain access to the PDS visit https://artable.com/shrvPDF51WJSzqcNet/. MWCCS PDS will be available in 2022

Supplemental material

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