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Cohort profile: The multi-site prospective Liver Disease and Reproductive Aging (LIVRA) study in U.S. women living with and without HIV

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3 **Cohort profile: The multi-site prospective Liver Disease and Reproductive Aging (LIVRA)**
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5 **study in U.S. women living with and without HIV**
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Abbreviations:

Ab	antibody
BMI	body mass index
CAP	controlled attenuation parameter
CI	confidence interval
dB/m	decibels/meter
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HOMA-IR	homeostatic model assessment of insulin resistance
IQR	interquartile range
kPa	kilopascals
LIVRA	Liver Disease and Reproductive Aging
LS	liver stiffness
MWCCS	MACS-WIHS Combined Cohort Study
PLWH	persons living with HIV
QA	quality assurance
US	United States
VCTE	vibration controlled transient elastography
WIHS	Women's Interagency HIV Study

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28 **Author contributions:** PCT and YM contributed the original concept and design of the study.
29 PCT, AAA, MAF, ALF, EG, EKP, MHK, IO, MP, and AS were involved in data collection and
30 sample management. YM, JCP, and PCT performed data analysis and all authors interpreted
31 the data interpretation. JCP and PCT article. All authors have critically revised this article and
32 approved the final version to be published.
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40 **Data sharing statement:** Access to individual-level data from the MACS/WIHS Combined
41 Cohort Study Data (MWCCS) may be obtained upon review and approval of a MWCCS concept
42 sheet. Links and instructions for online concept sheet submission are on the study website
43 (<http://mwccs.org/>).
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ABSTRACT

Purpose: The Liver Disease and Reproductive Aging (LIVRA) study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS) to examine the contributions of HIV, HCV, and aging to liver disease progression in women.

Participants: From 2013-2018, LIVRA enrolled 1576 participants (77 HCV-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 program 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 WIHS.

Findings to date: Prevalence of CAP-measured steatosis ($CAP \geq 248 \text{ dB/m}$) and LS-measured significant fibrosis ($LS \geq 7.1 \text{ kPa}$) was 48% and 24%, respectively. Waist circumference (WC) and insulin resistance, but not HIV or HCV seropositivity, were independently associated with higher CAP, whereas older age, WC, CAP, and HCV seropositivity (with and without HIV) were associated with higher LS. Excluding scans that were invalid based on traditional criteria for unreliability did not affect the results.

Future plans: Enrolled women undergo VCTE at three-year intervals unless LS is $\geq 9.5 \text{ kPa}$, 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 aging/ovarian function, host genetics, immune function, and contemporary HIV and HCV treatments on liver disease progression.

ARTICLE SUMMARY

Strengths and limitations of this study

- The Liver Disease and Reproductive Aging (LIVRA) study is designed to examine the contributions of HIV, hepatitis C virus (HCV), aging and ovarian function, immune function, host genomics, and contemporary antiviral treatments on hepatic steatosis (or fatty liver) and fibrosis progression measured via longitudinal vibration controlled transient elastography (VCTE).
- The study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS), which collects biological specimens, socio-demographic 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 program was developed to ensure consistency and accuracy of hepatic steatosis and fibrosis assessments.
- Though limited to women, the LIVRA VCTE protocol is currently being implemented in men enrolled into the newly integrated MACS-WIHS 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)¹. However, with the advent of direct acting antiviral agents to treat hepatitis C virus (HCV), the landscape of liver disease in PLWH is broadening with hepatic steatosis (fatty liver) being frequently observed among PLWH². The determinants of hepatic steatosis and fibrosis progression among PLWH are not well known, especially in the modern era of HIV and HCV treatment. Seminal cohort studies of people with hepatic steatosis have excluded PLWH and those with cleared HCV^{3,4}, and studies of hepatic steatosis in PLWH are often small in sample size.

Although women represent over 50% of all PLWH worldwide⁵ and close to 25% of all PLWH in the United States (US)⁶, the vast majority of studies of hepatic steatosis among PLWH have been conducted in men. 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 50⁷. Understanding the factors associated with steatosis and its impact on fibrosis among women in general, and in those living with HIV in particular, is of essential importance. Furthermore, few studies have examined the additional contribution of HCV coinfection in women. The Liver Disease and Reproductive Aging (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 aging 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 Study⁸ [MWCCS]) was a multicenter prospective cohort study established to investigate the course of HIV and associated conditions among women living with and without HIV. The WIHS enrolled 4,982 women (3,678 living with HIV infection and 1,304 living without HIV infection) during four recruitment waves: 1994-1995, 2001-2002, 2011-2012, and 2013-2015 from 10 U.S. cities (Bronx and Brooklyn, NY; Chicago, IL; San Francisco, CA; Los Angeles, CA; Washington D.C.; Atlanta, GA; Chapel Hill, NC; Miami, FL; Jackson, MS; and Birmingham, AL). Full details of recruitment, retention, and demographics have been published previously.⁹ 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 (e.g. serum, plasma, peripheral blood mononuclear cells, urine), and completed interviewer-administered questionnaires to obtain information regarding socio-demographics, lifestyle data (e.g. 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 to 60 years old for women without chronic HCV. All women with chronic HCV infection were eligible for enrollment, regardless of age, as long as they were not currently receiving anti-HCV therapy. Women with positive hepatitis B surface antigen, hemochromatosis, autoimmune hepatitis or primary biliary cholangitis were excluded, as were women who reported using medications associated with steatosis (i.e. systemic corticosteroids, amiodarone, methotrexate), signs of decompensated cirrhosis, current cancer, or severe renal insufficiency. Women who were pregnant or had an

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3 implantable cardiac device were excluded per the VCTE manufacturer (Fibroscan®; Echosens,
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5 Paris, France).
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11 As part of the study protocol, longitudinal VCTE examinations are performed at three-year
12 intervals after their baseline measurement unless liver stiffness (LS) values are ≥ 9.5 kilopascals
13 (kPa), consistent with advanced fibrosis, in which case VCTE are performed annually.
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15 Additionally, participants who report HCV treatment after enrollment undergo VCTE every 6
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17 months until 18 months after HCV treatment initiation. The study was approved by the
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19 Institutional Review Boards of all participating sites, and all participants provided written
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21 informed consent.
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29 **VCTE Performance and Quality Assurance**

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32 VCTE reliability is operator-dependent¹⁰, and therefore proper operator training and experience
33 is important, especially in multicenter studies involving multiple operators. VCTE operators at
34 each site were first trained by Echosens to: 1) operate the device, 2) obtain at least 10
35 measurements that the device deemed as valid per scan, and 3) recognize images that the
36 device deemed valid but required manual adjustment of probe placement by the operator due to
37 interference from vessel, breathing, or lung, and not being completely in the liver (e.g. probe
38 placed too low or needing to switch to the XL probe due to subcutaneous fat interference).
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40 Operators at each WIHS site were then certified by Echosens after demonstrating proficiency in
41 scanning 3 to 5 volunteers.
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3 A Quality Assurance (QA) program was implemented which required operators to complete at
4 least 50 additional training examinations on subjects with a wide range of body types and within
5 the age range of eligible participants. This benchmark was selected because a large multicenter
6 study indicated that at least 50 examinations were needed before an operator was considered
7 proficient to consistently acquire valid scans¹¹. Each of the first 25 examinations was reviewed
8 by the QA team, which was comprised of two clinicians and an experienced VCTE operator
9 (Table 1). Upon completion of the review, a written report was provided with the additional
10 option of individualized verbal feedback or an in-person hands-on training and observation. A
11 similar procedure was followed for the next 25 examinations. After completion of 50 training
12 examinations, the final report indicated whether: 1) the operator was certified; 2) additional
13 examinations were needed and/or 3) a site visit for in-person training was warranted. Twenty-
14 nine operators submitted a median of 55 scans (interquartile range [IQR] 51,62) for review
15 before being certified. To maintain certification, operators are required to perform at least 20
16 examinations per year.
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36 **Assessment of Hepatic Steatosis and Fibrosis**

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39 Hepatic steatosis was estimated in decibels/meter (dB/m) using the VCTE-controlled
40 attenuation parameter (CAP) software and fibrosis was estimated using LS measurements in
41 kPa. Participants were instructed to fast for at least three hours prior to VCTE. Operators were
42 instructed to manually switch from the M probe to the XL probe if images suggested
43 subcutaneous fat interference in the measurement range of the probe, i.e. >2.5 cm distance
44 from skin to liver capsule¹². All examinations had at least 10 successful measurements. At the
45 time of analyses, examinations were flagged as having abnormal validity if they had an
46 IQR/median LS ratio >30% and/or success rate <60% as per prior published criteria¹³, or poor
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3 image quality. The QA team manually reviewed scan images and data on all VCTE
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5 examinations with $LS \geq 9.3$ kPa, a random selection of 10% of examinations with $LS < 9.3$ kPa,
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7 and those with $CAP < 150$ dB/m. This latter criterion was established after image review
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9 suggested that some scans with very low CAP values, including those with the lower bound
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11 CAP value of 100 dB/m, may partly capture data from outside of the liver.
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17 Of the 1576 VCTE's performed, 56 had an IQR/median $> 30\%$ (3.6%), 129 had a success rate
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19 $< 60\%$ (8.2%), and 49 had poor image quality on review (3.1%). These categories were not
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21 mutually exclusive; 193 had at least one of these indices (12%). Due to an early technical error
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23 with the VCTE device, CAP values were missing for 33 women. Steatosis was defined by
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25 $CAP \geq 248$ dB/m, which was the optimal cut-off for selecting mild or greater steatosis in a meta-
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27 analysis including data from 2735 patients with liver biopsies¹⁴ and was 83% sensitive and 72%
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29 specific in detecting mild or greater steatosis in a subset of our cohort when compared to
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31 magnetic resonance spectroscopy (MRS)-measured liver fat fraction¹⁵. The following fibrosis
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33 categories were used: significant fibrosis ($LS \geq 7.1$ kPa), advanced fibrosis ($LS \geq 9.5$ kPa), and
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35 cirrhosis ($LS \geq 12.5$ kPa)¹⁶.
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43 **Covariates**

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45 Race/ethnicity was self-reported as White, Black, or Hispanic (including White and Black
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47 Hispanic). Menopause stage was determined based on the Stages of Reproductive Aging
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49 Workshop criteria¹⁷. Self-reported alcohol consumption was categorized as: none; light ($> 0-7$
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51 drinks/week); moderate (7-12 drinks/week); or heavy (> 12 drinks/week). Additional demographic
52
53 and behavioral covariates were obtained through self-report. Body mass index (BMI) was
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55 calculated in kg/m^2 , waist circumference was measured in cm, and the homeostatic model
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3 assessment of insulin resistance (HOMA-IR) was calculated using 8-hour fasting insulin and
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5 glucose values.
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11 HIV and HCV seropositivity were defined by documentation of a reactive HIV enzyme
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13 immunoassay and reactive second or third-generation HCV enzyme immunoassay,
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15 respectively). At WIHS study entry, women underwent testing for HCV antibody (Ab) with HCV
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17 RNA if positive. If negative, HCV Ab testing was repeated at last WIHS study visit. For those
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19 who became HCV Ab positive, additional retrospective HCV Ab testing was performed to
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21 determine when HCV incidence occurred, and then HCV RNA testing was performed at least 2
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23 years later to determine whether they had spontaneously cleared or had chronic infection. For
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25 all chronic infections, HCV RNA testing was performed at later visits to determine which had
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27 cleared following treatment. HCV RNA was performed using either the COBAS Amplicor
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29 Monitor 2.0 or the COBAS Taqman assay, as previously described (both from Roche
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31 Diagnostics, Branchburg, New Jersey, USA)¹⁸. HCV genotyping was conducted on a subset of
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33 HCV viremic women using the NC TRUGENE HCV 5' NC Genotyping Kit (Bayer HealthCare
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35 LLC, Tarrytown, NY), as previously described¹⁹.
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45 **Statistical Analysis**

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48 For the purposes of this analysis, HIV and HCV infection categories were defined by serostatus.
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50 We compared participant characteristics by infection category using ANOVA or Kruskal-Wallis
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52 tests for continuous variables and Chi-square or Fisher's exact tests for categorical variables, as
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54 appropriate. We used unadjusted and multivariable adjusted linear regression models to
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3 examine associations with CAP and LS. LS had a right-skewed distribution and therefore was
4 log-transformed to normalize its distribution, and the regression coefficients and their confidence
5 intervals (CI) were exponentiated to calculate percentage differences attributable to each
6 covariate. These analyses were performed for all women with successful VCTE's, and
7 sensitivity analyses were performed excluding the scans with IQR/median >30%, success rate
8 <60%, poor image quality on QA review, or any one of these criteria. In linear regression
9 models where there were missing covariate information, we used the full information maximum
10 likelihood approach in the setting of path analysis instead of the multiple imputation approach
11 for its efficiency²⁰. All analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary,
12 NC).

23 24 25 26 27 28 **Baseline characteristics**

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31 Among the 1576 WIHS women enrolled in LIVRA, 77 had HCV mono-infection, 248 HIV/HCV
32 coinfection, 868 HIV infection alone, and 383 neither infection (Table 2). About three quarters
33 were Black, and median age was 51 years (IQR 44,56), with 31% premenopausal, 13%
34 perimenopausal, and 56% postmenopausal. Nearly half reported an annual household income
35 of ≤\$12,000, and one-third of the cohort completed less than a high school education. Most
36 women with HIV were taking ART and had undetectable HIV RNA.

37 38 39 40 41 42 43 44 45 46 47 **FINDINGS TO DATE**

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50 Women with neither HIV nor HCV had higher median CAP values than women with HCV
51 mono-infection (247 dB/m [IQR 204, 281] vs 232 dB/m [IQR 191, 285]), and women with HIV
52 mono-infection had higher values than women with HIV/HCV coinfection (246 dB/m [IQR 208,
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3 290] vs 237 dB/m [IQR 200, 272]). Similarly, the prevalence of hepatic steatosis based on CAP
4 ≥ 248 dB/m was highest in women with neither infection (50%), followed by those with HIV
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7 monoinfection (49%), HIV/HCV coinfection (43%), and HCV monoinfection (38%) (Figure 1a).
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9 The overall prevalence of steatosis was 48%, which is slightly higher than the 43% prevalence
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11 observed among women in the 2017-2018 NHANES study²¹, which used a CAP cut-off of 263
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13 dB/m, and higher than the 39% reported in a retrospective study of 1511 PLWH from Canada
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15 and Italy, which used the same 248 dB/m CAP cut-off as our study²². The latter study included
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17 mostly White men, whereas our study included mostly Black women, a group that has
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19 traditionally been found to have lower risk of hepatic steatosis.
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26 On multivariable analysis, greater waist circumference (a marker of visceral obesity) and greater
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28 HOMA-IR were significantly associated with higher CAP values (Table 3). Our findings are
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30 consistent with other studies that have demonstrated visceral adiposity and insulin resistance to
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32 be key mediators in the pathogenesis of fatty liver disease^{23,24}. However, HIV infection was not
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34 associated with CAP, suggesting that the clinical phenotype of fatty liver disease in PLWH is
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36 predominantly caused by metabolic dysregulation that appears to be independent of HIV
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38 infection. By contrast, HCV seropositivity was associated with significantly lower CAP values in
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40 analyses adjusted for alcohol use, smoking status, and menopause stage. Further research on
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42 the potential mechanisms underlying this observation as well as the impact of HCV clearance
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44 on fatty liver disease are warranted.
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51 As expected, women with HCV had higher median LS values compared to women without HCV,
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53 with the highest LS observed among those with HCV monoinfection (6.9 kPa [IQR 5.0, 10.7]),
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55 followed by those with HIV/HCV coinfection (6.2 kPa [IQR 4.6, 9.1]), HIV monoinfection (5.0 kPa
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[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 to those without HCV (Figure 1b). In multivariable analysis, HIV/HCV coinfection, HCV mono-infection, 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 age²⁵. Estrogen depletion occurs during the late phase of perimenopause and is associated with increased levels of inflammatory cytokines²⁶ and mucosal barrier dysfunctions²⁷. These changes may result in increased gut microbial translocation and inflammation²⁸, 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.

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 (Supplemental Tables 1 and 2).

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 generalizable to men,

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3 but our VCTE protocol is currently being implemented in men enrolled in the newly integrated
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5 MWCCS allowing us to address sex differences.
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11 A major strength of the LIVRA study is that it is conducted in a large, nationally representative
12 cohort of women living with HIV in the US³¹ where racial and ethnic minorities, people living
13 below the poverty line, and those with less than a high school education are at higher risk for
14 liver disease³². Second, LIVRA has detailed longitudinal HIV and HCV data allowing the study of
15 liver steatosis and fibrosis trajectories in women with active HCV viremia, spontaneous HCV
16 clearance, and treated HCV clearance. Third, the inclusion of women with and without HIV and
17 HCV infections will allow us to evaluate the association of viral factors with liver disease utilizing
18 seronegative controls. Finally, due to our rigorous QA program, a low proportion of VTCE scans
19 were invalid based on traditional criteria for unreliability and excluding these scans did not
20 impact our results. With longitudinal assessments, the LIVRA study will enable evaluation of
21 hepatic steatosis and fibrosis progression in women and support investigation of mechanistic
22 pathways and potentially modifiable risk factors for liver disease in high-risk women.
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40 **PATIENT INVOLVEMENT**

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43 Participants of the LIVRA Study were not involved in the development of the study question or
44 the liver disease outcomes. However, the national community advisory board of the WIHS (now
45 MWCCS) approved the study of liver disease progression using VCTE as a high scientific
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Table 1. LIVRA Study Quality Assurance Program for VCTE Operator Training Certification

Exams	Guidelines	Training Goals
1-25	<p>Subjects: Varied body types and ages, discrete subjects</p> <p>Parameter goals to work toward: IQR<30% of median, >60% of measurements deemed valid by device</p> <p>REQUIRED: 10 valid measurements obtained per participant</p>	<ul style="list-style-type: none"> • Get comfortable with positioning • Familiarize with Fibroscan® screen and available tools; Observe how age and abdominal fat affect Fibroscan® acquisition • Work on consistently achieving valid assessments
Scans are reviewed by QA team and operator receives written feedback report. Check-in call and additional in-person training optional.		
26-50	<p>Subjects: Varied body types and ages, discrete subjects</p> <p>Parameters: REQUIRED: IQR<30% of median, >60% of measurements deemed valid by device, 10 valid measurements per participant</p>	<ul style="list-style-type: none"> • Focus on achieving valid assessments consistently • Utilize deletion to meet parameter requirements
Scans are reviewed by QA team and operator receives written feedback report. Additional examinations and site visit may be required prior to certification.		
<p>Maintenance certification:</p> <ul style="list-style-type: none"> • Operator must perform at least 20 examinations per year • Ongoing written feedback provided for studies reviewed by QA team 		

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Table 2. Characteristics of the study population by HIV and HCV antibody status.

Median (IQR) or %	HIV-/HCV+ (n=77)	HIV+/HCV+ (n=248)	HIV+/HCV- (n=868)	HIV-/HCV- (n=383)	p-value
Sociodemographic					
Age (years)	56 (49, 60)	56 (53, 60)	49 (43, 54)	47 (41, 54)	<0.001
Race/Ethnicity					0.001
Black	64%	67%	76%	76%	
White	7%	15%	9%	7%	
Hispanic	22%	17%	11%	12%	
Other	7%	2%	4%	5%	
Menopause stage					<0.001
Premenopause	16%	7%	33%	44%	
Perimenopause	13%	8%	14%	14%	
Postmenopause	71%	85%	53%	43%	
Household income					<0.001
<\$6,000	29%	16%	12%	19%	
\$6,000-\$12,000	44%	49%	35%	26%	
\$12,001-\$36,000	7%	13%	15%	13%	
>\$36,000	9%	9%	17%	23%	
Education					0.02
Less than high school	43%	39%	31%	32%	
High school	35%	29%	34%	28%	
More than high school	22%	32%	35%	41%	
Lifestyle					
Alcohol use					<0.001
None	51%	63%	51%	42%	
Light	27%	25%	40%	42%	
Moderate	4%	3%	3%	4%	
Heavy	18%	8%	6%	12%	
Current smoking	71%	54%	35%	42%	<0.001
Current marijuana use	30%	26%	20%	27%	0.05
Ever injection drug use	36%	23%	1%	4%	<0.001
Metabolic					
BMI (kg/m ²)	30 (25, 36)	28 (23, 32)	31 (26, 36)	32 (27, 38)	<0.001
Waist Circumference (cm)	99 (88, 115)	97 (86, 108)	99 (89, 111)	100 (88, 113)	0.12
HOMA-IR	3.2 (1.6, 6.8)	2.48 (1.3, 4.9)	2.1 (1.3, 3.8)	1.8 (1.1, 3.3)	<0.001
Liver-related					
AST (U/L)	26 (19, 37)	24 (18, 35)	19 (15, 23)	17 (14, 20)	<0.001
ALT (U/L)	23 (15, 34)	18 (12, 30)	15 (12, 21)	14 (11, 18)	<0.001
HIV-related					
Undetectable HIV RNA		69%	72%		0.60

CD4 current (cells/mm ³)	616 (428, 869)	656 (435, 870)	0.35
CD4 nadir (cells/mm ³)	215 (107, 320)	222 (103, 361)	0.95
History of clinical AIDS	42%	28%	<0.001
Current ART	89%	89%	0.81

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2 **Figure 1a.** Prevalence of VCTE-estimated hepatic steatosis ($CAP \geq 248$ dB/m) by HIV and HCV
3 antibody status. **1b.** Prevalence of VCTE-estimated significant fibrosis ($LS \geq 7.1$ kPa), advanced fibrosis
4 ($LS \geq 9.5$ kPa), and cirrhosis ($LS \geq 12.5$ kPa), as estimated by VCTE-measured liver stiffness, by HIV
5 and HCV antibody status.
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Table 3. Independent associations of clinical and demographic characteristics with CAP and LS

	CAP difference (dB/m) (CI)**	p-value	Liver stiffness % change (CI)***	p-value
Infection Status (ref=HIV-/HCV-)				
HIV+/HCV-	1.1 (-5.3, 7.5)	0.74	4.4% (-1.1%, 10.3%)	0.12
HIV+/HCV+	-9.2 (-18.2, -0.3)	0.04	33.4% (23.5%, 44.1%)	<0.001
HIV-/HCV+	-10.4 (-23.8, 3.1)	0.13	43.2% (27.8%, 60.4%)	<0.001
Age (per 10 years)	2.4 (-2.0, 6.9)	0.29	4.4% (0.4%, 8.4%)	0.03
Race/Ethnicity (ref=white non-Hispanic)				
Black	-6.1 (-14.2, 2.1)	0.14	2.8% (-4.0%, 10.1%)	0.43
Hispanic	-3.1 (-12.6, 6.4)	0.52	3.1% (-5.0%, 11.7%)	0.47
Other	2.9 (-8.2, 14.0)	0.61	2.5% (-6.7%, 12.7%)	0.60
Waist circumference (per 10 cm)	17.8 (16.2, 19.4)	<0.001	5.0% (3.3%, 6.6%)	<0.001
HOMA-IR (per doubling)	1.2 (0.8, 1.6)	<0.001	0.3% (-0.1%, 0.7%)	0.11
CAP (per 10 dB/m)			0.6% (0.1%, 1.0%)	0.01

*All variables shown in the table are included in a single multiple linear regression model (one model for CAP and one for LS). Also adjusted for alcohol use, smoking status, and menopause stage (parameter estimates not shown).

**N=1543

***N=157

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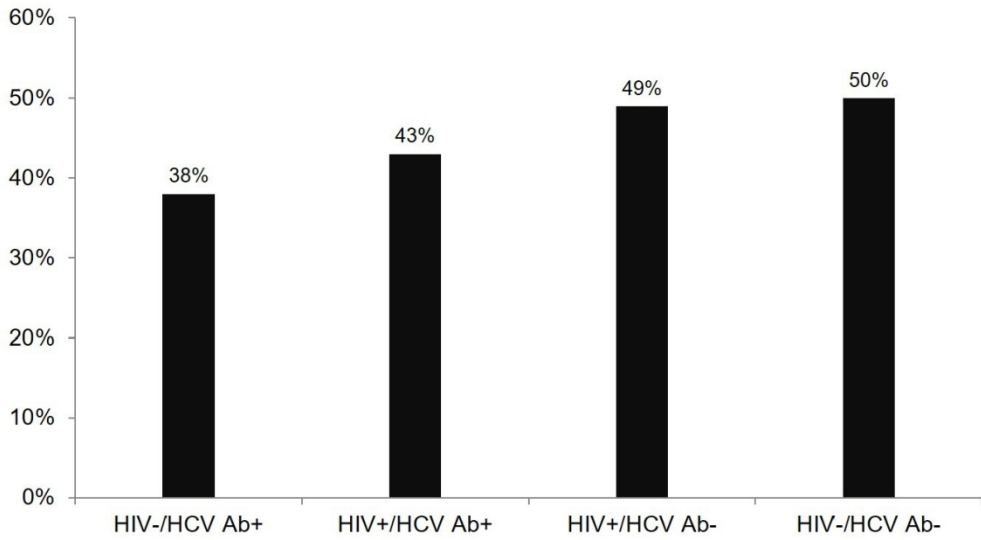


Figure 1a. Prevalence of VCTE-estimated hepatic steatosis (CAP \geq 248 dB/m) by HIV and HCV antibody status.

279x216mm (123 x 123 DPI)

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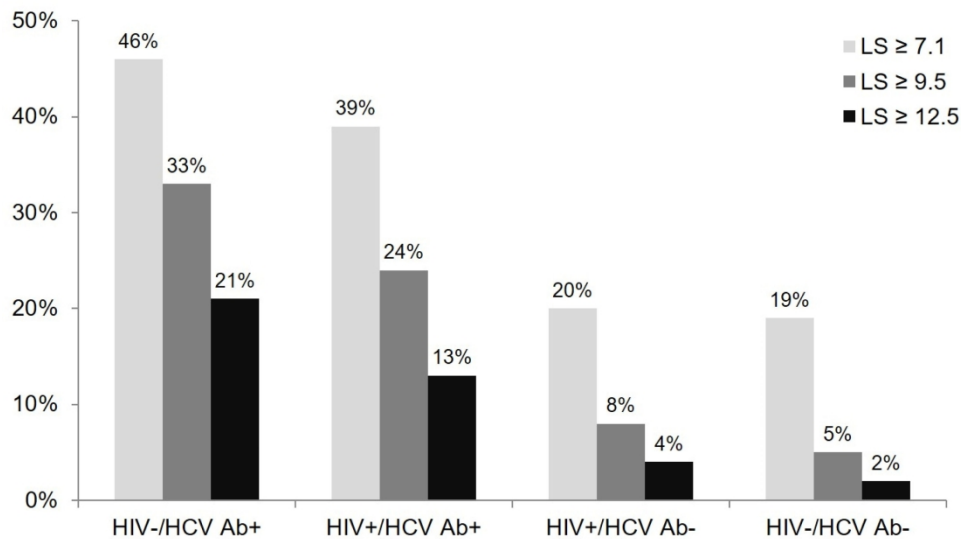


Figure 1b. Prevalence of VCTE-estimated significant fibrosis (LS ≥ 7.1 kPa), advanced fibrosis (LS ≥ 9.5 kPa), and cirrhosis (LS ≥ 12.5 kPa), as estimated by VCTE-measured liver stiffness, by HIV and HCV antibody status.

266x184mm (300 x 300 DPI)

Supplemental Table 1. Factors associated with CAP in multivariable analysis*, excluding VCTE scans with abnormal validity criteria.

	Excluding if IQR/Med >30% (N=1520)		Excluding success rate <60% (N=1447)		Excluding poor image quality (N=1527)		Excluding any abnormal validity criteria (N=1383)	
	CAP difference (dB/m)	p-value	CAP difference (dB/m)	p-value	CAP difference (dB/m)	p-value	CAP difference (dB/m)	p-value
Infection Status (ref=HIV-/HCV-)								
HIV+/HCV-	0.3 (-6.2, 6.8)	0.92	-1.1 (-7.7, 5.5)	0.74	1.4 (-4.8, 7.6)	0.65	-0.2 (-6.7, 6.3)	0.95
HIV-/HCV+	-11.7 (-25.3, 2)	0.09	-16.6 (-30.5, -2.7)	0.02	-11.2 (-24, 1.7)	0.09	-17.8 (-31.5, -4.1)	0.01
HIV+/HCV+	-8.4 (-17.5, 0.7)	0.07	-12.1 (-21.2, -3)	0.01	-8.5 (-17.2, 0.2)	0.06	-9.5 (-18.5, -0.5)	0.04
Age (per 10 years)	2.4 (-2.2, 6.9)	0.31	1.6 (-3, 6.2)	0.50	1.3 (-3.1, 5.6)	0.56	0.9 (-3.7, 5.5)	0.70
Race/Ethnicity (ref=white non- Hispanic)								
Black	-5.6 (-13.9, 2.7)	0.18	-8.2 (-16.5, 0)	0.05	-5.7 (-13.5, 2.2)	0.16	-7.3 (-15.5, 0.9)	0.08
Hispanic	-4.2 (-13.8, 5.4)	0.40	-4.4 (-13.9, 5)	0.36	-3.6 (-12.7, 5.6)	0.45	-4.9 (-14.2, 4.5)	0.31
Other	4.4 (-6.8, 15.6)	0.44	3.6 (-7.5, 14.7)	0.53	2.9 (-7.8, 13.5)	0.60	4.6 (-6.3, 15.6)	0.40
WC (per 10 cm)	17.8 (16.2, 19.5)	<0.001	17.5 (15.9, 19.2)	<0.001	17.8 (16.3, 19.4)	<0.001	17.6 (15.9, 19.2)	<0.001
HOMA-IR (per doubling)	1.2 (0.8, 1.6)	<0.001	1.5 (1, 2)	<0.001	1.2 (0.8, 1.5)	<0.001	1.5 (1, 2)	<0.001

*Also adjusted for alcohol use, smoking status, and menopause stage

Supplemental Table 2. Factors associated with liver stiffness in multivariable analysis, excluding VCTE scans with abnormal validity criteria.

	Excluding if IQR/Med >30% (N=1520)		Excluding success rate <60% (N=1447)		Excluding poor image quality (N=1527)		Excluding any abnormal validity criteria (N=1383)	
	LS % change	p-value	LS % change	p-value	LS % change	p-value	LS % change	p-value
Infection Status (ref=HIV-/HCV-)								
HIV+/HCV-	4.4% (-1.2%, 10.3%)	0.12	4.5% (-1.2%, 10.5%)	0.12	4.1% (-1.4%, 9.9%)	0.14	5.2% (-0.6%, 11.3%)	0.08
HIV-/HCV+	44.2% (28.6%, 61.6%)	<0.001	45.1% (29%, 63.1%)	<0.001	44.5% (29.3%, 61.5%)	<0.001	46.8% (30.5%, 65.2%)	<0.001
HIV+/HCV+	34.4% (24.3%, 45.2%)	<0.001	35.2% (25%, 46.2%)	<0.001	31.1% (21.5%, 41.6%)	<0.001	33.6% (23.4%, 44.6%)	<0.001
Age (per 10 years)	4.6% (0.6%, 8.7%)	0.02	3.4% (-0.6%, 7.5%)	0.10	3.3% (-0.6%, 7.3%)	0.10	3.1% (-0.9%, 7.3%)	0.13
Race/Ethnicity (ref=white non-Hispanic)								
Black	2.2% (-4.7%, 9.6%)	0.54	2.3% (-4.6%, 9.7%)	0.52	1% (-5.7%, 8.1%)	0.78	0.2% (-6.6%, 7.6%)	0.95
Hispanic	3.7% (-4.5%, 12.4%)	0.39	2.2% (-5.8%, 10.8%)	0.60	1% (-6.8%, 9.4%)	0.82	0.5% (-7.4%, 9.1%)	0.90
Other	1.9% (-7.4%, 12%)	0.70	2.8% (-6.5%, 13%)	0.57	1.3% (-7.7%, 11.2%)	0.79	1.5% (-7.7%, 11.6%)	0.76
WC (per 10 cm)	4.4% (2.7%, 6.1%)	<0.001	3.8% (2.1%, 5.5%)	<0.001	4.4% (2.7%, 6.1%)	<0.001	3.4% (1.7%, 5.2%)	<0.001
log2HOMA-IR	0.3% (0%, 0.7%)	0.08	0.1% (-0.4%, 0.5%)	0.80	0.3% (-0.1%, 0.6%)	0.14	0.1% (-0.4%, 0.5%)	0.79
CAP (per 10 dB/m)	0.6% (0.1%, 1%)	0.01	0.7% (0.2%, 1.2%)	0.004	0.6% (0.1%, 1.1%)	0.01	0.7% (0.2%, 1.2%)	0.01

*Also adjusted for alcohol use, smoking status, and menopause stage

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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	1 5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria and number of exposed and unexposed	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	12 11 11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	13
Outcome data	15*	Report numbers of outcome events or summary measures over time	13

1	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14
2			(b) Report category boundaries when continuous variables were categorized	14
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
4	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
5	Discussion			
6	Key results	18	Summarise key results with reference to study objectives	13
7	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
8	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
9	Generalisability	21	Discuss the generalisability (external validity) of the study results	
10	Other information			
11	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.

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Cohort profile: The multi-site prospective Liver Disease and Reproductive Aging (LIVRA) study in U.S. women living with and without HIV

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3 **Cohort profile: The multi-site prospective Liver Disease and Reproductive Aging (LIVRA)**
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5 **study in U.S. women living with and without HIV**
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Abbreviations:

Ab	antibody
BMI	body mass index
CAP	controlled attenuation parameter
CI	confidence interval
dB/m	decibels/meter
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HOMA-IR	homeostatic model assessment of insulin resistance
IQR	interquartile range
kPa	kilopascals
LIVRA	Liver Disease and Reproductive Aging
LS	liver stiffness
MWCCS	MACS-WIHS Combined Cohort Study
PLWH	persons living with HIV
QA	quality assurance
US	United States
VCTE	vibration controlled transient elastography
WIHS	Women's Interagency HIV Study

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29 PCT, AAA, MAF, ALF, EG, EKP, MHK, IO, MP, and AS were involved in data collection and
30 sample management. YM, JCP, and PCT performed data analysis and all authors interpreted
31 the data interpretation. JCP and PCT article. All authors have critically revised this article and
32 approved the final version to be published.

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40 **Data availability statement:** Access to individual-level data from the MACS/WIHS Combined
41 Cohort Study Data (MWCCS) may be obtained upon review and approval of a MWCCS concept
42 sheet. Links and instructions for online concept sheet submission are on the study website
43 (<http://mwccs.org/>).

ABSTRACT

Purpose: The Liver Disease and Reproductive Aging (LIVRA) study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS) to examine the contributions of HIV, HCV, and aging to liver disease progression in women.

Participants: From 2013-2018, LIVRA enrolled 1576 participants (77 HCV-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 program 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 WIHS.

Findings to date: At baseline, 737 of 1543 women with CAP measurements had steatosis ($CAP \geq 248$ dB/m) and 375 of 1576 women with LS measurements had significant fibrosis ($LS \geq 7.1$ 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:16.2,19.5] and 1.2 dB/m per doubling [95%CI:0.8,1.6], respectively). By contrast, HIV/HCV seropositivity and HCV seropositivity alone were associated with less steatosis compared to HIV/HCV seronegative women, although the latter did not reach statistical significance (-9.2 dB/m [95%CI:-18.2,-0.3] and -10.4 dB/m [95%CI:-23.8,3.1], respectively). Factors independently associated with higher LS were age (4.4% per 10 years [95%CI:0.4%,8.4%], WC (5.0% per 10cm [95%CI:3.3%,6.6%], CAP-steatosis (0.6% per 10dB/m [95%CI:0.1%,1.0%]), HIV/HCV seropositivity (33% [95%CI:24%,44%]), and HCV seropositivity

alone (43% [95%CI:28%,60%]). Excluding scans that were invalid based on traditional criteria for unreliability did not affect the results.

Future plans: Enrolled women undergo VCTE at three-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 aging/ovarian function, host genetics, immune function, and contemporary HIV and HCV treatments on liver disease progression.

ARTICLE SUMMARY

Strengths and limitations of this study

- The Liver Disease and Reproductive Aging (LIVRA) study is designed to examine the contributions of HIV, hepatitis C virus (HCV), aging and ovarian function, immune function, host genomics, and contemporary antiviral treatments on hepatic steatosis (or fatty liver) and fibrosis progression measured via longitudinal vibration controlled transient elastography (VCTE).
- The study leverages the infrastructure of the decades-long multi-center prospective Women's Interagency HIV Study (WIHS), which collects biological specimens, socio-demographic 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 program was developed to ensure consistency and accuracy of hepatic steatosis and fibrosis assessments.
- Though limited to women, the LIVRA VCTE protocol is currently being implemented in men enrolled into the newly integrated MACS-WIHS 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)¹. 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^{2,3}, 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 worldwide⁴ and close to 25% of all PLWH in the United States (US)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 50⁶.

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 Aging (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 aging 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 Study⁷ [MWCCS]) was a multicenter prospective cohort study established to investigate the course of HIV and associated conditions

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3 among women living with and without HIV. The WIHS enrolled 4,982 women (3,678 living with
4 HIV infection and 1,304 living without HIV infection) during four recruitment waves: 1994-1995,
5 2001-2002, 2011-2012, and 2013-2015 from 10 U.S. cities (Bronx and Brooklyn, NY; Chicago,
6 IL; San Francisco, CA; Los Angeles, CA; Washington D.C.; Atlanta, GA; Chapel Hill, NC; Miami,
7 FL; Jackson, MS; and Birmingham, AL). Full details of recruitment, retention, and demographics
8 have been published previously.⁸ Eligibility criteria varied with each wave of enrollment mainly to
9 recruit women with earlier HIV disease stage and who had never taken older antiretroviral drugs
10 associated with increased metabolic perturbations during later waves (i.e., didanosine and
11 stavudine). Eligible women had documentation of reactive anti-HIV serology and if positive, a
12 confirmatory test or if they were HIV-seronegative had risk factor(s) for HIV exposure. Baseline
13 sociodemographic characteristics and HIV risk factors were similar between HIV-seropositive
14 and HIV-seronegative women. At semi-annual research visits, participants completed a physical
15 examination, provided biological specimens (e.g. serum, plasma, peripheral blood mononuclear
16 cells, urine), and completed interviewer-administered questionnaires to obtain information
17 regarding socio-demographics, lifestyle data (e.g. substance use, food security, physical
18 activity), and clinical, psychosocial, and medication history. Host genetic data and genome-wide
19 association studies are also available in the WIHS, as are markers of immune activation and
20 inflammation and hormone levels.
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44 From December 2013 to December 2018, 1576 of the approximately 2100 active WIHS women
45 were enrolled into the LIVRA ancillary study. Eligibility included age 35 to 60 years old for
46 women without chronic HCV. All women with chronic HCV infection were eligible for enrollment,
47 regardless of age, as long as they were not currently receiving anti-HCV therapy. Women with
48 positive hepatitis B surface antigen, hemochromatosis, autoimmune hepatitis or primary biliary
49 cholangitis were excluded, as were women who reported using medications associated with
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3 steatosis (i.e. systemic corticosteroids, amiodarone, methotrexate), signs of decompensated
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5 cirrhosis, current cancer, or severe renal insufficiency. Women who were pregnant or had an
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7 implantable cardiac device were excluded per the VCTE manufacturer (Fibroscan®; Echosens,
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9 Paris, France).
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15 As part of the study protocol, longitudinal VCTE examinations are performed at three-year
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17 intervals after their baseline measurement unless liver stiffness (LS) values are ≥ 9.5 kilopascals
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19 (kPa), consistent with advanced fibrosis, in which case VCTE are performed annually.
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21 Additionally, participants who report HCV treatment after enrollment undergo VCTE every 6
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23 months until 18 months after HCV treatment initiation. The study was approved by the
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25 Institutional Review Boards (IRB) of all participating sites, and all participants provided written
26
27 informed consent to undergo longitudinal VCTE exam during the WIHS study visit. Each
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29 participating institution's IRB has been formally designated to review and monitor biomedical
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31 research involving human subjects, with the primary responsibility being the protection of
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33 subjects from undue risk and from deprivation of personal rights and dignity, which are the
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35 touchstones of ethical research.
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45 **VCTE Performance and Quality Assurance**

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48 VCTE reliability is operator-dependent⁹, and therefore proper operator training and experience
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50 is important, especially in multicenter studies involving multiple operators. VCTE operators at
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52 each site were first trained by Echosens to: 1) operate the device, 2) obtain at least 10
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54 measurements that the device deemed as valid per scan, and 3) recognize images that the
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3 device deemed valid but required manual adjustment of probe placement by the operator due to
4 interference from vessel, breathing, or lung, and not being completely in the liver (e.g. probe
5 placed too low or needing to switch to the XL probe due to subcutaneous fat interference).
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9 Operators at each WIHS site were then certified by Echosens after demonstrating proficiency in
10 scanning 3 to 5 volunteers.
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17 A Quality Assurance (QA) program was implemented which required operators to complete at
18 least 50 additional training examinations on subjects with a wide range of body types and within
19 the age range of eligible participants. This benchmark was selected because a large multicenter
20 study indicated that at least 50 examinations were needed before an operator was considered
21 proficient to consistently acquire valid scans¹⁰. Each of the first 25 examinations was reviewed
22 by the QA team, which was comprised of two clinicians and an experienced VCTE operator
23 (Table 1). Upon completion of the review, a written report was provided with the additional
24 option of individualized verbal feedback or an in-person hands-on training and observation. A
25 similar procedure was followed for the next 25 examinations. After completion of 50 training
26 examinations, the final report indicated whether: 1) the operator was certified; 2) additional
27 examinations were needed and/or 3) a site visit for in-person training was warranted. Twenty-
28 nine operators submitted a median of 55 scans (interquartile range [IQR] 51,62) for review
29 before being certified. To maintain certification, operators are required to perform at least 20
30 examinations per year.
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51 **Assessment of Hepatic Steatosis and Fibrosis**

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53 Hepatic steatosis was estimated in decibels/meter (dB/m) using the VCTE-controlled
54 attenuation parameter (CAP) software and fibrosis was estimated using LS measurements in
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3 kPa. Participants were instructed to fast for at least three hours prior to VCTE. Operators were
4 instructed to manually switch from the M probe to the XL probe if images suggested
5 subcutaneous fat interference in the measurement range of the probe, i.e. >2.5 cm distance
6 from skin to liver capsule¹¹. All examinations had at least 10 successful measurements. At the
7 time of analyses, examinations were flagged as having abnormal validity if they had an
8 IQR/median LS ratio >30% and/or success rate <60% as per prior published criteria¹², or poor
9 image quality. The QA team manually reviewed scan images and data on all VCTE
10 examinations with $LS \geq 9.3$ kPa, a random selection of 10% of examinations with $LS < 9.3$ kPa,
11 and those with $CAP < 150$ dB/m. This latter criterion was established after image review
12 suggested that some scans with very low CAP values, including those with the lower bound
13 CAP value of 100 dB/m, may partly capture data from outside of the liver.
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30 Of the 1576 VCTE's performed, 56 had an IQR/median >30% (3.6%), 129 had a success rate
31 <60% (8.2%), and 49 had poor image quality on review (3.1%). These categories were not
32 mutually exclusive; 193 had at least one of these indices (12%). Due to an early technical error
33 with the VCTE device, CAP values were missing for 33 women. Steatosis was defined by
34 $CAP \geq 248$ dB/m, which was the optimal cut-off for selecting mild or greater steatosis in a meta-
35 analysis including data from 2735 patients with liver biopsies¹³ and was 83% sensitive and 72%
36 specific in detecting mild or greater steatosis in a subset of our cohort when compared to
37 magnetic resonance spectroscopy (MRS)-measured liver fat fraction¹⁴. The following fibrosis
38 categories were used: significant fibrosis ($LS \geq 7.1$ kPa), advanced fibrosis ($LS \geq 9.5$ kPa), and
39 cirrhosis ($LS \geq 12.5$ kPa)¹⁵.
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55 **Covariates**

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3 Race/ethnicity was self-reported as White, Black, or Hispanic (including White and Black
4 Hispanic). Menopause stage was determined based on the Stages of Reproductive Aging
5 Workshop criteria¹⁶. Self-reported alcohol consumption was categorized as: none; light (>0-7
6 drinks/week); moderate (7-12 drinks/week); or heavy (>12 drinks/week). Additional demographic
7 and behavioral covariates were obtained through self-report. Body mass index (BMI) was
8 calculated in kg/m², waist circumference was measured in cm, and the homeostatic model
9 assessment of insulin resistance (HOMA-IR) was calculated using 8-hour fasting insulin and
10 glucose values.
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24 HIV and HCV seropositivity were defined by documentation of a reactive HIV enzyme
25 immunoassay and reactive second or third-generation HCV enzyme immunoassay,
26 respectively). At WIHS study entry, women underwent testing for HCV antibody (Ab) with HCV
27 RNA if positive. If negative, HCV Ab testing was repeated at last WIHS study visit. For those
28 who became HCV Ab positive, additional retrospective HCV Ab testing was performed to
29 determine when HCV incidence occurred, and then HCV RNA testing was performed at least 2
30 years later to determine whether they had spontaneously cleared or had chronic infection. For
31 all chronic infections, HCV RNA testing was performed at later visits to determine which had
32 cleared following treatment. HCV RNA was performed using either the COBAS Amplicor
33 Monitor 2.0 or the COBAS Taqman assay, as previously described (both from Roche
34 Diagnostics, Branchburg, New Jersey, USA)¹⁷. HCV genotyping was conducted on a subset of
35 HCV viremic women using the NC TRUGENE HCV 5' NC Genotyping Kit (Bayer HealthCare
36 LLC, Tarrytown, NY), as previously described¹⁸.
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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 ANOVA or Kruskal-Wallis tests for continuous variables and Chi-square 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 normalize its distribution, and the regression coefficients and their confidence intervals (CI) were exponentiated to calculate percentage differences attributable to each covariate. These analyses were performed for all women with successful VCTE's, 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 efficiency¹⁹. All analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC).

Baseline characteristics

Among the 1576 WIHS women enrolled in LIVRA, 77 had HCV mono-infection, 248 HIV/HCV coinfection, 868 HIV infection alone, and 383 neither infection (Table 2). About three quarters were Black, and median age was 51 years (IQR 44,56), with 31% premenopausal, 13% perimenopausal, and 56% postmenopausal. Nearly half reported an annual household income of ≤\$12,000, and one-third of the cohort completed less than a high school education. Most women with HIV were taking ART and had undetectable HIV RNA. Among the 325 women with

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3 HCV seropositivity, 136 were HCV viremic at the baseline LIVRA visit (42%), 98 had cleared
4 HCV with treatment (30%), and 91 had cleared HCV spontaneously (28%).
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10 11 **PATIENT AND PUBLIC INVOLVEMENT** 12

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14 Participants of the LIVRA Study were not involved in the development of the study question or
15 the liver disease outcomes. However, the national community advisory board of the WIHS (now
16 MWCCS) approved the study of liver disease progression using VCTE as a high scientific
17 priority.
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26 27 **FINDINGS TO DATE** 28

29 At baseline, women with neither HIV nor HCV had higher median CAP values than women with
30 HCV mono-infection (247 dB/m [IQR 204, 281] vs 232 dB/m [IQR 191, 285]), and women with
31 HIV mono-infection had higher values than women with HIV/HCV coinfection (246 dB/m [IQR
32 208, 290] vs 237 dB/m [IQR 200, 272]). Similarly, the prevalence of hepatic steatosis based on
33 CAP \geq 248 dB/m was highest in women with neither infection (50%), followed by those with HIV
34 mono-infection (49%), HIV/HCV coinfection (43%), and HCV mono-infection (38%) (Figure 1a).
35 The overall prevalence of steatosis was 48%, which is slightly higher than the 43% prevalence
36 observed among women in the 2017-2018 NHANES study²⁰, which used a CAP cut-off of 263
37 dB/m, and higher than the 39% reported in a retrospective study of 1511 PLWH from Canada
38 and Italy, which used the same 248 dB/m CAP cut-off as our study²¹. The latter study included
39 mostly White men, whereas our study included mostly Black women, a group that has
40 traditionally been found to have lower risk of hepatic steatosis.
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3 On multivariable analysis, greater waist circumference (a marker of visceral obesity) and greater
4 HOMA-IR were significantly associated with higher CAP values (Table 3). Our findings are
5 consistent with other studies that have demonstrated visceral adiposity and insulin resistance to
6 be key mediators in the pathogenesis of fatty liver disease^{22,23}. However, HIV infection was not
7 associated with CAP, suggesting that the clinical phenotype of fatty liver disease in PLWH is
8 predominantly caused by metabolic dysregulation that appears to be independent of HIV
9 infection. By contrast, HCV seropositivity was associated with significantly lower CAP values in
10 analyses adjusted for alcohol use, smoking status, and menopause stage. This is consistent
11 with our prior finding that non-genotype 3 HCV infection was associated with significantly lower
12 magnetic resonance spectroscopy-measured liver fat fraction among 356 men and women with
13 or without HIV and HCV²⁴. Further research on the potential mechanisms underlying this
14 observation as well as the impact of HCV clearance on fatty liver disease are warranted.
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32 As expected, women with HCV had higher median LS values compared to women without HCV,
33 with the highest LS observed among those with HCV mono-infection (6.9 kPa [IQR 5.0, 10.7]),
34 followed by those with HIV/HCV coinfection (6.2 kPa [IQR 4.6, 9.1]), HIV mono-infection (5.0 kPa
35 [IQR 3.9, 6.7]) and those with neither infection (4.9 kPa [IQR 3.9, 6.2]). Prevalence of significant
36 fibrosis (LS \geq 7.1 kPa), advanced fibrosis (LS \geq 9.5 kPa), and cirrhosis (LS \geq 12.5 kPa) was also
37 higher among women with HCV compared to those without HCV (Figure 1b). In multivariable
38 analysis, HIV/HCV coinfection, HCV mono-infection, older age, greater waist circumference, and
39 greater CAP values were each independently associated with higher LS (Table 3). These
40 findings have important implications for women as visceral adiposity and metabolic
41 abnormalities increase during the menopausal transition, independent of chronological age²⁵.
42 Estrogen depletion occurs during the late phase of perimenopause and is associated with
43 increased levels of inflammatory cytokines²⁶ and mucosal barrier dysfunctions²⁷. These changes
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3 may result in increased gut microbial translocation and inflammation²⁸, factors that have also
4 been linked with steatosis and fibrosis^{29,30}. Studies are underway evaluating the impact of
5 ovarian follicular reserve on steatosis and fibrosis among LIVRA participants.
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13 In analysis restricted to women living with HIV, HIV related factors such as CD4 count, HIV
14 RNA, and ART regimen, were not significantly associated with CAP or LS on cross-sectional
15 analysis. However, the potential impact of ART class, including integrase strand transfer
16 inhibitors, on incident steatosis and fibrosis will be an important area of research within the
17 cohort prospectively. Finally, a notable finding was that the factors associated with steatosis and
18 fibrosis were similar after excluding VCTE examinations with IQR/median >30%, success rate
19 <60%, poor image quality, or any of these three criteria (Supplemental Tables 1 and 2). In the
20 setting of a large multicenter epidemiologic study, these findings provide assurance about the
21 rigor of our data collection approach. In clinical practice, currently accepted VCTE validity
22 criteria should be adhered to.
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38 **STRENGTHS AND LIMITATIONS**

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41 To our knowledge, this is the most comprehensive cohort study evaluating liver disease among
42 US women using VCTE and the largest study of VCTE in women living with HIV. However, our
43 cohort has limitations. First, there are demographic differences by HIV and HCV serostatus, and
44 thus unmeasured confounders may remain despite adjustment for multiple variables. Second,
45 we rely on VCTE to assess liver disease, rather than liver biopsy. However, liver biopsy is
46 infeasible in large cohorts such as ours. Finally, our findings may not be generalizable to men,
47 but our VCTE protocol is currently being implemented in men enrolled in the newly integrated
48 MWCCS allowing us to address sex differences.
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6 A major strength of the LIVRA study is that it is conducted in a large, nationally representative
7 cohort of women living with HIV in the US³¹ where racial and ethnic minorities, people living
8 below the poverty line, and those with less than a high school education are at higher risk for
9 liver disease³². Second, LIVRA has detailed longitudinal HIV and HCV data allowing the study of
10 liver steatosis and fibrosis trajectories in women with active HCV viremia, spontaneous HCV
11 clearance, and treated HCV clearance. Third, the inclusion of women with and without HIV and
12 HCV infections will allow us to evaluate the association of viral factors with liver disease utilizing
13 seronegative controls. Finally, due to our rigorous QA program, a low proportion of VTCE scans
14 were invalid based on traditional criteria for unreliability and excluding these scans did not
15 impact our results. With longitudinal assessments, the LIVRA study will enable evaluation of
16 hepatic steatosis and fibrosis progression in women and support investigation of mechanistic
17 pathways and potentially modifiable risk factors for liver disease in high-risk women.
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35 **COLLABORATION**

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38 The datasets generated and analyzed during the current study are not publicly available but are
39 available from the principal investigator of the LIVRA Study on reasonable request and upon
40 approval by the Executive Committee of the MACS-WIHS Combined Cohort Study. Please see
41 [42 https://statepi.jhsph.edu/mwccs/work-with-us/](https://statepi.jhsph.edu/mwccs/work-with-us/) for further information on how to work with our
43 cohort.
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Table 1. LIVRA Study Quality Assurance Program for VCTE Operator Training Certification

Exams	Guidelines	Training Goals
1-25	<p>Subjects: Varied body types and ages, discrete subjects</p> <p>Parameter goals to work toward: IQR<30% of median, >60% of measurements deemed valid by device</p> <p>REQUIRED: 10 valid measurements obtained per participant</p>	<ul style="list-style-type: none"> • Get comfortable with positioning • Familiarize with Fibroscan® screen and available tools; Observe how age and abdominal fat affect Fibroscan® acquisition • Work on consistently achieving valid assessments
Scans are reviewed by QA team and operator receives written feedback report. Check-in call and additional in-person training optional.		
26-50	<p>Subjects: Varied body types and ages, discrete subjects</p> <p>Parameters: REQUIRED: IQR<30% of median, >60% of measurements deemed valid by device, 10 valid measurements per participant</p>	<ul style="list-style-type: none"> • Focus on achieving valid assessments consistently • Utilize deletion to meet parameter requirements
Scans are reviewed by QA team and operator receives written feedback report. Additional examinations and site visit may be required prior to certification.		
<p>Maintenance certification:</p> <ul style="list-style-type: none"> • Operator must perform at least 20 examinations per year • Ongoing written feedback provided for studies reviewed by QA team 		

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Table 2. Characteristics of the study population by HIV and HCV antibody status.

Median (IQR) or %	HIV-/HCV+ (n=77)	HIV+/HCV+ (n=248)	HIV+/HCV- (n=868)	HIV-/HCV- (n=383)	p-value
Sociodemographic					
Age (years)	56 (49, 60)	56 (53, 60)	49 (43, 54)	47 (41, 54)	<0.001
Race/Ethnicity					0.001
Black	64%	67%	76%	76%	
White	7%	15%	9%	7%	
Hispanic	22%	17%	11%	12%	
Other	7%	2%	4%	5%	
Menopause stage					<0.001
Premenopause	16%	7%	33%	44%	
Perimenopause	13%	8%	14%	14%	
Postmenopause	71%	85%	53%	43%	
Household income					<0.001
<\$6,000	29%	16%	12%	19%	
\$6,000-\$12,000	44%	49%	35%	26%	
\$12,001-\$36,000	7%	13%	15%	13%	
>\$36,000	9%	9%	17%	23%	
Education					0.02
Less than high school	43%	39%	31%	32%	
High school	35%	29%	34%	28%	
More than high school	22%	32%	35%	41%	
Lifestyle					
Alcohol use					<0.001
None	51%	63%	51%	42%	
Light	27%	25%	40%	42%	
Moderate	4%	3%	3%	4%	
Heavy	18%	8%	6%	12%	
Current smoking	71%	54%	35%	42%	<0.001
Current marijuana use	30%	26%	20%	27%	0.05
Ever injection drug use	36%	23%	1%	4%	<0.001
Metabolic					
BMI (kg/m ²)	30 (25, 36)	28 (23, 32)	31 (26, 36)	32 (27, 38)	<0.001
Waist Circumference (cm)	99 (88, 115)	97 (86, 108)	99 (89, 111)	100 (88, 113)	0.12
HOMA-IR	3.2 (1.6, 6.8)	2.48 (1.3, 4.9)	2.1 (1.3, 3.8)	1.8 (1.1, 3.3)	<0.001
Liver-related					
AST (U/L)	26 (19, 37)	24 (18, 35)	19 (15, 23)	17 (14, 20)	<0.001
ALT (U/L)	23 (15, 34)	18 (12, 30)	15 (12, 21)	14 (11, 18)	<0.001
HIV-related					
Undetectable HIV RNA		69%	72%		0.60

CD4 current (cells/mm ³)	616 (428, 869)	656 (435, 870)	0.35
CD4 nadir (cells/mm ³)	215 (107, 320)	222 (103, 361)	0.95
History of clinical AIDS	42%	28%	<0.001
Current ART	89%	89%	0.81
NNRTI	30%	29%	0.84
PI	26%	25%	0.80
INSTI	44%	48%	0.22
EI	1.6%	0.8%	0.26

NNRTI= non-nucleoside reverse transcriptase inhibitor; PI= protease inhibitor; INSTI= Integrase strand transfer inhibitor; EI= entry inhibitor

1
2 **Figure 1a.** Prevalence of VCTE-estimated hepatic steatosis ($CAP \geq 248$ dB/m) by HIV and HCV
3 antibody status. **1b.** Prevalence of VCTE-estimated significant fibrosis ($LS \geq 7.1$ kPa), advanced fibrosis
4 ($LS \geq 9.5$ kPa), and cirrhosis ($LS \geq 12.5$ kPa), as estimated by VCTE-measured liver stiffness, by HIV
5 and HCV antibody status.
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Table 3. Independent associations of clinical and demographic characteristics with CAP and LS

	CAP difference (dB/m) (CI)**	p-value	Liver stiffness % change (CI)***	p-value
Infection Status (ref=HIV-/HCV-)				
HIV+/HCV-	1.1 (-5.3, 7.5)	0.74	4.4% (-1.1%, 10.3%)	0.12
HIV+/HCV+	-9.2 (-18.2, -0.3)	0.04	33.4% (23.5%, 44.1%)	<0.001
HIV-/HCV+	-10.4 (-23.8, 3.1)	0.13	43.2% (27.8%, 60.4%)	<0.001
Age (per 10 years)	2.4 (-2.0, 6.9)	0.29	4.4% (0.4%, 8.4%)	0.03
Race/Ethnicity (ref=white non-Hispanic)				
Black	-6.1 (-14.2, 2.1)	0.14	2.8% (-4.0%, 10.1%)	0.43
Hispanic	-3.1 (-12.6, 6.4)	0.52	3.1% (-5.0%, 11.7%)	0.47
Other	2.9 (-8.2, 14.0)	0.61	2.5% (-6.7%, 12.7%)	0.60
Waist circumference (per 10 cm)	17.8 (16.2, 19.4)	<0.001	5.0% (3.3%, 6.6%)	<0.001
HOMA-IR (per doubling)	1.2 (0.8, 1.6)	<0.001	0.3% (-0.1%, 0.7%)	0.11
CAP (per 10 dB/m)			0.6% (0.1%, 1.0%)	0.01

*All variables shown in the table are included in a single multiple linear regression model (one model for CAP and one for LS). Also adjusted for alcohol use, smoking status, and menopause stage (parameter estimates not shown).

**N=1543

***N=157

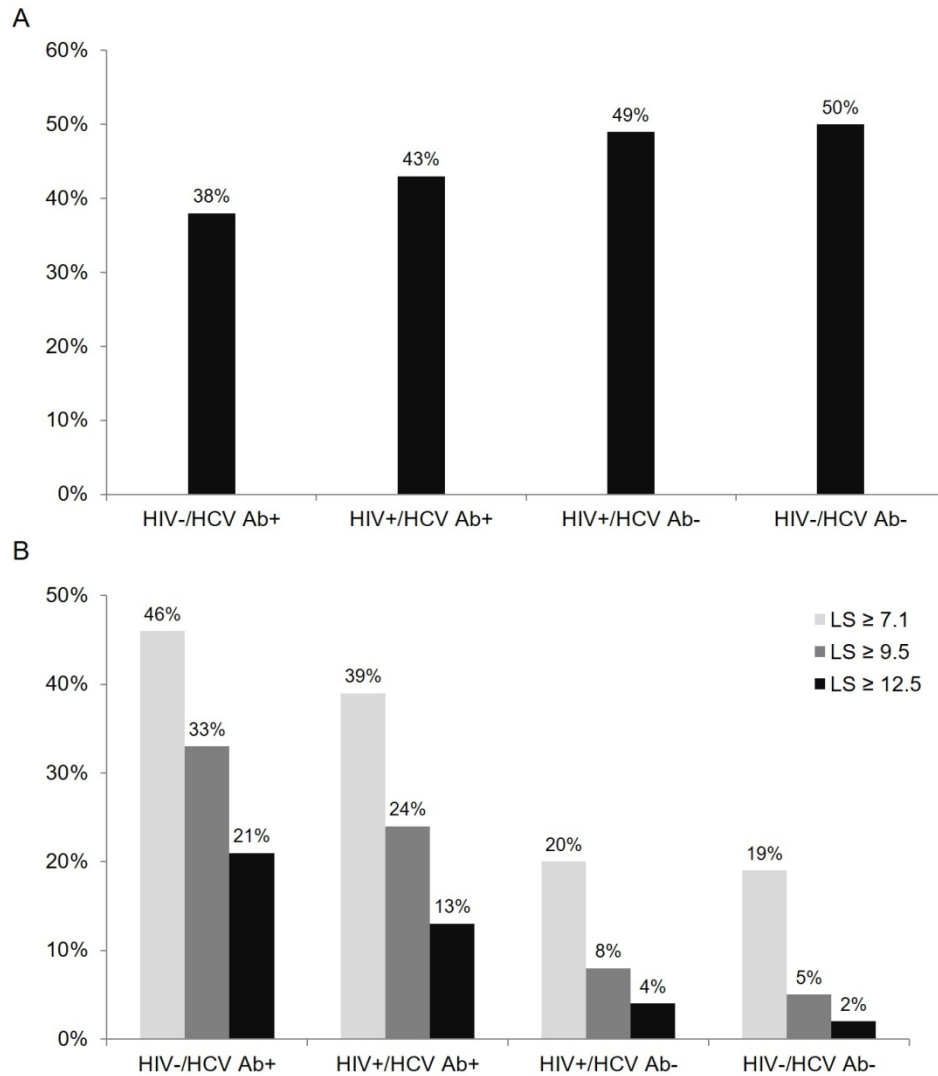


Figure 1A Prevalence of VCTE-estimated hepatic steatosis (CAP≥248 dB/m) by HIV and HCV antibody status. 1B Prevalence of VCTE-estimated significant fibrosis (LS ≥7.1 kPa), advanced fibrosis (LS ≥9.5 kPa), and cirrhosis (LS ≥12.5 kPa), as estimated by VCTE-measured liver stiffness, by HIV and HCV antibody status.

247x269mm (150 x 150 DPI)

Supplemental Table 1. Factors associated with CAP in multivariable analysis*, excluding VCTE scans with abnormal validity criteria.

	Excluding if IQR/Med >30% (N=1520)		Excluding success rate <60% (N=1447)		Excluding poor image quality (N=1527)		Excluding any abnormal validity criteria (N=1383)	
	CAP difference (dB/m)	p-value	CAP difference (dB/m)	p-value	CAP difference (dB/m)	p-value	CAP difference (dB/m)	p-value
Infection Status (ref=HIV-/HCV-)								
HIV+/HCV-	0.3 (-6.2, 6.8)	0.92	-1.1 (-7.7, 5.5)	0.74	1.4 (-4.8, 7.6)	0.65	-0.2 (-6.7, 6.3)	0.95
HIV-/HCV+	-11.7 (-25.3, 2)	0.09	-16.6 (-30.5, -2.7)	0.02	-11.2 (-24, 1.7)	0.09	-17.8 (-31.5, -4.1)	0.01
HIV+/HCV+	-8.4 (-17.5, 0.7)	0.07	-12.1 (-21.2, -3)	0.01	-8.5 (-17.2, 0.2)	0.06	-9.5 (-18.5, -0.5)	0.04
Age (per 10 years)	2.4 (-2.2, 6.9)	0.31	1.6 (-3, 6.2)	0.50	1.3 (-3.1, 5.6)	0.56	0.9 (-3.7, 5.5)	0.70
Race/Ethnicity (ref=white non- Hispanic)								
Black	-5.6 (-13.9, 2.7)	0.18	-8.2 (-16.5, 0)	0.05	-5.7 (-13.5, 2.2)	0.16	-7.3 (-15.5, 0.9)	0.08
Hispanic	-4.2 (-13.8, 5.4)	0.40	-4.4 (-13.9, 5)	0.36	-3.6 (-12.7, 5.6)	0.45	-4.9 (-14.2, 4.5)	0.31
Other	4.4 (-6.8, 15.6)	0.44	3.6 (-7.5, 14.7)	0.53	2.9 (-7.8, 13.5)	0.60	4.6 (-6.3, 15.6)	0.40
WC (per 10 cm)	17.8 (16.2, 19.5)	<0.001	17.5 (15.9, 19.2)	<0.001	17.8 (16.3, 19.4)	<0.001	17.6 (15.9, 19.2)	<0.001
HOMA-IR (per doubling)	1.2 (0.8, 1.6)	<0.001	1.5 (1, 2)	<0.001	1.2 (0.8, 1.5)	<0.001	1.5 (1, 2)	<0.001

*Also adjusted for alcohol use, smoking status, and menopause stage

Supplemental Table 2. Factors associated with liver stiffness in multivariable analysis, excluding VCTE scans with abnormal validity criteria.

	Excluding if IQR/Med >30% (N=1520)		Excluding success rate <60% (N=1447)		Excluding poor image quality (N=1527)		Excluding any abnormal validity criteria (N=1383)	
	LS % change	p-value	LS % change	p-value	LS % change	p-value	LS % change	p-value
Infection Status (ref=HIV-/HCV-)								
HIV+/HCV-	4.4% (-1.2%, 10.3%)	0.12	4.5% (-1.2%, 10.5%)	0.12	4.1% (-1.4%, 9.9%)	0.14	5.2% (-0.6%, 11.3%)	0.08
HIV-/HCV+	44.2% (28.6%, 61.6%)	<0.001	45.1% (29%, 63.1%)	<0.001	44.5% (29.3%, 61.5%)	<0.001	46.8% (30.5%, 65.2%)	<0.001
HIV+/HCV+	34.4% (24.3%, 45.2%)	<0.001	35.2% (25%, 46.2%)	<0.001	31.1% (21.5%, 41.6%)	<0.001	33.6% (23.4%, 44.6%)	<0.001
Age (per 10 years)	4.6% (0.6%, 8.7%)	0.02	3.4% (-0.6%, 7.5%)	0.10	3.3% (-0.6%, 7.3%)	0.10	3.1% (-0.9%, 7.3%)	0.13
Race/Ethnicity (ref=white non-Hispanic)								
Black	2.2% (-4.7%, 9.6%)	0.54	2.3% (-4.6%, 9.7%)	0.52	1% (-5.7%, 8.1%)	0.78	0.2% (-6.6%, 7.6%)	0.95
Hispanic	3.7% (-4.5%, 12.4%)	0.39	2.2% (-5.8%, 10.8%)	0.60	1% (-6.8%, 9.4%)	0.82	0.5% (-7.4%, 9.1%)	0.90
Other	1.9% (-7.4%, 12%)	0.70	2.8% (-6.5%, 13%)	0.57	1.3% (-7.7%, 11.2%)	0.79	1.5% (-7.7%, 11.6%)	0.76
WC (per 10 cm)	4.4% (2.7%, 6.1%)	<0.001	3.8% (2.1%, 5.5%)	<0.001	4.4% (2.7%, 6.1%)	<0.001	3.4% (1.7%, 5.2%)	<0.001
log2HOMA-IR	0.3% (0%, 0.7%)	0.08	0.1% (-0.4%, 0.5%)	0.80	0.3% (-0.1%, 0.6%)	0.14	0.1% (-0.4%, 0.5%)	0.79
CAP (per 10 dB/m)	0.6% (0.1%, 1%)	0.01	0.7% (0.2%, 1.2%)	0.004	0.6% (0.1%, 1.1%)	0.01	0.7% (0.2%, 1.2%)	0.01

*Also adjusted for alcohol use, smoking status, and menopause stage

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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	1 5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria and number of exposed and unexposed	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	12 11 11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	13
Outcome data	15*	Report numbers of outcome events or summary measures over time	13

1	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14
2			(b) Report category boundaries when continuous variables were categorized	14
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
4	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
5	Discussion			
6	Key results	18	Summarise key results with reference to study objectives	13
7	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
8	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
9	Generalisability	21	Discuss the generalisability (external validity) of the study results	
10	Other information			
11	Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.