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MASLD in persons with HIV is associated with high cardiometabolic risk as evidenced by altered advanced lipoprotein profiles and targeted metabolomics

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Abstract

Background Metabolic dysfunction associated steatotic liver disease (MASLD) is associated with increased cardiovascular disease (CVD) risk in persons with HIV (PWH). The lipidomic and metabolomic alterations contributing to this risk are poorly understood. We aimed to characterize the advanced lipoprotein and targeted metabolomic profiles in PWH and assess if the presence and severity of MASLD influence these profiles.

Methods This is a cross-sectional analysis of a prospectively enrolled multicenter cohort. PWH without alcohol abuse or known liver disease underwent vibration-controlled transient elastography for controlled attenuation parameter (CAP) and liver stiffness measurement (LSM). Lipidomic and metabolomic profiling was undertaken with nuclear magnetic resonance (NMR) spectroscopy. Hepatic steatosis was defined as CAP ≥ 263 dB/m and clinically significant fibrosis (CSF) as LSM ≥ 8 kPa. Logistic regression models assessed associations between MASLD, CSF and lipidomic and metabolic parameters.

Results Of 190 participants (71% cisgender male, 96% on antiretroviral therapy), 58% had MASLD and 12% CSF. Mean (SD) age was 48.9 (12.1) years and body mass index (BMI) 29.9 (6.4) kg/m^2. Compared to PWH without MASLD (controls), PWH with MASLD had lower HDL-C but higher total triglyceride, VLDL-C, branched-chain amino acids, GlycA, trimethylamine N-oxide levels, Lipoprotein-Insulin Resistance and Diabetes Risk Indices. There were no significant differences in these parameters between participants with MASLD with or without CSF. In a multivariable regression analysis, MASLD was independently associated with changes in most of these parameters after adjustment for age, gender, race/ethnicity, type 2 diabetes mellitus, BMI, and lipid lowering medications use.

Conclusions MASLD in PWH is independently associated with altered advanced lipoprotein and targeted metabolic profiles, indicating a higher CVD risk in this population.

Keywords HIV, MASLD, Cardiovascular disease, GlycA, TMAO, BCAA

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Introduction

With improving access to antiretroviral therapy (ART), people with HIV (PWH) are aging and becoming at higher risk for cardiovascular disease (CVD) than the general population [1]. Established CVD risk factors, such as central obesity, hypertension, hyperglycemia, smoking, and abnormal lipids can help identify some patients at risk for CVD who may benefit from interventions. However, cardiovascular events may develop in people classified as being at low or intermediate CVD risk, or in patients already achieving target lipids goals with lipid-lowering therapy [2]. Recent lipidomic and metabolomic studies using quantitative nuclear magnetic resonance (NMR) spectroscopy have demonstrated an association between increased lipoprotein subclasses, such as triglyceride (TG)-rich lipoprotein (TRL), small low density lipoprotein (LDL) particles, as well as raised metabolites GlycA and branched chain amino acids (BCAA) with poor cardiometabolic outcomes [3–7]. In PWH, two nested case-controlled studies documented the adverse effects of small dense LDL and protective impact of large high-density lipoprotein (HDL) particles on CVD [8, 9].

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease worldwide, affecting an estimated 30% of the world population [10]. MASLD is also the most common liver disease in PWH, estimated to affect 50-64% of this population [11, 12]. Obesity, insulin resistance, metabolic syndrome, and genetic polymorphisms influence risk of development and progression of MASLD [13]. Variants in *PNPLA3* and *TM6SF2* are associated with the risk of steatosis, fibrosis, steatohepatitis, and liver cancer in the general population [14, 15]. *PNPLA3* variants impede lipolysis, while the *TM6SF2* variants impair lipidation and export of very low-density lipoproteins (VLDL) [16, 17]. In PWH, however, only a few limited studies investigated the effects of these genetic polymorphisms on MASLD risk and severity with conflicting signals [18–20] .

Patients with MASLD in either the general population or PWH are enriched with coexisting metabolic conditions and as a result are vulnerable to CVD [21, 22]. Lipidomic studies in patients with MASLD using NMR documented a characteristic atherogenic pattern, i.e. enrichment and enlargement of TRL, as well as a shift of LDL and HDL from large to small particles [23–25]. Among these lipoprotein derangements, small LDL particles had been documented to correlate with higher CVD risk [26]. A recent study demonstrated MASLD was associated with higher CVD risk in PWH as determined by Atherosclerotic Cardiovascular Disease Risk score [27]. However, little is known about lipidomic and metabolomic alterations associated with increased CVD risk in PWH who have MASLD.

Our aims were to investigate whether MASLD in PWH is associated with changes in lipidomic and metabolomic parameters linked to CVD, and whether MASLD severity, defined by absence or presence of clinically significant fibrosis (CSF), influenced these changes.

Methods

Study participants

We prospectively enrolled consecutive adult PWH who agreed to participate and provided written informed consent from outpatient HIV clinics at three centers (Indiana University, Massachusetts General Hospital, and UTHealth-Houston) between 2018 and 2022. The inclusion criteria included age 18 years and more; documented HIV infection defined by a positive HIV antibody assay and/or detectable HIV-1 RNA; stable antiretroviral (ART) regimen for 3 months before enrollment for those on ART at the time of study enrolling. We excluded those with evidence of or established hepatitis B or C coinfection or other liver diseases. To evaluate the amount of alcohol consumption, Alcohol Use Disorders Identification Test (AUDIT) questionnaire was provided to each participant. AUDIT is a well validated, simple test which contains 10 questions and allows the investigator to quantify participants' daily drinks [28, 29], and to determine if a person has a risky drinking pattern or alcohol use disorder. Participants with a score of 8 or above were considered to have a strong likelihood of hazardous drinking, and therefore, were excluded from analysis. For this analysis, we included participants with fasting plasma samples. This study protocol had been reviewed and approved by each site's the Institutional Review Board.

Lipoprotein and metabolite profiling by NMR spectroscopy

Venous blood was collected after a minimum fasting period of six hours. Ethylenediaminetetraacetic acid (EDTA) plasma samples were obtained and aliquots were frozen at -80 °C until analysis. NMR LipoProfile® testing [30, 31] was deployed on a Vantera® NMR Clinical Analyzer (Morrisville, NC) which provides average sizes for three lipoprotein classes, 33 concentrations of 24 lipoprotein particle subclasses, several small molecule metabolites, and GlycA in a single proton NMR spectrum from a plasma or serum specimen [30–32]. The results for these parameters were reported using the latest LP4 deconvolution algorithm [33]. Descriptions and diameter ranges of the lipoprotein classes and subclasses have been reported previously. Linear regression of the lipoprotein subclass signal areas against serum lipid and apolipoprotein levels measured by chemical assays in a large health study population ($n=698$) provided the conversion

factors to generate NMR-derived concentrations of total cholesterol, triglycerides (TG), the TG and cholesterol in the triglyceride-rich lipoproteins or TRL (TRL-TG and TRL-C). Results for LDL-C and HDL-C, apolipoprotein B (ApoB) and apolipoprotein A-I (ApoA-I) levels were determined using partial least square (PLS) regression method [34]. NMR-derived concentrations of these parameters are highly correlated with those measured by standard chemistry methods. Details regarding the NMR quantification of the BCAAs, citrate, and ketone bodies have been previously reported [35, 36]. Trimethylamine N-oxide (TMAO), betaine, and choline were quantified as previously described. Assay development, analytical performance evaluation and clinical validation of the Lipoprotein Insulin Resistance Index (LP-IR) (0−100; least to most insulin resistant), Diabetes Risk Index (DRI) (1−100; least to greatest risk of developing T2D), Metabolic Malnutrition Index (MMX), Inflammation Vulnerability Index (IVX), and Metabolic Vulnerability Index (MVX) have also been reported [6, 7, 37]. LP-IR is based on particle numbers of very large, large TRL, small LDLP, large HDLP, and mean sizes of TRL, HDL, and LDL [6]. DRI is based on LP-IR, valine and leucine [7]. IVX combines GlycA and small HDL particles. MMX combines valine, leucine, isoleucine, and citrate. MVX combines IVX and MMX [37].

Data collection and vibration controlled transient elastography (VCTE)

The study physicians took history and performed physical examinations for each participant. Medical problems, including hypertension, diabetes, hyperlipidemia, were collected from patients' interview, and were verified in their electronic medical records. Extensive data collection was done, including demographic (age, sex, race, and ethnicity), anthropometrics (body mass index [BMI] and waist circumference), vital signs, medical and medicinal history, latest laboratory and HIV data (CD4+T cell count, HIV-1 RNA) within 6 months of enrollment, and current and prior ART classes [ritonavir-boosted protease inhibitors (PI/r), non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside reverse transcriptase inhibitors (NRTI), and integrase inhibitors (INSTI)]. Adequate viral suppression was defined as participants with HIV-1 RNA < 200 copies/mL. The participants also underwent VCTE by Fibroscan® (Echosens, Paris, France) for controlled attenuation parameters (CAP) and liver stiffness measurement (LSM) by trained study staff. Fasting for at least 3 h before VCTE was required. Fibroscan® was performed by a maximum of 2 trained study staff. The use of the M versus XL probe was notified by Fibroscan® automatically. The final CAP and LSM measurements were recorded as the median values of 10 consecutive valid measurements, and they were expressed in dB/m and kPa, respectively. The LSM values were considered unreliable as medians with the interquartile range (IQR)/median > 30%. Participants with CAP ≥ 263 dB/m and an AUDIT < 8 were determined to have MASLD [38]. Those with LSM ≥ 8.0 kPa were classified to have clinically significant fibrosis [39].

Statistical methods

Numbers and percentages for categorical variables and mean ± standard deviation (SD) were used to describe the baseline characteristics of the participants. Differences between groups were evaluated using One-Way ANOVA or Kruskal-Wallis Test if not normal distribution. Our composite and ordinal categorical study outcome is as follows: Controls (non-MASLD and non-CSF) (0) vs. MASLD including MASLD and non-CSF [1], MASLD and CSF [2]. Ordinal logistic regression models were implemented to examine the association between the study outcome and the select participants' variables. Non-MASLD and non-CSF was the comparator for all logistic regression analyses. We adjusted for age, sex at birth, and race/ethnicity in model 1, and in model 2 we included variables in model 1 in addition to BMI, diabetes, and lipid-lowering agents use. A p-value < 0.05 was considered statistically significant All statistical analyses were performed using Stata software, version 17 (StataCorp, College Station, TX, US).

Results

Patient characteristics

A total 190 patients were included (Table 1), with 135 cisgender males (71%), 59 (31%) Blacks, 67 (35%) Hispanic participants, mean (SD) age 48.9 (12.1) years, duration of HIV infection 14.1 (9.3) years, CD4 count 732.5 cells/mm^3 (334.4), and BMI 29.9 (6.4) kg/m^2. The prevalence of MASLD was 58%, CSF 12% and cirrhosis (LSM > 13 kPa) 3%. The majority of patients (96%) were on antiretroviral therapy.

There were 80 (42%) controls, 87 (46%) participants with MASLD without CSF, and 23 (12%) with MASLD and CSF (Table 1). There were significant differences between the groups in mean (SD) BMI, waist circumference, frequency of hypertension, diabetes and hyperlipidemia, use of statins or fibrates, levels of alanine transaminase (ALT), aspartate transaminase (AST), TG and HDL-C. There were no significant differences in mean age, sex at birth, ethnicity, time elapsed since diagnoses of HIV, HIV-RNA viral loads, mean CD4+cell counts, the percentages of exposure to each ART class, or other laboratory parameters.

Lipidomic changes associated with MASLD

Compared to controls (Table 2), participants with MASLD (with or without CSF) had significantly higher

Table 1 Characteristics of study participants

Variables	Entire cohort *N* = 190 Mean ± SD or *N* (%)	Control *N* = 80 Mean ± SD or *N* (%)	MASLD without CSF *N* = 87 Mean ± SD or *N* (%)	MASLD with CSF *N* = 23 Mean ± SD or *N* (%)	*P* value*
Age, y	48.9 ± 12.1	45.7 ± 12.8	51.4 ± 11.3	49.5 ± 9.9	0.11
Sex at birth (male)	135 (71)	57 (70)	63 (72)	15 (65)	0.68
Transgender (female)	16 (8)	9 (11)	6 (7)	1 (4)	0.64
Race/ethnicity					0.24
Non-Hispanic White	58 (31)	19 (24)	32 (36)	7 (30)	
Non-Hispanic Black	59 (31)	33 (41)	20 (23)	7 (30)	
Hispanic	67 (35)	26 (32)	33 (38)	9 (31)	
Other/multiracial	6 (3)	3 (4)	3 (3)	0 (0)	
Body mass index (kg/m^2)	29.9 ± 6.4	26.4 ± 4.6	31.7 ± 5.8	35.3 ± 7.0	< 0.01
Waist circumference (cm)	102.5 ± 15.9	92.9 ± 12.4	107.5 ± 13.7	117.9 ± 14.6	< 0.01
Hypertension	73 (38)	24 (30)	35 (40)	14 (61)	0.02
Diabetes mellitus	24 (13)	5 (6)	14 (16)	5 (22)	0.05
Hyperlipidemia	62 (33)	19 (23)	36 (41)	7 (30)	0.05
Taking statins or fibrates†	49 (26)	13 (16)	30 (34)	6 (26)	0.03
HIV related features					
Time since HIV diagnosis (years)	14.1 ± 9.3	12.4 ± 8.8	15.4 ± 9.1	14.9 ± 10.9	0.10
HIV-RNA (copies/mL)	20 (IQR: 20–30)	20 (IQR: 20–32)	20 (IQR: 20–30)	20 (IQR: 20–20)	0.70
CD4 + cell count (cells/mm^3)	732.5 ± 334.4	678.2 ± 323.8	774.1 ± 322.7	803.0 ± 406.7	0.11
ART classes					
NRTI	170 (93)	71 (93)	81 (95)	20 (87)	0.36
NNRTI	32 (18)	13 (17)	14 (17)	5 (22)	0.84
PI/r	182 (100)	76 (100)	85 (100)	23 (100)	1.00
INSTI	144 (79)	59 (78)	69 (81)	17 (74)	0.71
Liver function tests					
Albumin (g/dL)	4.31 ± 0.36	4.31 ± 0.36	4.32 ± 0.36	4.25 ± 0.36	0.69
Total bilirubin (mg/dL)	0.57 ± 0.38	0.65 ± 0.51	0.54 ± 0.33	0.54 ± 0.25	0.21
AST (U/L)	26.9 ± 17.2	22.3 ± 11.8	28.9 ± 18.9	38.4 ± 23.5	< 0.01
ALT (U/L)	33.6 ± 30.9	22.3 ± 11.8	39.5 ± 38.8	49.4 ± 30.8	< 0.01
ALP (U/L)	77.3 ± 23.8	75.1 ± 21.1	79.7 ± 27.5	77.8 ± 19.0	0.47
Platelet (1000 cells/mm^3)	241 ± 63	239 ± 58	243 ± 65	236 ± 74	0.89
Lipid panel					
Total cholesterol (mg/dl)	178.7 ± 40.2	175.5 ± 40.7	181.7 ± 38.5	178.9 ± 44.1	0.63
Triglycerides (mg/dl)	159.6 ± 121.8	130.8 ± 74.5	175.5 ± 148.3	208.3 ± 117.9	< 0.01
HDL-cholesterol (mg/dl)	47.1 ± 14.3	49.7 ± 16.1	45.5 ± 12.9	43.1 ± 10.9	0.05
LDL-cholesterol (mg/dl)	101.2 ± 34.0	101.2 ± 33.8	102.9 ± 33.6	92.9 ± 32.1	0.53
Fibrosis and steatosis measures					
CAP (median, IQR)	280 (IQR: 229–316)	223 (IQR: 200–245)	308 (IQR: 289–331)	325 (IQR: 294–366)	< 0.01
LSM (median, IQR)	4.9 (IQR: 3.9–6.1)	4.1 (IQR: 3.5–5.1)	5.3 (IQR: 4.3–6.2)	9.7 (IQR: 8.6–11.8)	< 0.01
FIB-4 > 2.67 N (%)	8 (4)	3 (3.9)	4 (4.8)	1 (4.6)	0.55

* One-Way ANOVA or Kruskal-Wallis Test if not normal distribution

† 48 patients on statins and 4 on fibrates (3 of them were also taking statins)

Abbreviations: PWH, patients with HIV infection; MASLD: metabolic dysfunction-associated steatotic liver disease; CSF, clinically significant fibrosis; NRTI, Nucleoside reverse transcriptase inhibitors; NNRTI, Non-nucleoside reverse transcriptase inhibitors; PI/r, Ritonavir-Boosted Protease Inhibitors; INSTI: Integrase inhibitors, AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; FIB-4, fibrosis 4 score

total, very large, large, and medium TRL-P levels, higher mean TRL sizes, TRL-TG, TRL-C, TG, and VLDL-C levels, lower concentrations of large LDL-P, large HDL-P; H7P, H5P, and HDL, and lower mean sizes of LDL and HDL (all $P<0.05$).

Differences in most of these lipidomic parameters between MASLD and controls remained significant after adjustment for age, sex at birth, race/ethnicity, BMI, diabetes, and statins/fibrates use (Table 3).

In a sensitivity analysis (Supplemental Table 1), no significant differences were observed in lipoprotein

Table 2 Lipidomic parameters derived from NMR spectrometry, stratified by MASLD. Results based on univariate binary logistic regression models

Variables	Non-MASLD *N* = 80	MASLD *N* = 110	Univariate Analysis	
			Crude OR*	*P* value
Triglyceride-Rich Lipoprotein Particle (TRLP) Concentrations				
Total TRLP (nmol/L)	149.83 ± 69.36	173 ± 74.3	1.004	0.03
Very Large TRLP (nmol/L)	0.27 ± 0.31	0.52 ± 0.68	3.08	< 0.01
Large TRLP (nmol/L)	2.09 ± 3.52	6.51 ± 6.44	1.29	< 0.01
Medium TRLP (nmol/L)	12.86 ± 13.02	24.14 ± 23.03	1.04	< 0.01
Small TRLP (nmol/L)	47.50 ± 40.89	44.54 ± 30.00	0.99	0.56
Very small TRLP (nmol/L)	87.09 ± 61.90	97.27 ± 56.01	1.00	0.24
Calibrated LDL Particle (cLDLP) Concentrations				
Total cLDLP (nmol/L)	1420.58 ± 411.9	1468.1 ± 422.25	1.00	0.44
Large cLDLP (nmol/L)	302.11 ± 199.46	207.27 ± 184.24	0.99	< 0.01
Medium cLDLP (nmol/L)	406.68 ± 275.42	404.9 ± 310.42	0.99	0.97
Small cLDLP (nmol/L)	711.81 ± 358.14	855.9 ± 438.45	1.0009	0.02
Calibrated HDL Particle (cHDLP) Concentrations				
Total cHDLP (µmol/L)	19.72 ± 2.99	19.84 ± 3.43	1.01	0.80
Large cHDLP (µmol/L)	1.88 ± 1.14	1.32 ± 1.13	0.65	< 0.01
Medium cHDLP (µmol/L)	5.19 ± 2.22	4.59 ± 2.57	0.90	0.09
Small cHDLP (µmol/L)	12.65 ± 3.34	13.93 ± 4.06	1.09	0.02
HDL Subspecies				
H7P (µmol/L)	0.29 ± 0.37	0.17 ± 0.24	0.24	0.02
H6P (µmol/L)	0.68 ± 0.79	0.55 ± 0.71	0.80	0.25
H5P (µmol/L)	0.89 ± 0.79	0.59 ± 0.69	0.56	< 0.01
H4P (µmol/L)	1.66 ± 1.11	1.55 ± 1.22	0.93	0.53
H3P (µmol/L)	3.54 ± 2.06	3.04 ± 2.23	0.90	0.12
H2P (µmol/L)	11.69 ± 2.68	13.08 ± 3.56	1.15	< 0.01
H1P (µmol/L)	0.96 ± 1.51	0.85 ± 1.65	0.96	0.65
Mean Lipoprotein Sizes				
TRL Size (nm)	43.33 ± 7.70	50.34 ± 0.82	1.12	< 0.01
LDL Size (nm)	20.90 ± 0.40	20.62 ± 0.52	0.28	< 0.01
HDL Size (nm)	8.97 ± 0.38	8.76 ± 0.35	0.20	< 0.01
Derived Triglyceride (TG) and Cholesterol (C) Concentrations				
TRL-TG (mg/dL)	75.2 ± 46.53	134.17 ± 85.76	1.02	< 0.01
TRL-C (mg/dL)	26.44 ± 11.85	36.01 ± 17.47	1.05	< 0.01
Derived Apolipoprotein Concentrations				
ApoB (mg/dL)	103.94 ± 21.21	107.06 ± 21.18	1.00	0.32
ApoA1 (mg/dL)	128.90 ± 20.52	125.46 ± 23.16	0.99	0.29
Extended lipoprotein by partial least squares regression				
TC (mg/dL)	186.25 ± 39.76	185.83 ± 35.96	0.99	0.94
HDL-C (mg/dL)	52.29 ± 11.58	46.52 ± 13.13	0.96	< 0.01
TG (mg/dL)	99.51 ± 48.83	163.24 ± 91.96	1.02	< 0.01
LDL-C (mg/dL)	115.80 ± 35.62	110.46 ± 29.75	0.99	0.26
VLDL-C (mg/dL)	18.16 ± 7.86	28.84 ± 15.26	1.10	< 0.01
Non-HDL-C (mg/dL)	133.96 ± 37.11	139.30 ± 34.45	1.00	0.31

* Crude ORs were calculated through binary logistic regression models. NMR, Nuclear magnetic resonance; MASLD: metabolic dysfunction-associated steatotic liver disease; CSF, clinical significant fibrosis; OR, odds ratio; CI, confidence interval; TRL(P), triglyceride-rich lipoprotein (particle); LDL, low-density lipoprotein; cLDLP, calibrated LDL particle; HDL, high-density lipoprotein; cHDLP, calibrated HDL particle; TG, triglyceride; C, cholesterol; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; TC, total cholesterol; VLDL-C, very low-density lipoprotein cholesterol

Table 3 Lipidomic parameters derived from NMR spectrometry, stratified by MASLD. Results based on multivariable ordinal logistic regression models

Variables	Model 1*		Model†	
	Adjusted OR (95% CI)	*P* value‡	Adjusted OR (95% CI)	*P* value‡
Triglyceride-Rich Lipoprotein Particle (TRLP) Concentrations				
Total TRLP (nmol/L)	1.005 (1.001–1.009)	0.02	1.007 (1.001–1.01)	0.02
Very Large TRLP (nmol/L)	3.64 (1.51–8.80)	< 0.01	3.01 (1.07–8.55)	0.04
Large TRLP (nmol/L)	1.32 (1.18–1.47)	< 0.01	1.26 (1.12–1.41)	< 0.01
Medium TRLP (nmol/L)	1.04 (1.02–1.07)	< 0.01	1.05 (1.02–1.08)	< 0.01
Small TRLP (nmol/L)	-	-	-	-
Very small TRLP (nmol/L)	-	-	-	-
Calibrated LDL Particle (cLDLP) Concentrations				
Total cLDLP (nmol/L)	-	-	-	-
Large cLDLP (nmol/L)	0.99 (0.99–0.99)	< 0.01	0.99 (0.99–0.99)	< 0.01
Medium cLDLP (nmol/L)	-	-	-	-
Small cLDLP (nmol/L)	1.00 (1.0002–1.001)	0.01	1.00 (1.000-1.002)	0.04
Calibrated HDL Particle (cHDLP) Concentrations				
Total cHDLP (µmol/L)	-	-	-	-
Large cHDLP (µmol/L)	0.57 (0.42–1.77)	< 0.01	0.64 (0.46–0.90)	0.01
Medium cHDLP (µmol/L)	-	-	-	-
Small cHDLP (µmol/L)	-	-	-	-
HDL Subspecies				
H7P (µmol/L)	0.22 (0.07–0.68)	< 0.01	-	-
H6P (µmol/L)	-	-	-	-
H5P (µmol/L)	0.52 (0.34–0.82)	< 0.01	-	-
H4P (µmol/L)	-	-	-	-
H3P (µmol/L)	-	-	-	-
H2P (µmol/L)	1.13 (1.02–1.25)	0.02	-	-
H1P (µmol/L)	-	-	-	-
Mean Lipoprotein Sizes				
TRL Size (nm)	1.12 (1.07–1.18)	< 0.01	1.11 (1.06–1.18)	< 0.01
LDL Size (nm)	0.26 (0.13–0.53)	< 0.01	0.29 (0.13–0.66)	< 0.01
HDL Size (nm)	0.15 (0.06–0.38)	< 0.01	0.15 (0.05–0.45)	< 0.01
Derived Triglyceride (TG) and Cholesterol (C) Concentrations				
TRL-TG (mg/dL)	1.02 (1.01–1.03)	< 0.01	1.02 (1.01–1.03)	< 0.01
TRL-C (mg/dL)	1.05 (1.03–1.08)	< 0.01	1.06 (1.03–1.10)	< 0.01
Derived Apolipoprotein Concentrations				
ApoB (mg/dL)	-	-	-	-
ApoA1 (mg/dL)	-	-	-	-
Extended lipoprotein by partial least squares regression				
TC (mg/dL)	-	-	-	-
HDL-C (mg/dL)	0.95 (0.93–0.98)	< 0.01	0.96 (0.93–0.99)	0.02
TG (mg/dL)	1.02 (1.01–1.02)	< 0.01	1.02 (1.009–1.03)	< 0.01
LDL-C (mg/dL)	-	-	-	-
VLDL-C (mg/dL)	1.11 (1.07–1.16)	< 0.01	1.11 (1.06–1.17)	< 0.01
Non-HDL-C (mg/dL)	-	-	-	-

* Model 1: Adjusted for age, gender, race/ethnicity

† Model 2: Adjusted for variables included in model 1 plus type 2 diabetes mellitus, BMI (kg/m2), and statins/fibrates

‡ Adjusted ORs were calculated through ordinal logistic regression models

NMR, Nuclear magnetic resonance; MASLD: metabolic dysfunction-associated steatotic liver disease; CSF, clinical significant fibrosis; OR, odds ratio; CI, confidence interval; TRL(P), triglyceride-rich lipoprotein (particle); LDL, low-density lipoprotein; cLDLP, calibrated LDL particle; HDL, high-density lipoprotein; cHDLP, calibrated HDL particle; TG, triglyceride; C, cholesterol; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; TC, total cholesterol; VLDL-C, very low-density lipoprotein cholesterol

Minus (-) sign means not significant

parameters between participants with MASLD with or without CSF.

Metabolomic changes associated with MASLD

Compared to controls (Table 4), participants with MASLD (with or without CSF) had significantly higher levels of total BCAA, valine, leucine, isoleucine, alanine, GlycA, LP-IR index, and DRI, TMAO and choline concentrations.

The differences in levels of total BCAA, valine and alanine, as well as LP-IR and DRI remained significantly higher in participants with MASLD after adjustment for age, sex at birth, race/ethnicity, BMI, diabetes, and statins/fibrates use (Table 5).

In a sensitivity analysis (Supplemental Table 2), no significant differences were observed in the metabolites levels between participants with MASLD with or without CSF.

Discussion

Both MASLD and HIV are associated with increased risk of CVD [40, 41]. Indeed, CVD is a leading cause of death in patients with MASLD [42, 43]. Thus, understanding the lipidomic and metabolomic alterations associated with CVD risk in PWH and MASLD is important. This study provides a detailed characterization of changes in advanced lipoprotein profiles and targeted metabolites associated with MASLD in a diverse cohort of PWH. Atherogenic alterations in lipids and lipoprotein parameters are observed in PWH who have MASLD but fibrosis severity in MASLD does not appear to accentuate these changes. Together with increases in different metabolite levels, these changes are associated with hepatic and peripheral insulin resistance and elevated risk for CVD in PWH.

In addition to rising serum TG and VLDL-C levels, lipidomic profiling in this study showed TG-rich lipoproteins increase, enlarge, and carry more TG and cholesterol in PWH with MASLD. It also showed a reduction in the number of large HDL and large LDL particles and size of HDL and LDL. These lipidomic derangements are characteristic of atherogenic dyslipidemia which is driven by insulin resistance [44]. Overproduction of VLDL/TRL and raised plasma TG levels enhance production of TG-rich LDL and HDL, both catabolized rapidly, resulting in a shift from larger towards small LDL and HDL particles,

Table 4 Metabolites derived from NMR spectrometry, stratified by MASLD. Results based on univariate binary logistic regression

Variables	Non-MASLD *N* = 80	MASLD *N* = 110	Univariate Analysis	
			Crude OR*	*P* value
Amino Acid Concentrations				
Branched-Chain Amino Acids (BCAA)				
Total BCAA (μmol/L)	344.35 ± 70.56	394.84 ± 91.25	1.008	< 0.01
Valine (μmol/L)	193.19 ± 37.51	222.25 ± 43.53	1.02	< 0.01
Leucine (μmol/L)	92.16 ± 26.30	104.11 ± 34.72	1.01	0.01
Isoleucine (μmol/L)	58.88 ± 16.94	68.42 ± 21.21	1.03	< 0.01
Alanine (μmol/L)	302.56 ± 76.59	350.92 ± 78.54	1.008	< 0.01
Small Molecule Metabolites				
Glucose (mg/dL)	94.28 ± 37.49	98.39 ± 25.43	1.00	0.39
Citrate (μmol/L)	1.93 ± 0.54	1.95 ± 0.50	1.08	0.79
Ketone Body Concentrations				
Total ketone body (μmol/L)	207.80 ± 180.87	209.49 ± 163.70	1.00	0.95
Beta-hydroxy-butyrate (μmol/L)	125.10 ± 120.60	118.42 ± 106.86	0.99	0.69
Acetoacetate (μmol/L)	56.33 ± 43.83	62.52 ± 44.74	1.00	0.35
Acetone (μmol/L)	26.58 ± 22.09	28.64 ± 21.80	1.00	0.52
Inflammation marker				
GlycA (μmol/L)	387.33 ± 74.54	417.49 ± 76.35	1.005	< 0.01
Other markers				
LP-IR Index (0-100)	46.04 ± 14.68	60.48 ± 15.93	1.06	< 0.01
Diabetes risk index (0-100)	24.70 ± 15.42	42.76 ± 17.55	1.06	< 0.01
Inflammation vulnerability index (0-100)	39.28 ± 13.08	41.00 ± 12.88	1.01	0.36
Metabolic malnutrition index (0-100)	56.27 ± 6.37	55.21 ± 6.76	0.98	0.28
Metabolic vulnerability index (0-100)	46.21 ± 10.66	46.68 ± 10.94	1.00	0.77
Trimethylamine N-Oxide (μmol/L)	3.07 ± 2.00	4.79 ± 4.60	1.18	0.01
Betaine (μmol/L)	39.99 ± 12.63	39.03 ± 12.65	0.99	0.64
Choline (μmol/L)	9.74 ± 3.99	11.07 ± 3.80	1.10	0.04

* Crude ORs were calculated through binary logistic regression models. NMR, Nuclear magnetic resonance; MASLD: metabolic dysfunction-associated steatotic liver disease; CSF, clinically significant fibrosis; OR, odds ratio; CI, confidence interval; GlycA, glycoprotein acetylation; LP-IR index; lipoprotein-insulin resistance index

Table 5 Metabolites derived from NMR spectrometry, stratified by MASLD and clinically significant fibrosis. Results based on multivariable ordinal logistic regression models

Variables	Model 1*		Model 2†	
	Adjusted OR (95% CI)	*P* value‡	Adjusted OR (95% CI)	*P* value‡
Amino Acid Concentrations				
Branched-Chain Amino Acids (BCAA)				
Total BCAA (μmol/L)	1.005 (1.002–1.008)	< 0.01	1.004 (1.000-1.007)	0.04
Valine (μmol/L)	1.01 (1.006–1.02)	< 0.01	1.009 (1.002–1.02)	0.01
Leucine (μmol/L)	1.008 (1.000-1.02)	0.05	-	-
Isoleucine (μmol/L)	1.02 (1.003–1.03)	0.01	1.02 (1.00-1.03)	0.05
Alanine (μmol/L)	1.006 (1.003–1.01)	< 0.01	1.006 (1.002–1.009)	< 0.01
Small Molecule Metabolites				
Glucose (mg/dL)	-	-	-	-
Citrate (μmol/L)	-	-	-	-
Ketone Body Concentrations				
Total ketone body (μmol/L)	-	-	-	-
Beta-hydroxy-butyrate (μmol/L)	-	-	-	-
Acetoacetate (μmol/L)	-	-	-	-
Acetone (μmol/L)	-	-	-	-
Inflammation marker				
GlycA (μmol/L)	1.004 (1.000-1.007)	0.04	-	-
Other markers				
LP-IR Index (0-100)	1.06 (1.04–1.08)	< 0.01	1.05 (1.03–1.08)	< 0.01
Diabetes risk index (0-100)	1.05 (1.03–1.07)	< 0.01	1.04 (1.02–1.06)	< 0.01
Inflammation vulnerability index (0-100)	-	-	-	-
Metabolic malnutrition index (0-100)	-	-	-	-
Metabolic vulnerability index (0-100)	-	-	-	-
Trimethylamine N-Oxide (μmol/L)	1.07 (1.00-1.15)	0.04	-	-
Betaine (μmol/L)	-	-	-	-
Choline (μmol/L)	-	-	-	-

* Model 1: Adjusted for age, gender, and race/ethnicity

† Model 2: Adjusted for variables included in model 1 plus type 2 diabetes mellitus, BMI (kg/m2), and statins/fibrates

‡ Adjusted ORs were calculated through ordinal logistic regression models

NMR, Nuclear magnetic resonance; MASLD: metabolic dysfunction-associated steatotic liver disease; CSF, clinically significant fibrosis; OR, odds ratio; CI, confidence interval; GlycA, glycoprotein acetylation; LP-IR index; lipoprotein-insulin resistance index

and a decrease in HDL [45, 46]. These derangements had been linked to CVD risk in patients with MASLD and other conditions such as diabetes, obesity, and polycystic ovarian syndrome [5, 26, 47].

While some studies had investigated the associations between HIV or ART and atherogenic dyslipidemia [8], detailed characterization of the lipidomic profile of PWH and MASLD has not been explored. Khalili et al. recently reported that in patients with HIV-hepatitis B virus coinfection, fatty liver was significantly associated with higher concentrations of TG and small dense LDL particles [48]. The results from our HIV mono-infection cohort support these findings and more comprehensively demonstrate the alterations of TRL parameters and sizes of lipoprotein fractions.

It is interesting to note that the strength of association between MASLD and lipidomic and metabolomic parameters in this study showed minimal to small changes from the univariate to the multivariate models. This may suggest in this population of PWH on ART, MASLD is likely the main independent driver of these associations and highlights the key role of the liver in lipid metabolism and metabolite processing.

While some studies had demonstrated more pronounced atherogenic dyslipidemia in MASLD patients compared with those without, in either general population or PWH [24, 48], studies addressing the differences of lipidomic profile in MASLD patients with variable severity are sparse. DeFilippis et al. categorized 3362 participants in the Multi-Ethnic Study of Atherosclerosis cohort to no, mild, moderate, and severe MASLD according to their liver/spleen attenuation ratios on computed tomography and demonstrated an associations between the severity of steatosis with lipoprotein particle concentrations and sizes [49]. Amor et al. similarly reported more pronounced atherogenic lipidomic profile in the participants with more severe steatosis as estimated by the fatty liver index [23]. In this study, we assessed the

association of MASLD severity as defined by presence or absence of CSF with lipids and lipoprotein parameters. While we show similar atherogenic lipidomic changes with the presence of MASLD, we did not observe a significant association between increasing severity of fibrosis in the setting of MASLD and these changes.

In patients with MASLD, elevation of some amino acids, including glutamate, tyrosine, alanine, and BCAAs correlated with disease severity, and was thought of as an adaptative response to hepatic inflammation and stress [50, 51]. Elevated circulating BCAA levels were also considered to reflect upregulation of muscle catabolism associated with insulin resistance [52]. In line with these studies, we observed increased circulating total BCAAs, valine, alanine, and TMAO levels in PWH who had MASLD in this study, but we did not detect an association between increasing levels of these metabolites and severity of MASLD. Data on the association between BCAA levels and severity of hepatic fibrosis are conflicting. In a study of patients with MASLD [52], BCAA levels showed a trend of increase with progression of fibrosis but differences between F1-2 and F3-4 group were not significant. In contrast, another study reported decreased BCAA levels with increasing fibrosis stage in patients with steatohepatitis [53].

Similarly, LP-IR, an index reflecting both hepatic and peripheral insulin resistance, was significantly higher in PWH with MASLD versus controls in this study. DRI, which correlates with risk of developing diabetes, was higher in PWH who had MASLD compared to controls. Other NMR-derived indices that were previously linked to long term all-cause mortality, IVX, MMX and MVX (combing IVX and MMX) were not significantly different among the study groups.

Data from a recent randomized trial in PWH who are on ART showed pitavastatin reduced both LDL levels and risk of major adverse cardiovascular events (MACE) compared to placebo [54]. Our data highlight the importance of MASLD as a factor associated with increased risk for CVD in PWH. PWH and MASLD may be prioritized for pitavastatin therapy or other interventions that reduce the risk of CVD and MACE.

Recently, a new nomenclature for steatotic liver disease has been proposed [55]. MASLD has been suggested as a replacement term for non-alcoholic fatty liver disease (NAFLD) with the diagnostic criteria requiring the coexistence of at least one cardiometabolic factor in addition to hepatic steatosis. We have recently shown that the new nomenclature definitions have had minimal effect on classification of NAFLD to MASLD in PWH [56] as well as in the general population with MASLD [57]. Therefore, the results of this analysis are probably applicable when either the MASLD or NAFLD terminology is used to characterize non-alcohol associated steatotic liver disease in PWH in this cohort.

Study strengths

The strengths of this study include the systematic phenotyping of all participants, use of reliable noninvasive tools, CAP and LSM, to assess MASLD severity, and the comprehensive lipidomic and metabolomic profiling using NMR, which allowed interrogation of large number of variables and indices relevant to CVD risk in PWH.

Study limitations

As with other cross-sectional studies, associations observed here do not reflect causative relationship with MASLD. PWH in this study were on suppressive ART, whether our findings apply to PWH not on ART is unclear. The study may be underpowered to reveal associations between altered lipidomic and metabolomic profiles and liver fibrosis. Finally, data on lifestyle factors such as diet quality and physical activity, which could confound the results, were not captured as part of this study.

Conclusions

PWH with MASLD have altered lipidomic and metabolomic profiles indicating a higher CVD risk in this population. To reduce their CVD risk, PWH and MASLD should be screened for and offered interventions to address these untoward lipidomic and metabolomic changes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02317-4.

Supplementary Material 1

Author contributions

Study concept (KL, NC, SG); manuscript preparation (KL, SG); data acquisition (All authors), statistical analysis (EV), data interpretation and critical review of manuscript (All authors).

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

This study protocol had been reviewed and approved by each site's the Institutional Review Board (Indiana University School of Medicine, Massachusetts General Hospital, and, UTHealth Science Center). Each participant provided written informed consent.

Conflict of interest

Dr. Lin and Dr. Vilar-Gomez declare no conflicts of interest. Dr. Corey serves on the scientific advisory board for Theratechnologies, Novo Nordisk and BMS and has received grant funding from Boehringer-Ingelheim, BMS and Novartis, Dr. Connelly is an employee of and holds stock in Labcorp, Dr. Gupta receives consultancy fees from ViiV Healthcare and Gilead Sciences and unrestricted research grant funding from ViiV Healthcare, Dr. Lake serves as a consultant to CytoDyn and Theratechnologies, and receives research support from Gilead Sciences and Zydus Pharmaceuticals, Dr. Chalasani declares none for this paper. For full disclosure, he has had paid consulting agreements with Madrigal, GSK, Galectin, Zydus, Altimune, Foresite, Ventyx, Merck and Pfizer. He has research grants from DSM and Exact Sciences. He has equity ownership in Avant Sante Therapeutics, a contract research organization., Dr. Gawrieh consulting: TransMedics, Pfizer. Research grant support: Viking and Zydus.

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