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Distinct metabolic perturbations link liver steatosis and incident CVD in lean but not obese PWH

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Abstract

Background Metabolic dysfunction-associated steatotic liver disease (MASLD) is a key risk factor for cardiovascular disease (CVD), potentially driven by shared metabolic mechanisms. Metabolic perturbations associated with MASLD and CVD remain underexplored in people with HIV (PWH).

Methods We used data from the longitudinal multicenter 2000HIV study comprising 1895 virally suppressed PWH, out of which 970 had available liver and carotid artery measurements. Transient elastography with controlled attenuation parameter (CAP) was performed for the assessment of liver steatosis (CAP > 263 dB/m) and fibrosis (LSM ≥ 7.0). Historic and future incident CVD within 2-year follow-up, defined as myocardial infarction, stroke, peripheral arterial disease, and angina pectoris, were extracted from the medical files, while atherosclerotic plaque(s) in the carotid arteries were assessed using ultrasonography. Metabolic perturbations were analyzed using mass spectrometry-based untargeted metabolomics ($n = 500$ metabolites) and nuclear magnetic resonance spectroscopy for targeted lipids and other metabolites ($n = 246$ metabolites).

Results PWH with liver steatosis were more likely to have arterial plaques (47% vs. 36%; *P* value = 0.003) and CVD history (11% vs. 6.8%; *P* value = 0.021) than PWH without liver steatosis. These associations were only significant in lean PWH, in contrast to those with BMI ≥ 25 kg/m^2. Metabolic pathways associated with liver steatosis and fibrosis primarily involved lipid and amino acid metabolism, and they were validated by targeted lipoproteomic measurements. Interestingly, metabolomic pathways and lipoproteomic signatures associated with MASLD were mostly distinct from those associated with CVD parameters. However, several metabolic pathways were shared, especially in lean PWH. These include arachidonic acid metabolism and formation of prostaglandin, purine metabolism, cholecalciferol metabolism, and glycine, serine, alanine, and threonine metabolism.

Conclusion Metabolic disturbances linked to liver steatosis and CVD diverge across BMI categories in PWH. Lean PWH, unlike their overweight/obese counterparts, show common metabolic perturbations between MASLD and CVD, particularly involving arachidonic acid metabolism. This suggests that lean PWH with liver steatosis may face

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a heightened risk of CVD due to shared metabolic pathways, potentially opening avenues for targeted interventions, such as aspirin therapy, to mitigate this risk.

Keywords HIV, CVD, MASLD, Steatosis, Fibrosis

Background

The aging population of people with HIV (PWH) faces increased risks for non-AIDS-related morbidities [1, 2]. Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD) [3], is the most common liver disease in the general population [4] and an emerging cause of liver disease among PWH [5]. MASLD encompasses a spectrum ranging from simple steatosis, the accumulation of fat in the liver, to more severe manifestations including metabolic dysfunction-associated steatohepatitis (MASH, formerly known as non-alcoholic steatohepatitis), fibrosis, cirrhosis, and eventually hepatocellular carcinoma [6]. The progression of MASLD is heterogeneous and influenced by various factors such as metabolic dysregulation, insulin resistance, and inflammation [6].

MASLD is a risk factor for atherosclerotic cardiovascular disease (CVD) [6–8] and amplifies the risk of CVD threefold in PWH, even after correction for risk factors such as insulin resistance and hypertension [9, 10]. Of note, lean MASLD represents a distinct entity accompanied by an increased risk for CVD in the general population [11]. The mechanistic associations between MASLD and CVD are intricate and not fully understood. In PWH, unique HIV-associated factors like persistent inflammation and HIV medication may play pivotal roles in MASLD disease progression [12]. Although metabolic dysregulation is recognized as a leading factor to the development and progression of both MASLD and CVD [9], no studies have specifically examined the shared and unique metabolic abnormalities associated with MASLD and CVD in PWH. Previous metabolomic studies in PWH have shown widespread metabolic dysregulation including changes in amino acid, fatty acid, sphingolipid, glycerolipid, and glutathione metabolism, depending on the progression of HIV infection and exposure to antiretroviral therapy (ART) [13–16]. Alterations in the latter two metabolic pathways have been associated with liver fibrosis of unknown etiology and subclinical CVD in PWH [13].

Here, we aim to investigate the metabolomic and lipoproteomic profile of nearly 1000 individuals with HIV-1 infection with long-term viral suppression using antiretroviral therapy. We specifically focus on how the metabolomic and lipoproteomic profile relate to two components of MASLD: liver steatosis and fibrosis (assessed by controlled attenuation parameter and transient elastography), as well as subclinical CVD (presence of plaques) and previous and incident CVD. Our study had two primary objectives: (1) to evaluate metabolomic and lipoproteomic signatures associated with steatosis and fibrosis and (2) to determine whether these metabolic signatures associated with liver steatosis and fibrosis overlap with those linked to cardiovascular disease. Additionally, we examined the potential differences between lean and overweight/obese PWH with liver steatosis and fibrosis for both objectives.

Methods

Study population and data collection

We investigated PWH from the 2000HIV study embedded in the Human Functional Genomics Project (HFGP) (http://humanfunctionalgenomics.org) [17]. This longitudinal multicenter observational study comprises 1895 people with HIV on combination antiretroviral therapy (cART) ≥ 6 months, and with a suppressed HIV-RNA load (< 200 copies/mL) and no acute symptoms or active hepatitis B or C infection. PWH were included in four HIV treatment centers in the Netherlands between October 2019 and October 2021.

For this study, we used the following data registered or measured at the baseline visit: demographics, medical history, including history of HIV and comorbidities, current ART regimen, ART history, and comedication, obtained during the study visit or extracted from medical files and the "Stichting HIV Monitoring" (SHM, a national registry). The SHM has prospectively collected demographic and clinical data from 98% PWH in the Netherlands (also known as the ATHENA cohort), ensuring comprehensive and standardized data collection [18].

As previously described, we calculated 10-year risk of cardiovascular events using the Framingham risk score and SCORE2 at baseline [19–21]. During the baseline study visit, liver and carotid artery measurements were performed. After 2 years of follow-up, data on new cardiovascular events was extracted from the medical files. All information was collected in electronic case report forms (CRF) in CastorEDC.

Liver and carotid artery measurements

Transient elastography (TE) using FibroScan® (Echosens, Paris, France) was performed for the assessment of liver stiffness measurement (LSM) and controlled attenuation

parameter (CAP), as previously reported [17, 22]. In addition, we performed standard ultrasonography of the liver using a low-frequency abdominal probe for the assessment of fat layer thickness, defined as the total thickness of superficial tissue layers (including skin, muscle, subcutaneous and visceral fat) between the transducer and the liver capsule obtained at the level of the right liver lobe [17]. Fat layer thickness was dichotomized at a threshold of 25 mm: measurements ≤ 25 mm were assigned a value of 0, and measurements > 25 mm were assigned a value of 1.

IMT (intima media thickness) of the carotid arteries and atherosclerotic plaque presence were measured using 2-dimensional ultrasound acquired using an L14-5WE transducer (DC80A; Mindray, Shenzhen, China) for the assessment of subclinical atherosclerosis, as previously described [17]. A plaque was defined as a focal IMT > 1.5 mm or > 50% thickening of the IMT compared with the mean IMT in the common carotid artery, carotid bulb, or internal carotid artery [19].

Liver and carotid artery measurements were conducted by four operators, one per participating center, who also served as readers to ensure consistent interpretation. All operators received standardized training at Radboud University Medical Center, with regular evaluations to maintain high-quality procedures. To further reduce variability, all centers used identical machines for ultrasound and FibroScan, standardized in brand, model, settings, and calibration. Regular expert meetings were held to uphold consistent imaging quality.

Case definitions

Steatosis and fibrosis were defined according to standard cut-off values. Cut-off values for steatosis grades were CAP < 263 dB/m for S0 (no steatosis), CAP ≥ 263 dB/m and < 280 dB/m for S1 (mild steatosis), and CAP ≥ 280 dB/m for S2/S3 (moderate to severe steatosis) [23, 24]. Cut-off values for fibrosis grades were LSM < 7.0 kPa for F0-F1 (no–mild fibrosis), LSM ≥ 7.0 and < 8.7 kPa for F2 (moderate fibrosis), as LSM ≥ 8.7 and < 10.3 kPa for F3 (severe fibrosis), and LSM ≥ 10.3 kPa for F4 (cirrhosis) [25]. Unsuccessful transient elastography measurements and measurements of participants with cirrhosis due to alcohol abuse, (past) viral hepatitis, or both were excluded from the analyses. For downstream data analysis, participants were classified into the following categories based on their liver measurements: PWH with normal liver measurements (S0 and F0-F1), those with simple steatosis (≥ S1 and F0-F1), those with liver fibrosis (≥ F2), and those with steatosis and fibrosis (≥ S1 and ≥ F2).

A distinction between lean and overweight/obese PWH was made according to BMI [26, 27]: PWH with a BMI < 25 kg/m^2 were defined as lean, whereas PWH with a BMI ≥ 25 kg/m^2 were defined as overweight/obese. Definitions were different in people of Asian descent, where PWH with BMI < 23 kg/m^2 were defined as lean and PWH with a BMI ≥ 23 kg/m^2 as overweight/obese.

In assessing CVD-related parameters, we defined a history of CVD as a history of myocardial infarction, angina pectoris, stroke, or peripheral arterial disease. Incident CVD was defined as a new CVD event occurring within the 2-year follow-up period, restricted to individuals without a prior history of CVD. CVD diagnoses were determined from participants' medical records.

Of the baseline characteristics that were compared between the cases, low level viremia was defined as an HIV-1 viral load above the quantification limit during any measurement taken 3 years prior to inclusion.

Untargeted metabolomic measurement

Untargeted metabolomics measurement was performed on baseline plasma samples using flow injection electrospray–time-of-flight mass spectrometry by General Metabolics, LLC according to the methodology described previously [28]. Metabolite was identified based on their mass-to-charge ratio (ion m/z) and quantified as metabolite intensities. Data were normalized using a moving median approach. To identify outliers, we employed principal component analysis (PCA) on metabolomic data. Participants deviating more than four standard deviations from PC1 and/or PC2 were excluded from the analysis, resulting in the exclusion of 2 individuals. For downstream data analysis, we specifically selected metabolites from the Human Metabolome Database (HMDB) serum metabolites database (database version 2021–10–24) ($n = 500$).

Targeted lipoproteomic measurement

Plasma samples were measured using Nightingale's Biomarker Analysis Platform through nuclear magnetic resonance spectroscopy to quantify a total of 246 lipids and metabolites [29, 30]. Given high correlation between lipid parameters, we employed unsupervised hierarchical Ward-linkage clustering based on Spearman correlation coefficients. This clustering encompassed 131 measured metabolites, including lipoprotein subclasses, lipoprotein particle sizes, apolipoproteins, and cholesterol (Additional file 1: Table S1). Through this process, we identified 12 clusters of closely interrelated lipids ($\rho > 0.75$). Guided by expert opinion, we selected a single lipid measurement per cluster as a representative for clearer interpretation and to lessen the burden of multiple testing corrections. In addition to these 12 cluster representatives, we simultaneously analyzed 26 additional metabolites, including groups of fatty acids, amino acids, glycolysis metabolites,

ketone bodies, markers of fluid balance, and inflammatory markers. Nine lipid measurements showing no intercorrelation with others were also included individually. In total, 47 lipid and metabolite measurements were used for downstream data analysis.

Genotyping in the 2000HIV cohort

Study participants were genotyped on the Illumina Infinium Global Screening Array [17]. After performing standard quality control, imputation was performed using the TOPMed (version r2 on GRCh38) reference panel on the TOPMed imputation server. After excluding genetic outliers due to excess heterozygosity, relatedness, and ancestry and SNPs with low MAF and imputation quality (MAF > 5% and $R^2 > 0.3$ or $ER^2 > 0.7$ for imputation quality), the genotypes of SNPs of interest (rs738409, rs58542926, rs641738, rs780094, and rs2642438) were extracted for 1022 individuals of European ancestry with liver steatosis or fibrosis data available.

Statistics

Metabolomic analysis

For metabolomics analysis, we employed linear models to compare log2-normalized metabolites intensity, accounting for relevant confounding variables. To identify these factors, we performed linear regression on the first 10 principal components derived from PCA analysis of 500 metabolites. The results were visually represented as a heatmap, showcasing the explained variance (R2) of the first 5 PCs by each covariate (Additional file 2: Figs. S1–S2). We considered potential confounding variables as those leading to a 10% increase in beta coefficients when added to the linear model in a stepwise manner. This process identified age, sex, Black ethnicity, creatinine levels, and the use of lipid-lowering drugs (statins) and acetylsalicylic acid as potential confounders for the CVD models, and age, sex, Black ethnicity, creatinine levels, and lipid-lowering drugs (statins) for liver steatosis and fibrosis models. Of note, when comparing PWH with and without liver steatosis, we incorporated additional correction for fat layer thickness. We established significance using FDR-corrected *P* values < 0.05.

For metabolic pathway analysis, we utilized the web-based platform MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/) employing mummichog and the gene set enrichment analysis (GSEA) algorithm [31, 32]. The rationale behind this approach is that the collective behavior of multiple metabolites in a pathway is less susceptible to random errors introduced through individual peak assignments [32]. We utilized the default human library (MFN), which integrates the Kyoto Encyclopedia of Genes and Genomes (KEGG), Biochemical, Genetic, and Genomic (BiGG), and Edinburgh Model libraries.

Lipoproteomic analysis

Prior to analysis, we normalized lipoproteomics data using inverse rank-based transformation. Next, to analyze the differences in lipoproteomic data, we employed a linear regression model correcting for age, sex, Black ethnicity, and the use of cholesterol-lowering drugs (statin). These covariates were identified through a similar approach as that used for the metabolomics analysis (Additional file 2: Figs. S3–S4). Additional correction for fat layer thickness was applied for the steatotic liver disease (SLD) models. Multiple testing correction was carried out using FDR methods, where an FDR-corrected P value < 0.05 was deemed statistically significant.

Results

Characteristics of study population

In this study, we analyzed data of 970 PWH with available liver and carotid artery measurements, encompassing 533 PWH with normal liver measurements (S0 and F0-F1), 289 PWH with simple steatosis (≥ S1 and F0-F1), and 88 PWH with fibrosis, including 49 with concomitant steatosis (≥ S1 and ≥ F2). The baseline characteristics of PWH with and without steatosis and fibrosis are shown in Table 1 and Additional file 1: Table S2 for additional characteristics such as ART history as well as distribution of several SNPs, i.e., PNPLA3, TM6SF2, MBOAT7, GCKR, and MTARC1.

In the total study population and regardless of available liver measurements, 9.4% of individuals (177 out of 1875) had a history of previous CVD and 50% (901 out of 1795) had arterial plaques. Notably, 2.2% of individuals (37 out of 1698) developed incident CVD during the 2-year follow-up period.

PWH with simple steatosis were more likely to have arterial plaques (46.5% vs. 35.8%; *P* value = 0.003) and CVD history (11.4% vs. 6.8%; *P* value = 0.02) than PWH with normal liver measurements. PWH with fibrosis, regardless of steatosis degree, exhibited a significant association with prior myocardial infarction (8% vs. 2.7%, *P* value = 0.017), but not with other CVD nor with the presence of plaques or the occurrence of incident CVD. Steatosis was associated with increased 10-year risk of cardiovascular events according to the Framingham risk score and SCORE2 (Table 1), but no relation was found between simple steatosis with incident CVD events during 2 years of FU (Fig. 1).

No associations were found between the presence of steatosis with fibrosis in PWH with any CVD-related parameters. Interestingly, among CVD parameters, the presence of plaques showed associations with a history of

Table 1 Baseline characteristics of PWH with and without simple steatosis (left columns) and PWH with and without fibrosis (right columns)

Characteristic	Steatosis				Fibrosis			
	Overall, $N=822$[a]	S0, $N=533$[a]	S1 or higher, $N=289$[a]	*P* value[b]	Overall, $N=970$[a]	F0-F1, $N=882$[a]	F2 or higher, $N=88$[a]	*P* value[b]
Female sex	107 (13%)	76 (14%)	31 (11%)	0.15	129 (13%)	121 (14%)	8 (9.1%)	0.22
Age	52 (43, 59)	50 (40, 57)	55 (48, 61)	< 0.0001	52 (43, 59)	52 (43, 59)	55 (42, 63)	0.28
Ethnicity				0.12				0.40
Asian	33 (4.0%)	24 (4.5%)	9 (3.1%)		39 (4.0%)	37 (4.2%)	2 (2.3%)	
Black	85 (10%)	61 (11%)	24 (8.3%)		101 (10%)	96 (11%)	5 (5.7%)	
Hispanic	21 (2.6%)	13 (2.4%)	8 (2.8%)		23 (2.4%)	22 (2.5%)	1 (1.1%)	
Mixed	40 (4.9%)	32 (6.0%)	8 (2.8%)		50 (5.2%)	43 (4.9%)	7 (8.0%)	
Native American	3 (0.4%)	2 (0.4%)	1 (0.3%)		3 (0.3%)	3 (0.3%)	0 (0%)	
White	638 (78%)	399 (75%)	239 (83%)		752 (78%)	679 (77%)	73 (83%)	
BMI (kg/m^2)	24.9 (22.6, 27.6)	23.9 (21.7, 26.1)	26.8 (24.7, 29.9)	< 0.0001	25.1 (22.7, 27.8)	24.9 (22.5, 27.5)	27.6 (24.3, 30.9)	< 0.0001
Fat layer thickness > 25 mm	340 (43%)	156 (31%)	184 (66%)	< 0.0001	423 (46%)	366 (43%)	57 (69%)	< 0.0001
Current smoker (*N*)	249 (30%)	175 (33%)	74 (26%)	0.03	292 (30%)	266 (30%)	26 (30%)	0.90
Cholesterol-lowering drugs	160 (19%)	76 (14%)	84 (29%)	< 0.0001	200 (21%)	174 (20%)	26 (30%)	0.03
Acetylsalicylic acid	42 (5.1%)	21 (3.9%)	21 (7.3%)	0.04	54 (5.6%)	44 (5.0%)	10 (11%)	0.02
HIV duration	12 (6, 17)	11 (6, 17)	12 (7, 19)	0.09	11 (6, 17)	11 (6, 17)	10 (5, 15)	0.12
CD4 nadir	0.27 (0.16, 0.41)	0.28 (0.17, 0.41)	0.25 (0.12, 0.41)	0.06	0.27 (0.16, 0.41)	0.27 (0.16, 0.41)	0.26 (0.16, 0.40)	0.84
CD4:CD8 ratio at enrollment	0.87 (0.59, 1.16)	0.89 (0.64, 1.16)	0.84 (0.56, 1.17)	0.25	0.87 (0.59, 1.17)	0.88 (0.59, 1.17)	0.82 (0.59, 1.16)	0.46
Low level viremia	190 (23%)	117 (22%)	73 (25%)	0.31	221 (23%)	201 (23%)	20 (23%)	0.99
ART duration	9 (6, 15)	9 (5, 14)	10 (6, 15)	0.01	9 (5, 15)	9 (6, 15)	8 (5, 13)	0.22
CVD history (MI, stroke, PAD, or AP)	69 (8.4%)	36 (6.8%)	33 (11%)	0.02	88 (9.1%)	75 (8.5%)	13 (15%)	0.05
MI in MH	24 (2.9%)	12 (2.3%)	12 (4.2%)	0.12	31 (3.2%)	24 (2.7%)	7 (8.0%)	0.02
Stroke in MH	28 (3.4%)	17 (3.2%)	11 (3.8%)	0.64	36 (3.7%)	32 (3.6%)	4 (4.5%)	0.56
PAD in MH	11 (1.3%)	6 (1.1%)	5 (1.7%)	0.53	13 (1.3%)	12 (1.4%)	1 (1.1%)	1.00
AP in MH	16 (2.0%)	6 (1.1%)	10 (3.5%)	0.02	21 (2.2%)	17 (1.9%)	4 (4.5%)	0.12
Incident CVD 2-year FU	19 (2.5%)	14 (2.8%)	5 (2.0%)	0.47	23 (2.6%)	22 (2.7%)	1 (1.3%)	0.71
Mean of 3 IMT measurements	0.67 (0.57, 0.77)	0.65 (0.56, 0.75)	0.70 (0.61, 0.79)	< 0.0001	0.67 (0.57, 0.77)	0.67 (0.57, 0.77)	0.67 (0.56, 0.80)	0.87
Plaque in carotid artery	323 (40%)	189 (36%)	134 (47%)	0.003	380 (40%)	344 (39%)	36 (42%)	0.64
Hypertension in MH	202 (25%)	105 (20%)	97 (34%)	< 0.0001	241 (25%)	216 (24%)	25 (28%)	0.42
T2DM in MH	38 (4.6%)	16 (3.0%)	22 (7.6%)	0.003	51 (5.3%)	38 (4.3%)	13 (15%)	0.0003
Framingham risk score	12 (6, 19)	10 (6, 17)	15 (9, 22)	< 0.0001	12 (6, 19)	12 (6, 19)	12 (7, 21)	0.26
SCORE2	4.4 (2.8, 6.5)	3.8 (2.5, 5.9)	5.1 (3.6, 7.5)	< 0.0001	4.5 (2.8, 6.7)	4.4 (2.8, 6.6)	4.9 (3.5, 7.9)	0.09
ALT (U/I)	25 (20, 34)	24 (18, 31)	29 (22, 37)	< 0.0001	25 (19, 34)	25 (19, 33)	31 (20, 42)	0.007

Abbreviations: CAP Controlled attenuation parameter, *LSM* Liver stiffness measurement, *CVD* Cardiovascular disease, *MI* Myocardial infarction, *PAD* Peripheral arterial disease, *AP* Angina pectoris, *FU* Follow-up, *IMT* Intima media thickness, *MH* Medical history, *T2DM* Diabetes mellitus type 2. The Framingham risk score and SCORE2 both estimate 10-year risk of cardiovascular disease

The total study population with available liver measurements ($n = 970$ PWH) included 882 PWH without fibrosis, encompassing 533 PWH with normal liver measurements (S0 and F0-F1), 289 PWH with simple steatosis (≥ S1 and F0-F1), and 60 PWH without fibrosis with unknown steatosis status (S? and F0-F1), and 88 PWH with fibrosis, encompassing 31 with fibrosis without steatosis (S0 and ≥ F2), 49 with both fibrosis and steatosis (≥ S1 and ≥ F2), and 8 with fibrosis with unknown

Table 1 (continued)

steatosis status (S? and ≥ F2). The results are shown as [1]n (%) or Median (IQR); and derived from [2]Pearson's Chi-squared test; Wilcoxon rank sum test; or Fisher's exact test

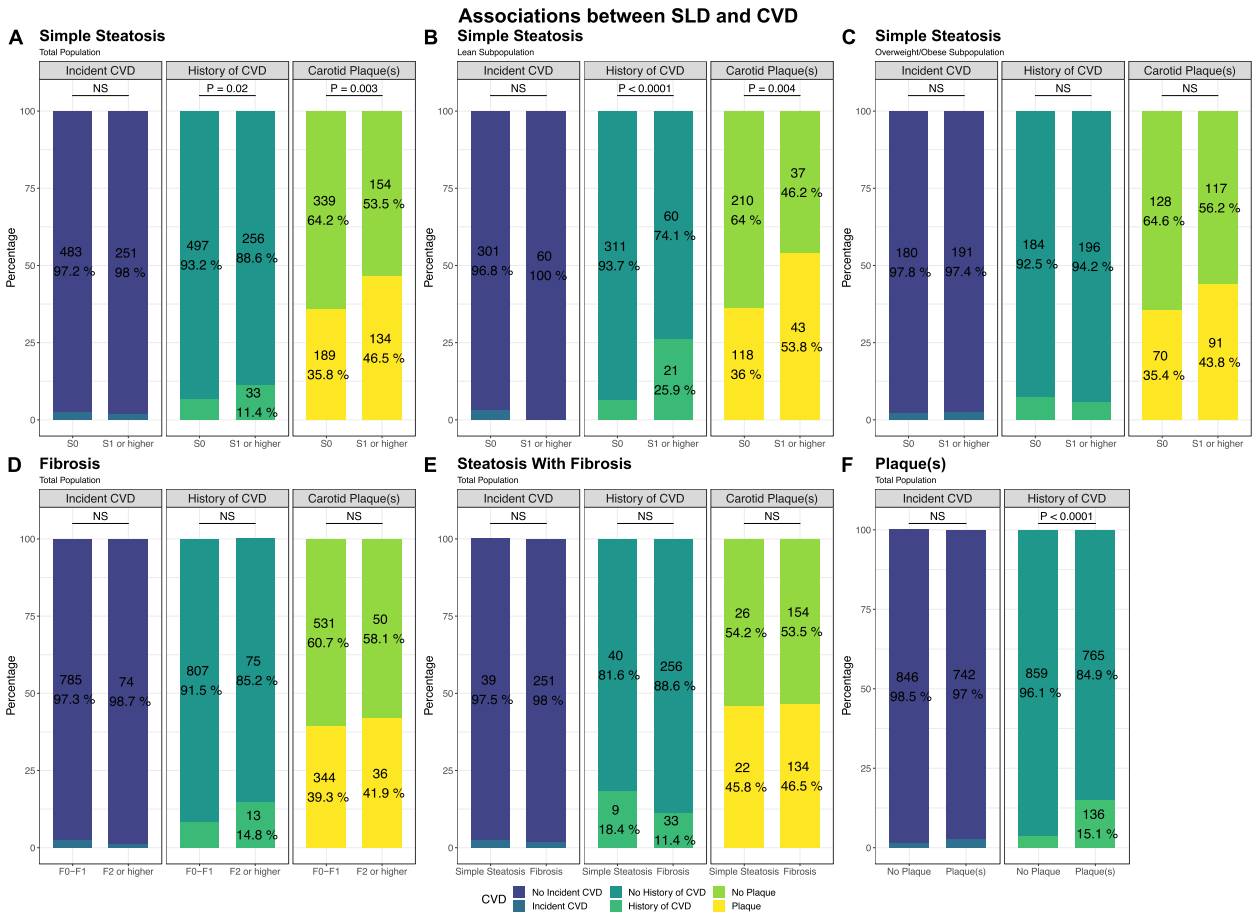

Fig. 1 Associations between liver steatosis and fibrosis and CVD-related parameters. *P* values were derived from chi-squared or Fisher's exact test, not corrected for confounders. For the readability of the plot, percentages < 10 are not shown in the corresponding bars

previous CVD (15.1% vs. 3.9%; *P* value < 0.0001), but not with incident CVD events (Fig. 1).

Baseline characteristics stratified by BMI category are provided in Additional file 1: Table S3. In contrast to overweight/obese PWH, liver steatosis in lean PWH is associated with HIV-specific factors including longer HIV duration, lower CD4 nadir, and ART history including previous exposure to dideoxynucleosides (stavudine and didanosine), protease inhibitors (indinavir), as well as the integrase strand-transfer inhibitor raltegravir, and the nucleoside reverse transcriptase inhibitor zidovudine.

Importantly, associations between liver steatosis and fibrosis and clinical parameters of CVD were more evident in lean than in overweight/obese PWH (Fig. 1, Additional file 1: Table S3). In lean PWH, simple steatosis was significantly associated with history of CVD including any CVD (26% vs. 6.3%, *P* value < 0.0001), myocardial infarction (11% vs. 2.1%, *P* value = 0.001), and angina pectoris (6.2% vs. 1.2%, *P* value = 0.017). Additionally, simple steatosis was also associated with measures of subclinical CVD, i.e., thicker intima media thickness (*P* value = 0.0003) and a higher frequency of carotid plaque(s) (*P* value = 0.004) (Additional file 1: Table S3). In contrast, in PWH with overweight or obesity, simple steatosis was only associated with intima media thickness (*P* value = 0.014). To verify our results, we performed additional analyses using logistic models to allow for adjustment of the effects of current smoking, hypertension, and T2DM. The results remained similar: history of CVD and presence of plaque(s) were associated with liver steatosis in lean PLHIV (history of CVD: *P* value = 2.10e − 05; plaque(s): *P* value = 0.0484), but not with liver steatosis in overweight/obese PLHIV (history of CVD: *P* value = 0.28772; plaque(s): *P* value = 0.19791). For the

total population, correction for these variables changed the significance of the associations; liver steatosis was not associated with history of CVD (*P* value = 0.20752) and presence of plaque(s) (*P* value = 0.0649) anymore when correcting for confounders.

However, liver steatosis was associated with increased 10-year risk of CVD according to the Framingham and SCORE2 risk scores in both lean (Framingham risk score: 15% vs. 10%, *P* value = 0.003; SCORE2: 5.3% vs. 3.9%, *P* value = 0.006) and overweight/obese PWH (Framingham risk score: 15% vs. 9%, *P* value < 0.0001; SCORE2: 5.0% vs. 3.6%, *P* value = 0.0003) (Additional file 1: Table S3), and the scores were similar between lean and overweight/obese PWH with steatosis (Framingham risk score: *P* value = 0.59; SCORE: *P* value = 0.84).

Metabolomic disturbances in liver steatosis and fibrosis

Initially, we assessed the variance in metabolomic measurements among PWH with normal liver measurements, simple steatosis, and fibrosis using principal component analysis. A considerable overlap was observed in the plasma metabolic profile among the three groups (Additional file 2: Fig. S5).

Next, we aimed to assess metabolite differences associated with steatosis and fibrosis using a linear model adjusting for the effects of age, sex, ethnicity, fat layer thickness, the use of cholesterol-lowering drugs, and creatinine levels (Fig. 2). First, we compared PWH with normal liver measurements with PWH with simple steatosis. Of 500 analyzed metabolites, 5 were up- and 20 were downregulated (Fig. 2A, Additional file 1: Table S4). Pathway analysis using both mummichog and GSEA revealed 13 enriched metabolic pathways (*P* value GSEA, mummichog, and/or meta-*P* value < 0.05), including several pathways related to lipids (i.e., "Squalene and cholesterol metabolism," "Omega-3 fatty acid metabolism," "Bile acid biosynthesis," "De novo fatty acid biosynthesis," and "Fatty acid activation"), pathways related to amino acid metabolism (i.e., "Glycine, serine, alanine and threonine metabolism," "Nitrogen metabolism," "Tryptophan metabolism," "Tyrosine metabolism," and "Urea cycle/amino group metabolism"), and "Prostaglandin formation from arachidonate," "Vitamin A (retinol) metabolism," and "Vitamin D3 (cholecalciferol) metabolism" (Fig. 2B; Additional file 1: Table S5).

Second, we compared the metabolites in PWH without fibrosis with PWH with fibrosis, regardless of steatosis degree. In total, five metabolites were up- and four were downregulated. Of the down regulated metabolites, three could be annotated as 1-acyl-sn-glycero-3-phosphocholine, and the fourth as lysophosphatidylcholine (Fig. 2C; Additional file 1: Table S6). All are lysophospholipids, which play a role in lipid signaling by binding to lysophospholipid receptors. In addition, three of the upregulated metabolites could be annotated as bile acids, i.e., glycodeoxycholate, chenodeoxycholic acid sulfate, and taurodeoxycholate. Significantly enriched pathways include "vitamin E metabolism," "urea cycle/amin group metabolism," "squalene and cholesterol biosynthesis," and "hyaluronan metabolism" (Fig. 2D; Additional file 1: Table S5).

Third, we compared PWH with simple steatosis (≥ S1 and F0-F1) with those with concomitant fibrosis (≥ S1 and ≥ F2). None of the metabolites remained significantly altered after correction for multiple testing (Additional file 1: Table S7). However, significant metabolites at nominal *P* value < 0.05 overlapped with those identified for fibrosis regardless of steatosis degree, including downregulated lysophospholipid and upregulated bile acids (Fig. 2E). Multiple pathways were significantly enriched, "alanine and aspartate metabolism," "arachidonic acid metabolism," "arginine and proline metabolism," "beta-alanine metabolism," "bile acid biosynthesis," "chondroitin sulfate degradation," "glutamate metabolism," "glycine, serine, alanine and threonine metabolism," "heparan sulfate degradation," "hyaluronan metabolism," "linoleate metabolism," "pentose and glucoronate interconversions," "prostaglandin formation from arachidonate," "urea cycle/amino group metabolism," "vitamin A (retinol) metabolism," "vitamin B5 – CoA biosynthesis from pantothenate," and "vitamin E metabolism" (Fig. 2F; Additional file 1: Table S5).

(See figure on next page.)

Fig. 2 Plasma metabolites and metabolic pathways associated with liver steatosis and fibrosis. **A** Individual metabolite comparison between PWH with simple steatosis ($n = 289$) vs. normal liver measurements ($n = 533$), **C** PWH with fibrosis ($n = 88$) vs. PWH without fibrosis ($n = 882$), regardless of steatosis status, and **E** PWH with steatosis with fibrosis ($n = 53$) vs. PWH with simple steatosis ($n = 289$). Analysis was performed using linear regression adjusting for the effects of age, sex, ethnicity, fat layer thickness, the use of cholesterol-lowering drugs, and creatinine levels. Red and blue points indicate up and downregulated metabolites, respectively. X-axis depicts log fold change and Y-axis depicts − log10 adjusted (FDR) *P* values for **A** and **C**, and unadjusted *P* value for **E**. Metabolic pathways associated with simple steatosis (**B**), liver fibrosis (**D**), and steatosis with fibrosis (**F**). Metabolic pathway analysis was performed using web-based platform MetaboAnalyst (https://www.metaboanalyst.ca/) employing mummichog and the gene set enrichment analysis (GSEA) algorithm. Only pathways with *P* value < 0.05 based on mummichog, GSEA, and/or meta-analysis were visualized. Several metabolic pathways in **B** and **F** were not annotated to enhance readability of plot (see more in Fig. 3 and Table S4)

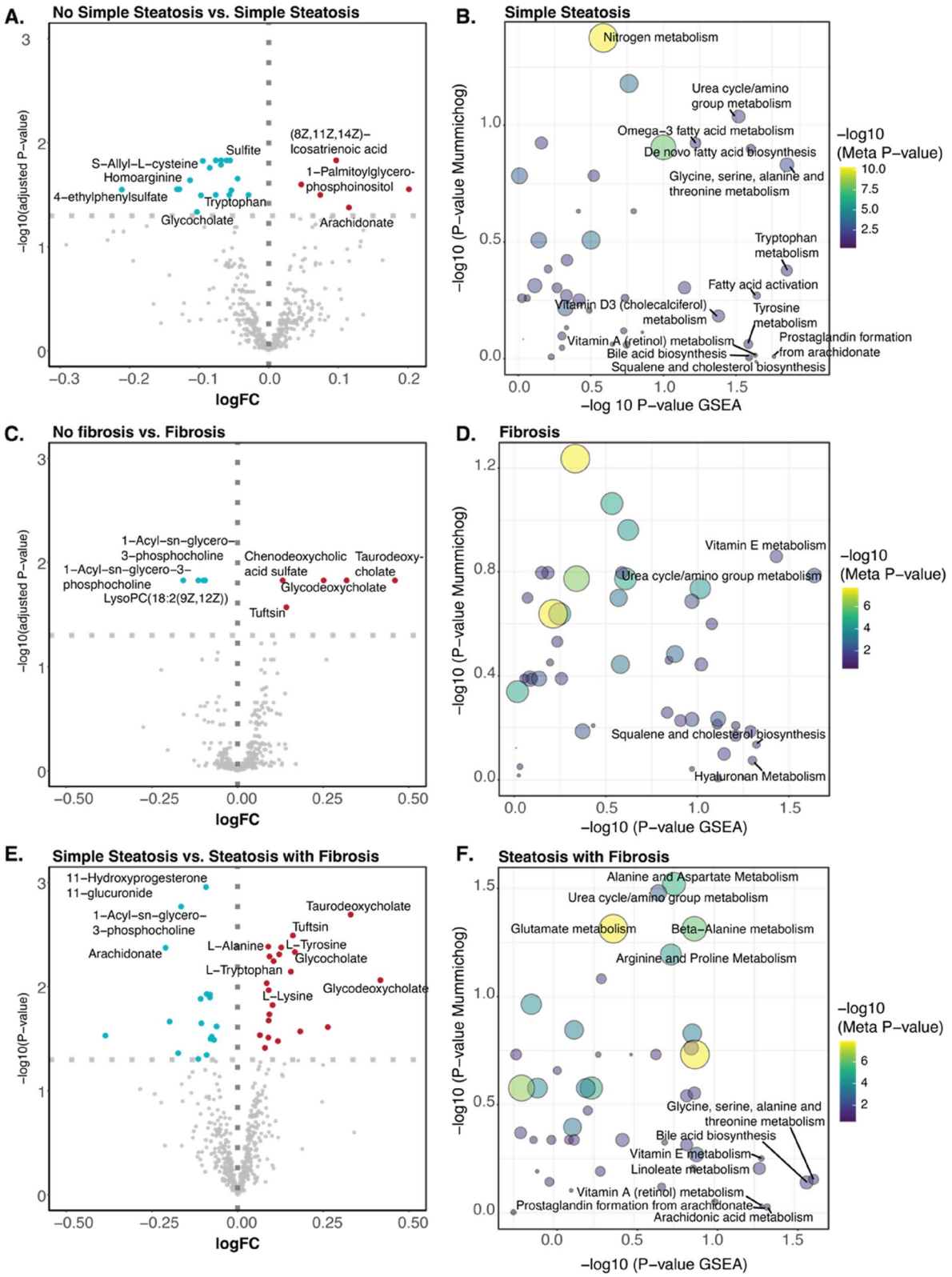

Fig. 2 (See legend on previous page.)

Fourth, since biological mechanisms driving liver steatosis and fibrosis may differ between PWH with a lean compared to those with an overweight or obese phenotype, we analyzed associations with metabolites and liver steatosis (insufficient number of cases with fibrosis) separately in these subpopulations employing the same linear model and confounder selection as for the total study population. In lean PWH, one metabolite was down- and two were upregulated in lean PWH with steatosis compared to those without steatosis (Additional file 1: Table S8). In addition, fourteen metabolic pathways were enriched in lean PWH with steatosis, i.e., "ascorbate (vitamin C) and aldarate metabolism," "bile acid biosynthesis," "butanoate metabolism," "C21-steroid hormone biosynthesis and metabolism," "de novo fatty acid biosynthesis," "fatty acid activation," "glycine, serine, alanine and threonine metabolism," "polyunsaturated fatty acid biosynthesis," "propanoate metabolism," "prostaglandin formation from arachidonate," "purine metabolism," "tryptophan metabolism," "vitamin (A) retinol metabolism," and "vitamin D3 (cholecalciferol) metabolism" (Additional file 1: Table S5). "Bile acid biosynthesis," "glycine, serine, alanine and threonine metabolism," "prostaglandin formation from arachidonate," and "vitamin (A) retinol metabolism" were also enriched in PWH with fibrosis.

In contrast, no metabolites were differentially expressed in overweight/obese PWH with and without steatosis (Additional file 1: Table S9). However, we identified few enriched metabolic pathways related to amino acids, including "alanine and aspartate metabolism," "histidine metabolism," "selenoamino acid metabolism," and "urea cycle/amino group metabolism" (Additional file 1: Table S5). Notably, none of the pathways was shared between lean and overweight/obese PWH (Fig. 3).

Overlap between metabolic disturbances associated with liver steatosis and fibrosis and subclinical, prior, and incident cardiovascular disease

Considering the established pathophysiological links between MASLD as a cardiovascular disease risk factor, we aimed to pinpoint shared metabolic disturbances associated with liver steatosis and fibrosis and incident and prior cardiovascular events as well as the presence of plaque(s) in PWH.

First, we assessed the metabolic disturbances associated with subclinical, prior, and incident cardiovascular disease. The results are shown in Additional file 3: Supplemental data, Additional file 1: Tables S10–S12, and Table S5.

Next, we compared metabolic pathways associated with liver steatosis and fibrosis with those associated with CVD, aiming to identify both distinct and common enriched pathways (Fig. 3 and Additional file 1: Table S5). In general, we observed limited overlap between metabolic pathways associated with liver steatosis and fibrosis and those associated with CVD. Specifically, we identified four overlapping pathways between liver steatosis with fibrosis and incident CVD. These included "prostaglandin formation from arachidonate," "arachidonic acid metabolism," "glycine, serine, alanine and threonine metabolism," and "vitamin B5 – CoA biosynthesis from pantothenate." In addition, we identified five overlapping pathways between liver steatosis in total and/or lean population and incident CVD, including "squalene and cholesterol biosynthesis," "glycine, serine, alanine and threonine metabolism," "prostaglandin formation from arachidonate," "vitamin D3 (cholecalciferol) metabolism," and "purine metabolism."

Lipoproteomic disturbances in liver steatosis and fibrosis

In addition to metabolic disturbances, we assessed lipoproteomic changes associated with steatosis and fibrosis in PWH. Using principal component analysis, we first assessed variance in lipoproteomic measurements of PWH with normal liver measurements, simple steatosis, and fibrosis. Clear separations were evident in the lipoproteomic profile among the three groups (Additional file 2: Fig. S6).

We first compared PWH with normal liver measurements with PWH with simple steatosis. PWH with simple steatosis exhibited extensive disturbances in the lipoproteomic profile with alterations of 31 of 47 metabolites, including 9 down- and 22 upregulated lipoproteins and other metabolites (Fig. 4A; Additional file 1: Table S13). Specifically, in PWH with simple steatosis, we observed lower concentrations of various lipids (i.e., HDL, LDL size, PUFA/MUFA, omega-6/omega-3, and unsaturation) and the amino acid glycine. Conversely, elevated concentrations were found in lipids (i.e., phosphoglycerides, cholines, phosphatidylcholine, total phospholipids, and TG/PG ratio), lipoproteins (i.e., VLDL and LDL), inflammation marker GlycA, branched-chain amino acids (i.e., valine, leucine, and isoleucine), and other amino acids (i.e., alanine, tyrosine, histidine).

Second, we compared PWH with and without fibrosis, regardless of the degree of steatosis. We found 16 down- and 6 upregulated lipoproteins and other metabolites in PWH with fibrosis (Fig. 4A; Additional file 1: Table S14). More specifically, PWH with fibrosis showed reduced concentrations of HDL and unsaturation, alongside increased concentrations of amino acids such as tyrosine, phenylalanine, and isoleucine. In contrast to the findings for steatosis, PWH had decreased levels of other lipids including sphingomyelins, cholines, phosphoglycerides, and phosphatidylcholines.

Fig. 3 Shared metabolic pathways in PWH with liver steatosis and fibrosis and subclinical, prior, and incident cardiovascular disease. Metabolic pathway analysis was performed using web-based platform MetaboAnalyst (https://www.metaboanalyst.ca/) employing mummichog and the gene set enrichment analysis (GSEA) algorithm. Only pathways with *P*-value < 0.05 based on mummichog, GSEA, and/or meta-analysis were visualized

Third, we compared PWH with simple steatosis (≥S1 and F0-F1) with those with concomitant fibrosis (≥S1 and ≥F2) (Fig. 4A; Additional file 1: Table S15). The results, including lower levels of HDL, unsaturation, and membrane lipids, and increased phenylalanine, tyrosine, and isoleucine, were largely similar to those observed for fibrosis regardless of steatosis.

Finally, we assessed differences in lipoproteomic measurements between PWH with normal liver measurements and those with simple steatosis, separately for lean and overweight/obese individuals (Fig. 4B; Additional file 1: Tables S16–S17). For both groups, PWH with simple steatosis exhibited increased VLDL, LDL, and TG/PG ratio along with decreased PUFA/MUFA and unsaturation. These findings were consistent with the results obtained from the combined population analysis. Additionally, lean PWH with steatosis had increased branched-chain amino acids as well as histidine and decreased XL-HDL and glycine levels, whereas overweight/obese PWH with steatosis had increased alanine, lipoproteins (LDL-TG, M-LDL-C, L-HDL-TG, and XS-VLDL-C), ApoB/ApoA1, omega-3, GlycA, and membrane lipids, and decreased LDL size and L-HDL-C. However, when comparing the levels of each of these metabolites between lean PWH without steatosis, overweight/obese PWH without steatosis, lean PWH with steatosis, and overweight/obese PWH with steatosis, similar trends were observed between lean and overweight/obese PWH (Additional file 2: Figs. S7–S10).

Overlap between lipidomic profiles associated with liver steatosis and fibrosis and subclinical, prior, and incident cardiovascular disease

Next, we compared plasma concentration of various lipoproteins and other metabolites in PWH with and without subclinical, prior, and incident CVD. The results are shown in Fig. 4A, Additional file 1: Tables S18–S20, and Additional file 2: Fig. S11. When comparing lipoproteins and other metabolites linked to liver steatosis and/or fibrosis with those associated with CVD, we observed shared downregulation, primarily between PWH with fibrosis and those with CVD history. These differences included decreased circulating concentrations of HDL, total phospholipids, sphingomyelins, phosphoglycerides, phosphatidylcholines, and cholines (Fig. 4A).

Discussion

The current understanding of the relationship between MASLD and cardiovascular disease, particularly in PWH, is limited. In this study, we comprehensively assessed the metabolomic and lipoproteomic profiles related to liver steatosis and fibrosis, as well as subclinical, prior, and incident CVD, in nearly 1000 PWH on suppressive ART. We observed that associations between liver steatosis and fibrosis with CVD were most evident for steatosis in lean PLIHV. Metabolomic and lipoproteomic profiles associated with liver steatosis and fibrosis were largely distinct from those associated with CVD. However, we identified overlapping enriched metabolic pathways associated with liver steatosis and fibrosis with those associated with incident CVD, particularly notable in lean PWH with steatosis.

Metabolomic changes linked to liver steatosis and fibrosis were notably distinct from those associated with CVD parameters. PWH with liver steatosis and/or fibrosis mainly had enrichment of pathways related to lipid and amino acid metabolism (10/33 related to amino acid and 8/33 to lipid metabolism) which was also confirmed by the lipoproteomic results. In contrast, PWH with subclinical, prior, and/or incident CVD mainly had enrichment cofactors and vitamin metabolism (9/27 pathways). Of note, enriched metabolic pathways across the three parameters of CVD (i.e., prior, incident, and subclinical) were highly heterogenous with only one commonly enriched pathway (i.e., omega-6 fatty acid metabolism). This discrepancy is likely due to heterogeneity among CVD traits (e.g., we were not able to differentiate between stable and vulnerable plaques). Consistent with results from metabolomic analysis, we observed limited overlap in lipoproteomic profiles associated with liver steatosis and fibrosis and CVD parameters. The most notable overlap was observed in lower concentrations of HDL, IDL, LDL, and membrane lipids in PWH with liver fibrosis, as well as PWH with a history of CVD. Unexpectedly, several lipids (i.e., XS-VLDL-C, VLDL-TG, total phospholipids, M-LDL-C, and L-VLDL-C) were associated

(See figure on next page.)

Fig. 4 Plasma lipoproteins associated with liver steatosis and fibrosis and subclinical, prior, and incident cardiovascular disease. **A** Heatmap summarizing the results of the lipoproteomic analysis for PWH with liver steatosis and PWH with liver fibrosis, as well as PWH with CVD-related traits, including incident CVD, CVD history, and presence of plaque(s). Analyses were performed using linear regression models correcting for age, sex, ethnicities, and the use of cholesterol-lowering drugs. Additional correction for fat layer thickness was applied for MASLD. Asterisks indicate adjusted *P* values (FDR) < 0.05 (*), < 0.001 (**), and < 0.0001 (***). **B** Plasma metabolites alterations in PWH with simple steatosis compared to PWH with normal liver measurements specific for lean PWH (left column), overweight/obese PWH (middle column), or in both lean and overweight/obese PWH

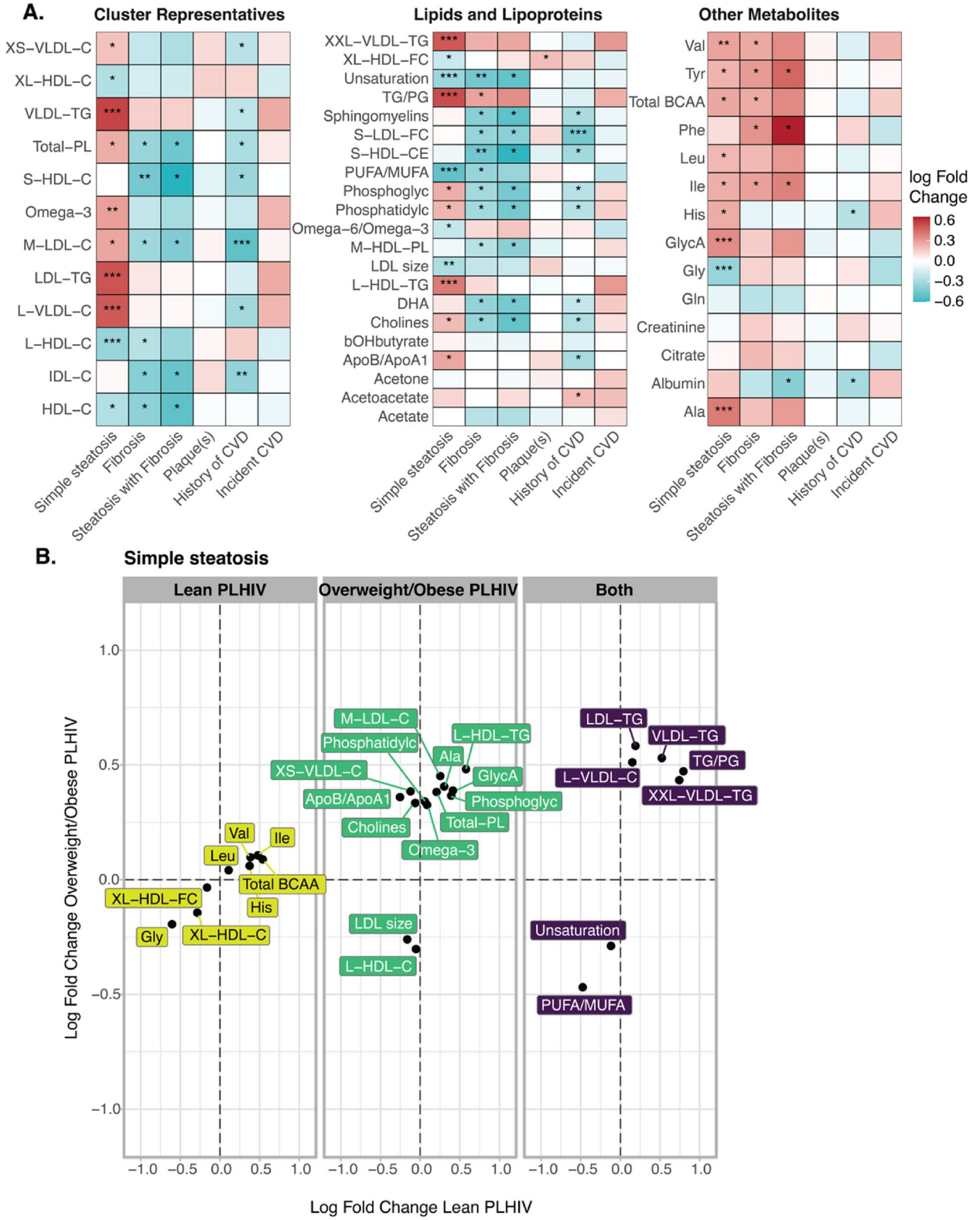

Fig. 4 (See legend on previous page.)

in opposite directions with liver steatosis and history of CVD. This may be explained by several mechanisms. First, although we corrected for this, lipid-lowering therapy may affect the lipoproteomic profile. PWH who experienced a cardiovascular event may better adhere to prescribed lipid-lowering medications compared to those who are not known with CVD. Second, PWH with a history of CVD may have modified their lifestyle in positive manner. Notably, recent research found a U-shaped association between HDL and LDL lipoproteins and cardiovascular risk [33, 34], implying that both very low and high levels are associated with adverse cardiovascular outcomes. Consequently, the observed associations in opposite direction may both predispose to cardiovascular events.

However, the differences in metabolomic and lipoproteomic profiles associated with liver steatosis and fibrosis and CVD may also suggest that other or additional mechanisms underlie the increased risk for CVD in individuals with liver steatosis and fibrosis in PWH. Several mechanisms have been proposed: MASLD may exacerbate insulin resistance, promote the release of pro-inflammatory, vasoactive, and thrombogenic factors, and affect cardiac substrate metabolism, all resulting in increased CVD risk [8, 35]. However, no evidence has yet demonstrated a cause-and-effect relationship. Moreover, the role of HIV-related factors on the link between MASLD and CVD remains to be uncovered [7]. Future studies should further explore these mechanisms.

Despite the reported differences, we identified 12 shared metabolic pathways associated with liver steatosis and/or fibrosis as well as with CVD-related variables, including nine pathways enriched in incident CVD. Of the pathways enriched in incident CVD, two amino acids pathways (i.e., selenoamino acid and histidine metabolism) were commonly associated with steatosis in overweight/obese PWH, and four metabolic pathways were commonly associated with steatosis in lean PWH.

The overlap in enriched metabolic pathways associated with simple steatosis in lean PWH with incident CVD is notable, especially considering the stronger associations between simple steatosis and the presence of plaque(s) and history of CVD in lean PWH. Up to 26% of lean PWH with simple steatosis experienced any CVD (myocardial infarction, stroke, peripheral arterial disease, or angina pectoris) in contrast to 5.8% of overweight/obese PWH with simple steatosis. Although the estimated risks according to the Framingham risk score and SCORE2 indicate similarly increased risk of CVD in lean and overweight/obese PWH with liver steatosis, the stronger associations with clinical parameters of CVD suggest that particularly in lean PWH with liver steatosis, additional mechanisms independent from the traditional CVD risk factors, which are included in the Framingham/SCORE risk scores, exist that drive CVD development. Increased cardiovascular risk has been suggested to be present in lean individuals with MASLD [11], but to date, no studies have addressed differences in cardiovascular risk in lean compared to overweight/obese individuals with MASLD specifically in a population living with HIV. Given the convincing associations with CVD history and presence of carotid plaque(s) in lean PWH with steatosis, associations with incident CVD might also have been expected. However, with a sample size of ten lean PWH with incident CVD, we could not detect significant differences. Future studies are needed to assess risk of incident CVD in lean PWH specifically.

The four shared metabolic pathways between lean PWH with steatosis and PWH with incident CVD include "prostaglandin formation from arachidonate," "cholecalciferol metabolism," "purine metabolism," and "glycine-, serine-, alanine-, and threonine metabolism." Notably, all of these are related to inflammation: vitamin D has anti-inflammatory properties [36], glycine and serine are involved in glutathione synthesis and hence, oxidative stress [37], whereas the products of arachidonic acid and purine metabolism have pro-inflammatory properties [38, 39]. Two of these pathways capture our specific attention: arachidonic acid metabolism and the formation of its derivates, and purine metabolism. Both pathways are well-known to influence CVD [38, 40, 41], but their role in MASLD is less established.

Accumulating evidence shows involvement of arachidonic acid metabolism in MASLD, including ambivalent effects of prostaglandin E2 (PGE2): Kupffer cells of the liver may produce pro-inflammatory PGE2, which on one hand exerts pro-steatogenic effects by promoting lipogenesis, while on the other hand PGE2 probably exerts anti-fibrotic effects by inhibiting tissue growth factor beta 1-mediated collagen production by activated stellate cells [39]. It is unknown whether SLD itself affects arachidonic acid metabolism. Notably, HIV infection is associated with changes in arachidonic acid metabolism. It has been reported that, compared to matched controls, PWH on suppressive ART have lower levels of prostaglandins and thromboxanes, both derivates of arachidonic acid metabolism [42]. In another study, enrichment of arachidonic acid metabolism was associated with HIV status [13]. In addition, purine metabolism was associated with incident CVD and lean SLD. Uric acid is the final product of purine metabolism. Uric acid affects inflammation, oxidative stress, insulin resistance, endoplasmic reticulum stress, and endothelial dysfunction [38]. Inflammation, oxidative stress, and insulin resistance may in turn cause

liver steatosis. Hence, with our current understanding, it seems reasonable that these pathways are involved in both liver steatosis and fibrosis as well as CVD.

An unresolved question that remains to be addressed is why, among other metabolic pathways, prostaglandin formation and purine metabolism are enriched in lean, but not in overweight and obese PWH with simple steatosis. Possibly, levels of metabolites of concerning pathways are more discriminative for liver steatosis in lean compared to overweight/obese individuals. At least for several of the lipids (e.g., L-HDL-C and L-HDL-TG), we found similar concentrations in lean and overweight/obese PWH with simple steatosis, but the difference in concentrations was enhanced lean PWH with and without steatosis. In addition, lean PWH with liver steatosis has distinct features including associations with exposure to older antiretroviral drugs including stavudine, raltegravir, and indinavir. Some may have long-lasting (> 10 years) metabolic effects such as impaired mitochondrial function. Indeed, exposure to stavudine, didanosine, and protease inhibitors has been associated with elevated uric acid, possibly due to mitochondrial toxicity [43]. Mitochondrial dysfunction increases the formation of lactate. Lactate competes with uric acid for tubular secretion by the kidneys, resulting in decreased secretion and thus increased serum levels of uric acid [43]. It is also possible that mitochondrial toxicity caused by ART affects arachidonic acid metabolism. All in all, our findings suggest that simple steatosis in lean PWH is more atherogenic than simple steatosis in overweight/obese PWH, possibly mediated by common dysregulation of arachidonic acid, purine, cholecalciferol, and amino acid metabolism.

The current findings suggest that the presence of liver steatosis, especially in lean PWH, needs to be considered in the guidelines for CVD prevention, which is presently not the case. The current guidelines advise a combination of a statin and aspirin for secondary prevention while for primary prevention, aspirin is reserved for PWH with > 20% 10-year risk with a low risk of bleeding, in addition to a statin [44]. Aspirin, also known as acetylsalicylic acid, inhibits platelet activation, platelet-derived growth factor, and -cyclooxygenase-2 signaling, the latter explaining the effect of acetylsalicylic acid on arachidonic acid metabolism [45]. As dysregulation of arachidonic acid metabolism was evident in both liver steatosis in lean PWH and incident CVD, prescription of medication that interferes with arachidonic acid metabolism such as aspirin may be further studied for the prevention of incident CVD in lean PWH with liver steatosis. Additionally, it has been shown in the general population that daily acetylsalicylic acid is associated with reduction of liver steatosis and the risk of developing hepatocellular carcinoma [45, 46].

Limitations of our study include the cross-sectional nature of the liver steatosis and fibrosis data. Second, we used transient elastography with controlled attenuation parameter for the assessment of steatosis and fibrosis. With this technique, we cannot assess inflammation, meaning that we lack information on MASH. Third, untargeted metabolomics hinders annotation of specific metabolites, meaning that we can only draw conclusions on enriched metabolic pathways. Fourth, lean and overweight/obese PWH differed in cardiovascular risk factors including smoking and history of CVD. However, this may have limited effect on our results. The percentage of smokers was higher among lean PWH compared to overweight/obese PWH (35% vs. 25%), but the proportion of smokers was comparable between lean PWH with and without liver steatosis. Consequently, the imbalance in smokers does not explain the pronounced association between liver steatosis and history of CVD in lean PWH. This was also reflected in an additional analysis in which we adjusted for the effects of smoking: the association between liver steatosis and history of CVD remained significant in lean PLHIV. In addition, the metabolomic analysis suggests that the association between history of CVD and liver steatosis in lean PWH had limited impact on the metabolomic profile of lean PWH with liver steatosis, as no overlap was observed in enriched metabolic pathways with CVD history. Fifth, the time intervals between the liver measurements and the incident CVD events cannot be specified more precisely than the 2-year interval between baseline and follow-up, as exact dates of incident CVD events were not collected. Lastly, as we did not investigate a population of individuals without HIV, we were not able to assess whether our observations are unique to PWH.

Conclusions

Liver steatosis is a recognized risk factor for CVD, also in PWH, but the underlying mechanisms are unclear. Our study demonstrates that metabolomic and lipoproteomic profiles associated with liver steatosis and fibrosis were largely distinct from those with subclinical, prior, and incident CVD. This suggests that the link between liver steatosis and fibrosis with CVD may depend on other mechanisms or additional factors. Nevertheless, we identified a commonality of inflammatory-related metabolic pathways associated with steatosis and fibrosis, particularly in lean individuals, and incident CVD. Additionally, in lean PWH, stronger associations between the presence of liver steatosis and history of CVD and presence of plaque(s) were observed. Taken together, our findings suggest that especially lean PWH with liver steatosis may be at increased risk for developing CVD, which may be attributed to common metabolic perturbations. Our

findings highlight the importance of investigating aspirin in preventing CVD among lean PWH with liver steatosis.

Abbreviations

AP	Angina pectoris
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
ART	Antiretroviral therapy
BiGG	Biochemical, Genetic, and Genomic library
CAP	Controlled attenuation parameter
CRF	Case report form
CVD	Cardiovascular disease
FDR	False discovery rate
FU	Follow-up
GlycA	Glycoprotein acetyls
GSEA	Gene set enrichment analysis
HDL	High-density lipoprotein
HGFP	Human Functional Genomics Project
HMDB	Human Metabolome Database
IDL	Intermediate-density lipoprotein
IMT	Intima media thickness
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDL	Low-density lipoprotein
LDL-TG	Triglycerides in LDL
L-HDL-C	Cholesterol in large HDL
L-HDL-TG	Triglycerides in large HDL
LSM	Liver stiffness measurement
MASH	Metabolic dysfunction-associated steatohepatitis
MASLD	Metabolic dysfunction-associated steatotic liver disease
MH	Medical history
MI	Myocardial infarction
M-LDL-C	Cholesterol in medium LDL
MUFA	Monounsaturated fatty acid
NAFLD	Non-alcoholic fatty liver disease
PAD	Peripheral arterial disease
PCA	Principal component analysis
PC1	Principal component 1
PC2	Principal component 2
PG	Phosphoglycerides
PGE2	Prostaglandin E2
PWH	People with HIV
PUFA	Polyunsaturated fatty acid
SHM	Stichting HIV Monitoring
TG	Triglycerides
T2DM	Diabetes mellitus type 2
VLDL	Very low-density lipoprotein
XL-HDL	Extra-large HDL
XS-VLDL-C	Cholesterol in very small VLDL

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-03914-5.

Additional file 1: Tables S1–S20. Table S1. Clusters of metabolites of the lipoproteomic panel including the group representatives. Table S2. Additional baseline characteristics of PWH with and without simple steatosis and PWH with and without fibrosis. Table S3. Baseline characteristics of lean and overweight/obese PWH with and without simple steatosis. Table S4. Metabolomic profile of liver steatosis in the total study population. Table S5. Enriched metabolic pathways in PWH with carotid plaque(s), history of CVD, incident CVD, liver steatosis, or fibrosis. Table S6. Metabolomic profile of liver fibrosis in the total study population. Table S7. Metabolomic profile of liver fibrosis in PWH with steatosis. Table S8. Metabolomic profile of liver steatosis in lean PWH. Table S9. Metabolomic profile of liver steatosis in overweight/obese PWH. Table S10. Metabolomic profile of CVD history in the total study population. Table S11. Metabolomic profile of incident CVD in the total study population. Table S12. Metabolomic profile of carotid plaque(s) in the total study population. Table S13. Lipoproteomic profile of liver steatosis in the total study population. Table S14. Lipoproteomic profile of liver fibrosis in the total study population. Table S15. Lipoproteomic profile of liver fibrosis in the subpopulation PWH with S1 or higher. Table S16. Lipoproteomic profile of liver steatosis in lean PWH. Table S17. Lipoproteomic profile of liver steatosis in overweight/obese PWH. Table S18. Lipoproteomic profile of carotid plaque(s) in the total study population. Table S19. Lipoproteomic profile of prior CVD in the total study population. Table S20. Lipoproteomic profile of incident CVD in the total study population.

Additional file 2: Figures S1–S11. Fig. S1. Principal component analysis on potential confounders of the metabolome for steatosis and fibrosis. Fig. S2. Principal component analysis on potential confounders of the metabolome for CVD variables. Fig. S3. Principal component analysis on potential confounders of the lipoproteome for SLD variables. Fig. S4. Principal component analysis on potential confounders of the lipoproteome for CVD variables. Fig. S5. Principal component analysis showing the variance in metabolomics between PWH with unaffected liver measurements, PWH with simple steatosis, and PWH with fibrosis regardless of steatosis. Fig. S6. Principal component analysis showing the variance in lipoproteomics between PWH with normal liver measurements, PWH with simple steatosis, and PWH with fibrosis regardless of steatosis. Fig. S7. Boxplots showing the levels of amino acids, glycolysis-related metabolites, and inflammation markers for PWH of different BMI (lean vs. overweight/obese) and steatosis group (no vs. yes). Fig. S8. Boxplots showing the levels of lipoproteins, cholesterol, triglycerides, and total lipids for PWH of different BMI (lean vs. overweight/obese) and steatosis group (no vs. yes). Fig. S9. Boxplots showing the levels of membrane lipids for PWH of different BMI (lean vs. overweight/obese) and steatosis group (no vs. yes). Fig. S10. Boxplots showing the levels of fatty acids and apolipoproteins for PWH of different BMI (lean vs. overweight/obese) and steatosis group (no vs. yes). Fig. S11. Plasma lipoproteins associated with liver steatosis and fibrosis and subclinical, prior, and incident cardiovascular disease.

Additional file 3: Supplemental data. Metabolic disturbances associated with subclinical, prior, and incident cardiovascular disease.

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Authors' contribution
LvE, NV, and AvdV contributed to the study design. LvE, NV, MB1, AG, and WV performed the investigation. LvE and NV drafted the manuscript. MB1, AG, WV, EN, AV, JS, MB2, JvL, MN, GW, NR, JR, QdM, ET, LJ, and AvdV revised and approved the manuscript. All authors had full access to the data, all authors read and approved the final manuscript, and all authors had final responsibility for the decision to submit the manuscript for publication.

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Data availability
The data used in this publication are part of 2000HIV study, which is a large collaborative effort. For this collaboration, analyses of the datasets and preparation of publications are still ongoing. After 31 December 2026, the 2000HIV dataset collection will be made available in the Radboudumc Research Data Repository (RDR, Radboud Data Repository (ru.nl)) in pseudonymized form and under restricted access. Submissions requesting data will be reviewed by the collection manager, after which a Data Use Agreement will be set up.

Declarations

Ethics approval and consent to participate
The 2000-HIV study was approved by the Independent Review Board Nijmegen (ref. NL68056.091.81) and the study protocol was published at clinicaltrials.gov (ID: NCT03994835). Written informed consent was obtained

from all study participants and experiments were conducted according to the principles of the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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