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Original article

Long-term effects of HCV eradication on lipid profiles associated with MASLD among people with HIV with advanced fibrosis or cirrhosis

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ABSTRACT

Background: Despite successful hepatitis C virus (HCV) clearance, some individuals continue to experience liver disease progression. Metabolic dysfunction-associated steatotic liver disease (MASLD) may play a key role in this ongoing progression. This study aims to characterize the lipidomic profiles associated with MASLD in individuals coinfected with human immunodeficiency virus (HIV) and HCV with advanced fibrosis or cirrhosis after sustained virologic response (SVR).

Methods: We conducted cross-sectional studies in fifty-two HIV/HCV-coinfected individuals. Untargeted lipidomics was performed on plasma samples collected at 1 year and 6 years post-SVR using liquid chromatography-mass spectrometry. The primary outcome was MASLD. Statistical analyses included orthogonal partial least squares discriminant analysis (OPLS-DA) and generalized linear models (GLM), with corrections for multiple comparisons.

Results: The prevalence of MASLD was 28.9 % one year after SVR, increasing to 44.8 % six years after SVR. OPLS-DA models identified 225 lipids at 1 year and 167 at 6 years, with a VIP score ≥ 1, distinguishing individuals based on MASLD status. Adjusted GLMs confirmed significant associations between MASLD and 116 lipids at 1 year and 49 at 6 years. At 1 year, most significant lipids were glycerophospholipids (GP), with increased phosphatidylcholines (PC) and phosphatidylethanolamines (PE), and decreased lysophosphatidylcholines (LPC) and lysophosphatidylethanolamines (LPE). By 6 years, LPC was the most abundant differential lipid, while triglycerides increased significantly.

Conclusions: MASLD was common during follow-up, with changes in lipidomic profiles over time suggesting ongoing metabolic disturbances that may contribute to liver disease progression despite SVR. These findings highlight the need for long-term metabolic and liver health monitoring after HCV eradication in these individuals.

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Introduction

Currently, antiviral therapy constitutes an effective treatment for managing chronic hepatitis C virus (HCV) infection, with HCV infection on the verge of eradication. Multiple studies have reported improved clinical outcomes in HCV-infected individuals who achieve sustained virologic response (SVR) after HCV therapy [1]. However, a significant number of individuals, particularly people with human immunodeficiency virus (HIV) (PWH), show a lack of liver disease regression after HCV eradication [2, 3]. Hence, the emergence of liver-related complications after HCV eradication is becoming an important issue.

In particular, steatosis is a common condition found in the liver of HCV-infected individuals [4]. HCV replication is closely related to an increase in lipid biosynthesis and a decrease in its degradation, favoring the accumulation of intracellular lipids (steatosis) [5] and, thus, promoting the development of steatotic liver disease [4, 6]. This condition is highly prevalent among HCV-infected individuals, with its prevalence increasing over time [6]. This metabolic phenotype involves a complex and diverse range of causes. It occurs alongside metabolic dysfunctions such as overweight, type 2 diabetes mellitus, insulin resistance, liver inflammation, or other metabolic risk abnormalities resulting from altered lipid metabolism [7, 8]. In addition, liver steatosis after HCV eradication is an independent risk factor for severe liver disease, such as cirrhosis, liver failure, and hepatocellular carcinoma [9]. Also, it increases the risk of all-cause mortality [9, 10], particularly in HIV/HCV-coinfected individuals [11].

A newly suggested term by international liver experts, metabolic dysfunction-associated steatotic liver disease (MASLD), is intended to replace earlier terminologies, such as non-alcoholic fatty liver disease and metabolic dysfunction-associated fatty liver disease [12]. The goal is to encompass the various causes of liver steatosis while avoiding potentially stigmatizing language [12]. As previously described by the new international consensus definition of MASLD, the diagnosis framework for MASLD requires two components: the detection of hepatic steatosis (by imaging, histology, or validated non-invasive biomarkers) coupled with the presence of at least one cardiometabolic risk factor. This two-component definition establishes MASLD as a fundamentally metabolic condition and provides a robust framework for its identification in clinical research. While evidence indicates a 55 % prevalence of MASLD in individuals with untreated HCV [13], it's still not clear if eradicating HCV improves this liver condition. Some studies have reported a tendency towards decreased steatosis following SVR [14, 15], while others have shown an increase in steatosis beginning 1-year after SVR [1, 3, 16, 17] or even from baseline [16, 17].

Due to the limited and inconsistent knowledge of MASLD occurrence, this study aimed to characterize the lipid profile linked to MASLD in HIV/HCV-coinfected individuals with advanced fibrosis or cirrhosis at 1 and 6 years post-successful HCV therapy.

Material and methods

Study subjects

We conducted a cross-sectional study in HIV/HCV-coinfected individuals from 10 centers in Spain (Supplementary Data 1) who had advanced fibrosis or cirrhosis prior to HCV therapy and achieved SVR after successful HCV therapy. HCV treatment included either interferon (IFN)-free DAAs or IFN-based (peg-IFN-α/ribavirin or peg-IFN-α/ribavirin/DAAs), initiated between February 2012 and August 2016. SVR was defined by an undetectable level of HCV-RNA 12–24 weeks after the completion of anti-HCV therapy, with the specific duration depending on the treatment regimen used. The participants were followed up 1-year and 6-years after completing HCV treatment, with the 6-year follow-up between January 2019 and May 2021. All participants were on stable antiretroviral therapy for over six months and had an undetectable plasma HIV viral load (< 50 copies/mL). Individuals with hepatitis B virus coinfection, acute hepatitis C, hepatocellular carcinoma, or hepatic decompensation were excluded from the study.

Clinical data and samples

Clinical, epidemiological, and virological characteristics of participants were obtained from medical records using an online form, meeting all confidentiality requirements. This information was monitored.

Peripheral blood samples were collected 1-year and 6-years post-successful completion of HCV treatment in EDTA tubes, processed within 24 h post-extraction, and stored at −80ºC in the HIV HGM BioBank until use.

Outcome variable

The outcome variable was the MASLD, evaluated at 1-year and 6-years post-successful HCV treatment. Our operational definition was designed to strictly adhere to the principles of the new international consensus, which requires a two-step diagnosis: 1) evidence of hepatic steatosis, and 2) the presence of at least one cardiometabolic risk factor (full description in Supplementary Data 2).

For the first step, hepatic steatosis was assessed non-invasively. Given that advanced imaging techniques were not available for this cohort and liver biopsy is clinically contraindicated for steatosis assessment alone, we selected the Hepatic Steatosis Index (HSI). The HSI is a well-established index with good diagnostic accuracy for detecting fatty liver (AUC > 0.8) [18]. Furthermore, its components (BMI, sex, ALT/AST ratio) are particularly relevant to the underlying pathophysiology of this specific population. Both HIV infection itself and metabolic shifts following HCV clearance are known to be core drivers of changes in BMI and transaminase levels, which are directly associated with hepatic fat accumulation. Thus, the HSI represents a validated, objective, and reproducible tool for this purpose, with an established cut-off of ≥ 36 demonstrating good diagnostic accuracy for detecting steatosis.

Non-targeted lipidomics analysis

The list of reagents and standards, lipid extraction, quality management assurance, analytical conditions, lipid annotation, data reprocessing and normalization are available in Supplementary Data 2**.**

Statistical analysis

For the descriptive study, continuous variables were expressed as median (interquartile range, IQR) and categorical as absolute count (percentage). Independent groups for continuous and categorical variables were compared using the Mann–Whitney U and Chi-square tests, respectively.

For the multivariate lipidomic analysis, outliers were firstly treated according to the commonly used 1.5 ×IQR rule (75th percentile + 1.5 × IQR, and 25th percentile −1.5 ×IQR), and data were log-transformed (log10) and auto-scaled. Subsequently, orthogonal partial least squares discriminant analysis (OPLS-DA) was carried out, and the variable importance in projection (VIP) scores were determined. Those plasma lipids with VIP > 1 were analyzed using Generalized Linear Models (GLM) with gamma distribution (log-link) to evaluate the association between individual plasma lipids (dependent variable) and MASLD (independent variable) at 1-year and 6-year post-successful HCV treatment (cross-sectional studies).

This test provides the arithmetic mean ratio, the 95 % confidence interval, and its level of significance (p-value), which was corrected for multiple testing using the False Discovery Rate with Benjamini and Hochberg procedure (q-value). In addition, the GLM models were ajusted for the primary available clinical and epidemiological variables, including gender, age, and the specific HCV treatment (IFN-based or DAAs). These covariates were previously selected by a stepwise method (forward), according to the specific model's lowest AKAike information criteria. To study the predictive role of lipid species, we analyzed the association of significant plasma lipids at 1-year with MASLD at 6-years, including covariates such as HSI value at 1-year and time elapsed between the two time points.

The significance level was defined as p-value < 0.05 (two-tailed) and q-value < 0.20. The statistical analysis was done using MetaboAnalyst 5.0 software (http://www.metaboanalyst.ca/) and R statistical package version v3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

Lipid network and pathways

The Lipid Network Explorer (LINEX) platform and The BioPAN web tool served as tools for visualizing and analyzing the functional relationships of lipids showing a significant association with MASLD in networks, and to explore quantitative lipid data and lipidome metabolic pathways, respectively (full description in Supplementary Data 2).

Results

Individuals' characteristics

The characteristics of 52 PWH at 1-year post-successful HCV treatment are shown in Table 1. Overall, 43 (82.7 %) were male, 27 (56.2 %) were current smokers, and 19 (39.6 %) and 40 (80.0 %) had a prior history of alcohol intake and injection drug use, respectively. The median age was 53, and the BMI was 25.4 kg/m2. Concerning liver markers, the median HSI was 32.4, and the median liver stiffness measurement was 14.1 kPa.

At the six-year follow-up, clinical data were available for 33 PWH, which allowed us to analyze changes in liver damage markers over time. Of these, 20 (60.6 %) showed an increase in HSI from year 1 to year 6. Similarly, among the 27 individuals with LSM measurements at both time points, 8 (29.6 %) experienced an increase in LSM. No significant differences for relevant variables were observed at 1-year post-successful completion of HCV treatment between patients who followed up at 6-years and those who did not (Supplementary Data 3).

Of the 33 PWH with clinical data, 29 PWH had available plasma samples 6-years post-successful HCV treatment for lipidomic analysis. The characteristics of these 29 PWH are shown in Supplementary Data 4. The characteristics of these 29 PWH are shown in Supplementary Data 4**.** No significant differences were found in clinical, epidemiological and virological characteristics of PWH between individuals with and without MASLD 1-year and 6-years after completion of successful HCV treatment, with the exception of BMI and HSI, as these variables are used to define MASLD.

Overall, there was an upward trend in HSI value from 1-year (32.4 (29.6–36.8)) to 6-years (34.8 (29.1–39.1)), although the change was not significant (p = 0.306) because of the variability between individuals (Supplementary Data 5). The prevalence of MASLD also increased from 28.9 % at 1-year to 44.8 % at 6-years, although it was not significant (p = 0.147).

Lipidome detection results

After mass spectrometry (MS) data reprocessing, 480 and 374 features were obtained in electrospray ionization (ESI) positive mode (+) and ESI negative mode (-), respectively. After data combination, normalization, and filtration, a total of 566 distinct plasma lipid species were detected (440 for liquid chromatography-mass spectrometry (LC-MS) ESI (+) and 126 for LC-MS ESI (-)). According to the LIPID MAPS structure database, four main categories of identified lipids were found: 112 glycerolipids (GL, 19.8 %), 286 glycerolphospholipids (GP, 50.6 %), 92 sphingolipids (SP, 16.3 %), and 74 fatty acyls (13.1 %) (Fig. 1A). These main categories were further divided into 17 subclasses, with triglycerides (TG), phosphatidylcholines (PC), sphingomyelins (SM), and fatty acyls having the highest relative abundance, respectively (Fig. 1B).

Characterization of the lipid profile associated with MASLD

One year after HCV therapy

The OPLS-DA models showed that lipids separated individuals according to the presence of MASLD (Supplementary Data 6A), identifying a total of 225 plasma lipids as most importantly related to MASLD (VIP > 1). Of them, significant associations were corroborated by adjusted GLMs for a total of 116 lipids, more than three quarters corresponding to GP (Fig. 1C): 16 decreased and 100 increased lipids in individuals with MASLD, compared with those without MASLD (Fig. 1D and Supplementary Data 7). In short, PC and phosphatidylethanolamines (PE) were the most abundant differential lipids in the GP category, being all increased in individuals with MASLD; in contrast, lysophosphatidylcholines (LPC) and lysophosphatidylethanolamines (LPE) were all decreased. Lipids corresponding to the GL, SP, and fatty acyl main classes increased.

Six-years after HCV therapy

Multivariate analysis identified a total of 167 plasma lipids with a high VIP score (VIP > 1) linked to the presence of MASLD (Supplementary Data 6B). Next, adjusted GLM models corroborated significant associations for 49 lipids (Fig. 1E). Individuals with MASLD had 35 decreased differential lipids and 14 increased lipids compared with individuals without MASLD (Fig. 1F, Supplementary Data 8). In short, at the subclass level, LPCs were the most abundant differential lipids in the GP category, with all of them decreasing in individuals with MASLD. LPE, lysophosphatidylinositols and acyl carnitine species also decreased. TGs in the GL category were mostly increased, with all of them classified as long-chain TGs.

Lipid network and pathways

Global lipid networks for the lipid species significantly associated with MASLD at 1-year and 6-years can be visualized in Fig. 2. At 1-year (Fig. 2A), an extensive part of the network corresponded to PC, and oxidized PC and SM species were interconnected by changes in the chain length and desaturation. The most active reactions were LPC→PC (Z-score=3.65), LPE→ PE→PC (Z-score=2.94) and ether-LPC (LPC-O) →ether-PC (PC-O) (Z-score=2.83) (Supplementary Data 9). However, no significant differences in these lipid species were observed at 6-years (Fig. 2B). A wide representation of the lipid network at 6-years was comprised of the metabolism of diglycerides (DG) and TG, being the most active pathway in the reaction DG→TG (Z-score=2.42) (Supplementary Data 10), leading to the accumulation of TG. Of note, DG and TG species were not differentially found at 1-year.

Fig. 2C-D provide quantitative information regarding alterations in lipid levels between PWH with and without MASLD at 1-year and

Table 1
Clinical, epidemiological, and virological profiles of people with HIV (PWH) one year post-successful HCV treatment, stratified by metabolic dysfunction-associated steatotic liver disease (MASLD) status.

	All individuals	MASLD	Not MASLD	*p*
No.	52	15 (28.9 %)	37 (71.1 %)	
Age (years)	53 [50–55]	52 [49–57]	53 [52–55]	0.350
Gender (male)	43 (82.7 %)	14 (93.3 %)	29 (78.4 %)	0.375
BMI (kg/m^2)	25.4 (22.4–28.2)	29.6 (28.6–31.8)	23.3 (21.2–25.5)	**< 0.001**
Blood pressure (> 130/85 mmHg)	6 (11.5 %)	3 (20.0 %)	3 (8.1 %)	0.461
Smoker (n = 48)	27 (56.2 %)	5 (38.5 %)	22 (62.9 %)	0.235
Alcohol intake (> 50 g/day) (n = 48)				0.520
Never	26 (54.2 %)	7 (53.8 %)	19 (54.3 %)	
Previous (> 6 months)	19 (39.6 %)	6 (46.2 %)	13 (37.1 %)	
Current	3 (6.2 %)	0 (0.0 %)	3 (8.6 %)	
Intravenous drug user (n = 50)				0.999
Never	10 (20.0 %)	3 (21.4 %)	7 (19.4 %)	
Previous (> 6 months)	40 (80.0 %)	11 (78.6 %)	29 (80.6 %)	
Current	0 (0 %)	0 (0 %)	0 (0 %)	
Previous HCV therapy	29 (55.8 %)	8 (53.3 %)	21 (56.8 %)	0.999
Previous AIDS	1 (1.9 %)	0 (0.0 %)	1 (2.7 %)	0.999
Biochemical markers (mg/dL)				
Glucose	98 (92–109)	102 (94–109)	97 (91–102)	0.327
Triglycerides (n = 51)	134 (94–182)	135 (85–186)	131 (97–180)	0.657
Total cholesterol	178 (157–190)	179 (161–188)	176 (158–192)	0.848
HDL cholesterol (n = 49)	40 (33–49)	41 (34–56)	40 (33–47)	0.492
LDL cholesterol (n = 48)	103 (84–124)	101 (85–116)	106 (86–126)	0.803
Liver markers				
HSI	32.4 (29.6–36.8)	38.3 (37.2–42.2)	30.9 (28.4–32.7)	**< 0.001**
LSM (kPa) (n = 45)	14.1 (8.1–19.2)	15.6 (13.6–22.6)	13.9 (8.0–19.2)	0.253
APRI (n = 51)	0.6 (0.4–0.9)	0.6 (0.4–1.0)	0.6 (0.4–0.9)	0.830
Previous HCV markers				
HCV genotype				0.904
1	39 (75.1 %)	12 (80.1 %)	27 (72.9 %)	
3	7 (13.5 %)	1 (6.7 %)	6 (16.2 %)	
4	5 (9.6 %)	2 (13.3 %)	3 (8.1 %)	
Others	1 (1.9 %)	0 (0.0 %)	1 (2.7 %)	
Log_{10} HCV-RNA (IU/mL)	6.3 (5.8–6.7)	6.1 (5.8–6.7)	6.4 (6.0–6.7)	0.378
HCV-RNA > 850.000 IU/mL)	37 (71.2 %)	8 (53.3 %)	29 (78.4 %)	0.142
HCV therapy				0.459
IFN-based	36 (69.2 %)	12 (80.0 %)	24 (64.9 %)	
DAAs	16 (30.8 %)	3 (20.0 %)	13 (35.1 %)	
HIV antiretroviral therapy (n = 49)				0.743
NRTI + NNRTI	17 (34.7 %)	7 (50.0 %)	10 (28.6 %)	
NRTI + II	18 (36.7 %)	4 (28.6 %)	14 (40.0 %)	
NRTI + PI	10 (20.2 %)	2 (14.3 %)	8 (22.8 %)	
Others	4 (8.1 %)	1 (7.1 %)	3 (8.6 %)	

Statistics: Categorical variables are reported as frequencies (percentages), while continuous variables are presented as medians (interquartile range).1 P-values were obtained through the Chi-square test and the Mann-Whitney *U* test.
Abbreviations: AIDS, acquired immune deficiency syndrome; APRI, AST to platelet ratio index; BMI, body mass index; DAAs, direct-acting antivirals; HCV, hepatitis C virus; HCV-RNA, viral load of hepatitis C; HDL, high-density lipoprotein; HSI, hepatic steatosis index; II, HIV integrase inhibitor; IU, international units; kPa, kilopascal; LDL, low-density lipoprotein; LSM, liver stiffness measurement; IFN, interferon; NRTI, nucleoside analogue HIV reverse transcriptase inhibitor; NNRTI, non-nucleoside analogue HIV reverse transcriptase inhibitor; PI, HIV protease inhibitor.

6-years. Higher PC, PE, oxidized PC, and SM species levels in PWH with MASLD were only observed at 1-year. Conversely, PWH with MASLD showed lower levels of LPC, LPE, and oxidized LPC at both the 1-year and 6-years after HCV treatment. The biological interpretation of metabolic alterations occurring in MASLD is shown in Fig. 3.

Plasma lipids persistently associated with MASLD

An association was observed between twelve common plasma lipids at both 1-year and 6-year and the presence of MASLD (Fig. 4A). Specifically, nine LPC (LPC 15:0/0:0, LPC 0:0/17:0, LPC 0:0/18:1, LPC 18:1/0:0, LPC 19:1/0:0, LPC 0:0/20:2, LPC 20:2/0:0, LPC 20:1/0:0, and LPC 17:1/0:0), one LPC-O (LPC O-18:1/0:0) and two LPE (LPE 20:3/0:0, and LPE 18:1/0:0) were inversely associated with MASLD, regardless of the time point post-successful HCV therapy (Fig. 4B, C), with the largest significant associations observed 6-years post-successful HCV treatment. These lipid species were part of the subclasses that exhibited higher VIP scores in the OPLS-DA analysis.

Lipid species predictive of long-term MASLD

Alternatively, we investigated the predictive role of the 12 common lipid species persistently associated with MASLD at 1-year, with long-term MASLD (6-years). Adjusted GLM models showed significant inverse associations of nine lipids (seven LPC, one LPC-O, and one LPE) at 1-year with a higher probability of having MASLD (Fig. 5, Supplementary Data 11).

Discussion

This is the first study describing the plasma lipid profile associated with MASLD in PWH years after achieving SVR. We found that plasma levels of PC, PE, and SM were higher in individuals with MASLD exclusively at 1-year post-successful HCV therapy but were not altered at 6-years. In contrast, fatty acyls increased at 1 year, driven specifically by fatty acids (FA), while at 6 years they decreased, due exclusively to reductions in acyl carnitine species. In addition, an association was observed between lower plasma levels

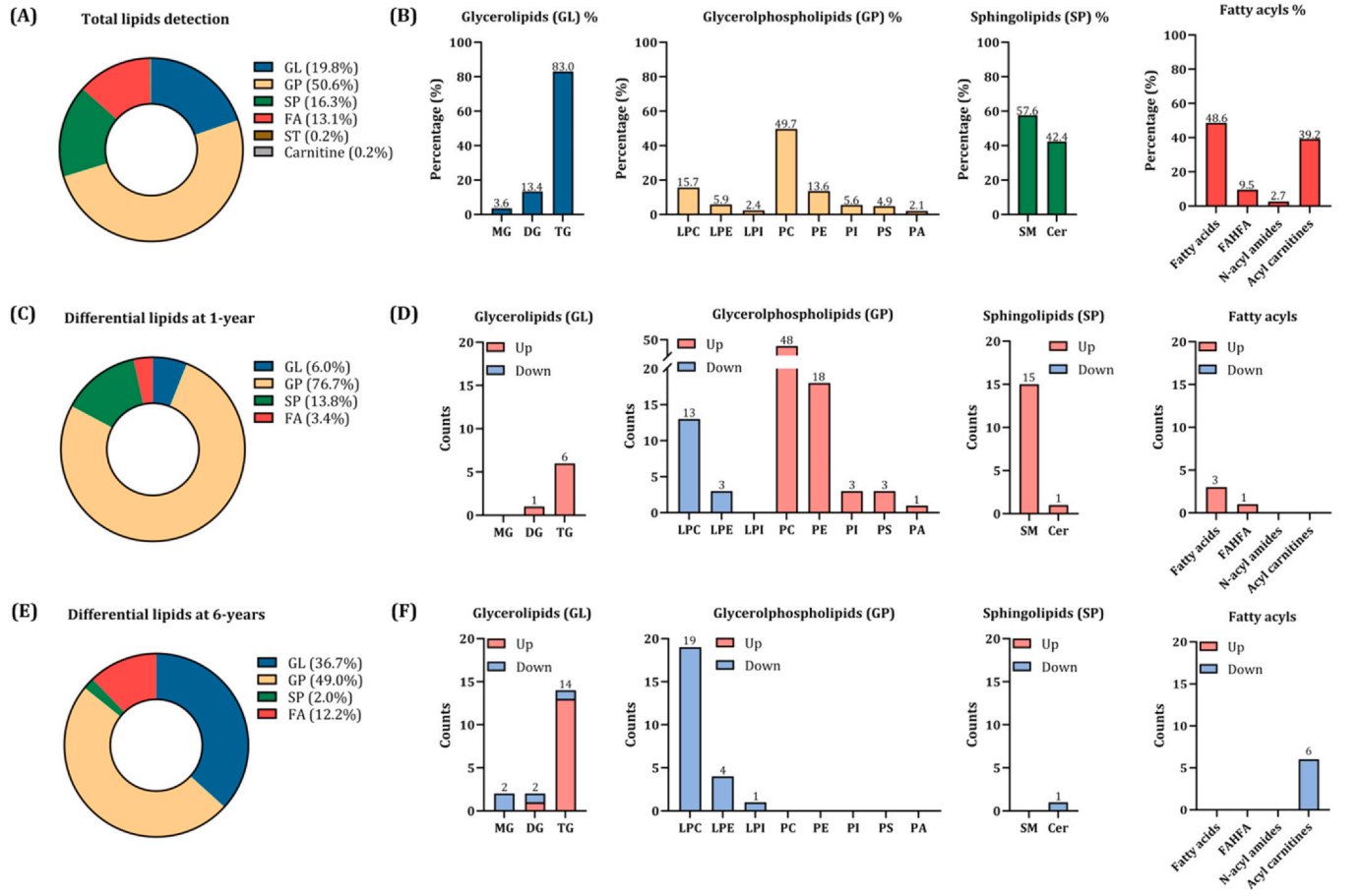

Fig. 1. Lipidomic analysis in PWH years after completion of HCV treatment according to MASLD. A) Proportion of main classes of identified lipids. B) Proportion of lipid subclasses in each main class. C) Proportion of main classes of differential lipids according to MASLD at 1-year. D) Counts of up- or down-regulated differential lipids in MASLD individuals vs without MASLD individuals at 1-year at the subclass level. E) Proportion of main classes of differential lipids according to MASLD at 6-years. F) Counts of up- or down-regulated differential lipids in MASLD individuals vs without MASLD individuals at 6-years at the subclass level. **Abbreviations:** MASLD, metabolic dysfunction-associated steatotic liver disease; PWH, people with HIV. Glycerolipids (GL) are referred to monoglycerides (MG), diglycerides (DG) and triglycerides (TG); glycerolphospholipids (GP) to lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), lysophosphatidylinositols (LPI), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acids (PA); sphingolipids (SP) to sphingomyelins (SM) and ceramides (Cer); and fatty acyls to fatty acids, fatty acyl ester of hydroxy fatty acid (FAHFA), N-acyl amides and Acyl carnitine; sterol lipids (ST).

LPC and LPE, along with higher plasma levels of most TGs, and the presence of MASLD after HCV eradication, regardless of the time elapsed since HCV treatment.

To date, few studies have explored the occurrence of MASLD after HCV eradication, and none in PWH. Huang et al. [19]. reported that HCV eradication mitigates MASLD, showing a lower proportion of affected individuals after treatment. Nevertheless, the difference did not reach statistical significance, and the follow-up period was short, so these findings could not accurately capture the long-term effects of HCV eradication on MASLD. In a study with a longer follow-up, Tokuchi et al. [20]. reported a significant increase in the prevalence of fatty liver 96 weeks after achieving SVR. This aligns with our results, which showed an upward trend in the proportion of PWH with MASLD from the short term (1-year) to the long term (6-years), although not significant, post-successful HCV therapy. This indicates that, as described in the literature, HCV infection could leave an aberrant fingerprint on lipid metabolism that may contribute to more severe pathologies after its eradication [5]. Given the substantial burden of MASLD in these individuals, it is essential to intensify research efforts on this issue and explore existing knowledge gaps, including the underlying metabolic abnormalities.

In our study, PC and PE were the most abundant differential lipids one year post-successful HCV therapy, increasing in PWH with MASLD, while LPC and LPE were all decreased. The potential role of these lipids has been widely studied in MASLD development, and our results align with those found in the literature. Specifically, LPC are biologically active lipids that play a crucial role in hepatic lipotoxicity [21]. They are found to be increased in the liver [22, 23] but decreased in the plasma [24, 25] of individuals with steatosis and seem to correlate with disease severity [26]. The reduction in plasma LPC levels is likely due to increased turnover of LPC to PC by LPC acyltransferase [27], leading to the observed rise in plasma PC levels and reduction in LPC levels. Indeed, the LPC to PC conversion was the most active reaction found 1-year after HCV eradication in our study.

Regarding LPE, their physiological functions have not been fully elucidated, but recent research suggests they are involved in forming hepatic lipid droplets, contributing to steatosis [28]. Several studies have shown that LPE levels are increased in the liver [29] but decreased in plasma [30, 31] in individuals with steatosis compared to healthy individuals. This supports the idea of enhanced uptake of LPC and LPE from plasma into the liver during MASLD, where they are converted into PC and PE, contributing to TG synthesis and fat accumulation. This condition induces the formation of lipid droplets in the liver, lined by a phospholipid monolayer, affecting hepatocyte cells' capacity to respond to insulin, resulting in a whole-body insulin-resistant condition [32].

Besides, plasma levels of FA were higher in individuals with MASLD at 1-year post-successful HCV therapy. Previous studies have described that disrupted FA metabolism, including increased synthesis and reduced β-oxidation in the liver, as well as the

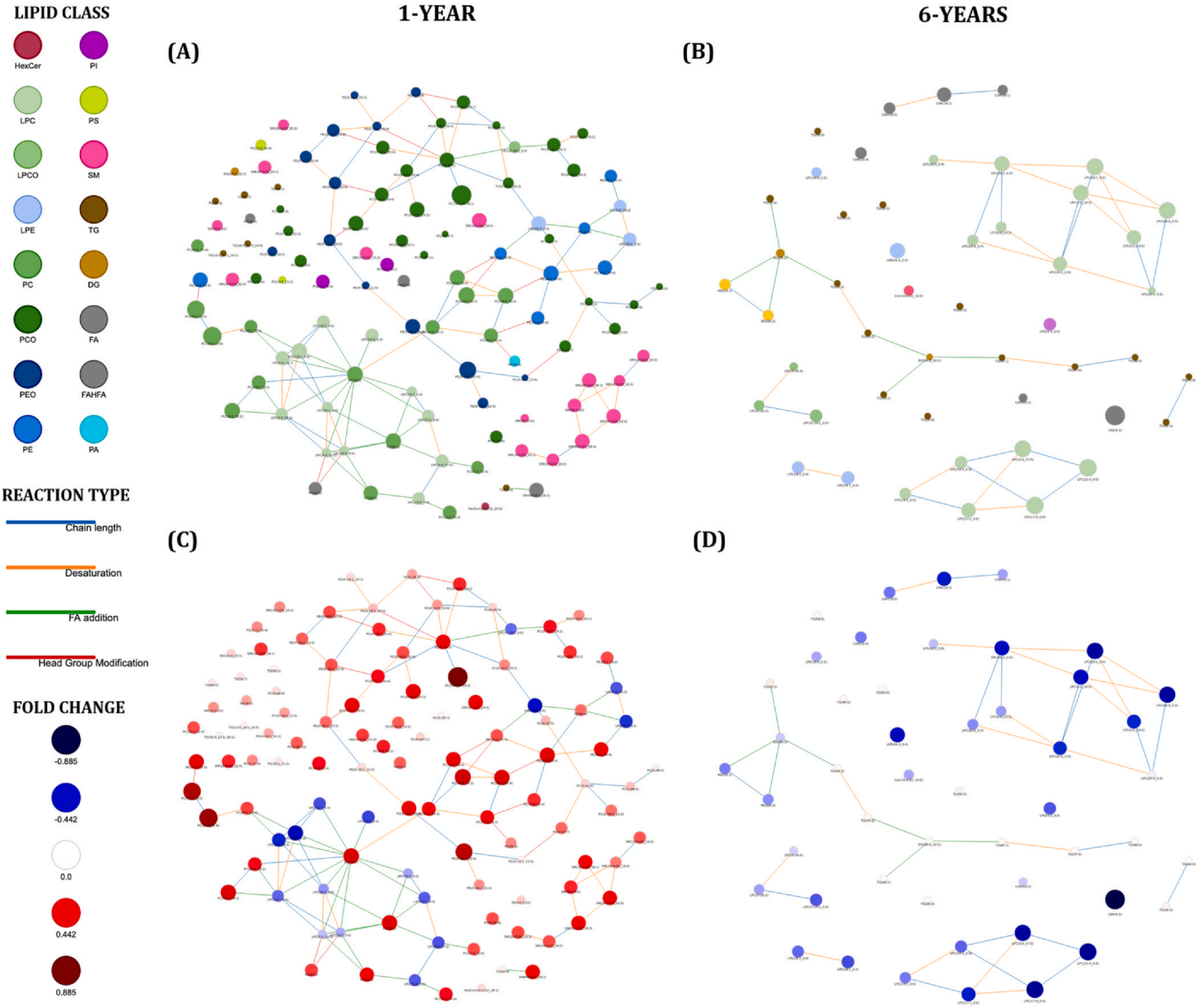

Fig. 2. Lipid network alterations in MASLD vs non-MASLD years after completion of HCV treatment. (A, C) 1-year and (B, D) 6-years after completion of HCV treatment. Nodes in the network represent distinct lipid species, while edges connecting these nodes illustrate a biochemical reaction that allows for the conversion of one lipid to another. Node colours represent the lipid subclass for Fig. 2A-B and the fold change for Fig. 2C-D, with red indicating increased and blue indicating decreased lipid levels in MASLD. Node sizes represent the log fold change between the two conditions, where more strongly altered lipids are displayed as larger nodes. **Abbreviations:** MASLD, metabolic dysfunction-associated steatotic liver disease; PWH, people with HIV.

overproduction of TG, can contribute to the MASLD development in individuals with dyslipidemia [10]. The increased circulating levels of plasma TGs and FAs promote insulin resistance, which activates the novo lipogenesis and the secretion of inflammatory cytokines inside the liver. This fat accumulation exacerbates hepatic lipotoxicity, resulting in oxidative stress and abnormal mitochondrial function, contributing to FA liver collection and activating immune-inflammatory pathways [33]. Similarly, when intracellular FA concentrations increase, endoplasmic reticulum stress occurs, which subsequently activates inflammation and increases reactive oxygen species [7].

As a consequence, an upregulation of the expression of sterol regulatory element binding protein 1c, which is vital for the *de novo* synthesis of FA, has been described as a contributing factor for HCV-associated steatosis [34]. This positive feedback loop supports disease progression, facilitating the development of more severe liver diseases. Alternatively, the accumulation of FA, as a consequence of an alteration in the elongase and desaturase activities, results in aberrant GP synthesis, which would influence the inflammatory and apoptotic processes in these individuals [32].

Concerning SM species, we also observed higher levels in individuals with MASLD in PWH at 1-year. This result is consistent with earlier research indicating that SM levels may serve as biomarkers for monitoring individuals with non-alcoholic steatohepatitis [35].

In the long term, about 6-years after the successful completion of HCV therapy, LPC and LPE remained decreased in PWH with MASLD. As commented before, a generalized reduction in circulating LPC species has been previously linked to more advanced stages of liver disease among PWH individuals with chronic hepatitis C [36], which aligns with our findings. It is also interesting to note that PC, PE, and SM levels were elevated exclusively at 1-year post-HCV eradication in PWH with MASLD but not detected at six years. The absence of significant PC levels at 6-years may be related to the substantial long-term accumulation of TG species, as TG synthesis from DG was the most active pathway at 6-year. PCs can be easily transformed

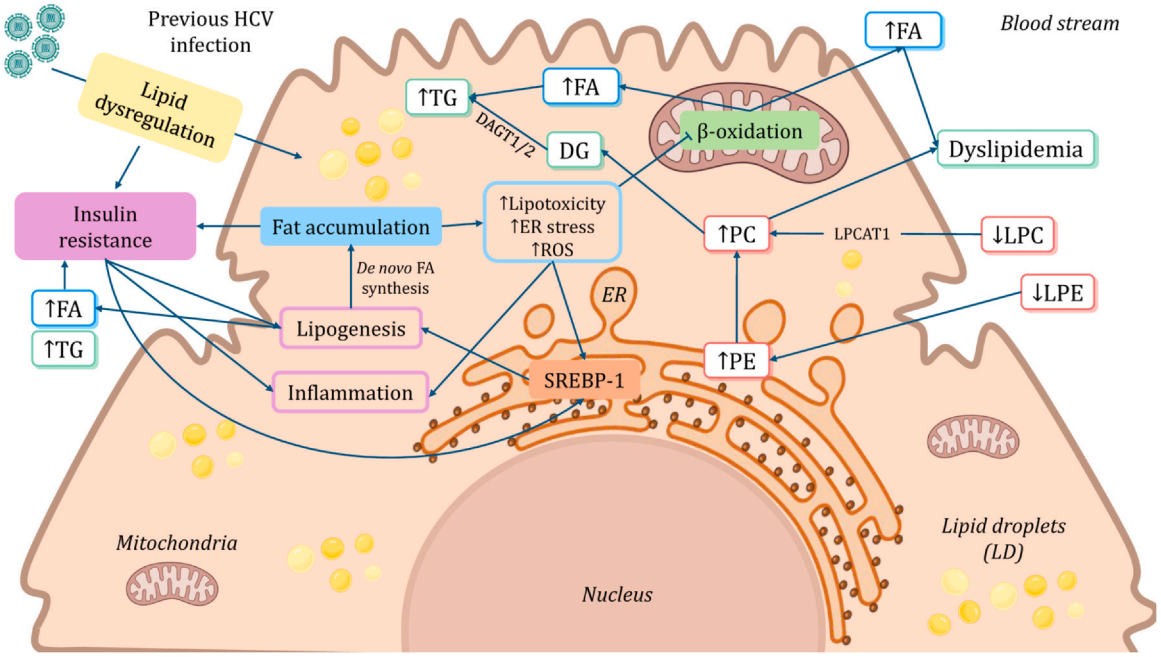

Fig. 3. Biological interpretation of metabolic changes in MASLD. **Abbreviations:** DAGT1/2, diacylglycerol acyltransferase 1/2; DG, diglyceride; ER, endoplasmic reticulum; FA, fatty acids; HCV, hepatitis C virus; HDL, high-density lipoproteins; LPC, lysophosphatidylcholines; LPCAT1, lysophosphatidylcholine acyltransferase 1; LPE, phosphatidylethanolamines; MASLD, metabolic dysfunction-associated steatotic liver disease; PC, phosphatidylcholines; PE, phosphatidylethanolamines; ROS, reactive oxygen species; SREBP-1c, sterol regulatory element binding protein 1c; TG, triglycerides.

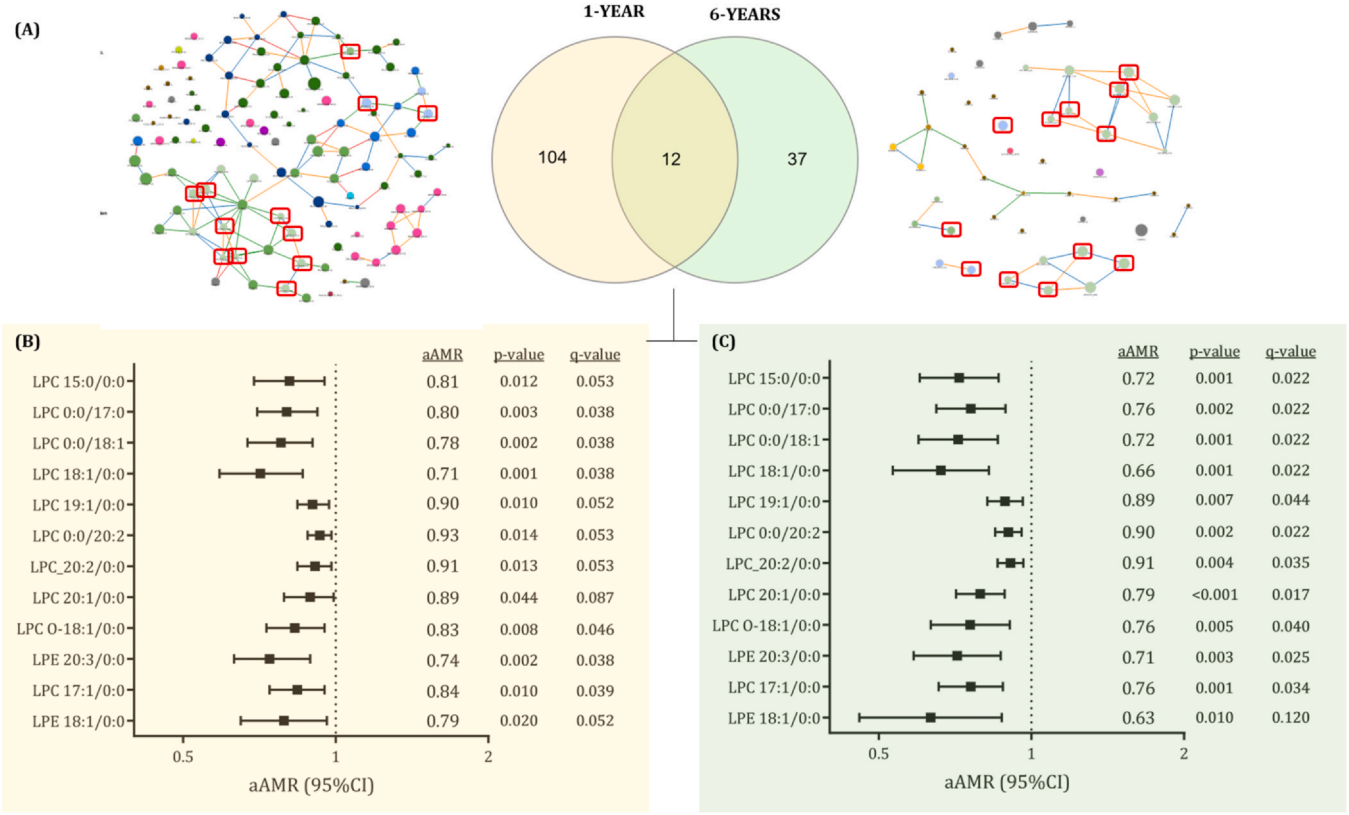

Fig. 4. Plasma lipids associated with MASLD after completion of HCV treatment in PWH. A) Plasma lipids associated with MASLD 1-year and 6-years after completion of successful HCV treatment in PWH. B) Association of common plasma lipids 1-year and 6-years after completion of successful HCV treatment with MASLD in PWH. **Statistics**: Generalized Linear Models (GLM) with a gamma distribution and a gamma distribution (log-link) were used to calculate the data, with adjustments made for gender, age, and the specific HCV treatment (IFN-based or DAAs), as detailed in the Materials and Methods section. The q-values are corrected p-values that account for multiple comparisons using the False Discovery Rate (FDR). **Abbreviations**: MASLD, metabolic dysfunction-associated steatotic liver disease; LPC, lysophosphatidylcholine; LPC O, oxidized lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; aAMR, adjusted arithmetic mean ratio; 95 %CI, 95 % of confidence interval; p, level of significance; q, corrected p-values.

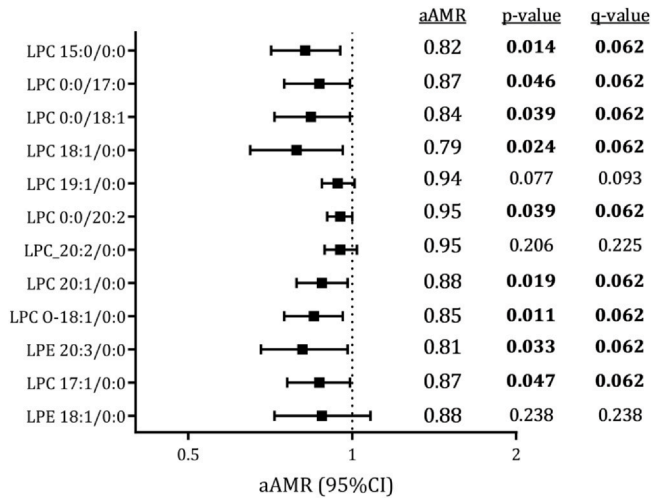

Fig. 5. Association of lipids 1-year after HCV treatment with the presence of long-term MASLD 6-years after treatment in PWH. **Statistics**: Generalized Linear Models (GLM) with a gamma distribution and a gamma distribution (log-link) were used to calculate the data, with adjustments made for gender, age, and the specific HCV treatment (IFN-based or DAAs), as detailed in the Materials and Methods section. The q-values are corrected p-values that account for multiple comparisons using the False Discovery Rate (FDR). Statistically significant differences are shown in bold. **Abbreviations**: MASLD, metabolic dysfunction-associated steatotic liver disease; PWH, people with HIV; LPC, lysophosphatidylcholine; LPC O, oxidized lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; aAMR, adjusted arithmetic mean ratio; 95 %CI, 95 % of confidence interval; p, level of significance; q, corrected p-values.

into TG via an intermediate DG synthesis step followed by the addition of free FA, a reaction catalyzed by diacylglycerol O-acyltransferase 1 (DGAT1) [37].

Recent studies highlight the pivotal role of mitochondrial dysfunction in MASLD pathogenesis, linking impaired free FA β-oxidation, and TG accumulation to persistent inflammation, production of reactive oxygen species (ROS), and the resulting oxidative stress, which contribute to the extensive liver injury and disease progression [33, 38]. Notably, all TG species altered in individuals with MASLD at 6-years were long-chain TGs, which can significantly contribute to liver fat accumulation. Due to their larger structure and higher energy content, long-chain TGs are more likely to be stored as fat instead of being used for energy obtention [39]. In PWH, metabolic dysregulation and chronic inflammation can further impair lipid metabolism, leading to increased retention of these triglycerides in liver tissue, favoring the formation of lipid droplets and insulin resistance, as mentioned above. This excessive fat deposition could increase the risk of long-term MASLD, potentially accelerating inflammation and liver fibrosis over time after HCV eradication in PWH.

In addition, a decreased level of acyl-carnitine species was detected at 6-years. Carnitine and acyl-carnitine species are responsible for long-chain FA translocation into the mitochondria for β-oxidation. A reduction in the levels of these species favors the intracellular long-chain FA accumulation in hepatocytes, which could exacerbate liver steatosis and disease progression, contributing to MASLD development, as observed in our study.

Additionally, in line with our findings, some specific plasma lipid species detected at both 1-year and 6-years have been previously associated with steatosis, such as oxidized phosphatidylethanolamine PE O-38:7 [40] and TG 50:1 [40, 41] at 1-year and DG 32:1, TG 48:1, TG 49:0, TG 49:2, TG 50:0 and TG 53:2 [41] at 6-years, which provides robustness to our study. Additionally, we identified 12 common lipids in the LPC and LPE subclasses that were persistently altered in individuals with MASLD, regardless of the time since HCV treatment. Several of these lipid species have been linked to steatosis in previous studies. Specifically, Flores et al. [24]. found that LPC 15:0, LPC 17:0, and LPC 18:1 were significantly lower in individuals with non-alcoholic fatty liver disease compared to healthy controls. Similarly, Tanaka et al. [27]. reported a correlation between reduced LPC 18:1 levels and increased LPC acyltransferase mRNAs expression in mice, which is concordant with our findings that the LPC to PC conversion by LPC acyltransferase was the most active reaction at 1-year. Additionally, associations were observed between 9 of the 12 common lipid species at 1-year and the presence of MASLD at 6-years, suggesting these specific lipids could be useful as early markers of long-term MASLD in PWH after HCV eradication. However, further studies with larger cohorts would be needed to confirm these results.

Several limitations should be considered for a correct interpretation of the data. First, although the sample size was limited, it did meet the necessary number as determined by MetSizeR, a tool specifically developed for sample size estimation in metabolomic research. Second, different HCV therapies (IFN-based and IFN-free) could have biased the results. However, we meticulously controlled this factor by including it as a covariate in GLM analyses. Third, liver biopsies were unavailable since this technique is avoided in clinical practice. Instead, hepatic steatosis was assessed using the non-invasive HSI score, which has shown excellent diagnostic performance in previous studies. Fourth, the study lacks a control group of HIV-negative individuals, which would help distinguish HIV-specific effects from those related to HCV. Fifth, the use of other indices, such as the Fatty Liver Index (FLI) or Controlled Attenuation Parameter (CAP), would have been valuable to assess the robustness of our findings. However, these were not available. Sixth, we did not stratify our analysis by fibrosis stage. While all patients presented with advanced fibrosis or cirrhosis at baseline, our study was not prospectively designed or powered for this sub-analysis, which was further complicated by the lack of complete LSM data at the 1-year follow-up. Clarifying how the initial degree of fibrosis influences long-term hepatic markers after viral cure requires specifically designed investigations with systematic follow-up.

Conclusions

MASLD was a common condition during follow-up in this cohort, with lipidomic profiles indicating significant changes over time. Increases in PCs and TGs, alongside decreases in LPCs and LPEs, suggest ongoing metabolic disturbances that may contribute to liver disease progression despite SVR. These findings highlight the potential role of lipid dysregulation in the pathogenesis of post-SVR liver disease, emphasizing the need for long-term metabolic and liver health monitoring after HCV eradication in HIV/HCV-coinfected individuals with advanced fibrosis or cirrhosis.

Author contributions

Conceptualization: MAJS. Data curation: BR, AVB, CGR and RME. Formal analysis: AVB, BR, CGR, CB, PMG, RME and MAJS. Funding acquisition: MAJS. Investigation: AVB, BR, CGR, JB, JGG, CD, VH and RME. Methodology: MAJS. Project administration: MAJS. Supervision: MAJS. Visualization: RME and MAJS. Writing – original draft: BR, RME and MAJS. Writing – review and editing: AVB, JB, JGG, CGR, AFR, PMG, CB, and SR. All authors have approved the final article.

Ethics approval

The Research Ethics Committee of the Institute of Health Carlos III approved this study (CEI PI 72_2021) and was conducted following the Declaration of Helsinki. Written informed consent was

obtained from all participants prior to their involvement in the study.

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Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2025.102981.

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