

HCV clearance by direct-acting antivirals reduces serum phosphatidylethanolamine and improves phospholipid balance

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ABSTRACT

Phosphatidylethanolamine (PE) contributes to hepatitis C virus (HCV) replication. Although direct-acting antivirals (DAAs) achieve rapid viral clearance and normalize circulating lipid abnormalities, their effects on the serum PE profile remain incompletely defined. We longitudinally investigated 18 serum PE species during DAA therapy in 178 patients with chronic HCV infection. PE species were quantified using direct-flow injection tandem mass spectrometry on a triple-quadrupole platform. Samples were collected before treatment and at weeks 4 and 12 of DAA therapy. DAA therapy was associated with declines in PE 36:4, PE 38:4, 38:6, 40:5, and 40:6, as well as total serum PE. In parallel, the serum phosphatidylcholine-to-phosphatidylethanolamine (PC/PE) ratio increased. Most PE species, total PE levels, and the PC/PE ratio did not correlate with routine laboratory markers of liver disease or with the Model for End-Stage Liver Disease score. Consistent with this, total PE levels and the PC/PE ratio were similar in patients with and without cirrhosis at the end of treatment. However, cirrhosis was associated with higher PE 36:3 and lower PE 38:4, 38:6, and 40:6. Patients infected with HCV genotype 3a had lower levels of eight PE species and lower total PE, whereas the PC/PE ratio was unchanged. These genotype-associated differences were no longer evident by the end of therapy. In summary, HCV clearance with DAA therapy is associated with marked remodeling of the serum PE profile, characterized by lower total PE levels and an improved PC/PE ratio. These findings suggest circulating phospholipid levels approaching normal after viral eradication.

1. Introduction

After phosphatidylcholine (PC), phosphatidylethanolamine (PE) is the second most abundant phospholipid in mammalian cells and has various functions [1]. The concentration of PC in serum is around 2000 μ M, whereas that of PE is around 40 μ M, indicating a significant difference between the levels of these lipid classes in the circulation [2]. PC and PE are abundant in high-density lipoprotein (HDL), and both phospholipids are also components of low-density lipoprotein (LDL) [2].

Chronic hepatitis C virus (HCV) infection is associated with hepatic and systemic inflammation, and remains a major cause of liver cirrhosis

[3,4]. It is well known that HCV uses host lipids for propagation [5,6] and LDL levels are reduced in patients with HCV [7]. Direct-acting antivirals (DAAs) have emerged as highly effective treatments for HCV elimination, with LDL levels normalizing during therapy [7].

Both PC and PE are involved in lipoprotein metabolism. A reduced hepatic PC/PE ratio results in reduced very-low-density lipoprotein (VLDL) secretion by the liver and lowers HDL levels [1]. It also increases LDL clearance [1] and may therefore be affected in chronic HCV.

Infection of Huh-7.5 cells with HCV provided evidence of increased PC synthesis in these cells, partly through conversion of PE to PC [8]. The enzyme phosphatidylethanolamine *N*-methyltransferase (PEMT)

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catalyzes the methylation of PE to produce PC, and is almost exclusively expressed in the liver [1]. HCV infection increases PEMT, thereby supporting viral replication [9]. Conversely, viral replication decreases upon PEMT knockdown [9]. Higher PEMT levels also contribute to liver steatosis, and their further increase leads to greater liver fat accumulation in patients infected with HCV genotype 3 than in those infected with genotype 1 [9,10]. However, at least serum PC species levels did not correlate with viral load, and 13 PC species were reduced in patients infected with genotype 3a compared with those infected with genotype 1 [11].

PEMT expression in the livers of patients with HCV was much higher than in those with HBV, who also commonly develop liver steatosis [9,12]. However, analysis of liver tissue from HCV patients revealed lower levels of both PE and PC in HCV patients than in HBV patients [13]. The PC/PE ratio, an indicator of liver health ranging between 1.5 and 2.0 in a healthy liver [1], was normal in the livers of patients with HBV and at the upper end of the range in the livers of patients with HCV [13].

Patients with liver cirrhosis mostly have a low hepatic PC/PE ratio. This is associated with impaired lipoprotein secretion, larger lipid droplet size, and endoplasmic reticulum stress [1]. The association of the serum PC/PE ratio with liver disease severity has received less attention. The serum PC/PE ratio decreased in mice that consumed alcohol, suggesting a correlation with liver injury [14]. A reduced PC/PE ratio has also been linked to a heightened risk of non-alcoholic steatohepatitis in patients with hereditary hemochromatosis [15]. Patients with HCV and cirrhosis had higher serum levels of PC 30:0 and 32:0 before and at the end of DAA therapy, whereas PC species containing polyunsaturated fatty acids decreased in cirrhosis [11]. In alcoholic liver disease, PC and PE levels in serum were not associated with cirrhosis [16].

The fatty acid and lipid composition of red blood cells is altered in patients with HCV [17]. A pilot study observed an increase in the PC/PE ratio due to a decline in PE levels in red blood cells following antiviral treatment, accompanied by reduced aminotransferase levels [18]. However, despite the effective elimination of HCV, the concentration of total PC in serum did not change significantly, and the levels of four of the 27 analyzed PC species were slightly higher at the end of therapy [11]. To our knowledge, serum PE levels in HCV patients have not been measured before and after viral cure, and it is unclear whether the PC/PE ratio in serum also improves.

Based on current studies, it is unclear whether liver cirrhosis, HCV infection, or both are associated with altered serum PE profiles and changes in the serum PC/PE ratio. This study aimed to longitudinally characterize 18 serum PE species and the PC/PE ratio in patients with chronic HCV infection before and during DAA therapy, and to determine whether these measures are associated with cirrhosis status and change following HCV clearance.

2. Materials and methods

2.1. Patients and sample collection

Between October 2014 and September 2019, serum samples were collected from adult patients with chronic HCV infection treated at the Department of Internal Medicine I, University Hospital Regensburg. All patients were treatment-naïve for HCV and met eligibility criteria for DAA therapy [19]. DAA regimens included sofosbuvir/velpatasvir, glecaprevir/pibrentasvir, sofosbuvir/daclatasvir, sofosbuvir/ledipasvir and elbasvir/grazoprevir. Patients with HIV or HBV co-infection and those with decompensated cirrhosis were excluded. Serum samples were analyzed before treatment initiation, at 4 weeks after therapy started, and at week 12 after starting DAA therapy, which is the end of treatment.

Because many patients were enrolled shortly after DAAs entered clinical practice, concomitant statin therapy was temporarily

discontinued during antiviral treatment, as potential adverse effects and drug–drug interactions had not yet been excluded [20].

Liver cirrhosis was diagnosed by ultrasonography on the basis of a nodular liver surface, reduced liver size, and coarse liver parenchymal echotexture. Laboratory parameters were measured at the Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg.

2.2. Quantification of PE species

Lipids were extracted from 10 µl of serum samples using the Bligh and Dyer method [21]. For quantification, PE 14:0/14:0 and PE 20:0/20:0 (diphytanoyl; Avanti Polar Lipids, AL, USA) were added prior to lipid extraction. The total volume of the chloroform phase was 2 ml, and 0.5 ml of the recovered chloroform phase was vacuum-dried and subsequently dissolved in a methanol (Merck, Darmstadt, Germany)/chloroform (Roth, Karlsruhe, Germany) mixture 3:1 (v/v), which contained 7.5 mM ammonium acetate [22]. Lipid analysis was conducted using direct flow injection analysis (FIA) coupled with a triple quadrupole mass spectrometer (FIA-MS/MS, QQQ) in positive ion mode. A neutral loss of 141 characteristic for PE was applied [23]. Lipid species were annotated according to the recently published guidelines for shorthand notation of lipid structures derived from mass spectrometry using the assumption that only diacyl species are present [24].

2.3. Statistical analysis

The Shapiro–Wilk test revealed that the PE species were not normally distributed ($p < 0.001$ for all). Therefore, the levels of the PE species are presented as box plots in the figures, with the median, minimum, and maximum values listed in the tables. The Mann–Whitney U test, the receiver operating characteristic curve, the Kruskal–Wallis test, and the repeated-measures ANCOVA were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA, 2019). Values were adjusted for multiple comparisons (p -values were multiplied by 18, the number of PE species analyzed), with p -values < 0.05 considered significant.

3. Results

3.1. PE species of HCV patients with liver cirrhosis

This study compared serum PE species levels in patients with chronic HCV before treatment with DAAs and at the end of therapy. Because it did not include healthy controls, the serum lipids of the patients and controls could not be compared. The study involved 178 patients with chronic HCV, 40 of whom were diagnosed with liver cirrhosis using ultrasound. Specifically, increased levels of PEs 34:2, 34:3, 36:1, and 36:3 were found in patients with cirrhosis (Table 1). Conversely, the levels of PEs 38:1, 38:4, 38:6, and 40:6 were significantly lower in this patient group (Table 1). Overall, total PE levels and the PC/PE ratio in the serum of patients with and without cirrhosis were comparable (Table 1).

PE 36:3 had an area under the receiver operating characteristic curve (AUROC) of 0.723 ± 0.048 ($p < 0.001$) to diagnose liver cirrhosis. A value of 1.9 nmol/ml had a sensitivity of 52% and a specificity of 88% to diagnose liver cirrhosis. The AUROCs of other species were lower and are not listed here.

The 40 patients with liver cirrhosis were excluded from further analysis due to the association of liver cirrhosis with altered levels of eight PE species. Table 2 provides a summary of the patients' characteristics with chronic HCV before treatment and at therapy end who did not have liver cirrhosis. Aminotransferase levels, procalcitonin, and viral load declined, and low-density lipoprotein (LDL) increased during treatment (Table 2).

Table 1

Median, minimum, and maximum serum PE species levels of patients with HCV before therapy. Data for patients with and without liver cirrhosis are shown. Not significant, ns; ↑ higher in cirrhosis, ↓ lower in cirrhosis.

PE nmol/ml	HCV without cirrhosis			HCV with cirrhosis			p-Value	Cirrhosis/no cirrhosis
	Median	Minimum	Maximum	Median	Minimum	Maximum		
32:1	0.09	0.02	0.54	0.11	0.04	0.32	ns	
34:1	0.82	0.12	3.23	1.02	0.43	2.44	ns	
34:2	1.69	0.43	7.02	2.21	0.93	4.97	<0.05	↑
34:3	0.10	0.04	0.47	0.13	0.05	0.29	<0.05	↑
36:1	0.68	0.16	4.59	0.91	0.32	2.54	<0.001	↑
36:2	3.51	1.34	18.93	4.74	1.65	10.67	ns	
36:3	1.12	0.33	6.08	1.97	0.51	4.40	<0.001	↑
36:4	1.57	0.50	4.56	1.43	0.72	3.42	ns	
36:5	0.12	0.05	0.35	0.12	0.06	0.29	ns	
38:1	0.25	0.10	0.55	0.22	0.07	0.38	<0.05	↓
38:2	0.23	0.11	0.70	0.23	0.14	0.43	ns	
38:3	1.07	0.28	4.15	1.09	0.52	2.63	ns	
38:4	9.02	2.37	28.16	6.69	2.92	16.50	<0.05	↓
38:5	2.78	0.85	6.83	2.49	1.21	5.71	ns	
38:6	4.55	0.53	16.35	2.80	0.90	10.53	<0.001	↓
40:4	0.31	0.10	0.88	0.28	0.12	0.73	ns	
40:5	0.96	0.17	2.86	0.72	0.20	1.85	ns	
40:6	3.24	0.34	15.13	1.83	0.52	8.56	<0.001	↓
Total PE	32.27	10.55	97.16	30.23	14.97	65.24	ns	
PC/PE	50.53	20.93	109.64	49.37	22.49	103.08	ns	

Table 2

Laboratory parameters of the patients before and at the end of antiviral therapy, excluding patients with liver cirrhosis (model of end-stage liver disease (MELD), not significant (ns)). The serum of two patients was unavailable at the end of therapy.

Laboratory parameter	Baseline (138 patients)	12 weeks of therapy (136 patients)	p-Value
Age, years	51 (24–82)	51 (24–82)	ns
Female/male	58/80	58/78	ns
Body mass index, kg/m ²	25.5 (17.6–40.4)	25.6 (17.6–40.4)	ns
MELD score	7 (6–21)	7 (6–20)	ns
Alanine aminotransferase, U/l	71 (19–305)	26 (6–388)	<0.001
Aspartate aminotransferase, U/l	44 (14–1230)	19 (6–836)	<0.001
Bilirubin, mg/dl	0.6 (0–1.7)	0.5 (0.2–1.8)	ns
International normalized ratio	1.0 (0.8–1.4)	1.0 (0.9–1.4)	ns
Creatinine, mg/dl	0.8 (0.5–14.0)	0.8 (0.5–14.7)	ns
Leukocytes, n/l	6.8 (2.2–72.4)	7.2 (2.6–62.9)	ns
C-reactive protein, mg/l	2.9 (2.8–55.0)	2.9 (2.9–20.3)	ns
Procalcitonin, ng/ml	0.06 (0–10.07)	0.03 (0.01–0.60)	0.03
Albumin, g/l	39 (29–46)	40 (32–93)	ns
High-density lipoprotein, mg/dl	53 (19–111)	50 (23–89)	ns
Low-density lipoprotein, mg/dl	100 (23–219)	128 (49–251)	<0.001
Viral Load	12 × 10 ⁵ (23–25 × 10 ⁶)	1 (1–29)	<0.001

3.2. Correlation of PE species with routine measures of inflammation, the MELD score, and parameters of liver disease

Total PE levels, the PC/PE ratio, and PE species were not associated with bilirubin, the international normalized ratio (INR), or creatinine, nor were they related to the MELD score (Table 3). There were also no significant associations with albumin or alanine aminotransferase (ALT). PE 34:1, 34:2, 36:1, 36:2, 36:3, 36:4, 38:3, 38:5, 38:6, 40:5, and 40:6, as well as total PE levels, positively correlated with aspartate aminotransferase (AST), which was negatively associated with the PC/PE ratio. PE 38:1 positively correlated with LDL, while PE 34:1 and 36:1 negatively correlated with HDL (Table 3). The PC/PE ratio was also negatively associated with HDL. Total PE levels, the PC/PE ratio, and individual PE species were not associated with leukocyte count, C-reactive protein, or procalcitonin ($p > 0.05$ for all).

3.3. Associations of PE species with viral genotype and correlation with viral load

Of the patients without liver cirrhosis, 43 were infected with genotype 1a, 53 with genotype 1b, 29 with genotype 3a, and 13 with less common genotypes of HCV, which have been categorized together.

PE 34:3, 36:4, 36:5, 38:3, 38:5, 40:4, 40:5, and total PE levels were lower in genotype 3a patients than in genotype 1a patients (Fig. 1a, b). PE 38:4 was lower in genotype 3a patients than in patients with genotypes 1a or 1b (Fig. 1a). HCV genotype 3a had a PC/PE ratio comparable to all of the other groups ($p > 0.05$).

The levels of serum PE species, total PE levels, and the PC/PE ratio were not correlated with viral load ($p > 0.05$ for all).

3.4. PE species at therapy end

At 12 weeks after the start of DAA therapy, the patients had lower levels of aminotransferases and procalcitonin. LDL was significantly increased (Table 2), consistent with previous reports [7,25–28].

At 4 and 12 weeks after therapy started, PE 36:4, 38:4, and 38:6 were reduced compared with pre-therapy levels (Fig. 2a). PE 40:5 and 40:6 were reduced at the end of therapy compared with levels before treatment (Fig. 2a).

To test whether changes in these PE species relate to changes in LDL, we performed a repeated-measures ANCOVA using data from before treatment and at the end of therapy, adjusting for changes in LDL. Changes in PE 36:4, 38:4, and 40:5 levels were independent of LDL changes ($p < 0.001$ for all). Decreases in PE 38:6 and 40:6 were

Table 3

Correlation of PE species with the model of end-stage liver disease (MELD) score, alanine aminotransferase (ALT), aspartate aminotransferase (AST), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and leukocyte count.

PE nmol/ ml	MELD score	ALT U/l	AST U/l	LDL mg/dl	HDL mg/dl	Leukocytes n/l
32:1	0.083	0.076	0.240	0.198	-0.073	0.034
34:1	0.094	0.164	0.307 ^b	0.082	-0.267 ^a	0.108
34:2	0.070	0.082	0.277 ^a	0.108	-0.115	-0.006
34:3	-0.017	-0.056	0.123	0.110	0.015	-0.070
36:1	0.021	0.114	0.277 ^a	-0.023	-0.283 ^a	0.063
36:2	0.036	0.078	0.267 ^a	0.064	-0.201	-0.014
36:3	0.030	0.049	0.258 ^a	-0.034	-0.120	-0.070
36:4	0.071	0.098	0.264 ^a	0.113	-0.095	-0.006
36:5	-0.048	-0.027	0.119	0.089	0.136	-0.072
38:1	-0.072	-0.070	0.024	0.286 ^a	0.256	-0.178
38:2	-0.079	0.090	0.149	0.117	0.027	-0.070
38:3	0.034	0.123	0.277 ^a	0.195	-0.156	0.022
38:4	0.046	0.086	0.230	0.142	-0.119	0.004
38:5	0.050	0.079	0.256 ^a	0.088	-0.001	-0.041
38:6	0.059	0.150	0.301 ^b	0.131	0.075	-0.096
40:4	0.014	0.114	0.233	0.151	-0.205	-0.026
40:5	0.074	0.151	0.296 ^b	0.140	-0.125	-0.007
40:6	0.074	0.162	0.308 ^b	0.185	-0.057	-0.081
Total	0.065	0.118	0.298 ^b	0.140	-0.113	-0.033
PE						
PC/PE	-0.148	-0.132	-0.285 ^a	0.060	0.278 ^a	-0.030

^a $p < 0.05$.

^b $p < 0.01$.

associated with changes in LDL levels and were not significant after adjusting for LDL changes ($p > 0.05$).

Total PE levels were lower at 4 and 12 weeks after starting therapy than at pretreatment (Fig. 2b). Serum total PC levels did not change significantly during treatment [11], and the serum PC/PE ratio was significantly increased at 4 weeks after the start of therapy and at the end of therapy (Fig. 2c).

In patients with liver cirrhosis, the levels of all PE species, total PE, and the PC/PE ratio before and at the end of therapy were similar ($p > 0.05$ for all comparisons).

At the end of therapy, total PE levels, the PC/PE ratio, and PE species did not correlate with the MELD score, bilirubin, INR, creatinine, ALT, or AST. PE species were not associated with C-reactive protein, procalcitonin, or leukocyte count. Total PE levels, the PC/PE ratio, and the PE species did not correlate with HDL. PE 36:2, 38:1, 38:2, 38:3, 38:4, 40:4, 40:5, 40:6, and total PE levels positively correlated with LDL (Table 4).

The genotype 3a-associated changes disappeared by the end of

therapy ($p > 0.05$ for all comparisons).

By the end of the therapy, the PE 36:3 level ($p = 0.017$) was increased in patients with liver cirrhosis, whereas the PE 38:4 ($p = 0.004$), 38:6 ($p = 0.003$), and 40:6 ($p < 0.001$) levels were decreased compared with patients without liver cirrhosis. However, the AUROCs of these PE species were all < 0.7 , making them unsuitable for diagnosing cirrhosis. Total PE levels and the PC/PE ratio were similar in patients with and without cirrhosis ($p > 0.05$).

Liver cirrhosis was characterized by higher levels of PE species with 32, 34, and 36 carbon atoms and 1, 2, or 3 double bonds, relative to total PE levels (Fig. 3). The relative levels of PEs with 38 or 40 carbon atoms and four or six double bonds declined in cirrhosis (Fig. 3).

3.5. Effect of age, sex, and body mass index

Age, sex, and body mass index may confound analyses of serum lipids. PE 34:3 levels were higher in women than in men before therapy ($p = 0.010$), but not at the end of therapy.

PE 32:1, 34:1, 34:2, 36:4, 38:3, 38:4, 35:5, 38:6, 40:5, 40:6, and total PE positively correlated with age before treatment. At the end of therapy, PE 32:1, 34:3, and 36:5 positively correlated with age (Table 5).

PE 34:1, 36:1, 38:3, 38:4, 40:4, and total PE levels positively correlated with body mass index before therapy started and at the end of therapy, only PE 36:1 was positively correlated with body mass index (Table 5).

4. Discussion

DAA-mediated HCV clearance was associated with a marked reduction in total serum PE, largely attributable to declines in PE species containing four to six double bonds. By contrast, serum PC levels remained largely unchanged during treatment [10], thereby increasing the PC/PE ratio. As this ratio did not differ between patients with and without cirrhosis before or after therapy, it does not appear to reflect liver disease severity in chronic HCV infection. These data, therefore, highlight serum PE remodeling as a novel lipid response to HCV eradication.

In liver cirrhosis, polyunsaturated PEs are reduced, and shorter-chain PE species are increased, indicating a grossly altered PE profile. The serum PC/PE ratio did not change in cirrhosis but improved during viral cure. Because these lipid species did not correlate significantly with measures of liver injury or inflammation markers, we suggest that HCV infection, rather than liver disease caused by this virus, induced these changes in the PE profile.

The decline of specific polyunsaturated PEs at therapy end raises the

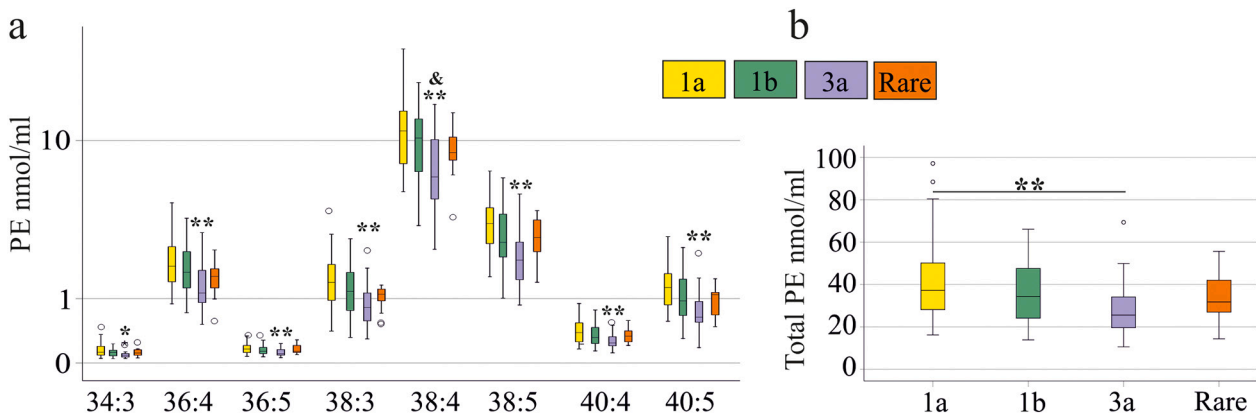


Fig. 1. Levels of serum PE species, which decline in HCV genotype 3a. (a) PE species of patients infected with genotype 1a (yellow boxes), 1b (green boxes), 3a (purple boxes) or less common variants, which are grouped together (orange boxes); (b) total PE levels of the patients described in a. * $p < 0.05$, ** $p < 0.01$ for comparison of 3a to 1a, & $p < 0.05$ for comparison of 3a to 1b. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

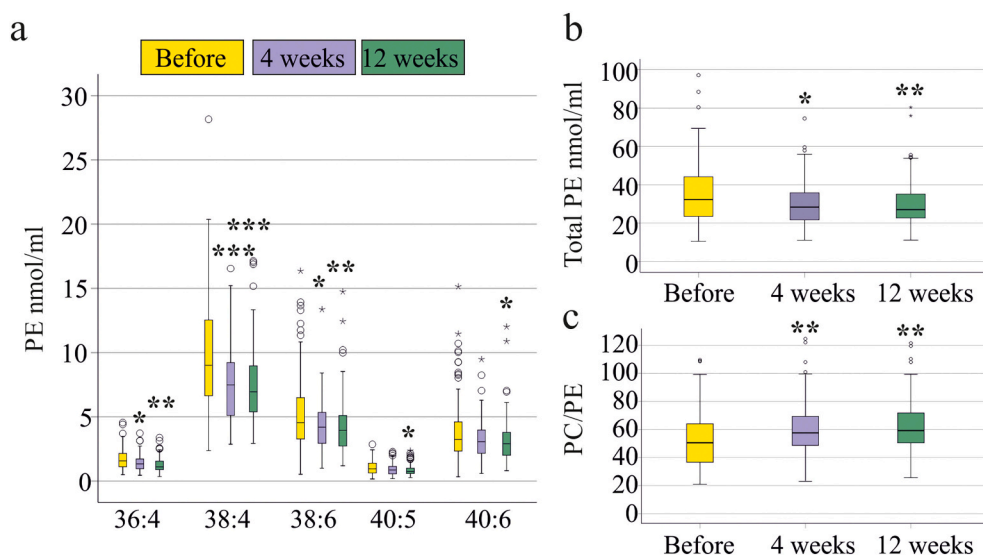


Fig. 2. PE species in serum, which decline during therapy with direct-acting antivirals. (a) PE species levels before (yellow boxes), at 4 weeks (purple boxes), and at 12 weeks (green boxes) after the start of therapy are shown; (b) total PE levels in serum before and during therapy; (c) the serum PC/PE ratio before and during treatment. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to levels before treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4

Correlation of PE species, total PE, and the PC/PE ratio with low-density lipoprotein at the end of therapy.

PE nmol/ml	Low-density lipoprotein
32:1	0.242
34:1	0.223
34:2	0.222
34:3	0.184
36:1	0.253
36:2	0.283 ^a
36:3	0.217
36:4	0.191
36:5	0.219
38:1	0.397 ^c
38:2	0.446 ^c
38:3	0.387 ^c
38:4	0.317 ^b
38:5	0.214
38:6	0.200
40:4	0.308 ^b
40:5	0.327 ^b
40:6	0.318 ^b
Total PE	0.336 ^b
PC/PE	-0.015

^a $p < 0.05$.

^b $p < 0.01$.

^c $p < 0.001$.

question of how this works. Polyunsaturated phospholipid levels do not consistently decline after viral cure. Polyunsaturated PC species were modestly higher after DAA treatment, although the fatty acid composition of the PC species that increased differed from that of the PE species that declined at therapy end. In addition, saturated, unsaturated, and polyunsaturated Lyso-PC species were modestly higher after viral cure [11,29]. Among phosphatidylinositol species, 36:3, 36:4, 38:3, 38:4, and 38:5 increased, whereas 40:5 and 40:6 species declined at the end of therapy [30].

In HCV-infected HepG2 cells, levels of the phospholipids PC, PE, and phosphatidylinositol are increased [31]. This study provided evidence that fatty acid desaturase 2, the rate-limiting enzyme in polyunsaturated fatty acid synthesis, may contribute to higher unsaturated fatty acids in

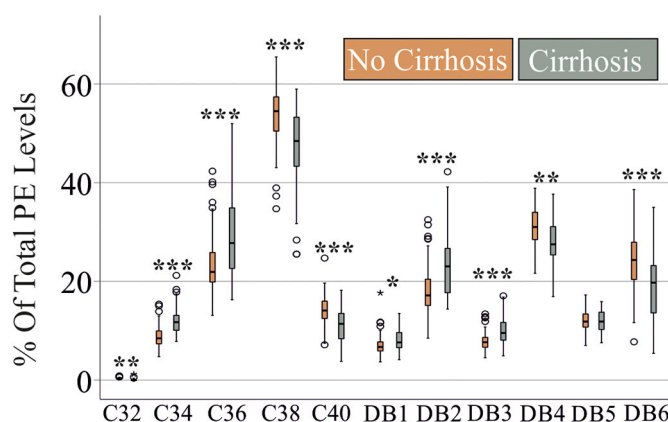


Fig. 3. PE species with an identical number of carbon atoms (C32, C34, C36, C38, C40) or with an identical number of double bonds (DB1–DB6) relative to total PE levels (%) are shown. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

HCV-infected cells, which is essential for the HCV lifecycle [31]. From this study, one might conclude that polyunsaturated lipids are increased in HCV and decline after viral cure. However, higher availability of PUFA lipids will not only affect PUFA-PE levels but may also change, for example, PUFA-PC levels [32]. Moreover, as discussed above, PUFA-phospholipid levels do not consistently decline after viral cure [11,29].

Another possibility is that HCV particles are enriched in polyunsaturated PEs and that effective antiviral therapy therefore lowers their serum levels. However, the PE content of HCV particles is comparatively low [33], making it unlikely that depletion of virion-associated PE accounts for the decline in serum PE levels.

PE can be acylated to form Lyso-PE, a lipid class not measured in our analysis, however, these mechanism is not specific to individual PE species [34]. Similarly, phosphatidylserine decarboxylation to PE occurs in mitochondria, but there is no evidence that these pathways are affected by HCV infection [34]. Thus, none of these pathways provides an obvious mechanism that specifically explains alterations in polyunsaturated PE metabolism.

Polyunsaturated PE-specific mechanisms have been described in the context of ferroptosis, a form of programmed cell death driven by the

Table 5

Correlation of PE species, total PE levels, and the PC/PE ratio with age and body mass index (BMI) before treatment and at the end of therapy.

PE nmol/ ml	Age (before therapy)	BMI (before therapy)	Age (therapy end)	BMI (therapy end)
32:1	0.270 ^a	0.143	0.410 ^c	0.127
34:1	0.261 ^a	0.298 ^a	0.238	0.168
34:2	0.271 ^a	0.234	0.255	0.118
34:3	0.230	0.144	0.333 ^b	0.107
36:1	0.120	0.310 ^a	0.045	0.264 ^b
36:2	0.218	0.269	0.142	0.198
36:3	0.199	0.167	0.171	0.068
36:4	0.284 ^a	0.258	0.241	0.115
36:5	0.253	0.030	0.310 ^b	0.059
38:1	0.052	0.156	0.009	0.070
38:2	0.067	0.137	0.046	−0.015
38:3	0.272 ^a	0.286 ^a	0.242	0.233
38:4	0.276 ^a	0.318 ^b	0.153	0.260
38:5	0.292 ^b	0.167	0.237	0.064
38:6	0.269 ^a	0.131	0.223	−0.003
40:4	0.191	0.345 ^b	0.216	0.231
40:5	0.265 ^a	0.263	0.200	0.206
40:6	0.273 ^a	0.272	0.224	0.191
Total PE	0.298 ^a	0.294 ^a	0.214	0.188
PC/PE	−0.241	−0.238	0.100	−0.160

^a $p < 0.05$.

^b $p < 0.01$.

^c $p < 0.001$.

oxygenation of polyunsaturated phospholipids. When complexed with PE-binding protein 1, 15-lipoxygenases shift their substrate specificity toward polyunsaturated PE species, generating hydroperoxy-PE species [35]. Yamane et al. showed that ferroptosis suppressed HCV replication [36], and higher polyunsaturated PE levels may protect from HCV. However, ferroptosis can also contribute to chronic liver disease and fibrosis [37]. Whether 15-lipoxygenase-mediated oxygenation of PE contributes to the changes in polyunsaturated PE levels during viral cure remains unknown. Moreover, our study cannot prove that the specific PE species that decline after viral cure are higher in HCV infection because we did not include healthy controls.

Liver cirrhosis is associated with low serum lipid levels, including reduced cholesterol, triglycerides, and lysophosphatidylcholines [38,39]. In patients with HCV cirrhosis, PC levels increased for species with 0, 1, and 2 double bonds and decreased for species with 4 to 6 double bonds [11]. Accordingly, levels of polyunsaturated PEs with 1–3 double bonds increased, and those with 4–6 double bonds declined, showing a shift toward more saturated phospholipids in cirrhosis. Changes in phospholipid saturation can profoundly affect cell membrane function. Membranes that become excessively rigid or overly fluid may hinder protein localization and function [40].

Total PE levels, along with ten individual PE species, were positively correlated with AST levels in non-cirrhotic patients with HCV. These lipid levels were not associated with other indicators of liver disease severity, such as MELD score or ALT, suggesting that they are not directly linked to hepatic functional status. While ALT is relatively specific for hepatocellular injury, AST is less specific and may also reflect damage to extrahepatic tissues, including cardiac and skeletal muscle [41]. Elevated AST levels have been reported in hematologic disorders, skeletal muscle injury, and cardiac damage [42], as well as in metabolic syndrome [43].

Chronic HCV infection is associated with a range of extrahepatic manifestations, including sarcopenia, insulin resistance, and neurocognitive impairment, some of which partially resolve following viral eradication [44,45]. However, the relationship between circulating PE levels and skeletal muscle mass or insulin resistance remains unclear [46]. Certain PE species—such as PE 16:0/16:1, PE 18:0/20:5, and PE 18:0/22:4—have been linked to adverse cardiovascular outcomes [47],

yet broader PE classes containing these species (e.g., PE 32:1, 38:5, and 40:4) did not change in our cohort following viral eradication. Additionally, 16 of the 20 measured PE species were elevated in diabetic patients [48], although it remains unknown whether reductions in specific PE species contribute to improved insulin sensitivity after DAA therapy.

This observational study cannot determine the pathophysiological significance of the observed positive correlations between PE species and AST in chronic HCV, which are no longer present after viral clearance. Both AST levels and PE species declined by the end of therapy. Elevated AST is commonly associated with systemic inflammation, which markedly improves following DAA treatment [26,49]. Nevertheless, the mechanisms driving the reduction in specific PE species during viral eradication remain unclear.

PC and PE are the most prevalent phospholipids in mammalian cell membranes, and both high and low PC/PE ratios in liver tissue are associated with chronic liver disease [1]. However, the serum PC/PE ratio has received less attention. Mice deficient in PEMT, which produces around 30% of hepatic PC [1,50], had a lower PC/PE ratio in VLDL and whole plasma [51], showing that hepatic synthesis greatly affects circulating levels. The PC/PE ratio in serum decreased in mice that consumed alcohol, indicating an association with liver disease [14]. A low PC/PE ratio in plasma has been associated with an increased risk of non-alcoholic steatohepatitis in patients with hereditary hemochromatosis [15]. However, the serum PC/PE ratio of HCV patients with and without cirrhosis was comparable both before and after therapy. Consistently, this ratio did not correlate with the MELD score or aminotransferase levels. Therefore, serum PC and PE levels decrease in parallel in patients with HCV-induced cirrhosis, and their ratio is not a suitable marker of liver disease severity in these patients.

Notably, the PC/PE ratio of HCV patients increased at the end of therapy compared to pretreatment levels. This is due to a decline in total PE levels, while total PC levels remained relatively unchanged during therapy [11]. Increased PEMT expression in the livers of patients with HCV suggests a higher conversion of PE to PC [9,12], which should result in higher serum PC levels and lower PE levels in chronic HCV and a decline in PC levels and an increase in PE levels during viral cure. However, this was not observed in our patient cohort, suggesting that altered PEMT expression in the HCV-infected liver [9] does not produce the expected changes in serum phospholipid levels.

Absolute lipid concentrations measured at different time points are not directly comparable due to minor methodological and analytical variability, and the absence of a healthy control group further limits interpretation. However, relative proportions—expressed as ratios between lipid classes—are expected to remain largely consistent between different measurements and therefore provide a more reliable basis for comparison. The PC/PE ratio in the serum of healthy donors was approximately 60 [2,52], which is similar to the median ratio of 60 observed in HCV patients at the end of therapy. Before treatment, the median ratio was 50, suggesting that the PC/PE ratio shifts toward the expected reference values in healthy controls during DAA therapy. However, the physiological consequence of this observation remains unclear, as this ratio does not appear to be associated with improved liver function or reduced inflammation in the current study.

HCV genotype 3a has been associated with lower LDL levels [10]. Eight PE species and total PE levels were reduced in patients with HCV genotype 3. The decline in PE species in genotype 3a-infected patients was not associated with lower LDL levels, suggesting this is not due to reduced lipoprotein levels. PEMT expression was over 50% higher in liver biopsies from individuals infected with genotype 3 than from those infected with genotype 1 [9]. Reduced serum PE levels in patients infected with genotype 3a do support the idea of higher PEMT activity [9]. Nevertheless, most PC species were also low in genotype 3 infection [11], and the PC/PE ratio remained unchanged.

The PE content of HDL is much higher than that of LDL [2], but the PE species did not correlate with HDL levels before or after treatment. PE

38:1 was positively correlated with LDL before therapy, and PE 38:1, 38:2, 38:3, 38:4, 40:4, 40:5, and 40:6, as well as total PE levels, were correlated with LDL levels at the end of therapy. PE 36:4, 38:4, 38:6, 40:5, and 40:6 declined, and LDL was induced during therapy, which may partly explain why these associations appeared at the end of therapy but not before treatment. Thus, higher LDL levels may confound the analysis of PE species levels across cohorts. Notably, changes in PE 36:4, 38:4, and 40:5 during DAA therapy were independent of LDL dynamics, whereas reductions in PE 38:6 and 40:6 were associated with LDL changes. This may suggest that the PE composition of LDL changes during HCV eradication. Because only total serum PE species were quantified, rather than lipoprotein-associated fractions, this analysis does not allow assessment of lipoprotein composition at baseline or at the end of therapy.

Statin treatment is well described as lowering LDL cholesterol levels and reducing levels of almost all lipid classes, including various PE and PC species, in users [53]. In our cohort, statins were discontinued during DAA therapy, but they should be considered in similar studies in which these drugs are administered alongside DAAs.

Two PE species were positively correlated with age, and a positive association of a few PE species with aging has been described before in Japanese women and men [54]. Higher body mass index may be a confounding factor for PE 36:1 in studies measuring serum PE species levels.

This study has several limitations. First, serum samples from healthy controls were not analyzed in parallel, precluding direct comparison with a contemporaneous reference population. Second, blood samples were collected under routine clinical conditions rather than at standardized time points or in the fasting state; therefore, pre-analytical variation may have influenced absolute serum PE levels and the PC/PE ratio. Fasting does not affect plasma PC levels [55], but this does not rule out the possibility that PC species levels differ between fed and fasted states, as has been shown for erythrocyte PC composition [56]. To our knowledge, serum PE levels have not been compared between fed and fasted states. A study of 6 patients showed a higher PC/PE ratio, attributable to a decline in PE levels in erythrocytes following DAA treatment [18], which agrees with the current observations in serum. However, this study did not specify whether blood was collected from fasted individuals [18]. Nonetheless, the longitudinal design strengthens the conclusion that DAA-induced HCV clearance is associated with remodeling of the serum PE profile and improvement of the PC/PE ratio. Because circulating PE species were not associated with standard markers of liver function, the physiological significance of their alterations following viral clearance remains uncertain. Accordingly, while these findings are of scientific interest, they do not currently translate into clinically actionable insights.

5. Conclusions

DAA-induced HCV clearance is associated with a distinct shift in circulating phospholipid composition, marked by reduced serum PE and an improved PC/PE ratio. This identifies serum PE remodeling as a novel metabolic consequence of viral eradication and suggests a shift toward normal lipid homeostasis after cure.

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
DAA	Direct-acting antiviral
HCV	Hepatitis C virus
HDL	High-density lipoprotein
INR	International normalized ratio
LDL	Low-density lipoprotein
MELD	Model of end-stage liver disease
PE	Phosphatidylethanolamine
PUFA	Polyunsaturated fatty acid

CRedit authorship contribution statement

Georg Peschel: Writing – review & editing, Resources. **Kilian Weigand:** Writing – review & editing, Resources. **Sabrina Krautbauer:** Writing – review & editing, Investigation. **Marcus Höring:** Writing – review & editing, Investigation. **Gerhard Liebisch:** Writing – review & editing, Formal analysis. **Martina Müller:** Writing – review & editing. **Christa Buechler:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Institutional review board statement

The study protocol was approved by the ethics committee of the University Hospital of Regensburg (14–101-0049, date of approval: 22 May 2014), and the study was conducted in accordance with the updated guidelines for good clinical practice and the updated Declaration of Helsinki.

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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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Data availability

Data will be made available on request.

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