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Interferon-α could induce liver steatosis to promote HBsAg loss by increasing triglyceride level

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ABSTRACT

Background: The correlation between metabolic syndrome (MetS) and hepatitis B surface antigen (HBsAg) loss remains to be further elucidated, particularly in patients receiving pegylated interferon-α (PEG-IFN) treatment.

Methods: 758 patients with low HBsAg quantification who had received nucleos(t)ide analog (NUC) therapy for at least one year and subsequently switched to or add on PEG-IFN therapy over an unfixed course were enrolled. 412 patients were obtained with baseline data matched. A total of 206 patients achieved HBsAg loss (cured group) within 48 weeks. Demographic and biochemical data associated with MetS were gathered for analysis. HepG2.2.15 cell line was used in vitro experiments to validate the efficacy of interferon-α (IFN-α).

Results: The proportion of patients with diabetes or hypertension in the uncured group was significantly higher than in the cured group. The levels of fasting blood glucose (FBG) and glycated albumin remained elevated in the uncured group over the 48 weeks. In contrast, the levels of blood lipids and uric acid remained higher in the cured group within 48 weeks. Triglycerides levels and liver steatosis of all patients increased after PEG-IFN therapy. Baseline elevated uric acid levels and hepatic steatosis may be beneficial for HBsAg loss. IFN-α could induce hepatic steatosis and indirectly promote HBsAg loss by increasing triglyceride level through upregulation of acyl-CoA synthetase long-chain family member 1(ACSL1).

Conclusions: IFN-α could induce liver steatosis to promote HBsAg loss by increasing triglyceride level through upregulation of ACSL1. Comorbid diabetes may be detrimental to obtaining HBsAg loss with PEG-IFN therapy in CHB patients.

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Abbreviations

ACSL1	acyl-CoA synthetase long-chain family member 1
MetS	metabolic syndrome
HBsAg	hepatitis B surface antigen
PEG-IFN	pegylated interferon
IFN-α:	interferon-α
NUC	nucleos(t)ide analog
FBG	fasting blood glucose FBG
CHB	chronic hepatitis B
T2DM	Type 2 diabetes mellitus
HDL:	high density lipoprotein
MAFLD	metabolic dysfunction-associated fatty liver disease
HCC	hepatocellular carcinoma
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
CHOL:	cholesterol
TRIG	triglycerides
LDL:	low-density lipoprotein
UA	uric acid
CAP	controlled attenuation parameter (CAP)
LSM	liver stiffness measurement
TBIL:	total bilirubin
ALB	albumin
ALT	alanine aminotransferase
AST	aspartate aminotransferase
GGT	gamma-glutamyl transpeptidase
BMI	body mass index
FT3	free triiodothyronine
FT4	free thyroxine
TSH	thyroid stimulating hormone

1. Introduction

Chronic HBV infection is a global public health concern estimated to impact approximately 300 million persons worldwide [1]. A functional cure for chronic hepatitis B (CHB) is considered the ultimate goal of antiviral therapy and may be achieved by hepatitis B surface antigen (HBsAg) loss with or without seroconversion to hepatitis B surface antibody (HBsAb) [2,3]. Studies have demonstrated that long-term nucleos(t)ide analog (NUC) treatment of CHB patients followed PEG-IFN therapy, whether added or prescribed alone, can significantly increase the rate of HBsAg loss [4–6].

Metabolic syndrome (MetS) is defined as a pathologic condition characterized by abdominal obesity, insulin resistance, hypertension and hyperlipidemia [7,8]. There is a strong correlation between MetS and a poor prognosis in liver disease [9,10]. Type 2 diabetes mellitus (T2DM) and liver steatosis are recognized risk factors for liver cirrhosis and hepatocellular carcinoma (HCC) in patients with liver disease, irrespective of the cause of their liver disease [10–15].

Studies have shown that MetS and T2DM at baseline are predictors of delayed hepatitis B e antigen (HBeAg) seroclearance after adjusting for viral load, antiviral therapy and necroinflammation [16]. Research suggests that metabolic dysfunction-associated fatty liver disease (MAFLD) has an inverse relationship with viral activity in CHB patients [17]; it is associated with lower hepatitis B viral load and antiviral response [18]. However, MAFLD aggravates hepatic inflammation and fibrosis, resulting in diverse effects on long-term outcomes, including HCC occurrence among CHB patients [17,19–21]. Another study suggested that among 356 treatment-naïve CHB patients, coexisting fatty liver disease leaded to worsening of liver fibrosis but also increases the chance of HBsAg loss [22].

However, the correlation between Mets and HBsAg loss remains to be further elucidated, particularly in HBeAg-negative CHB patients with low levels of HBsAg receiving PEG-IFN treatment. Meanwhile, the effects of PEG-IFN therapy on Mets remain unclear, and thus, this study aims to further elucidate these questions.

2. Material and methods

2.1. Patient enrollment

This study is a nonrandomized retrospective study. Consecutive patients with CHB from 2018 to 2020 who were treated in the

Third Affiliated Hospital of Sun Yat-sen University were included in our study. In brief, CHB patients who had already been treated with NAs for at least one year with HBsAg quantitation less than 1500 IU/mL, HBeAg seroconversion and hepatitis B virus deoxyribonucleic acid (HBV DNA) < 20 IU/mL were included. Patients with the following diseases were excluded from this study: (i) Co-infection with other viruses, including other hepatotropic viruses (such as hepatitis A, C, D, E; cytomegalovirus, Epstein–Barr virus) and human immunodeficiency virus; (ii) autoimmune diseases, including autoimmune liver disease, primary sclerosing cholangitis, primary biliary cholangitis, hyperthyroidism or hypothyroidism; (iii) Wilson's disease, haemochromatosis, or 1-antitrypsin deficiency; (iv) alcoholic liver disease, moderate to severe steatohepatitis, parasite-related liver damage; (v) currently active or suspected hepatocellular carcinoma or other malignant diseases, previous liver transplantation; (ix) contraindications related to interferon therapy; (x) combined with severe organ damage due to respiratory, cardiovascular, digestive, urinary or neurological diseases.

Patients' enrollment was shown in Fig. 1. In the order of entry into the study (ClinicalTrials.gov: NCT04035837), we sequentially chose a total of 758 patients with low HBsAg quantification after NUC therapy for at least one year and subsequently either switched their regimen to or added on PEG-IFN therapy.

The patients took PEG-IFN at a dose of 180 μg per week. The total course of PEG-IFN treatment was not fixed; after an HBsAg of <0.05 IU/ml was achieved, patients received 12–24 weeks of PEG-IFN consolidation therapy depending on the doctor's advice. If a patient's treatment for HBsAg was not satisfactory, their treatment plan was adjusted in accordance with the doctor's recommendation. The baseline point was identified as the time when the patient started PEG-IFN therapy. All patients were monitored every 12 weeks, with at least one follow-up time point for those who were enrolled. Blood samples at baseline, week 12 and week 24 of all patients were collected for research. A blood sample was retained during the follow-up for research use.

2.2. Data acquisition

Demographic information of all patients at baseline were collected. Data regarding the type of NUCs used was also collected, as was patients' comorbidities. We simultaneously collected Laboratory data during therapy on routine blood, liver and kidney function and metabolism-related indexes. Given that glycated hemoglobin was not included as a routine test item during the follow-up period, we retrieved serum samples over 0–24 weeks from the first 100 patients of the 758 patients selected in chronological order of enrollment for the purpose of testing glycated albumin as a reference. Data on waist circumference, Homeostasis Model Assessment Index and high sensitivity C reactive proteins were not available in the medical records.

2.3. Definitions

Metabolic dysregulation was described in our previous study [23]. High blood pressure (HBP): blood pressure ≥130/85 mmHg or hypotensive drug treatment. Type 2 diabetes mellitus was diagnosed using fasting plasma glucose (FPG) (≥7.0 mmol/L) or

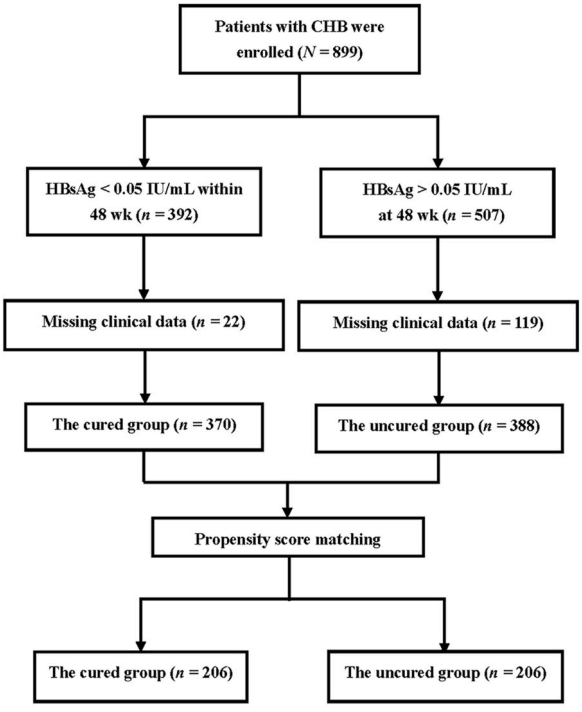

Fig. 1. Patient enrollment.

haemoglobin A1c (HbA1c) (≥6.5 %) or treatment of diabetes mellitus according to guidelines [24]. About drinking history, a history of long-term alcohol consumption was required, which was generally over 5 years, amounting to ethanol consumption of ≥40 g/day for men and ≥20 g/day for women according to guidelines of alcoholic liver disease [25].

2.4. Transient elastography

Hepatic steatosis and fibrosis were assessed using transient elastography to determine the controlled attenuation parameter (CAP) and liver stiffness measurement (LSM). LSM was expressed in kiloPascals (kPa), and CAP was expressed in dB/m. Refer to our past studies for specific operational details [26,27]. Patients were divided into four groups according to CAP value at baseline: no hepatic steatosis (CAP <240 dB/m), mild steatosis (240 dB/m ≤ CAP <265 dB/m), moderate steatosis (265 dB/m ≤ CAP <295 dB/m) and severe steatosis (CAP ≥295 dB/m).

2.5. HBV serological markers

Semiquantification of HBeAg was detected in serum using commercially available enzyme immunoassays by Cobas e601 (Roche; Geneva, Switzerland). The results are presented as log10 COI. Elecsys HBsAg II Quant reagent kit (Roche Diagnostics, Indianapolis, IN, USA; lower limit of quantification (LLOQ): 0.05 IU/mL) was used to measure HBsAg. Serum HBV DNA was tested using the Roche COBAS AmpliPrep/COBAS TaqMan HBV Test version 2.0 (Roche Diagnostics; LLOQ: 20 IU/mL). A Hitachi 7600 automatic analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) was used to test liver function, and the upper limit of normal of the alanine aminotransferase (ALT) level was set to 35 U/L.

2.6. Cell culture

HepG2.2.15 cells containing the complete HBV genome and capable of stable HBV expression and replication in culture, were maintained in exponential growth phase in DMEM (Gibco, USA) supplemented with 10 % fetal bovine serum, 100 units/ml penicillin, and 0.1 % (w/v) streptomycin at 37 °C in a humidified atmosphere of 5 % CO2. Human Interferon-Alfa-2A (cat: SRE0013-10UG) was obtained from Sigma-Aldrich, IFN-α-2a (0-1000-2000 U/mL) was added to the HepG2.2.15 culture for experiments for 48 h according to cell viability assay. Oil red O staining was used to detect intracellular triglyceride content.

2.7. ELISA

The levels of triglyceride (cat:MM-2101H1, MEIMIAN from China) as well as HBsAg (cat: KHHBSAG, KEHUA from China) in cell culture supernatants were measured by ELISA according to the manufacturer's protocols.

2.8. Western blot analysis

Total protein was isolated from cells samples using RIPA lysis buffer. Equal amounts of total protein were separated by SDS-PAGE electrophoresis, transferred to PVDF membranes, and blocked with western blocking buffer at room temperature for 15min. Next, the PVDF membranes were washed with TBST containing NaCl, Tris-HCl, and Tween-20 and incubated with primary antibodies against target proteins at 4 °C overnight, followed by three washes with TBST. Acyl-CoA synthetase long-chain family member 1(ACSL1, 1:1000, cat: 9189T), GAPDH (1:1000, cat: 2118S) antibody and alpha-Tubulin antibody (1:1000, cat: 2144S) was purchased from Cell Signaling Technology. HBsAg (1:1000, cat:MA17603) were obtained from ThermoFisher. PVDF membranes were incubated with the appropriate secondary antibodies at room temperature and then washed for three times with TBST. Protein bands were visualized by chemiluminescence (cat: WBKLS0500, Merck millipore).

2.9. Statistical analysis

Statistical analysis was performed using IBM SPSS 25.0 (IBM Corp, NY, USA). Continuous variables are expressed as the median (25th to 75th) deviation or mean ± SD according to the type of data distribution. Qualitative data are presented as numbers (%). The Mann–Whitney *U* test was used to compare the differences between different groups when the data did not conform to a normal distribution or were classified as categorical. Comparisons between two categorical variables were performed using the chi-square test. The Wilcoxon matched-pairs signed rank sum test was used to analyze the data from the same group at different time points. Experimental data between two groups were analyzed by 2-tailed Student t-test. A p value of <0.05 (two-tailed) was considered statistically significant.

3. Results

3.1. Patient enrollment and propensity score matching

In this study, 758 patients were included, of whom 370 achieved HBsAg levels of <0.05 IU/ml (cured) within 48 weeks, and 388 had the opposite result (uncured). To prevent the effect of virus indices and therapeutic protocols on the outcomes, we conducted a

propensity score matching of patients' age, sex, treatment plan, HBsAg quantification, and transaminase levels at baseline. Finally, we obtained 412 patients with baseline data that matched our criteria, including 206 patients in both the cured and the uncured groups. The detailed results can be seen in Table 1. We further analyzed the data from 12 to 48 weeks for the 412 patients. There was no significant difference in the types of NUC used during PEG-IFN therapy between the two groups of patients (Fig. 2A). The total duration of PEG-IFN treatment in patients of both groups was comparable (Fig. 2B). Detailed information of the paired patients in both groups can be found in Table 2. Among 412 patients matched with baseline characteristics, the results indicated that the proportion of patients with diabetes (Fig. 2C) or hypertension (Fig. 2D) was significantly higher in the uncured group than in the cured group. However, the proportion of patients with a history of alcohol consumption (Fig. 2E) or a family history of hepatitis B (Fig. 2F) showed no significant difference between the two groups.

3.2. Elevated fasting blood glucose (FBG) levels could be detrimental to HBsAg loss

After further investigation, it was revealed that the majority of the FBG levels in the two groups were within the normal range, but FBG levels of patients in the uncured group were consistently higher than those of patients in the cured group in 48 weeks (Fig. 3A). Simultaneously, we found that PEG-IFN treatment had little effect on the fluctuations in FBG levels except for a slight increase in the glucose levels of cured patients compared to baseline at 24 weeks, and there was no obvious fluctuation in the FBG levels of uncured patients within 48 weeks (Fig. 3B). Seeking further support for our results, we retrieved serum samples drawn from patients between 0 and 24 weeks and measured the glycated albumin levels. Similar to the results regarding FBG, the levels of glycated albumin were significantly higher in the uncured patients than in the cured patients within 24 weeks (Fig. 3C). However, it was clear that PEG-IFN therapy had a positive impact on the levels of glycated albumin in both groups, a result that was visible in both sets (Fig. 3D).

3.3. An increased level of blood lipids and uric acid may promote HBsAg loss

We delved deeper into the correlation between blood lipids and the interferon-α response. We found that the total cholesterol (CHOL) levels in the cured group were higher than those in the uncured group (Fig. 4A), and PEG-IFN treatment could reduce the level of CHOL. However, in the 48th week, the cholesterol levels had nearly reverted to their initial levels (Fig. 4B). The same outcome was observed regarding both high-density lipoprotein (HDL) (Fig. 4C–D) and low-density lipoprotein (LDL) (Fig. 4E–F). Despite the fact that triglycerides were elevated in the cured group compared to the uncured group (Fig. 4G), PEG-IFN therapy did not reduce the level of triglycerides but actually increased it, and by 48 weeks, triglyceride levels had dropped back close to baseline levels (Fig. 4H). The difference in UA levels between the two groups and the effect of PEG-IFN treatment on those levels were similar to the corresponding difference and effect observed regarding CHOL levels (Fig. 4I–J).

3.4. Sustained increase in CAP values during PEG-IFN treatment

During PEG-IFN treatment, patients underwent a FibroTouch examination every six months to determine the controlled attenuation parameter (CAP) and the liver stiffness measurement (LSM). Additionally, each patient's body mass index (BMI) was recorded. We examined the follow-up results of the patients within 48 weeks of PEG-IFN treatment and one year after PEG-IFN discontinuation.

Table 1
Patients enrolled in the study had their baseline data matched using propensity score matching[a].

	Before match (*N=758*)			After match (*N=412*)		
	Ucured	Cured	*p*	Uncured	Cured	*p*
	N = 388	N = 370		N = 206	N = 206	
Sex			**0.003**			**0.50**
Male	362 (93.3 %)	321 (86.7 %)		189 (91.7 %)	184(89.3 %)	
Female	26 (6.7 %)	49 (13.3 %)		17 (8.3 %)	22 (10.7 %)	
Age, year	43.2 ± 8.4	39.3 ± 8.5	**<0.001**	41.2 ± 8.2	39.7 ± 7.8	**0.06**
Treatment			**0.33**			**0.48**
IFN	55	67		34	40	
NUC + IFN (12W) →IFN	96	90		60	50	
NUC + IFN	237	213		112	116	
HBsAg, IU/ml			**<0.001**			**0.06**
<0.05–20	1 (0.3 %)	83 (22.4 %)		1 (0.5 %)	8(3.9 %)	
20–100	9 (2.3 %)	87 (23.5 %)		9 (4.4 %)	18 (8.7 %)	
100–500	138 (35.6 %)	134 (36.2 %)		120 (58.3 %)	114 (55.3 %)	
500–1000	165 (42.5 %)	58 (15.7 %)		68 (33.0 %)	58 (28.2 %)	
>1000	75 (19.3 %)	8 (2.2 %)		8 (3.8 %)	8 (3.9 %)	
ALT, U/L	24.0 (19.0–32.0)	25.0 (19.0–34.0)	**0.31**	25.0 (18.5–32.0)	27.0 (20.0–34.0)	**0.11**
AST, U/L	23.0 (20.0–27.0)	23.0 (20.0–28.0)	**0.35**	23.0 (20.0–27.0)	24.0 (21.0–28.0)	**0.07**

Abbreviations: IFN: interferon-α; NUC, nucleos(t)ide analogue; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[a] Values are expressed as median (25th to 75th), mean ± SD or number (%).

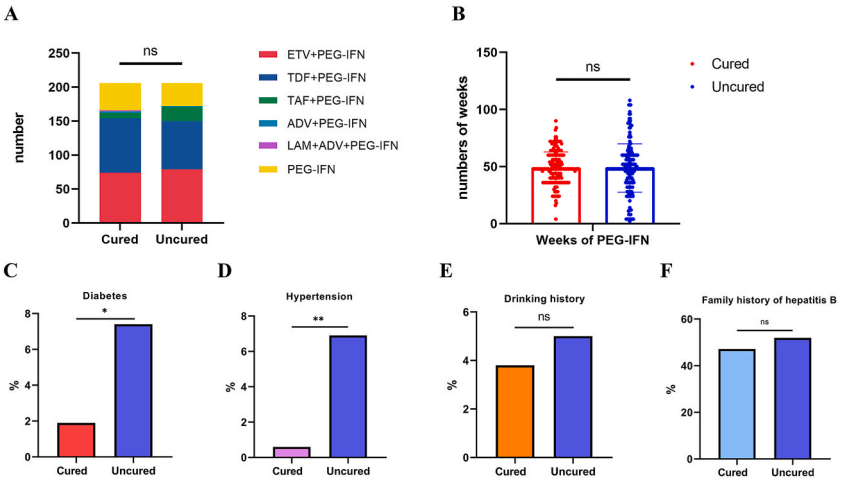

Fig. 2. Differences in medication regimens and comorbidities between the two groups of patients. Medication regimens for the two groups of patients (A–B). The proportion of patients with type 2 diabetes (C) or hypertension (D) in the two groups. The proportion of patients with a history of alcohol consumption (E) and family history (F) in the two groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, "ns" indicates no significance. PEG-IFN, pegylated interferon-α; ETV: entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; ADV, adefovir dipivoxil; LAM, lamivudine.

Table 2
Biochemical indicators of the 412 matched patients differed between the cured group and uncured group[a].

	Time	Cured(*N*=*206*)	Uncured(*N*=*206*)	*p*
BMI, kg/m^2	0W	22.8 (20.8–24.7)	23.7 (21.9–25.4)	**0.16**
TRIG, mmol/L	0W	1.0 (0.7–1.5)	1.0 (0.8–1.5)	**0.93**
CHOL, mmol/L	0W	4.7 (4.3–5.4)	4.5 (3.8–5.0)	**0.04**
HDL, mmol/L	0W	1.1 (0.9–1.3)	1.1(0.9–1.3)	**0.59**
LDL, mmol/L	0W	2.9 (2.5–3.5)	2.9 (2.3–3.3)	**0.70**
Uric acid, umol/L	0W	421.0(361.0–473)	391.0 (336.0–464.0)	**0.04**
Weeks of PEG-IFN		48.0 (40.0–60.0)	48.0 (38.0–60.0)	**0.98**
HBsAg, log10 IU/ml	0W	2.5 (2.2–2.8)	2.4 (2.6–2.8)	**0.06**
HBsAg decline, log10 IU/ml	12W	1.1 (0.42–2.41)	−0.002 (−0.13-0.17)	**<0.001**
	24W	3.58 (2.39–4.30)	0.14 (−0.19-0.36)	**<0.001**
	48W	4.49 (4.16–4.76)	0.29 (0.10–0.64)	**<0.001**
ALT, U/L	12W	73.0 (51.3–111.8)	49.0 (35.0–71.0)	**<0.001**
	24W	54.0 (38.0–78.0)	42.0 (29.5–65.0)	**<0.001**
	48W	40.0 (29.0–59.0)	32.0 (23.0–45.0)	**0.001**
Maximum ALT, U/L		105.5 (71.0–170.3)	69.0 (45.0–111.0)	**<0.001**
AST, U/L	12W	60.0 (43.3–87.0)	43.0 (31.0–58.0)	**<0.001**
	24W	48.0 (36.0–69.0)	37.0 (29.0–55.0)	**<0.001**
	48W	37.0 (28.0–52.0)	32.0 (24.0–41.0)	**0.001**
GGT, U/L	12W	73.0 (46.0–115.0)	55.0 (35.0–87.5)	**<0.001**
	24W	70.0 (44.0–117.3)	59.5 (40.0–80.3)	**0.003**
	48W	54.0 (36.0–95.0)	45.0 (30.3–70.3)	**0.04**
TBIL, umol/L	12W	10.2 (8.5–12.4)	10.5 (8.5–13.7)	**0.48**
	24W	9.0 (7.4–10.9)	9.4 (7.5–12.0)	**0.14**
	48W	8.4 (7.1–11.1)	9.0 (7.3–12.2)	**0.30**
ALB, g/L	12W	46.2 ± 2.7	46.2 ± 2.7	**0.87**
	24W	46.2 ± 2.8	46.3 ± 2.7	**0.83**
	48W	46.8 ± 2.9	46.7 ± 3.0	**0.74**

Abbreviations: BMI, body mass index; TRIG: triglycerides; CHOL: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PEG-IFN: pegylated interferon; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT: gamma-glutamy transpeptidase; TBIL: total bilirubin; ALB: albumin.

[a] Values are expressed as median (25th to 75th), mean ± SD.

The data indicated that the baseline BMI of patients in the uncured group was higher than that of patients in the cured group (Fig. 5A). PEG-IFN therapy resulted in a marked decline in BMI of patients in both groups at 24 and 48 weeks, after ceasing PEG-IFN therapy, the BMI increased (Fig. 5B). Moreover, there was no significant difference between the CAP and LSM values in the two groups (Fig. 5C and E). Surprisingly, despite the drastic drop in patients' weights, the CAP value of patients in the two groups increased drastically, and CAP value continued to rise even after discontinuing PEG-IFN therapy after one year in both groups. (Fig. 5D). The noticeable

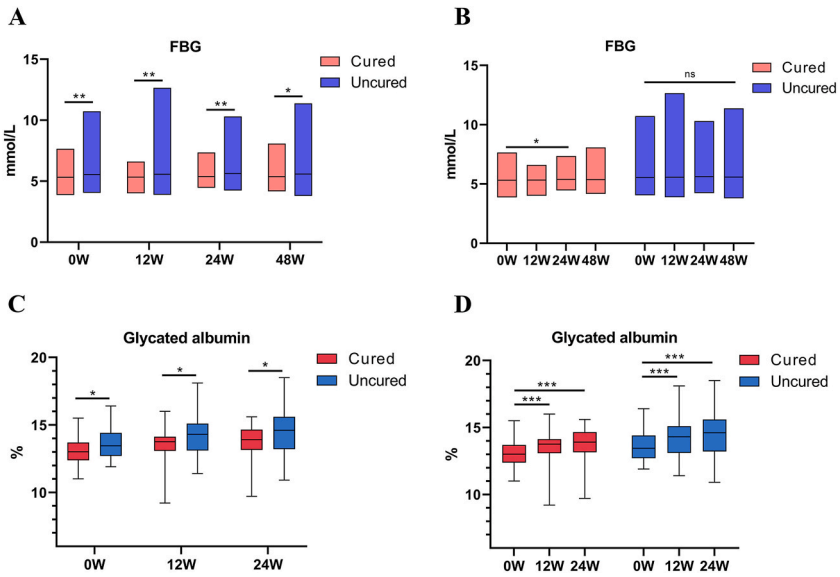

Fig. 3. Difference in blood glucose between the two groups. The difference and the dynamic changes in fasting blood glucose (A–B) and glycated albumin (C–D) levels between the cured and uncured patients at different time points. "ns" indicates no significance, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

difference was that the LSM value rose temporarily during PEG-IFN treatment but then decreased after discontinuation of PEG-IFN (Fig. 5F).

3.5. *Interferon-α could induce liver steatosis*

In conjunction with the CAP values, we graded the liver steatosis of the patients. Consistent with the CAP values, no significant differences were seen between the different liver steatosis gradations of the two groups of patients before and after PEG-IFN treatment, but PEG-IFN therapy significantly increased the proportion of mild and moderate steatosis (Fig. 6A–B).

Concurrently, we scrutinized the results of the ultrasound examination of the patient's liver. No substantial variation in the prevalence of liver steatosis diagnosed through ultrasound was observed between the two groups of patients over a period of 48 weeks (Fig. 6C). The proportion of liver steatosis diagnosed by ultrasound at 24 weeks and 48 weeks was significantly increased from baseline in both groups of patients (Fig. 6D). The outcomes closely matched the results obtained regarding the CAP value.

3.6. *High UA levels and CAP values at baseline may be beneficial for HBsAg loss*

Furthermore, we examined the relationship between baseline transaminase levels, metabolic indices and HBsAg quantification. The baseline UA levels (Fig. 6E) and CAP values (Fig. 6F) were negatively correlated with HBsAg quantification, while no significant correlation was observed between the ALT level or other metabolic indices and HBsAg quantification at baseline (data not shown). The above results indicate that a higher UA level at baseline and liver steatosis may be associated with a beneficial effect on HBsAg clearance.

3.7. *Thyroid function does not affect HBsAg loss*

Thyroid function has a close correlation to the metabolism of patients. We analyzed differences in thyroid function free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) levels between the two groups simultaneously. The FT3 levels in patients of the cured group were slightly higher than those in patients of the uncured group at 24 weeks (Fig. 7A), but there was no notable difference in FT4 and TSH between the two groups (Fig. 7B–C). PEG-IFN therapy itself can lead to a rise in TSH and FT3 concentrations and a slight decline in FT4 levels (Fig. 7D–F).

3.8. *IFN-α promoted triglyceride synthesis by upregulating acyl-CoA synthetase long-chain family member 1 (ACSL1)*

ACSL1 is the most abundant acyl-coenzyme A synthetases subtype in adipose tissue, liver and heart, and has a wide range of fatty acid specificity. It has been reported that ACSL1 promotes hepatic triglyceride storage [28], overexpression of ACSL1 promotes hepatocyte triglyceride synthesis [29]. Therefore, we hypothesized whether IFN-α could promote triglyceride synthesis by up-regulating ACSL1. We used HepG2.2.15 cells (with HBV genomic DNA) for in vitro experiments. Our results showed that IFN-α significantly

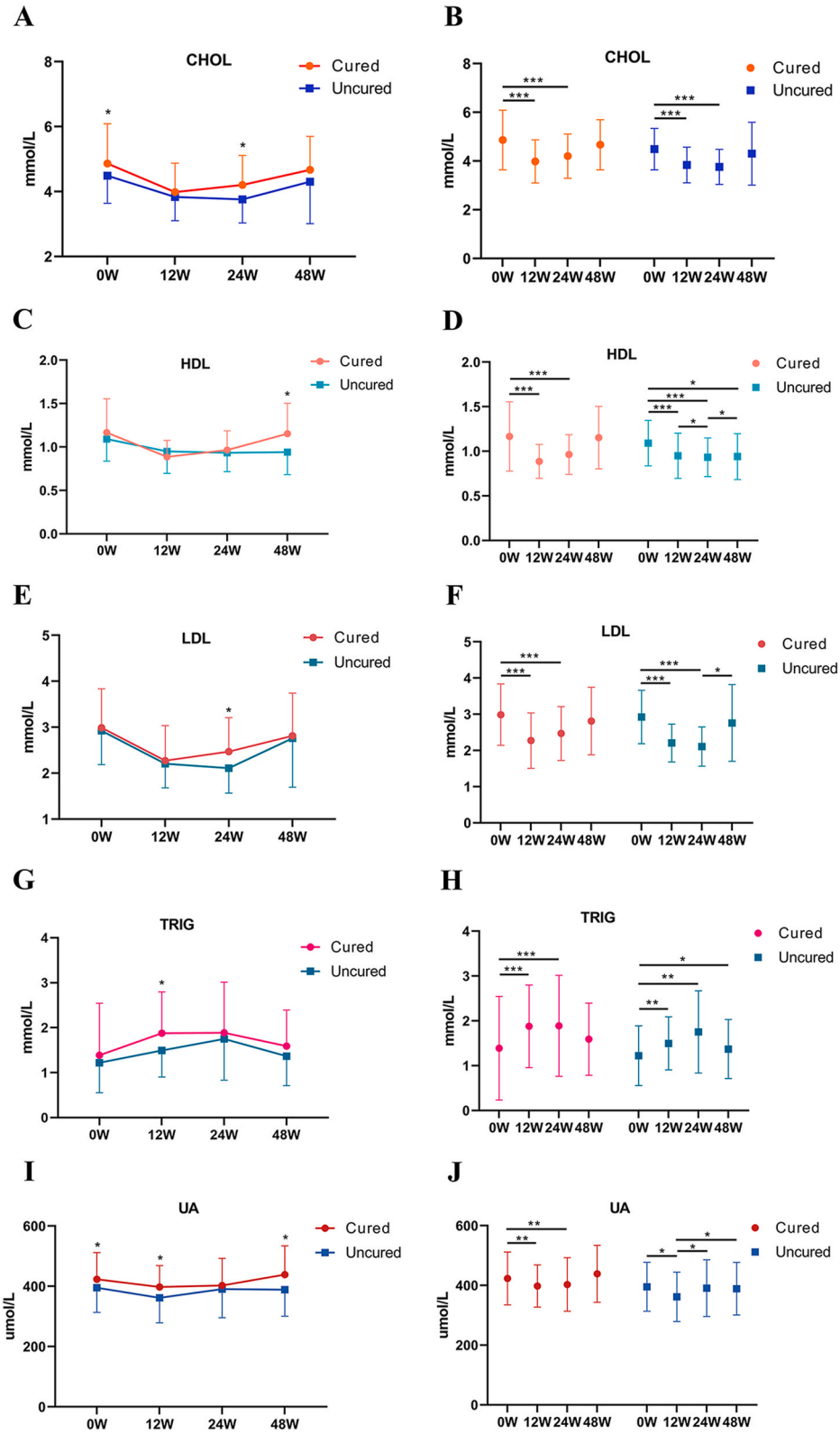

Fig. 4. Differences in lipids and uric acid between the two groups of patients. The difference and dynamic changes in total cholesterol (CHOL) (A–B), high-density lipoprotein (HDL) (C–D), low-density lipoprotein (LDL) (E–F), triglyceride (TRIG) (G–H) and uric acid (UA) (I–J) levels between cured and uncured patients at different time points. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

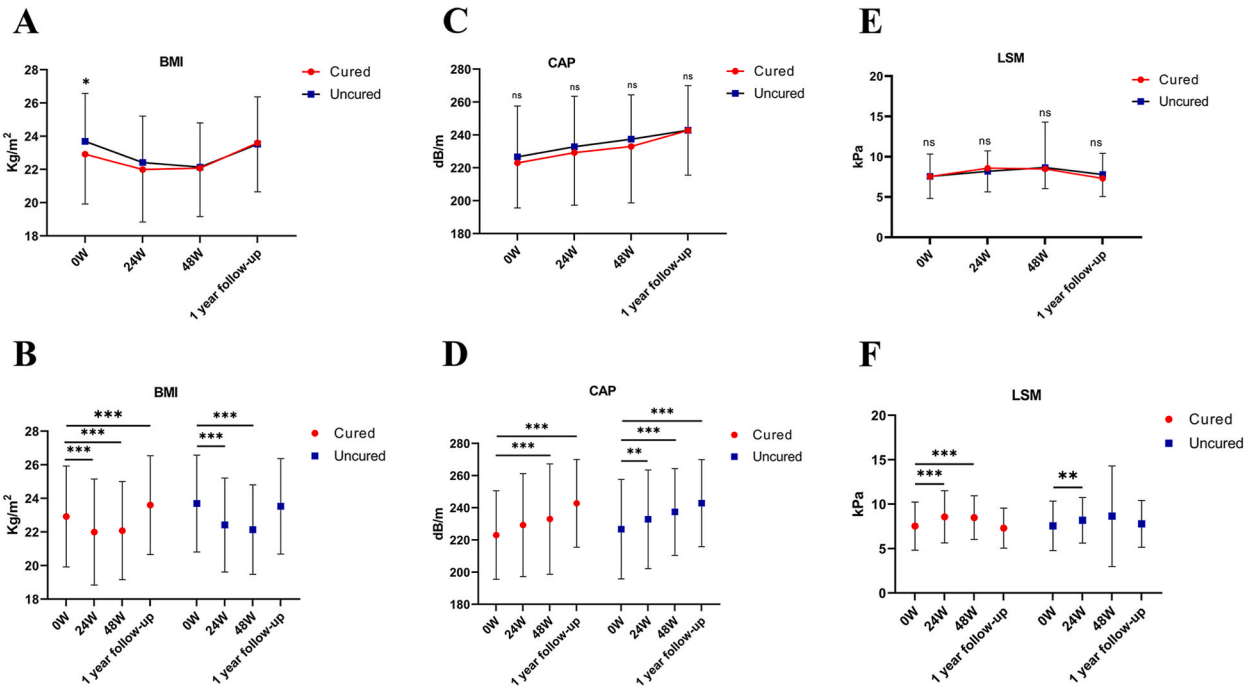

Fig. 5. Differences in the level of hepatic steatosis as well as fibrosis between the two groups of patients. The difference and dynamic changes in body mass index (BMI) (A–B), controlled attenuation parameter (CAP) (C–D) and liver stiffness measurement (LSM) (E–F) levels between cured and uncured patients at different time points. "ns" indicates no significance, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

down-regulated intracellular and extracellular HBsAg levels while up-regulating ACSL1 expression (Fig. 8A–D). Consistent with this, IFN-α treatment did upregulate intra- and extracellular triglyceride levels (Fig. 8E–F). Further results suggested that the intracellular and extracellular HBsAg levels could be down-regulated by high-fat medium conditions compared to normal medium (Fig. 8G–I). IFN-α could induce hepatocyte steatosis by upregulating ACSL1 expression, which induced elevated triglyceride level, and the high-fat intracellular environment further inhibited HBsAg levels. The present study revealed a novel mechanism of HBsAg inhibition by IFN-α.

4. Discussion

Our work is a retrospective study. This research included patients with low HBsAg quantification under NUC treatment who were subsequently treated with either monotherapy or combination therapy with PEG-IFN. The results suggested that metabolic syndrome had a role in interfering with HBsAg loss in CHB patients. Generally, PEG-IFN treatment had a minimal impact on FBG levels, but our results suggested that a combination of diabetes and higher levels of FBG levels may not be beneficial for HBsAg loss. Increasing evidence suggests that metabolic syndrome increases the risk of HCC [30], and diabetes further increases the risk of developing HCC in CHB patients [31]. Studies have reported that diabetes and higher blood glucose levels among those without known diabetes are associated with higher risks of liver cancer and major chronic liver diseases in Chinese adults [32]. HBV was proved to promote its own replication by utilizing cellular autophagy [33], and diabetes could induce unregulated autophagy activation [34], so we hypothesized that hyperglycemia may promote HBV replication through aberrant activation of autophagy to the detriment of HBsAg loss. Despite this, the mechanism by which HBsAg loss is affected by the combination of diabetes and higher glucose levels is not yet clear, and further research is needed.

In contrast to results regarding blood glucose, our data indicated that there was a correlation between liver steatosis, increased levels of blood lipids and HBsAg loss, especially triglycerides. Although it has been proved that hepatic steatosis did not affect the efficiency of a 48-week course of PEG-IFN treatment for CHB patients [35–38]. Whereas most of the above studies have investigated the response to viral or biochemical responses in CHB patients with HBeAg-positive or HBV DNA-positive, but the relationship between hyperlipidemia or liver steatosis and HBsAg loss in HBeAg-negative CHB patients with low levels of HBsAg treated with PEG-IFN has yet to be elucidated. Although Li et al. [52] showed that HBsAg clearance was significantly higher in patients with moderate steatosis than in those without steatosis (74.07 % vs. 48.15 %, $P = 0.008$) at week 96 after PEG-IFN therapy, but the number of patients enrolled in this study was limited (n = 174) and the population studied was primarily inactive HBsAg carriers with low levels of HBsAg (<200 IU/ml) treated with PEG-IFN, whose conclusions needed to be further substantiated.

Previous studies have suggested that there is an inverse relationship between HBV infection and metabolic syndrome among lean subjects [14,39,40]. Those suffering from HBV infection may be less likely to experience MetS and fatty liver [41]. Moreover, the study conducted by Yu et al. [42]indicated that HBsAg seroclearance was associated with a 1.41-fold increased risk of liver steatosis, and the

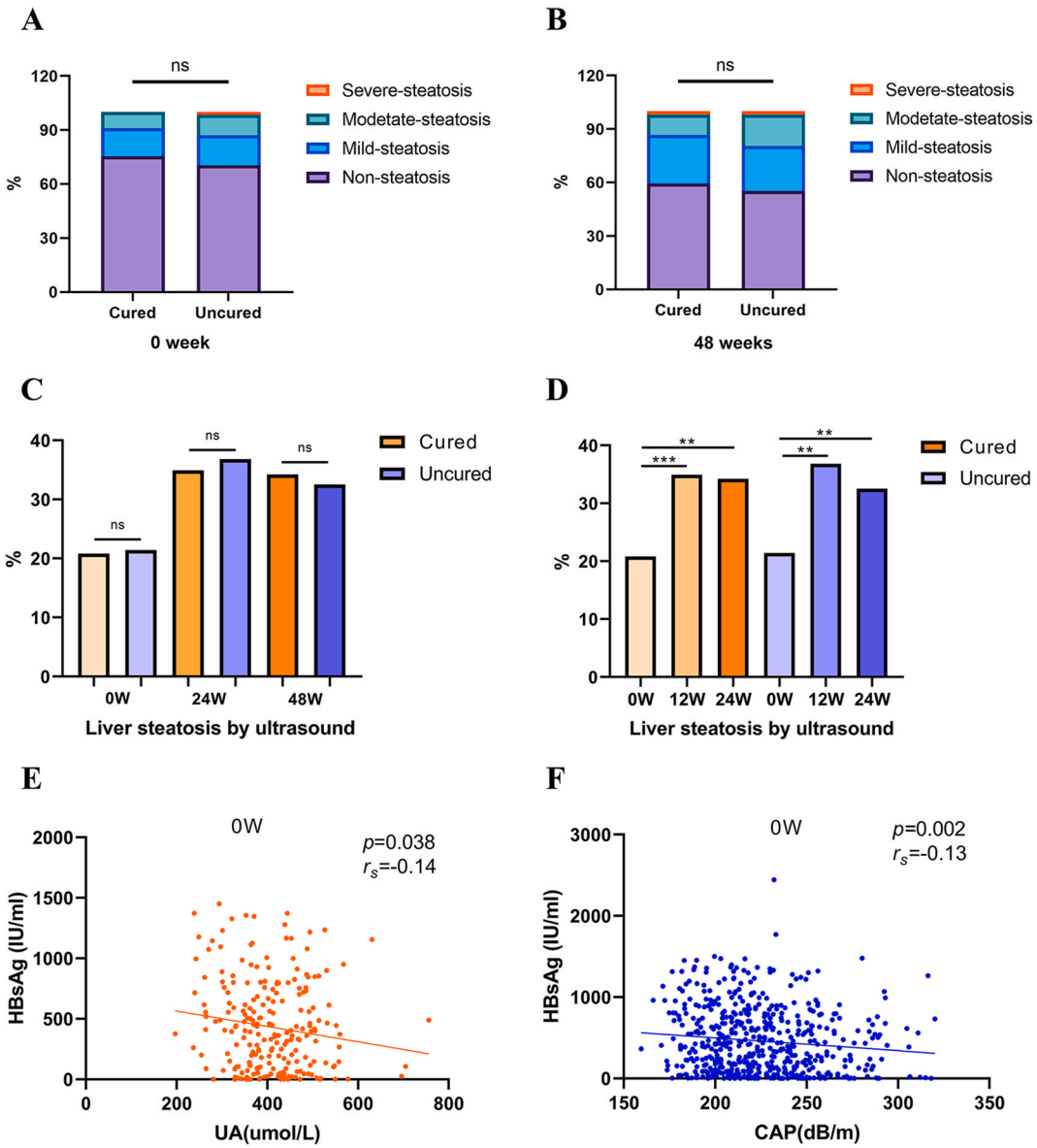

Fig. 6. Increased hepatic steatosis after interferon-α therapy. Differences in liver steatosis grading before and after PEG IFN treatment in two groups of patients (A–B). The difference and dynamic changes in the proportion of liver steatosis diagnosed by ultrasound (C–D) between cured and uncured patients at different time points. The baseline uric acid (UA) level (E) and controlled attenuation parameter (CAP) value (F) were negatively correlated with HBsAg quantification.

increased risk for progressive impairment of glucose metabolism due to steatosis was especially prominent after HBsAg seroclearance.

On the contrary, results from both clinical studies and animal experiments pointed to hepatic steatosis limiting HBV replication [40]. Lin et al. [43] conducted a study that revealed that lipid peroxidation can cause endoplasmic reticulum stress in hepatocytes, thereby suppressing the release of hepatitis B surface antigen and HBV DNA. Zhou et al. [44] found that hyperlipidemia conferred a nearly 3-fold increased risk of phase transition or treatment initiation in e-Antigen-Negative CHB patients and low-level viremia. Other studies suggested that patients with hepatic steatosis were more likely to experience an advancement of fibrosis, yet the HBsAg seroclearance rate was three times higher in treatment-naïve patients [22,45]. Given that neither the CAP values nor the gradations of liver steatosis were significantly different between the two groups of patients in our study during the treatment period, our study was unable to directly answer the question of the predominant role of liver steatosis on the clearance of HBsAg by IFN-α.

IFN-α is known to modify lipid metabolism, increasing triglyceride level and partially reducing cholesterol value [35,46,47]. As we all know, the two main ways in which the body obtains cholesterol are endogenous synthesis from scratch and absorption from food. During the course of PEG-IFN treatment, the patient's appetite and body weight were significantly reduced, resulting in the inhibition of cholesterol synthesis, which to a certain extent affected the release of triglycerides in the liver [48]. Triglycerides then aggregated in

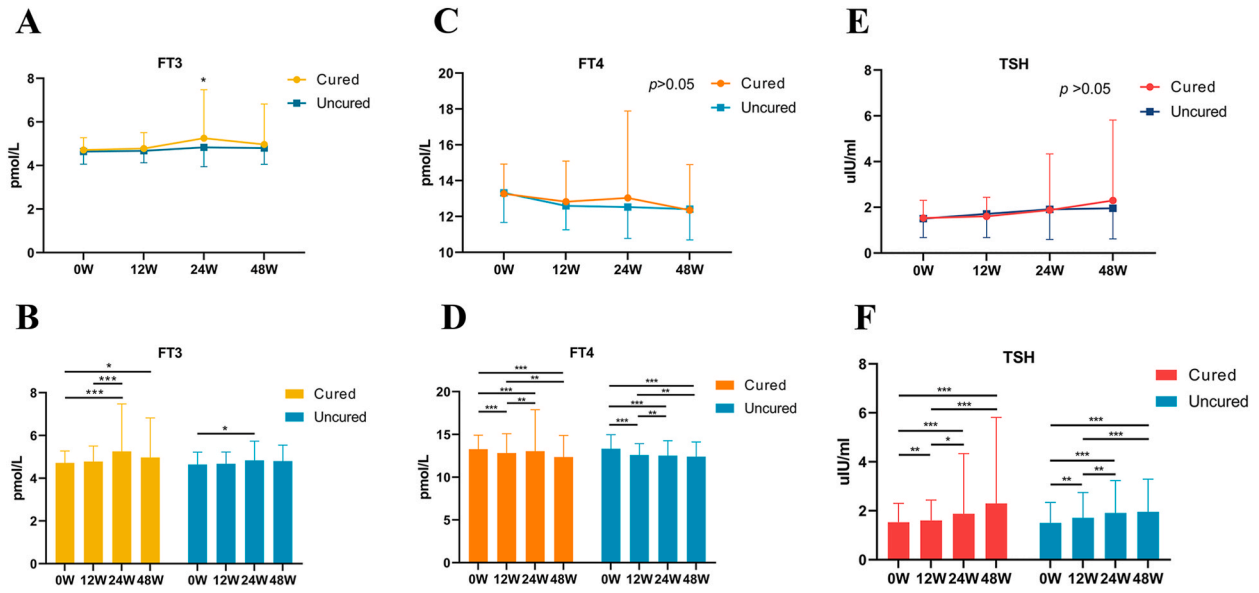

Fig. 7. Differences in thyroid function between the two groups. The difference and dynamic changes in free triiodothyronine (FT3) (A–B), free thyroxine (FT4) (C–D) and thyroid stimulating hormone (TSH) (E–F) levels between cured and uncured patients at different time points. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

the form of lipid droplets in the liver cytoplasm, promoting liver steatosis. As for the HDL and LDL mainly carrying cholesterol, will naturally have a corresponding decreasing trend similar to cholesterol, which supports the conclusions of our results. With the cessation of PEG-IFN treatment, the patient's appetite is restored, weight is regained, and HBV is suppressed to some extent, all of which may favor intrahepatic fat accumulation. Tominaga et al. [49] found that the interferon-α production was positively correlated with triglyceride and UA levels but negatively correlated with age and FBG levels. Wu et al. [50] proved that type I interferon signaling could accelerated liver regeneration by metabolic modulation and promoted lipid accumulation during liver regeneration. Based on the above mechanisms, elevated ALT during PEG-IFN therapy implies destruction of infected hepatocytes and subsequent regeneration of the remaining hepatocytes, and since PEG-IFN therapy could lead to elevated triglyceride level, which in turn causes hepatic steatosis and thus increases CAP level.

Our work further elucidated that IFN-a promotes triglyceride synthesis by upregulating ACSL1 expression, which causes hepatic steatosis. To some extent, this explains the persistent elevation of CAP values during the course of PEG-IFN therapy and during the discontinuation follow-up phase, which cannot be explained by the transient increase in ALT levels. Given that hepatic steatosis inhibits viral replication [40,43,44], suggesting that IFN-α could downregulate HBsAg levels by promoting hepatic steatosis in all patients. The present study elucidated a new potential mechanism of HBsAg inhibition by IFN-α, which partly explained the decrease in serum HBsAg levels during 48 weeks of PEG-IFN therapy even in patients who could not achieve HBsAg loss. The findings also indicated that increased levels of blood lipids in the cured group may aid in the response to PEG-IFN therapy.

Therefore, it is conceivable that the inhibition of HBV replication by IFN-α, along with the decrease in HBsAg quantification, may result in an increase in liver fat and a mild elevation of blood glucose. Further research and more solid evidence are needed to confirm the aforementioned inference. Drawing upon previous research findings and our data, we postulated that IFN-α could downregulate HBsAg levels by promoting hepatic steatosis.

Our study further suggests that an inverse relationship exists between UA level and HBsAg quantification, implying that higher UA levels may lead to a decrease in HBsAg. Consequently, we hypothesize that purine metabolism may be involved in the synthesis and secretion of HBsAg. Research conducted by Hong Tang's team has revealed a link between UA levels and the elimination of HBsAg and serological conversion [14]. The findings of a study have revealed that uric acid increases the immunity of mice to hepatitis B surface antigen-activated dendritic cells [51]. The above study suggested that uric acid may be able to modulate the functions of dendritic cells, which could then have a beneficial impact on the functions of effector T cells for HBsAg loss. Therefore, further research is needed to explore the specific mechanisms by which uric acid may facilitate HBsAg loss.

In terms of limitations, the shortcomings of this study were primarily due to the abundance of missing data regarding patient lipid and uric acid levels during the follow-up period although the existing data still validate our conclusion. Hence, further research is necessary to corroborate our findings. In addition, we did not have information on waist circumference, data related to the Homeostasis Model Assessment Index and high sensitivity C reactive proteins were not available. Therefore, further analysis of metabolic syndrome and MAFLD is needed. We were not yet able to ascertain whether the functional cure of CHB would result in an increase in liver fat and an accompanying higher risk of diabetes. Further research is needed to confirm the above speculation. We are simultaneously conducting longer follow-ups on patients to address the queries in the study.

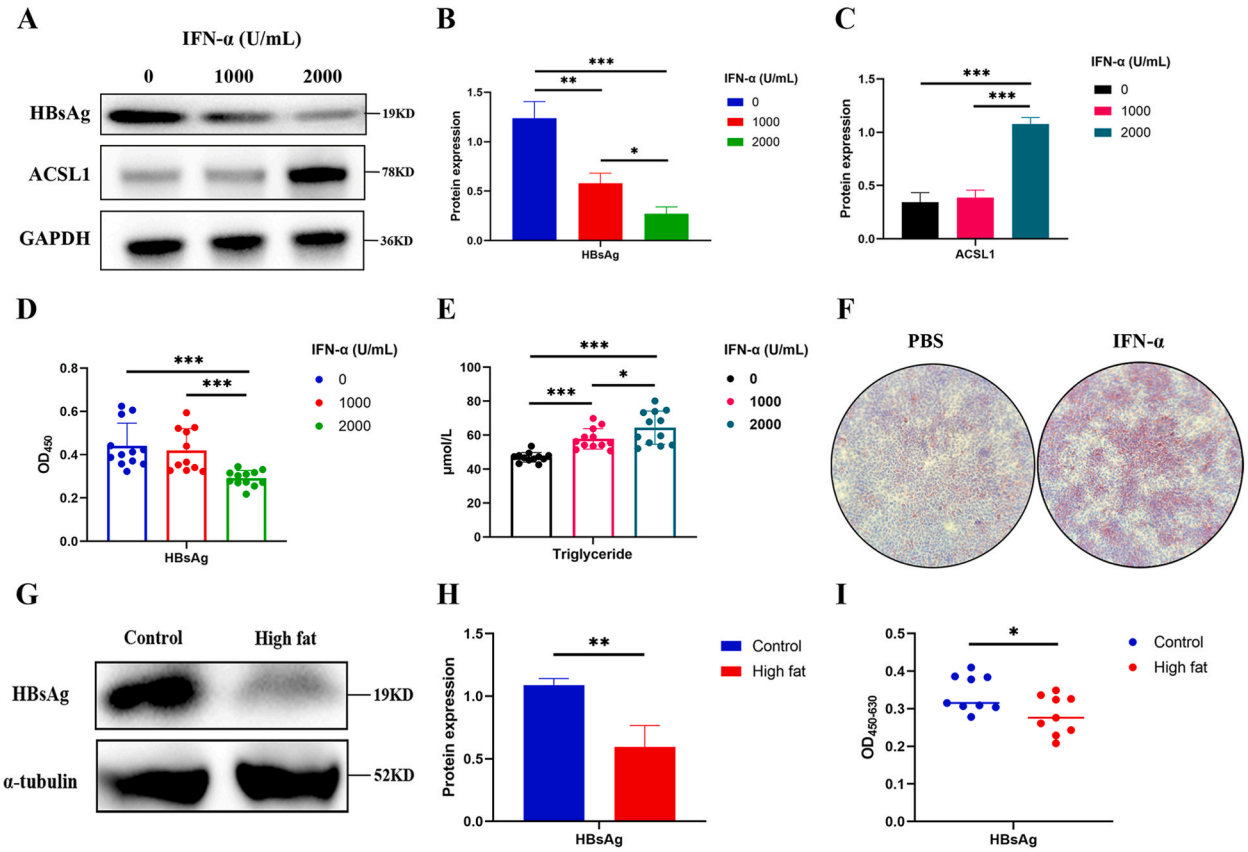

Fig. 8. IFN-α could induce liver steatosis to promote HBsAg loss by increasing triglyceride level through upregulation of ACSL1. IFN-α significantly down-regulated intracellular and extracellular HBsAg levels and up-regulated ACSL1 expression (A–D). IFN-α treatment upregulated intra- and extracellular triglyceride levels (E–F). The intracellular and extracellular HBsAg levels could be down-regulated by high-fat medium conditions compared to normal medium (G–I). IFN-α: interferon-alfa; ACSL1: acyl-CoA synthetase long-chain family member 1; HBsAg: hepatitis B surface antigen. "ns" indicates no significance, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

5. Conclusions

Metabolic syndrome certainly contributes to the response of CHB patients to IFN-α treatment. However, the prognosis may vary according to various factors. Inclusion of diabetes or impaired glucose tolerance in the patient profile is an unfavorable factor for HBsAg loss, but higher levels of blood lipids and uric acid may be beneficial. Triglyceride level and liver steatosis increased after PEG-IFN therapy, IFN-α could induce hepatic steatosis and indirectly promote HBsAg loss by increasing triglyceride level through upregulation of acyl-CoA synthetase long-chain family member 1(ACSL1).

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Disclosure of ethical Statements

Written informed consent was obtained from each participant. The study protocol was in line with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Independent Central Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University (zssy [2016] 2–129 and zssy [2018]02-218-02). The clinical trial of our study was registered in Chinese Clinical Trials Registry and ClinicalTrials.gov: NCT04035837. Animal Studies: N/A. Research involving recombinant DNA: N/A.

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Lili Wu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Supervision, Writing – original draft, Writing – review & editing. **Zhihui Li:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Na Gao:** Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation. **Hong Deng:** Data curation, Formal analysis, Investigation, Resources, Validation, Writing – review & editing. **Qiyi Zhao:** Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Writing – review & editing. **Zhaoxia Hu:** Data curation, Methodology, Resources, Validation, Writing – review & editing. **Junfeng Chen:** Data curation, Investigation, Methodology, Resources, Validation. **Ziying Lei:** Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Jinhua Zhao:** Data curation, Investigation, Software, Validation. **Bingliang Lin:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Zhiliang Gao:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32730.

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