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Risk Factors for Fatty Liver in the Multicenter AIDS Cohort Study

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Abstract

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CONFLICT OF INTEREST

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DISCLAIMER

The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH).

OBJECTIVES—Human immunodeficiency virus (HIV) infection and antiretroviral therapy (ART) may increase the risk of fatty liver disease. We determined the prevalence of and risk factors for fatty liver by comparing HIV-infected men with HIV-uninfected men who have sex with men in the Multicenter AIDS Cohort Study (MACS).

METHODS—In 719 MACS participants who consumed less than three alcoholic drinks daily, fatty liver was defined as a liver-to-spleen attenuation ratio < 1 on noncontrast computed tomography (CT). We genotyped single nucleotide polymorphisms in the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene and in other genes previously associated with nonalcoholic fatty liver disease. Risk factors for fatty liver were determined using multivariable logistic regression.

RESULTS—Among 254 HIV-uninfected men and 465 HIV-infected men, 56 % were White with median age 53 years and median body mass index 25.8 kg/m². The vast majority of HIV-infected men (92 %) were on ART, and 87 % of the HIV-infected men were treated with a nucleoside reverse transcriptase inhibitor for a median duration of 8.5 years. Overall, 15 % of the cohort had fatty liver, which was more common in the HIV-uninfected compared with the HIV-infected men (19 vs. 13 %, *P* = 0.02). In multivariable analysis, HIV infection was associated with a lower prevalence of fatty liver (odds ratio (OR) = 0.44, *P* = 0.002), whereas a higher prevalence of fatty liver was seen in participants with *PNPLA3* (rs738409) non-CC genotype (OR = 2.06, *P* = 0.005), more abdominal visceral adipose tissue (OR = 1.08 per 10 cm², *P* < 0.001), and homeostatic model assessment of insulin resistance (HOMA-IR) 4.9 (OR = 2.50, *P* = 0.001). Among HIV-infected men, *PNPLA3* (rs738409) non-CC genotype was associated with a higher prevalence of fatty liver (OR = 3.30, *P* = 0.001) and cumulative dideoxynucleoside exposure (OR = 1.44 per 5 years, *P* = 0.02).

CONCLUSIONS—CT-defined fatty liver is common among men at risk for HIV infection and is associated with greater visceral adiposity, HOMA-IR, and *PNPLA3* (rs738409). Although treated HIV infection was associated with a lower prevalence of fatty liver, prolonged exposure to dideoxynucleoside analogs is associated with higher prevalence.

INTRODUCTION

Liver disease is one of the most common non-AIDS-related causes of death among human immunodeficiency virus (HIV)-infected individuals, accounting for 14 % of all deaths and 50 % of hospital deaths (1–3). Although hepatitis C virus (HCV) coinfection is a well-recognized cause of liver disease among HIV-infected persons, mounting evidence suggests that hepatic steatosis is common among HIV-infected individuals with or without HCV coinfection (4–6). Nonalcoholic fatty liver disease refers to hepatic steatosis in individuals with little or no alcohol use and affects ~ 30 % of the adult US population (7). Well-established risk factors for fatty liver disease include hyperglycemia, diabetes mellitus, hypertriglyceridemia, and obesity, particularly abdominal visceral adiposity (8,9). Metabolic abnormalities are common among antiretroviral therapy (ART)-treated HIV-infected persons and have previously been associated with hepatic steatosis in this group (6,10,11).

The impact of additional host and viral factors on liver steatosis and fibrosis risk in the setting of HIV infection is poorly understood. The rs738409 single nucleotide

polymorphism (SNP) in the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene is strongly associated with steatosis, steatohepatitis, and fibrosis in HIV-uninfected persons, including patients with chronic HCV infection (12–15). Polymorphisms in other genes such as *NCAN* (rs2228603), *GCKR* (rs780094), *LYPLALI* (rs12137855), and *PPP1R3B* (rs4240624) have also been associated with steatosis in HIV-uninfected individuals, but these have not been studied in HIV-infected persons (16). HIV infection itself may lead to hepatic steatosis by direct interaction with the sterol regulatory element-binding protein-1, which stimulates lipogenesis, and peroxisome proliferator-activated receptor gamma, which is involved in insulin signaling (17). In addition, antiretrovirals of the nucleoside reverse transcriptase inhibitor (NRTI) class may cause steatosis via inhibition of mitochondrial polymerase γ and protease inhibitors by inducing hepatic overexpression of sterol regulatory element-binding protein-1 (18,19).

It has been suggested that HIV-infected persons comprise a population at high risk for fatty liver disease (20). However, it is unclear whether HIV *per se* increases fatty liver risk as studies of hepatic steatosis and HIV infection lack HIV-uninfected controls. The objective of this study was to determine the prevalence of and risk factors for fatty liver by comparing HIV-infected participants with HIV-uninfected participants in the Multicenter AIDS Cohort Study (MACS).

METHODS

Study population

We conducted a cross-sectional study nested within the MACS, an ongoing prospective cohort study involving HIV-infected and HIV-uninfected men who have sex with men. Details of MACS participant recruitment and study design have been described elsewhere (21,22). In brief, men were enrolled from four US metropolitan areas (Baltimore, MD/ Washington DC; Chicago, IL; Pittsburgh, PA; and Los Angeles, CA) during three recruitment periods (1984–1985, 1987–1990, and 2001–2003). Subjects were followed up every 6 months for interview, physical examination, laboratory testing, and collection of biological specimens for repository specimens.

Eligibility for the fatty liver study was participation in the MACS cardiovascular substudy, as these men underwent computed tomography (CT) imaging studies. The cardiovascular substudy recruited 1,002 men aged 40–71 years to undergo noncontrast cardiac CT imaging, cuts of which were extended to include liver and spleen imaging, between 1 January 2010 and 19 August 2013. Exclusion criteria were a history of cardiac surgery, history of coronary angioplasty, or weight > 300 pounds. After excluding 210 men whose CT images lacked adequate visualization of both the liver and spleen, 55 men who consumed more than or equal to three alcoholic drinks daily, and 18 men without stored cells or cell pellets for DNA extraction, 719 men were evaluated for the fatty liver study (**Figure 1**). Comparison of the 719 men who were included in the analysis and the 283 excluded men is detailed in **Supplementary Table S1** online. The study was approved by the Institutional Review Boards of all participating sites. All participants signed informed consent.

Assessment of hepatic steatosis

Noncontrast CT is a reliable noninvasive imaging method to detect hepatic steatosis of moderate or greater severity (23). Although the liver normally has a higher CT-attenuation value compared with the spleen, the presence of steatosis in the liver leads to a reversal of the liver/spleen attenuation ratio (24). Multidetector row CT scan was performed once for each participant. A single reader who was blinded to demographic data and HIV serostatus measured each scan. Liver and spleen attenuation values were measured by obtaining minimum, maximum, and mean Hounsfield units (HU) for regions of interest (ROI) > 100 mm². Two ROIs were placed in the right liver lobe, one ROI in the left hepatic lobe, and one ROI in the spleen. The mean hepatic HU was derived by averaging the mean HU across all liver ROIs. The liver/spleen ratio was calculated by dividing the mean hepatic HU measurements by the mean spleen HU measurements. Fatty liver was defined as a liver/spleen ratio < 1.0. For measurements of abdominal adipose tissue, one axial image was obtained in the space between the fourth and fifth lumbar vertebrae. Abdominal adipose tissue areas were calculated in cm² for visceral adipose tissue (VAT) and subcutaneous adipose tissue. The reader was blinded to all clinical and demographic data.

Laboratory testing and definitions

For SNP genotyping, DNA was extracted from stored cells and cell pellets using the DNeasy Blood and Tissue kit (Qiagen, Germantown, MD). Genotyping of *PNPLA3* (rs738049), *NCAN* (rs2228603), *GCKR* (rs780094), *LYPLALI* (rs12137855), and *PPP1R3B* (rs4240624) was performed via TaqMan SNP genotyping assays (Life Technologies, Carlsbad, CA), which use unique primers and probes for each SNP. DNA extraction and SNP testing were both accomplished using standard protocols. HIV antibody testing was performed using enzyme immunoassay and confirmed with western blot, as previously described (22). HCV infection was defined as serum HCV antibody and HCV RNA positivity. HCV antibody testing was performed with a third-generation enzyme immunoassay (ADVIA Centura HCV assay, Siemens Healthcare Diagnostics, Tarrytown, NY). If positive, HCV RNA testing was conducted from the same serum sample using Taqman HCV assay (lower limit of detection 43 IU/ml; COBAS AmpliPrep COBAS TaqMan HCV assay, Roche Molecular Systems, Pleasanton, CA). HCV antibody was tested in all participants within 2 years of entry into MACS and at most recent follow-up, with additional HCV antibody testing performed to identify the timing of all HCV seroconversions (25). Among HIV-infected subjects, CD4 T-cell counts and plasma HIV RNA levels were measured at each visit using standard assays.

Eight-hour fasting blood tests collected and assayed at the time of CT scanning or, if not available, within 6 months of CT scanning included the following: insulin, glucose, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, triglycerides, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Impaired fasting glucose or diabetes was defined as a fasting glucose ≥ 100 mg/dl or use of diabetes medication. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using fasting insulin and glucose values. For analysis, the HOMA-IR was dichotomized as <4.9 or ≥ 4.9 . The 4.9 cutoff was used because it defined the 75th percentile in our cohort. Body mass index at the time of CT or, if not available, within 6 months of CT scanning was calculated as (body weight (kg)/height (m))² and was categorized by a

modification of the World Health Organization's classification system: normal, $<25 \text{ kg/m}^2$; overweight, $25\text{--}30 \text{ kg/m}^2$; or obese, $\geq 30 \text{ kg/m}^2$. Hepatic fibrosis was estimated using the AST-to-platelet ratio index and the FIB-4, which were calculated from labs assayed from specimens at the time of CT scan or, if not available, within 6 months of the scan (26,27). AST-to-platelet ratio index was calculated using the formula $((\text{AST}/\text{upper limit of normal AST}) \times 100)/\text{platelet count}$ ($10^9/\text{l}$), and FIB-4 was calculated using the formula $(\text{age (years)} \times \text{AST})/(\text{platelet count (} 10^9/\text{l)} \times \text{ALT}^{1/2})$. We used an upper limit of normal of 30 U/l for calculations.

Statistical analysis

Demographic and clinical factors were compared by HIV serostatus and the presence or absence of fatty liver using the χ^2 -test for categorical variables and the Wilcoxon rank-sum test for continuous variables. For each SNP, Hardy–Weinberg equilibrium was determined by the χ^2 -test using one degree of freedom. Multivariable logistic regression was used to evaluate the association between fatty liver and the covariates of interest, including HIV infection, *PNPLA3* (rs738049), other candidate genes, and metabolic factors. The final model included HIV serostatus, age, race (White, Black, or other), MACS site, abdominal VAT, and the *PNPLA3* (rs738049), ALT >40 U/l, HOMA-IR ≥ 4.9 , and HCV serostatus. Separate sensitivity analyses were performed excluding HCV-infected subjects, adjusting for AST-to-platelet ratio index and FIB-4, and using a fatty liver cutoff of liver/spleen ratio <0.9 (which is less sensitive but 97 % specific for fatty liver) (24). We repeated the multivariable logistic regression after stratification by HIV serostatus. Among the HIV-infected men, associations between HIV-specific variables and fatty liver were explored, including current and cumulative ART exposure. All statistical analyses were performed using STATA 12.1 (StataCorp, College Station, TX).

RESULTS

Study population

Among the 719 men included in the analysis, 465 were HIV infected and 254 were HIV uninfected. The HIV-infected men were younger (median age, 53 vs. 55 years, $P < 0.001$) and more likely to be non-White (**Table 1**). Although they had a lower median body mass index (25.5 vs. 26.3 kg/m^2 , $P=0.002$), there was no difference in abdominal VAT between the HIV-infected vs. HIV-uninfected men (median, 143 cm^2 vs. 139 cm^2 , $P=0.91$). The HIV-infected men had higher median HOMA-IR (3.3 vs. 2.9 , $P=0.007$), lower median high-density lipoprotein cholesterol (46 vs. 50 mg/dl , $P < 0.001$), lower median low-density lipoprotein cholesterol (103 vs. 108 mg/dl , $P=0.02$), and higher median triglyceride levels (135 vs. 106 mg/dl , $P < 0.001$). HIV-infected subjects were more likely to be HCV infected (12 vs. 4% , $P < 0.001$), and all of the HCV-infected men had HCV genotype 1 or 2. Thirtyfive percent of the entire cohort was receiving a lipid-lowering medication at the time of CT scanning; this percentage did not differ significantly by HIV serostatus. Similarly, there was no difference in use of diabetes medications at the time of CT scanning by HIV serostatus. Among the HIV-infected subjects, 95 % were ART exposed, 92 % were on ART at the time of the CT scan, and 90 % had an undetectable plasma HIV RNA level within 6 months of the CT scan. The median cumulative ART duration was 9 years (interquartile

range=6.5, 13). Eighty-seven percent of the HIV-infected men were treated with an NRTI for a median cumulative duration of 8.5 years (interquartile range=6, 12.5).

Of the men without HIV or HCV, only 1 (0.4 %) had advanced fibrosis as estimated by FIB-4 > 3.25 compared with 11 (2.7 %) of the HIV-infected and HCV-uninfected men ($P=0.04$). Two HCV-monoinfected men (22 %) and 12 HIV/HCV-coinfected men (23 %) had advanced fibrosis. In the HIV-infected men without HCV coinfection, advanced fibrosis was associated with older age (median, 59 vs. 52, $P=0.002$), higher HOMA-IR (median, 6.3 vs. 3.1, $P=0.04$), and receipt of lipid-lowering agents (73 vs. 39 %, $P=0.02$; **Supplemental Table S2**). They also had longer cumulative ART (median years, 14 vs. 9, $P=0.002$), NRTI (median years, 13.5 vs. 8.5, $P=0.002$), non-NRTI (median years, 7 vs. 3, $P=0.01$), and dideoxynucleoside exposure (median years, 13.1 vs. 7.5, $P=0.01$). In the HIV/HCV-coinfected men, those with advanced fibrosis were more likely to have fatty liver (17 vs. 0, $P=0.01$) and to have had a nadir CD4 count <200 ($P=0.048$) compared with those without advanced fibrosis.

Factors associated with fatty liver in the entire cohort

The prevalence of fatty liver was 15 % in the entire cohort and was higher in HIV-uninfected compared with HIV-infected men (19 vs. 13 %, $P=0.02$). In univariate analysis, subjects with fatty liver were less likely to be Black (15 vs. 36 %, $P < 0.001$) or HCV infected (4 vs. 10 %, $P=0.04$) and were more likely to have impaired fasting glucose or diabetes (63 vs. 41 %, $P < 0.001$) or hypertension (62 vs. 47 %, $P=0.006$; **Table 2**). They also had significantly higher median body mass index (28.4 vs. 25.4 kg/m², $P < 0.001$), abdominal VAT volume (median, 223 cm² vs. 134 cm², $P < 0.001$), abdominal sub-cutaneous adipose tissue (median, 224 cm² vs. 195 cm², $P=0.006$), HOMA-IR (4.8 vs. 3.0, $P < 0.001$), triglycerides (160 vs. 119 mg/dl, $P < 0.001$), and lower median high-density lipoprotein cholesterol (43 vs. 48 mg/dl, $P < 0.001$). Median ALT was higher among the men with fatty liver (32 vs. 23 mg/dl, $P < 0.001$), as was median AST (25 vs. 23 mg/dl, $P=0.03$).

Finally, we examined the distribution of potential genetic associations with fatty liver status. The *PNPLA3* (rs738409) CC genotype was most common in the cohort (60 %), followed by GC (35 %) and GG (4.4 %). This distribution was in Hardy–Weinberg equilibrium and did not differ by HIV serostatus ($P=0.46$). The genotypes varied by race, with Blacks having the highest frequency of the CC genotype ($P < 0.001$ across groups; **Table 3**). Men with fatty liver were more likely to have the non-CC genotype compared with men without fatty liver (56 vs. 37 %, $P < 0.001$). The *NCAN* (rs2228603) TT genotype was also more frequent among the men with fatty liver but was not included in the multivariable model owing to its low frequency (0.7 %). The additional SNPs, *GCKR* (rs780094), *LYPLAL1* (rs12137855), and *PPP1R3B* (rs4240624), were not associated with fatty liver in our cohort.

In multivariable analysis, HIV serostatus remained associated with a lower odds of fatty liver (odds ratio (OR)=0.44; 95 % confidence interval (CI)=0.26, 0.74; $P=0.002$; **Table 4**). Factors independently associated with increased odds of fatty liver were abdominal VAT (OR=1.08 per 10 cm²; 95 % CI=1.04, 1.11; $P < 0.001$), *PNPLA3* (rs738409) non-CC genotype (OR=2.06; 95 % CI=1.24, 3.33; $P=0.005$), ALT > 40 U/l (OR=2.99; 95 %

CI=1.65, 5.42; $P<0.001$), and HOMA-IR 4.9 (OR=2.50; 95 % CI=1.44, 4.36; $P=0.001$). Black race was not significantly associated with decreased odds of fatty liver after adjusting for the other variables in the model. Using a liver/spleen ratio < 0.9 as a cutoff for fatty liver, as expected, the overall prevalence of fatty liver was lower (60 subjects, 8 %). However, the factors associated with fatty liver on multivariable analysis remained the same.

The finding of lower odds of fatty liver among HIV-infected men was unexpected. To exclude confounding by HCV infection, we performed a sensitivity analysis limited to HCV-uninfected participants, and the results of the multivariable analysis were qualitatively unchanged. We also considered that, even in the absence of HCV coinfection, the HIV-monoinfected subjects might have increased hepatic fibrosis, which might alter the liver attenuation on noncontrast CT scan (28). However, the results were unchanged after adjusting for AST-to-platelet ratio index or FIB-4.

Factors associated with fatty liver among HIV-infected subjects

To determine whether HIV-specific factors or medications were associated with fatty liver, we restricted the remaining analysis to HIV-infected subjects. In univariate analysis, HIV-specific factors associated with fatty liver included longer cumulative ART exposure (median, 9.8 years vs. 8.5 years, $P=0.006$), NRTI exposure duration (median, 9.5 years vs. 8.5 years, $P=0.006$), NNRTI exposure duration (median, 5 years vs. 2.5 years, $P=0.02$), lamivudine exposure (median, 7.8 years vs. 5.4 years, $P=0.01$), and dideoxynucleoside exposure (median, 4.1 years vs. 1.3 years, $P=0.04$; **Table 2**). Nadir CD4 cell count, presence of detectable plasma HIV RNA, current ART, and current antiretroviral use by medication class were not associated with fatty liver.

In multivariable analysis, more abdominal VAT (OR=1.07 per cm^2 ; 95 % CI=1.03, 1.12; $P<0.001$), ALT > 40 U/l (OR=2.54; 95 % CI=1.20, 5.36; $P=0.02$), HOMA-IR 4.9 (OR=2.54; 95 % CI=1.24, 5.19; $P=0.01$), greater cumulative dideoxynucleoside exposure (OR=1.44 per 5 years; 95 % CI=1.06, 1.97; $P=0.02$), and *PNPLA3* non-CC genotype (rs738409) (OR=3.30; 95 % CI=1.66, 6.57; $P=0.001$) were independently associated with increased odds of fatty liver (**Table 4**). Cumulative ART, NRTI, and NNRTI exposure was no longer significantly associated with fatty liver after adjusting for other covariates.

Interestingly, *PNPLA3* (rs738409) was not significantly associated with fatty liver in analysis limited to the HIV-uninfected men (OR=1.14; 95 % CI=0.51, 2.54; $P=0.75$). This was also noted in univariate analysis, comparing the prevalence of fatty liver by *PNPLA3* (rs738409) non-CC genotype after stratifying by HIV serostatus (**Figure 2**). To test whether *PNPLA3* (rs738409) genotype modified the association of HIV and fatty liver (or *vice versa*), we constructed an HIV and *PNPLA3* (rs738409) non-CC genotype interaction term. The interaction term trended toward a higher impact of *PNPLA3* (rs738409) among the HIV-infected men, but it was not statistically significant in multivariable analysis (OR=2.56; 95 % CI=0.94, 6.98; $P=0.07$).

DISCUSSION

In this well-characterized cohort of HIV-infected men who were extensively ART experienced, we found that HIV-infected men had a higher frequency of factors commonly associated with fatty liver, but, unexpectedly, they had a lower prevalence of CT-defined fatty liver compared with the HIV-uninfected men from the same cohort. This lower risk of fatty liver among HIV-infected men persisted after excluding HIV/HCV-coinfected men, adjusting for possible fibrosis (which may alter liver attenuation on CT), and using a more specific cutoff for CT-measured fatty liver. The only ART medications independently associated with fatty liver were dideoxynucleoside analogs. In both HIV-uninfected and -infected men, abdominal visceral adiposity and ALT > 40 IU/ml were associated with fatty liver. Notably, on stratified analysis, the *PNPLA3* (rs738409) non-CC genotype was associated with fatty liver only in HIV-infected men. Thus, in the current ART era, fatty liver prevalence is not increased in HIV-infected men in the MACS, and traditional metabolic and genetic factors are correlates of fatty liver in this setting.

The overall prevalence of fatty liver was 15 % in our cohort, a finding that was consistent with a report from another US-based cohort study involving HIV-uninfected persons that demonstrated a 17 % prevalence of nonalcoholic fatty liver disease using the same CT definition (29), and similar to the National Health and Nutrition Examination Survey data indicating a prevalence of 19 % using ultrasonography (30). Although noncontrast CT has high sensitivity and specificity for steatosis affecting 30 % of hepatic parenchyma, it is less sensitive for detecting mild (5–30 %) hepatic steatosis (24). In addition, our study excluded men with a history of cardiac surgery or coronary angioplasty as well as men weighing >300 pounds; therefore, our sample is likely biased toward a lower prevalence of fatty liver. For these reasons, our estimate of fatty liver is likely to be conservative in both the HIV-infected and -uninfected groups.

Thirteen percent of the HIV-infected men in our cohort had CT evidence of fatty liver. Other studies of HIV-infected individuals have estimated a prevalence of hepatic steatosis ranging from 13 to 53 % (4,5,31,32). Three of these studies used ultrasound to diagnose hepatic steatosis (4,31,32), a modality that tends to yield a higher prevalence, and two of these studies did not exclude participants with a history of heavy alcohol use (31,32). A study of 225 HIV-monoinfected patients at a metabolic clinic in Italy found a 37 % prevalence of fatty liver, as defined by noncontrast CT liver/ spleen ratio < 1.1 (5). In addition to using a different liver/spleen ratio cutoff for fatty liver than our study, this cohort had a different racial distribution and a very high prevalence of lipodystrophy. Unlike our study, these studies did not include HIV-uninfected controls and therefore could not address whether fatty liver is more common in the setting of HIV infection.

A major strength of our study is that both the HIV-infected and HIV-uninfected men came from the same cohort, which eliminates cohort differences that can confound comparisons. To our knowledge, this is the first study to directly compare the prevalence of fatty liver between these groups in a predominantly HCV-uninfected cohort. One novel finding is the lower fatty liver prevalence in HIV-infected men compared with HIV-uninfected men despite adjusting for metabolic risk factors. The reason for this is unclear, but it is possible

that the HIV-infected men had more frequent physician encounters, thus allowing for better control of risk factors for fatty liver disease. Our findings should also be interpreted with caution, as the correlation between noncontrast CT and histologic steatosis has been validated primarily among healthy HIV-uninfected individuals (24,33). CT may be less accurate in detecting steatosis in the setting of HIV infection and may miss both microvesicular steatosis and milder degrees of macrovesicular steatosis. Nevertheless, our findings suggest that in the current ART era, moderate or greater steatosis is common but is not increased in HIV-infected individuals on ART compared with HIV-uninfected persons. Prior studies evaluating the role of HIV infection on liver steatosis have been limited to HCV-infected individuals; results have been conflicting, and a meta-analysis failed to demonstrate an increased risk of histologic steatosis associated with HIV-coinfection (11,34–36).

Our study is the first to examine the relationship between the rs738409 SNP in the *PNPLA3* gene and fatty liver in the setting of HIV infection. This SNP, which has been noted in previous studies of HIV-uninfected persons (12–15), was associated with an increased risk of fatty liver in our cohort. *PNPLA3* encodes a protein with triglyceride hydrolase activity. The rs738409 mutation leads to markedly reduced enzymatic activity, but susceptibility for steatosis is increased via a gain-of-function mechanism that appears to be independent of lipid abnormalities or insulin resistance (16,37). What was unexpected in our findings was that stratification by HIV serostatus revealed an association only in HIV-infected subjects. It has been postulated that this SNP is important as a secondary risk when an environmental stressor such as obesity or alcohol is present (38,42,43). Among HIV-infected subjects, HIV may constitute the environmental stressor, as we have shown that HIV mono-infection affects ALT levels (28). The effects of *PNPLA3* may also be modified by gender, with a stronger association of *PNPLA3* and steatosis among women (13,39). These findings may also explain, in part, why we did not see an association of *PNPLA3* rs738409 and fatty liver in the HIV-uninfected men in this relatively lean cohort.

In the whole cohort as well as in analytic models limited to HIV-infected men, we found a strong positive association between metabolic factors and fatty liver, with greater amounts of abdominal visceral adiposity and HOMA-IR, each independently associated with fatty liver. These findings are consistent with well-established literature correlating metabolic risk factors and hepatic steatosis in the general population as well as in HCV-infected individuals (with or without HIV coinfection) (8,9,11,40). Other studies of HIV-mono-infected individuals have also demonstrated an increased risk of hepatic steatosis with greater waist circumference, higher serum triglyceride level, and lower high-density lipoprotein levels (4,5). We did not find an association between HCV infection and fatty liver risk. All of the HCV-infected men in our cohort had HCV genotype 1 or 2. Although it is postulated that HCV genotype 3 causes steatosis via viral-mediated dysregulation of lipoprotein metabolism (41), in non-genotype 3, HCV infection steatosis appears to be primarily mediated by metabolic factors (40,42).

Among HIV-infected men, cumulative exposure to dideoxynucleoside analogs was the only ART factor that was significantly associated with an increased risk of fatty liver in multivariable analysis. The dideoxynucleoside analogs inhibit mitochondrial polymerase γ ;

subsequent accumulation of dysfunctional mitochondria leads to increased lipid accumulation within hepatocytes (43). Dideoxynucleoside analog use has previously been associated with increased risk of hepatic steatosis and steatosis progression in a cross-sectional and prospective study, respectively, of HIV/HCV-coinfected individuals (44,45). In an HIV-monoinfected cohort, cumulative NRTI exposure was independently associated with increased risk of nonalcoholic fatty liver disease, although no individual agents increased the risk of nonalcoholic fatty liver disease (5). It is encouraging that the newer antiretroviral agents which comprise the majority of current use were not associated with fatty liver.

One limitation of our study was the inability to assess whether HIV infection itself directly affects steatosis, as 92 % of the men were receiving ART. Another limitation is that, although we adjusted for numerous factors in our multivariable model, residual confounding is likely. Finally, our findings may not be applicable to the general HIV-infected population as the MACS is a male cohort, includes few injection drug users, and mostly comprises people with relatively high socioeconomic status. Further studies in other cohorts that have HIV-infected and -uninfected persons are needed.

In summary, in this large cohort of well-characterized HIV-infected and HIV-uninfected men, we found that CT-defined fatty liver was common and was positively associated with abdominal visceral adiposity volume, HOMA-IR, and *PNPLA3* rs738409, whereas ART-treated HIV infection was associated with a lower risk of fatty liver. Among the HIV-infected men, prolonged exposure to dideoxynucleoside analog drugs was associated with a higher risk of fatty liver but none of the newer agents, which are used commonly today, were associated with it. These findings may help clinicians identify HIV-infected patients who are at increased risk of developing fatty liver so that potentially modifiable fatty liver risk factors can be targeted for intervention. Future studies are needed to determine whether HIV infection modifies the risk for development of hepatic fibrosis and adverse liver-related outcomes in persons with fatty liver disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Nonalcoholic fatty liver disease is common in the general population.
- ✓ Metabolic abnormalities and abdominal visceral adiposity are associated with an increased risk of fatty liver and are also common in antiretroviral therapy (ART)-treated HIV-infected individuals.
- ✓ Whether individuals with HIV on ART are at increased risk of fatty liver is unclear.

WHAT IS NEW HERE

- ✓ HIV-infected men (92 % on ART) in the Multicenter AIDS Cohort Study (MACS) had a lower prevalence of CT-defined fatty liver compared with HIV-uninfected controls, even after adjusting for metabolic and genetic risk factors for fatty liver.
- ✓ This suggests that, in the current ART era, HIV-infected individuals are not at increased risk of fatty liver and that fatty liver is predominantly mediated by metabolic and genetic factors in the setting of HIV.
- ✓ Further research is needed to confirm these findings and to determine whether HIV modifies the risk of steatohepatitis and fibrosis in persons with fatty liver.

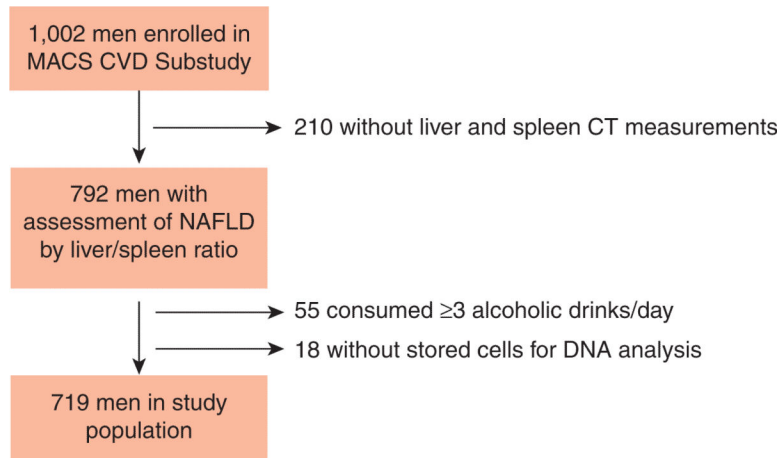


Figure 1.
Flow chart demonstrating inclusion in study sample.

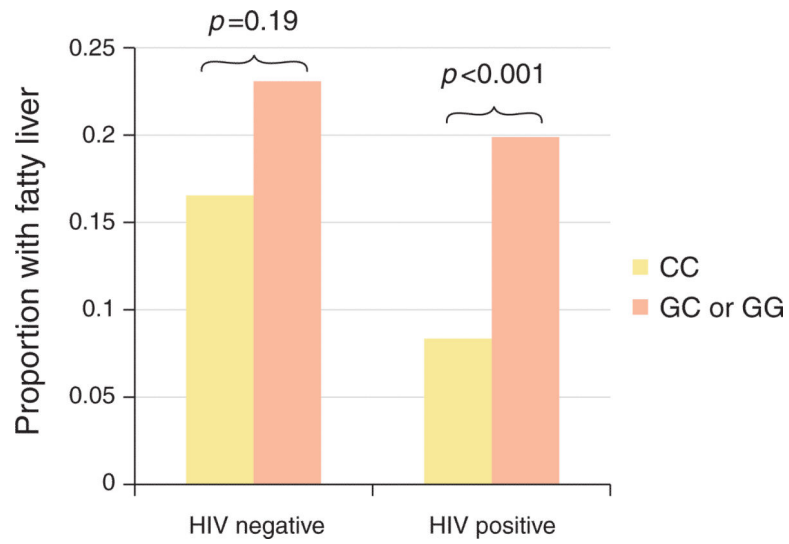


Figure 2. Proportion of participants with fatty liver by human immunodeficiency virus (HIV) and phospholipase domain-containing 3 (*PNPLA3*; rs738409) gene status.

Table 1

Characteristics of the study population^a

	HIV- (N=254)	HIV + (N=465)	P value
<i>Race</i>			
White non-Hispanic, N (%)	162 (64)	242 (52)	0.008
Black non-Hispanic	69 (27)	168 (36)	
Other ^b	22 (9)	55 (12)	
Age (years), median (IQR)	55 (51, 62)	53 (48, 58)	<0.001
BMI (kg/m ²), median (IQR)	26.3 (24.2, 29.9)	25.5 (23.3, 28.6)	0.002
Abdominal VAT (cm ²), median (IQR)	139 (88, 210)	143 (82, 211)	0.91
Abdominal SAT (cm ²), median (IQR)	231 (168, 304)	181 (114, 278)	0.007
IFG or diabetes ^c , N (%)	100 (43)	194 (45)	0.61
HOMA-IR, median (IQR)	2.9 (2.2, 4.5)	3.3 (2.3, 5.1)	0.007
HOMA-IR > 4.9, N (%)	48 (21)	114 (27)	0.08
On lipid-lowering agent, N (%)	76 (31)	167 (37)	0.11
Total cholesterol (mg/dl), median (IQR)	187 (162, 209)	182 (157, 212)	0.41
HDL (mg/dl), median (IQR)	50 (41, 60)	46 (37, 54)	< 0.001
LDL (mg/dl), median (IQR)	108 (89, 132)	103 (81, 130)	0.02
Triglycerides (mg/dl), median (IQR)	106 (80, 146)	135 (97, 208)	< 0.001
HTN ^d , N (%)	115 (47)	226 (50)	0.41
ALT (U/l), median (IQR)	21 (17, 28)	26 (19, 39)	< 0.001
ALT > 40 U/l	23 (9)	104 (23)	< 0.001
AST (U/l), median (IQR)	21 (18, 26)	24 (20, 34)	< 0.001
AST > 40 U/l	9 (4)	67 (15)	< 0.001
APRI > 1.5	1 (0.4) ^e	19 (4.2) ^f	0.004
FIB-4 > 3.25	3 (1.2) ^g	23 (5.1) ^h	0.01
HCV, N (%) ⁱ	9 (4)	56 (12)	< 0.001
<i>PNPLA3 (rs738409)</i>			
CC, N (%)	145 (57)	288 (62)	0.46
GC	96 (38)	157 (34)	
GG	12 (5)	19 (4)	
Detectable HIV VL, N (%)		47 (10)	
Nadir CD4 < 200 cells/ml, N (%)		147 (33)	
Current ART, N (%)		420 (92)	
Cumulative ART (years), median (IQR)		8.5 (6.0, 12.5)	
Current NRTI, N (%)		400 (87)	
Cumulative NRTI (years), median (IQR)		8.5 (6.0, 12.5)	
Current PI, N (%)		223 (49)	
Cumulative PI (years), median (IQR)		4.0 (0.5, 8.0)	
Current NNRTI, N (%)		205 (45)	

	HIV- (N=254)	HIV + (N=465)	P value
Cumulative NNRTI (years), median (IQR)		3.0 (0.5, 6.5)	

ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; HCV, hepatitis C virus; HOMA-IR, homeostatic model assessment of insulin resistance; HTN, hypertension; IFG, impaired fasting glucose; IQR, interquartile range; LDL, low-density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; VL, viral load.

^aMissing values: race=1, BMI=39, abdominal VAT=2, abdominal SAT=2, IFG or diabetes=52, HOMA-IR=63, lipid-lowering agent=15, total cholesterol=66, HDL=63, LDL=71, triglycerides=63, HTN=24, ALT=14, AST=14, HCV=12, PNPLA3 (rs738409)=2, HIV viral load=8, nadir CD4=16, ART medication use=6.

^b70/77 Self-identified as Hispanic.

^cDefined by fasting glucose or use of diabetes medication.

^dDefined by blood pressure or use of anti-hypertensive medication.

^e0 HCV infected.

^f13 HCV infected.

^g2 HCV infected.

^h12 HCV infected.

ⁱ58 Genotype 1, 7 Genotype 2.

Table 2

Characteristics of participants without and with fatty liver^a

	No fatty liver (N=611)	Fatty liver (N=108)	P value
HIV-infected, N (%)	406 (66)	59 (55)	0.02
<i>Race</i>			
White non-Hispanic, N (%)	325 (54)	79 (73)	< 0.001
Black non-Hispanic	221 (36)	16 (15)	
Other	64 (10)	13 (12)	
Age (years), median (IQR)	53 (49, 59)	54 (49, 59)	0.48
BMI (kg/m ²), median (IQR)	25.4 (23.4, 28.4)	28.4 (25.6, 31.8)	< 0.001
Abdominal VAT (cm ²), median (IQR)	134 (79, 197)	223 (134, 304)	< 0.001
Abdominal SAT (cm ²), median (IQR)	195 (125, 280)	224 (161, 339)	0.006
IFG or diabetes ^b , N (%)	228 (41)	66 (63)	< 0.001
HOMA-IR, median (IQR)	3.0 (2.2, 4.4)	4.8 (3.1, 6.8)	< 0.001
HOMA-IR > 4.9, N (%)	112 (20)	50 (50)	< 0.001
On lipid-lowering agent, N (%)	198 (33)	45 (43)	0.05
Total cholesterol (mg/dl), median (IQR)	184 (159, 212)	187 (158, 213)	0.82
HDL (mg/dl), median (IQR)	48 (40, 58)	43 (35, 51)	< 0.001
LDL (mg/dl), median (IQR)	105 (83, 131)	105 (81, 132)	0.85
Triglycerides (mg/dl), median (IQR)	119 (85, 178)	160 (110, 225)	< 0.001
HTN ^c , N (%)	277 (47)	64 (62)	0.006
ALT (U/I), median (IQR)	23 (17, 33)	32 (22, 47)	< 0.001
ALT > 40 U/I	93 (16)	34 (32)	< 0.001
AST (U/I), median (IQR)	23 (19, 30)	25 (20, 35)	0.010
AST > 40 U/I	58 (10)	18 (17)	0.03
HCV, N (%)	61 (10)	4 (4)	0.04
APRI > 1.5	17 (3)	3 (3)	0.99
FIB-4 > 3.25	23 (4)	3 (3)	0.62
<i>PNPLA3 (rs738409)</i>			
CC, N (%)	385 (63)	48 (44)	0.001
GC	198 (33)	55 (51)	
GG	26 (4)	5 (5)	
<i>NCAN (rs2228603)</i>			
CC, N (%)	554 (86)	92 (14)	0.01
TC	54 (82)	12 (18)	
TT	2 (40)	3 (60)	
<i>GCKR (rs780094)</i>			
CC, N (%)	263 (86)	43 (14)	0.78
TC	276 (84)	51 (16)	
TT	70 (83)	14 (17)	
<i>LYPLAL1 (rs12137855)</i>			

	No fatty liver (N=611)	Fatty liver (N=108)	P value
TT, N (%)	26 (93)	2 (7)	0.41
CT	177 (83)	35 (17)	
CC	406 (85)	70 (15)	
<i>PPP1R3B (rs4240624)</i>			
GG, N (%)	13 (100)	0	0.18
AG	138 (87)	20 (13)	
AA	459 (84)	88 (16)	
Detectable HIV VL ^d , N (%)	42 (11)	5 (9)	0.66
Nadir CD4 < 200 ^d , N (%)	130 (33)	17 (30)	0.69
Current ART ^d , N (%)	367 (92)	53 (92)	0.97
Current NRTI ^d , N (%)	349 (87)	51 (88)	0.85
Current PI ^d , N (%)	197 (49)	26 (45)	0.54
Current NNRTI ^d , N (%)	174 (43)	31 (54)	0.15
Cumulative ART (years) ^d , median (IQR)	8.5 (6.0, 12.5)	9.8 (8.0, 13.5)	0.006
Cumulative NRTI (years) ^d , median (IQR)	8.5 (5.5, 12.5)	9.5 (8.0, 13.0)	0.006
Cumulative PI (years) ^d , median (IQR)	4.0 (0.5, 7.5)	5.0 (1.0, 8.0)	0.23
Cumulative NNRTI (years) ^d , median (IQR)	2.5 (0.5, 6.5)	5.0 (1.0, 8.0)	0.02
Cumulative Lamivudine (years) ^d , median (IQR)	5.4 (1.2, 9.0)	7.8 (3.7, 11.0)	0.01
Cumulative Efavirenz (years) ^d , median (IQR)	1.0 (0, 4.7)	2.5 (0, 7.1)	0.25
Cumulative dideoxy (years) ^d , median (IQR)	1.3 (0, 5.8)	4.1 (0, 8.0)	0.04

ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HTN, hypertension; IFG, impaired fasting glucose; IQR, interquartile range; LDL, low-density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

^aMissing values: race=1, BMI=39, abdominal VAT=2, abdominal SAT=2, IFG or diabetes=52, HOMA-IR=63, lipid-lowering agent=15, total cholesterol=66, HDL=63, LDL=71, triglycerides=63, HTN=24, ALT=14, AST=14, HCV=12, PNPLA3 (rs738409)=2, HIV viral load=8, nadir CD4=16, ART medication use=6.

^bDefined by fasting glucose or use of diabetes medication.

^cDefined by blood pressure or use of anti-hypertensive medication.

^dAmong HIV + only.

Table 3Distribution of *PNPLA3* (rs738409) genotype by race

	<i>PNPLA3</i> (rs738409) genotype^{a,b}		
	CC	GC	GG
White, non-Hispanic, <i>N</i> (%)	222 (55)	158 (39)	23 (6)
Black, non-Hispanic, <i>N</i> (%)	180 (76)	54 (23)	3 (1)
Other ^c , <i>N</i> (%)	30 (39)	41 (54)	5 (7)
Total, <i>N</i> (%)	432 (60)	253 (35)	31 (4)

^aMissing=3.^b*P* value across groups < 0.001.^c70/77 (91%) Identified as Hispanic.

Table 4

Multivariable analysis of factors associated with fatty liver among the entire cohort and among the HIV-infected men only

	<u>Entire cohort (n=652)^a</u>		<u>HIV uninfected (n=230)^a</u>		<u>HIV infected (n=421)^a</u>	
	OR	P value	OR	P value	OR	P value
HIV	0.44 (0.26, 0.74)	0.002				
Abdominal VAT (per 10 cm ²)	1.08 (1.04, 1.11)	< 0.001	1.08 (1.03, 1.14)	0.002	1.07 (1.03, 1.12)	< 0.001
PNPLA3 (rs738409) non-CC	2.03 (1.24, 3.33)	0.005	1.14 (0.51, 2.54)	0.746	3.30 (1.66, 6.57)	0.001
ALT > 40 U/l	2.99 (1.65, 5.42)	< 0.001	7.21 (2.13, 24.41)	0.002	2.54 (1.20, 5.36)	0.015
HOMA-IR 4.9 ^b	2.50 (1.44, 4.36)	0.001	2.27 (0.89, 5.76)	0.084	2.54 (1.24, 5.19)	0.011
<i>Race (ref=white)</i>						
Black	0.49 (0.24, 1.03)	0.059	0.48 (0.13, 1.74)	0.266	0.54 (0.21, 1.39)	0.200
Other	0.95 (0.41, 2.24)	0.915	1.11 (0.27, 4.59)	0.883	0.61 (0.19, 2.02)	0.423
HCV	0.55 (0.17, 1.82)	0.329	0.40 (0.03, 5.95)	0.503	0.54 (0.14, 2.18)	0.394
Cumulative dideoxynucleoside (per 5 year exposure)					1.44 (1.06, 1.97)	0.021

ALT, alanine aminotransferase; HOMA-IR, homeostatic model assessment of insulin resistance; VAT, visceral adipose tissue.

^aAlso adjusted for age and MACS site.

^b75% Percentile for HOMA in the cohort.