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INFECTIOUS DISEASES

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Decreased intestinal abundance of *Akkermansia muciniphila* is associated with metabolic disorders among people living with HIV

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ABSTRACT

Background: Previous studies have shown changes in gut microbiota after human immunodeficiency virus (HIV) infection, but there is limited research linking the gut microbiota of people living with HIV (PLWHIV) to metabolic diseases.

Methods: A total of 103 PLWHIV were followed for 48 weeks of anti-retroviral therapy (ART), with demographic and clinical data collected. Gut microbiome analysis was conducted using metagenomic sequencing of fecal samples from 12 individuals. Nonalcoholic fatty liver disease (NAFLD) was diagnosed based on controlled attenuation parameter (CAP) values of 238 dB/m from liver fibro-scans. Participants were divided based on the presence of metabolic disorders, including NAFLD, overweight, and hyperlipidemia. *Akkermansia* abundance in stool samples was measured using RT-qPCR, and Pearson correlation and logistic regression were applied for analysis.

Results: Metagenomic sequencing revealed a significant decline in gut *Akkermansia* abundance in PLWHIV with NAFLD. STAMP analysis of public datasets confirmed this decline after HIV infection, while KEGG pathway analysis identified enrichment of metabolism-related genes. A prospective cohort study with 103 PLWHIV followed for 48 weeks validated these findings. *Akkermansia* abundance was significantly lower in participants with NAFLD, overweight, and hyperlipidemia at baseline, and it emerged as an independent predictor of NAFLD and overweight. Negative correlations were observed between *Akkermansia* abundance and both CAP values and body mass index (BMI) at baseline and at week 48. At the 48-week follow-up, *Akkermansia* remained a predictive marker for NAFLD.

Conclusions: *Akkermansia* abundance was reduced in PLWHIV with metabolic disorders and served as a predictive biomarker for NAFLD progression over 48 weeks of ART.

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1. Introduction

With the widespread implementation of highly active antiretroviral therapy (HAART) in clinical settings, there has been a notable improvement in the prognosis of people living with HIV (PLWHIV) [1]. This enhanced life expectancy has, however, given rise to a new set of challenges, particularly the emergence of chronic metabolic disease in HIV infection that have garnered considerable attention [2]. Among the prevalent metabolic disorders in PLWHIV are alterations in fat distribution, non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, and overweight [2,3]. Notably, the incidence of overweight or obesity among PLWHIV in the United States exhibited a substantial increase from 28% between 1985 and 1990 to 51% between 1996

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and 2004, a trend observed prior to the initiation of HAART [4]. Concurrently, the prevalence of NAFLD among Asian PLWHIV was reported to be 28.7% [5]. In contrast the prevalence of NAFLD among general Asian population was 5.09% [6].

The etiology of metabolic diseases among PLWHIV exhibits a multifaceted nature. Both general risk factors applicable to the broader population and factors unique to PLWHIV contribute to the onset and progression of HIV related metabolic diseases [3]. Economic factors, unhealthy lifestyles, and enetic predispositions collectively elucidate a portion of the escalating prevalence of metabolic disorders within PLWHIV, mirroring trends observed in the general population [7,8]. Additionally, certain components of HAART regimens, such as integrase strand transfer inhibitors (INSTIs), protease inhibitors (PIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs), have been identified as contributors to generalized weight gain following HAART initiation [9]. Notably, INSTI-based HAART regimens exert a particularly pronounced influence on weight gain [10]. Furthermore, the burgeoning recognition of gut dysbiosis as a significant factor in metabolic disorders, including obesity [11] and non-alcoholic fatty liver disease (NAFLD) [12], has garnered considerable attention. Interestingly, recent studies increasingly suggest that dysbiosis of gut microbiota is an overlooked but significant contributor to metabolic disorders [13,14].

The relationship between HIV infection and variation in gut microbiota of PLWHIV has been the focus of extensive research. Researchers found an HIV-1-specific signature in the gut microbiome of PLWHIV after acute HIV infection, including depletion of *Akkermansia*, *Anaerovibrio*, *Bifidobacterium*, and *Clostridium*, and these alterations were linked to chronic inflammation and CD8+ T cell anergy [15]. During the initial stages of HIV infection, HIV-1 replication occurs within the gut-associated lymphoid tissue, resulting in a substantial reduction of mucosal effector CD4+ T cells, including T helper (Th) 17 cells and Th22 cells, which are crucial for maintaining gut integrity [16]. These pathological changes, indicative of compromised gut barrier function, facilitate microbial translocation from the intestinal tract [17]. Continual immune activation triggered by commensal microbial products from gut is implicated in persistent chronic inflammation, a factor confirmed to be associated with the development of chronic metabolic diseases [18].

Akkermansia muciniphila (AKK), a confirmed characteristic depleted gut bacterium in PLWHIV [15], has been extensively investigated in diverse patient populations, including those with NAFLD, obesity, hyperlipidemia, and cardiovascular disease (CVD) [19–22]. This mucin-degrading bacterium resides within the intestinal mucus layer of humans, demonstrating the capability to restore mucus thickness and enhance intestinal barrier integrity [23]. Depletion of *AKK* has been observed in intestinal of individuals with chronic metabolic diseases, suggesting a potential protective role in the onset and progression of these conditions through various mechanisms. For instance, *AKK* has been implicated in the regulation of gut barrier function, establishing a connection with alcoholic liver disease (ALD) [19], a role that has been previously investigated [24]. A recent study highlighted *AKK*'s preventive effect on fatty liver disease, elucidating its ability to modulate the expression of genes governing fat synthesis and inflammation in the liver [25]. However, research on *AKK* in PLWHIV is severely lacking. Whether metabolic disorders in PLWHIV are associated with *AKK* abundance requires further investigation.

Given that HIV infection may diminish the abundance of *AKK* in the gut tract of PLWHIV [15], particularly considering its pivotal role in metabolic diseases such as NAFLD [12] and obesity [21]. Previous research has established a correlation between *AKK* abundance, microbial translocation, and inflammation in PLWHIV, identifying this relationship as a critical pathway in the pathogenesis of liver disease [26]. Furthermore, emerging perspectives posit *AKK* as a safeguard for gut permeability, thereby impeding microbial translocation, mitigating inflammation, and ultimately diminishing the risks of non-AIDS comorbidities in PLWHIV [27].

Building upon these insights, we hypothesize that intestinal *AKK* may exert an influence on metabolic disorders among PLWHIV. The primary objective of this study is to investigate the correlation between intestinal *AKK* abundance and the occurrence of metabolic disorders, specifically overweight (obesity), NAFLD, and hyperlipidemia among PLWHIV.

2. Materials and methods

2.1. Study design and participants enrollment

We performed metagenomic sequencing of gut microbiome in fecal samples from 12 PLWHIV receiving no anti-retroviral therapy, including 6 with NAFLD and 6 without. Differential bacterial species associated with NAFLD were identified. Subsequently, 103 PLWHIV were prospectively enrolled at first medical contact and followed-up at the Department of Infectious Diseases, Nanfang Hospital. HIV diagnosis was confirmed in accordance with the *2021 Chinese Guidelines for the Diagnosis and Treatment of AIDS* [28]. Diagnosis of liver steatosis (NAFLD) was established on controlled

attenuation parameter (CAP) values of 238 dB/m measured with Fibro-scan [29]. The BMI cutoff value was 24 to distinguish obese-NAFLD and 18.5 to distinguish lean-NAFLD [30]. Exclusion criteria for PLWHIV included: (1) the presence of primary intestinal diseases impacting on gut microbiota, such as inflammatory bowel disease (IBD) and colorectal cancer; (2) recent acute intestinal infections; (3) recent systemic antibiotic use; and (4) co-infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).

Ethical approval for the study was granted by the Ethics Committee of Nanfang Hospital, and written informed consent was diligently obtained from each participant. All procedures strictly adhered to the ethical standards set by the responsible committee on human experimentation and conformed to the principles outlined in the *Declaration of Helsinki*.

2.2. Demographic data and laboratory tests

Basic demographic information and clinical data of PLWHIV were collected. The HAART regimens were classified based on whether they contained INSTIs. The AIDS stage was diagnosed as HIV infection with CD4+ T cell counts below 200 cells/μl [28]. Blood cell counts were analyzed using a Sysmex SE9000 automatic blood cell analyzer. Serum biochemical tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), and other parameters, were measured using an Olympus AU5400 automatic biochemical analyzer. CD4+ and CD8+ T lymphocyte counts were determined by flow cytometry. HIV Ribonucleic Acid (RNA) viral load was detected by polymerase chain reaction (PCR) and expressed as $\log_{10}$ copies/ml.

2.3. Metagenomic sequencing and bioinformatic analysis

High-quality whole metagenomic sequencing was performed for 12 samples consisting of 6 participants with NAFLD and 6 without NAFLD. We constructed one DNA paired-end (PE) library with an insert size of 300–400 base pairs (bp) for each sample, following the manufacturer's instructions (Illumina, San Diego, California, USA). All samples were sequenced using the PE 150 strategy on the MGISEQ 2000. Host sequences were processed and removed to generate clean data, and non-redundant genes were aligned to the KEGG database to complete gene functional annotation. Kraken2 alignment was used to calculate the sequence number of species contained in the sample, and then Bracken2 was used to estimate the actual abundance of species in the sample, complete species annotation, and calculate its alpha diversity and beta diversity. The raw data was stored at Bioproject PRJNA1167913.

Raw 16S rRNA gene sequences of fecal samples from healthy controls and PLWHIV were downloaded from Bioproject PRJNA673102 [31] and Bioproject PRJNA450025 [15] following the policy about data availability. Principal component analysis (PCA) was performed to identify batch effect, and it was corrected by ComBat function. Bioinformatic analysis was then performed to identify changes in the microbiome after HIV infection. STAMP analysis was used to explore the differences in species distribution between the two groups.

2.4. Detection of abundance of AKK in fecal samples

Fecal samples were collected from 103 PLWHIV for PCR detection for *AKK* abundance at baseline before initiation of ART. After pre-processing by filtration and centrifugation of stool samples from PLWHIV, the purification and precipitation steps were carried out using the MolPure® Stool DNA Kit to extract bacterial DNA. The primers used to detect *AKK* were based on 16S rDNA gene sequences: *AKK* forward: CAG CAC GTG AAG GTG GGG AC; *AKK* reverse: CCT TGC GGT TGG CTT CAG AT [32]. Detection was achieved using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) with the ChamQ Universal SYBR qPCR Master Mix Kit (Vazyme). The cycle threshold of each sample was then compared with a standard curve, performed in duplicate, made by diluting standard genomic DNA from *AKK* (MBD0001) purchased from Sigma-Aldrich. The data are expressed as Ct values of *AKK* DNA copies in fecal content.

2.5. Controlled attenuation parameter (CAP) measured by transient elastography

The CAP value was assessed using liver transient elastography by a professionally trained technician, according to the manufacturer's instructions. Ultrasonic attenuation was measured at 3.5 MHz using signals assessed by transient elastography. The median CAP value was expressed in decibels per meter (dB/m). A CAP value was considered reliable with both an interquartile range/median <0.3 and a success rate of >60%. We considered CAP values of >238, 260, and 290 dB/m for > S0, >S1, and > S2, respectively, to indicate liver steatosis [29].

2.6. Statistical analysis

The data were expressed as mean ± SD for normally distributed data and median (range) for data showing non-normal distribution. Categorical data were expressed as a percentage. Paired *t*-test and one-way ANOVA were used to compare the differences, when appropriate. Pearson correlation analysis was conducted to determine the relationship between two variables. The area under the receiver operating characteristic (AUROC) curve and logistic regression were calculated. Sensitivity, specificity, and odds ratio (OR) values were calculated. All analyses were performed using SPSS (version 26.0) software with a significant level of 0.05. Figures were generated using GraphPad Prism 8.0, and the graphical abstract image was created online with BioRender.com.

3. Results

3.1. Abundance of AKK decreased in PLWHIV and further decreased in PLWHIV with the presence of NAFLD

Gut microbiome metagenomic sequencing was performed on fecal samples from 6 individuals diagnosed with NAFLD and 6 PLWHIV diagnosed without NAFLD and the baseline characteristics of these 12 individuals were shown in Supplementary Table 1. The β-diversity in the non-NAFLD group was notably lower than in the NAFLD group by Wilcoxon test ($p = 0.026$) (Figure 1A). However, no statistically significant difference was observed in α-diversity between the two groups (Figure 1B). Notably, STAMP analysis revealed that the abundance of *Akkermansia muciniphila (AKK)* decreased in participants with NAFLD (mean relative abundance: 0.012% versus 0.024%, $p = 0.026$) (Figure 1C). KEGG analysis revealed a substantial number of genes annotated in metabolism-related signaling pathways (Figure 1D). Additionally, STAMP analysis of data sourced from public databases identified the intestinal species declined in PLWHIV compared to healthy controls where *AKK* was one of them (mean relative abundance: 0.430% versus 1.445%, $p = 0.017$) (Supplementary Figure 1). The combined findings showed that *AKK* was a shared species decreased in both populations of PLWHIV (compared to healthy controls) and in populations of PLWHIV with NAFLD (compared to PLWHIV without NAFLD) (Figure 1E). Based on these results, we found that intestinal *AKK* may be associated with

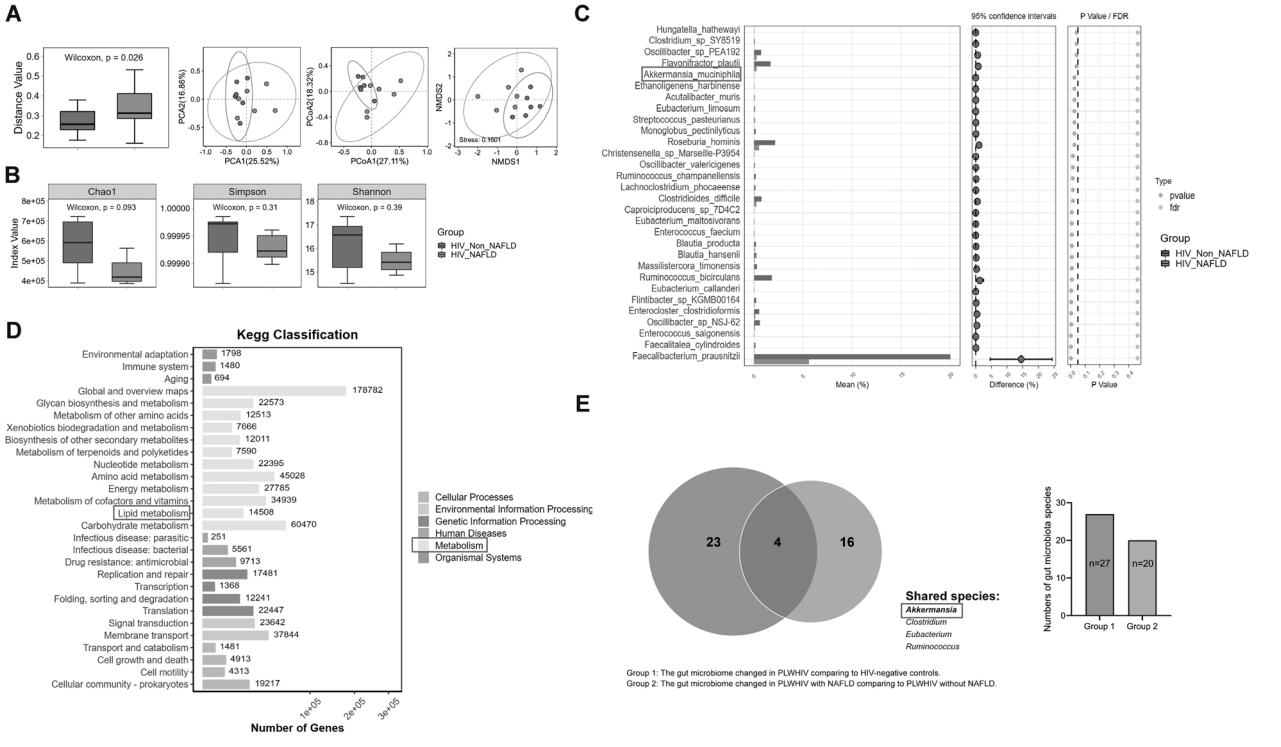

Figure 1. Analysis on gut microbiome of PLWHIV with NAFLD. (A) β-diversity of gut microbiome in groups of PLWHIV with and without NAFLD. (B) α-Diversity of gut microbiome in groups of PLWHIV with and without NAFLD. (C) STAMP analysis for species in fecal samples from PLWHIV. (D) KEGG Pathway analysis for genes annotation associated with different pathways. (E) Venn diagram showed shared gut species decreased both in PLWHIV (compared to healthy controls) and PLWHIV with NAFLD (compared to PLWHIV without NAFLD). *Abbreviations:* HIV: Human immunodeficiency virus; NAFLD: Nonalcoholic fatty liver disease; PLWHIV: People living with HIV.

NAFLD comorbidity in PLWHIV, but the findings need more evidence to confirm.

3.2. Confirmation of the relationship between abundance of AKK and NAFLD

A prospective cohort was constructed to further investigate the relationship between intestinal *AKK* abundance and metabolic disorder comorbidities in PLWHIV. A total of 103 PLWHIV were included and then grouped into the NAFLD group ($n=52$) and the non-NAFLD group ($n=51$) according to their baseline CAP values. The baseline characteristics were not significantly different between the two groups, except for baseline body mass index (BMI) ($p<0.001$), triglyceride levels ($p<0.001$), alanine aminotransferase (ALT) levels ($p=0.003$), albumin (ALB) levels ($p=0.013$) and uric acid (UA) levels ($p=0.013$) (Table 1). We found that the *AKK* abundance in PLWHIV with NAFLD group was lower than that in the non-NAFLD group ($p=0.049$) (Figure 2A). And there was a negative linear relationship between the Ct values of *AKK* and CAP values ($r=-0.241$, $p=0.014$) (Figure 2B). When PLWHIV with NAFLD was further categorized into liver steatosis severity of class S0–S3, based on CAP values, we found *AKK* abundance from S3 participants was significantly lower than it from S0 class ($p=0.043$) (Figure 2C). Considering the different causes and mechanisms between the two phenotypes of obese non-alcoholic fatty liver disease and lean non-alcoholic fatty liver disease, we next divided the NAFLD group into obese NAFLD and lean NAFLD groups based on baseline BMI. The abundance of *AKK* in the obese NAFLD group was lower than in the non-NAFLD group ($p=0.0233$), but the abundance of *AKK* in the lean NAFLD group was not different from the non-NAFLD group ($p=0.5282$) (Figure 2D).

Table 1. Baseline characteristics of the PLWHIV with and without NAFLD.

	People living with HIV		
Characteristic	Without NAFLD	With NAFLD	*p* Value
Sample size	51	52	
Age, years	32.78 ± 10.32	36.52 ± 12.84	0.107
Gender, %			1.000
Female	1 (1.9)	2 (3.8)	
Male	50 (98.1)	50 (96.2)	
Sex orientation			0.323
Heterosexuality	37 (72.5)	33 (63.5)	
Homosexuality	14 (27.5)	19 (36.5)	
Bisexuality	0	0	
BMI	19.51 ± 2.86	25.69 ± 3.16	<0.001
Disease stage, %			0.615
Non-AIDS stage	36 (70.6)	39 (75.0)	
AIDS stage	15 (29.4)	13 (25.0)	
ART regimen, %			0.952
INSTI based	14 (27.5)	14 (26.9)	
Others	37 (72.5)	38 (73.1)	
CD4+ T counts, cells/μl	295.88 ± 167.14	327.42 ± 161.73	0.333
CD8+ T counts, cells/μl	1276.37 ± 1067.56	1076.77 ± 463.31	0.220
HIV RNA, $\log_{10}$ copies/ml	4.16 ± 0.79	4.10 ± 0.79	0.735
ALT, U/L	21.57 ± 14.92	31.56 ± 17.78	0.003
AST,U/L	23.49 ± 14.09	25.02 ± 10.25	0.530
ALB, g/L	44.85 ± 4.42	46.78 ± 3.24	0.013
Cr, umol/L	77.63 ± 11.83	79.35 ± 15.26	0.525
UA, umol/L	403.06 ± 99.75	448.67 ± 83.37	0.013
CRP, mg/L	2.93 ± 5.18	2.90 ± 4.58	0.972
CHOL, mmol/L	4.10 ± 0.85	4.31 ± 0.80	0.205
LDL, mmol/L	2.57 ± 0.62	2.68 ± 0.63	0.370
TG, mmol/L	1.07 ± 0.39	1.94 ± 0.95	<0.001

Abbreviation: AIDS: Acquired immunodeficiency syndrome; ALB: Albumin; ALT: Alanine aminotransferase; ART: Anti-retroviral therapy; BMI: Body mass index; CHOL: Cholesterol; Cr: Creatinine; CRP: C-reactive protein; HIV: Human immunodeficiency virus; INSTI: Integrase strand transfer inhibitor; LDL-C: Low-density lipoprotein; TG: Triglycerides; RNA: Ribonucleic acid; UA: Uric acid.

3.3. Abundance of AKK as an independent variable for NAFLD and obese NAFLD

To further validate the role of *AKK* in NAFLD, variables associated with metabolic disorders in both the general population and PLWHIV, including age, disease stage, ART regimens, and biochemical parameters, along with baseline intestinal AKK abundance were included in the univariate and multivariate analysis. The multivariate analysis showed that age (OR = 1.090, $p=0.001$), ALT level (OR = 1.069, $p=0.001$), ALB level (OR = 1.405, $p<0.001$) and UA level (OR = 1.009, $p=0.005$) were risk factors for NAFLD. Abundance of *AKK* was the independentprotective factor for NAFLD (OR = 0.684, $p=0.039$) (Figure 3A). Similar results were found in multivariate analysis for obese NAFLD, and abundance of *AKK* remains the only protective factor for obese-NAFLD (OR = 0.582, $p=0.015$) (Figure 3B). Interestingly, abundance of *AKK* was investigated not an significant factor for lean NAFLD (Figure 3C).

3.4. Relationship between abundance of AKK and metabolic disorders among PLWHIV at baseline

Considering that liver steatosis is the specific manifestation of systemic metabolic disorders in liver, we next investigated the relationships between *AKK* abundance and weight, BMI and serum lipid levels. The variables included in the analysis are as described above. We found that there was a lower abundance of *AKK* in the overweight ($p=0.033$) and hypercholesterolemic ($p=0.038$) PLWHIV than their respective control groups, respectively (Figure 4A,B). No significant difference in Akkermansia abundance was observed between PLWHIV with and without hyper-low density

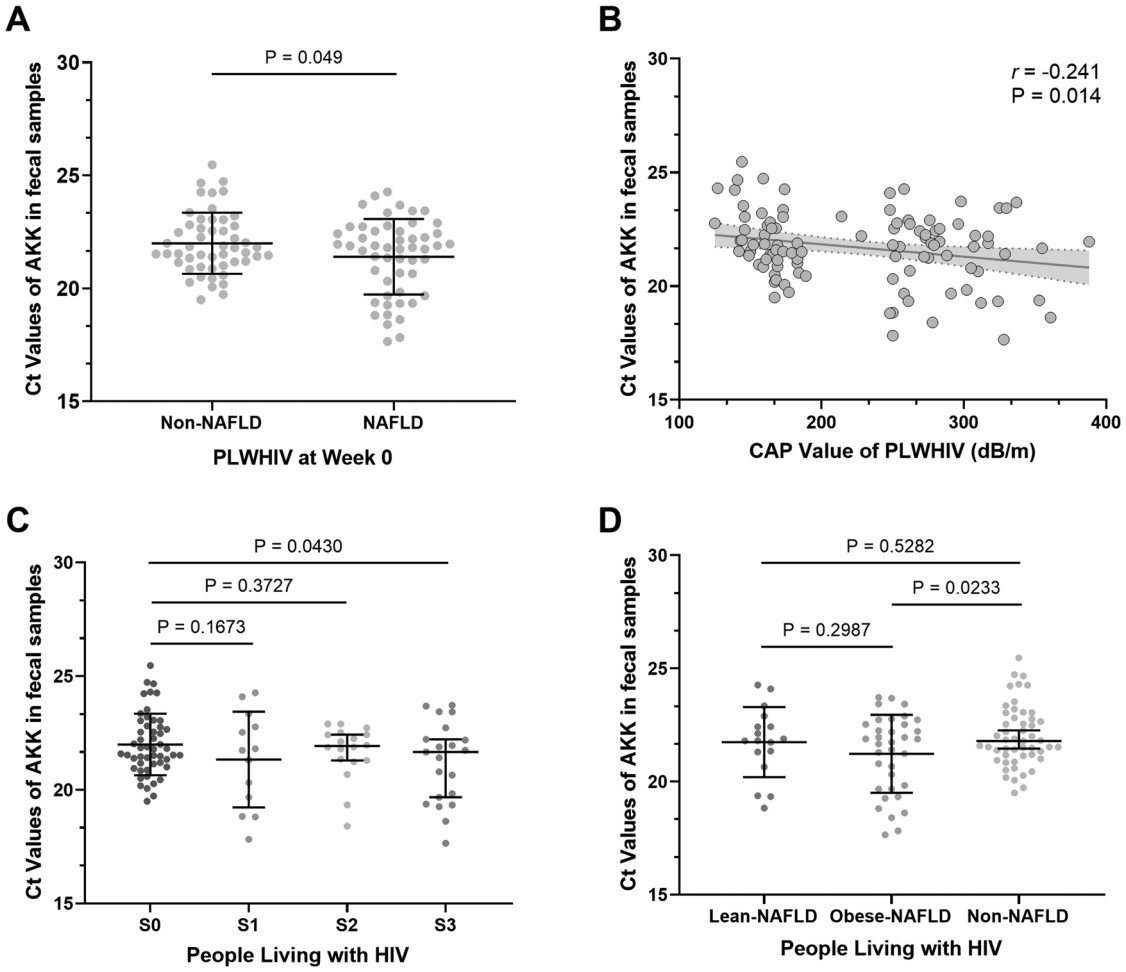

Figure 2. Abundance of AKK decreased in groups of PLWHIV with NAFLD. (A) Abundance of AKK in fecal samples of PLWHIV of non-NAFLD group and NAFLD group. (B) Pearson correlation analysis between liver CAP values and Ct values of gut AKK abundance. (C) Abundance of AKK in fecal samples of PLWHIV with level S0–S3 liver steatosis severity. (D) Abundance of AKK in fecal samples of PLWHIV of lean-NAFLD, obese-NAFLD and non-NAFLD sub-groups. *Abbreviations:* AKK: *Akkermansia muciniphila*; CAP: Controlled attenuation parameters; NAFLD: Nonalcoholic fatty liver disease; PLWHIV: People living with HIV.

lipoproteinemia and hypertriglyceridemia (Figure 4C,D). Moreover, there was a negative linear relationship between Ct values of *AKK* and CAP values among PLWHIV ($r=-0.263$, $p=0.007$) (Figure 4E). However, no linear relationship was observed between *AKK* Ct values and serum levels of the three lipids (Figure 4F–H). Multivariate analysis confirmed a protective association of *AKK* with obesity/overweight in PLWHIV (OR = 0.722, $p=0.034$) (Figure 4I), but not with hyperlipidemia (Figure 4J).

3.5. Dynamics of metabolic indicators from baseline to week 48

We next evaluated the dynamics of metabolic indicators including body weight, BMI, CAP values and serum lipids levels over the 48 weeks' ART. Our results indicated that there was no significant change in weight ($p=0.836$) or BMI ($p=0.917$), liver steatosis measured by CAP values ($p=0.868$) and serum lipid levels of triglyceride ($p=0.074$) and low-density lipoprotein cholesterol ($p=0.242$) except for cholesterol ($p=0.019$) (Figure 5).

3.6. Relationship between abundance of AKK and metabolic disorders among PLWHIV at week 48

Given that 48 weeks' ART did not change metabolic indicators of PLWHIV, we suppose that abundance of *AKK* at baseline may remain an association with metabolic disorders at week 48. The result of re-investigation showed that PLWHIV with persistent NAFLD at week 48 still had lower baseline abundance of *AKK* ($p=0.032$) (Figure 6A) and there remained a negative linear relationship between CAP values and Ct values of *AKK* ($r=-0.232$, $p=0.032$) (Figure 6F). Although *AKK* abundance was not significantly reduced in overweight

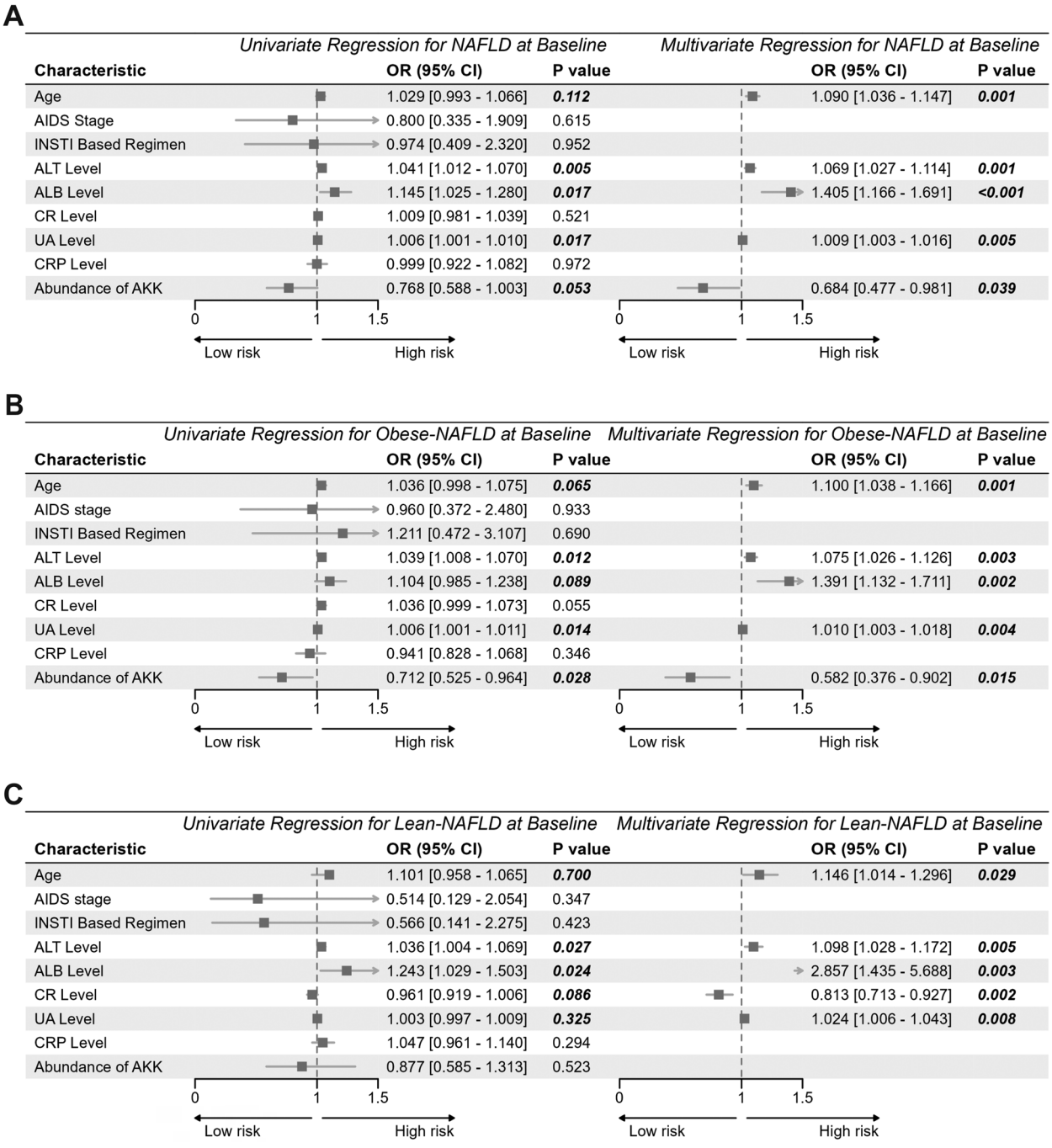

Figure 3. Abundance of AKK as a dependent variable for NAFLD and obese-NAFLD among PLWHIV. (A) Univariate and multivariate analysis for NAFLD among PLWHIV. (B) Univariate and multivariate analysis for obese-NAFLD among PLWHIV. (C) Univariate and multivariate analysis for lean-NAFLD among PLWHIV. *Abbreviations:* AIDS: Acquired immunodeficiency syndrome; AKK: *Akkermansia muciniphila*; ALT: Alanine transaminase; ALB: Albumin; CR: Creatinine; CRP: C-reactive protein; NAFLD: Non-alcoholic fatty liver disease; INSTI: Integrase strand transfer inhibitor; UA: Uric acid.

PLWHIV ($p=0.094$) (Figure 6B), we found there was a negative linear relationship between BMI and Ct values of *AKK* ($r=-0.326$, $p=0.027$) (Figure 6G). No significant association was observed between *AKK* abundance and serum concentrations of triglycerides, total cholesterol, and low-density lipoprotein cholesterol (Figure 6C–E,H–J).

3.7. Abundance of AKK can predict NAFLD after 48 weeks' ART

Thus, we aimed to explore the predictive ability of *AKK* abundance to predict metabolic disorders after 48 weeks of ART. All 103 participants were included in the predictive model analysis. The multivariate

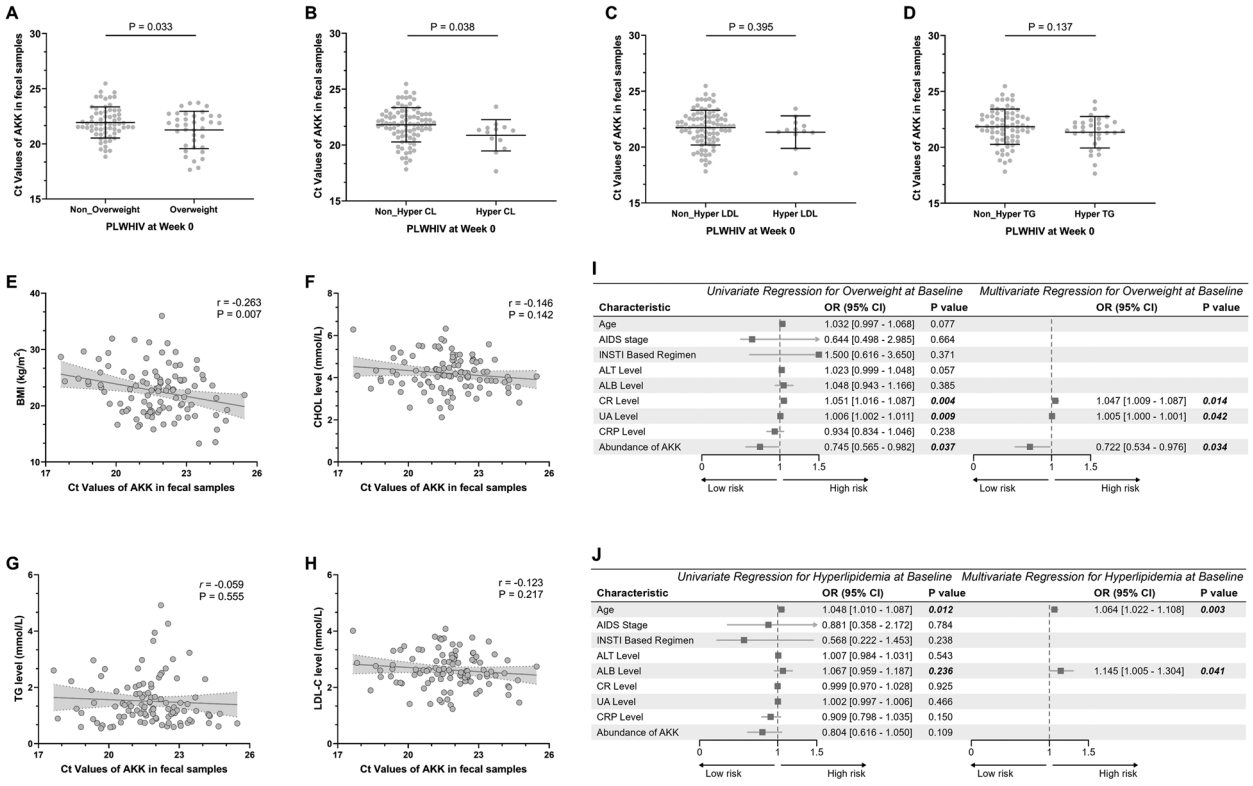

Figure 4. Relationship between abundance of AKK and BMI or serum lipid levels at baseline. (A) Abundance of AKK in fecal samples of PLWHIV of non-overweight group and overweight group. (B) Abundance of AKK in fecal samples of PLWHIV of non-hypercholesterolemia group and hypercholesterolemia group. (C) Abundance of AKK in fecal samples of PLWHIV of non-hyper LDL-C group and hyper LDL-C group. (D) Abundance of AKK in fecal samples of PLWHIV of non-hypertriglyceridemia group and hypertriglyceridemia group. (E) Pearson correlation analysis between BMI and Ct values of gut AKK abundance. (F) Pearson correlation analysis between CHOL levels and Ct values of gut AKK abundance. (G) Pearson correlation analysis between LDL-C levels and Ct values of gut AKK abundance. (H) Pearson correlation analysis between TG levels and Ct values of gut AKK abundance. (I) Univariate and multivariate analysis for overweight among PLWHIV. (J) Univariate and multivariate analysis for hyperlipidemia among PLWHIV. *Abbreviations:* AIDS: Acquired immunodeficiency syndrome; AKK: *Akkermansia muciniphila*; ALB: Albumin; ALT: Alanine transaminase; BMI: Body mass index; CL: Cholesterol; CR: Creatinine; CRP: C-reactive protein; INSTI: Integrase strand transfer inhibitor; LDL-C: Low density lipoprotein-cholesterol; NAFLD: Non-alcoholic fatty liver disease; TG: Triglycerides; UA: Uric acid.

analysis demonstrated that *AKK* abundance emerged as an independent protective factor for NAFLD (OR = 0.635, $p = 0.041$) (Figure 7A). Moreover, the receiver operating characteristic (ROC) curve revealed that *AKK* abundance had moderate discriminatory ability for predicting NAFLD risk, with an area under ROC (AUROC) of 0.688 (Figure 7B). The calibration curve (Figure 7C) indicated a good concordance between predicted and observed probabilities (Brier score = 0.212). Additionally, the decision curve analysis (DCA) showed that *AKK* abundance provided a net clinical benefit across a range of threshold probabilities compared to treating all or treating none (Figure 7D). Finally, the clinical impact curve (CIC) further confirmed that *AKK* reliably identified high-risk individuals, particularly when the threshold exceeded 0.4 (Figure 7E).

4. Discussion

In this research, *Akkermansia muciniphila* was identified as a target declined bacterium in intestinal through bioinformatic analysis in PLWHIV compared to healthy controls. Metagenomic sequencing of fecal samples obtained from PLWHIV, both with and without NAFLD comorbidity further confirmed its role in development of NAFLD among PLWHIV. Finally, we fully evaluated the role of *Akkermansia* in metabolic disorders in PLWHIV through a prospective cohort study.

In our study, we observed a further decrease in *AKK* abundance in PLWHIV with comorbid NAFLD, consistent with findings in the general population [12]. Subsequently, we conducted a prospective observational study involving 103 PLWHIV, categorizing them into NAFLD and non-NAFLD groups based on baseline CAP values. The NAFLD group exhibited significantly

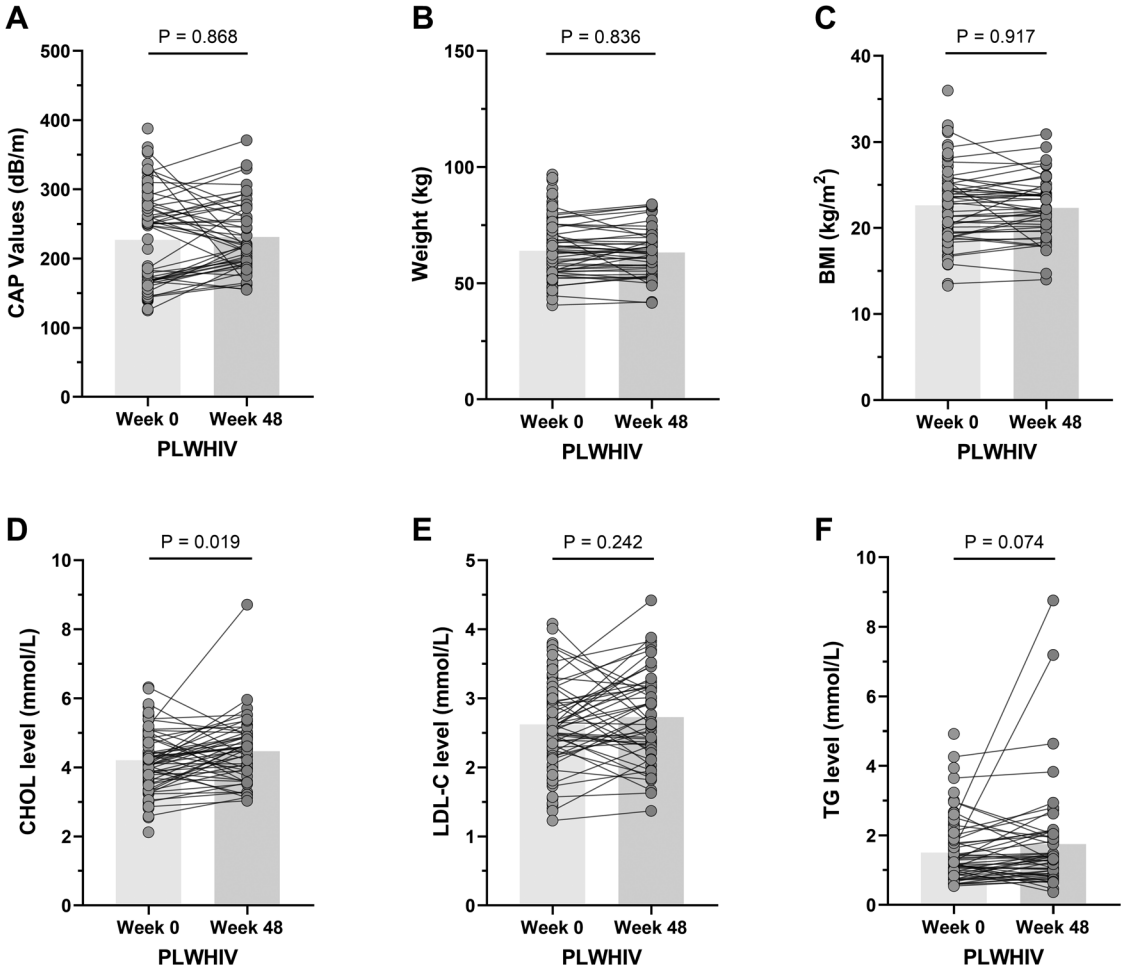

Figure 5. Dynamics of metabolic indicators from baseline to week 48. (A) Dynamics of CAP values among PLWHIV from Baseline to Week 48. (B) Dynamics of weight among PLWHIV from baseline to Week 48. (C) Dynamics of BMI among PLWHIV from baseline to Week 48. (D) Dynamics of CHOL levels among PLWHIV from baseline to Week 48. (E) Dynamics of LDL-C levels among PLWHIV from baseline to Week 48. (F) Dynamics of TG levels among PLWHIV from baseline to Week 48. *Abbreviations:* BMI: Body mass index; CAP: Controlled attenuation parameters; CHOL: Cholesterol; LDL-C: Low density lipoprotein-cholesterol; TG: Triglycerides.

higher BMI and serum lipid levels, aligning with previous research in both general population [33] and PLWHIV [34]. NAFLD, previously considered a local manifestation of metabolic syndrome in the liver, was officially renamed metabolic-associated fatty liver disease (MAFLD) in 2020 [35]. PLWHIV, due to their unique lipid metabolism influenced by HIV infection and ART, display increased susceptibility to NAFLD [36].

The crucial role of *AKK* in metabolic diseases within the general population has been extensively studied, revealing a decline in *AKK* abundance in individuals with metabolic disorders such as obesity, type 2 diabetes mellitus (T2DM), and hypertension [37–39]. Similar reductions in *AKK* abundance were identified in obese patients with NAFLD [40]. Our study confirmed this association in PLWHIV with both overweight and NAFLD. Furthermore, *AKK* abundance was inversely correlated with both CAP values and BMI, emerging as an independent factor for NAFLD and overweight. While a previous study noted a characteristic reduction of *AKK* in PLWHIV after acute HIV infection [15], the causal relationship between this reduction and subsequent susceptibility to NAFLD among PLWHIV remained uncertain. Our study clarified that *AKK* plays a crucial role in the development of NAFLD among PLWHIV, contributing to the existing body of research in this area.

Recent studies have classified NAFLD into two distinct phenotypes: obese NAFLD and lean NAFLD [41]. It is suggested that lean NAFLD may not be benign, exhibiting potentially worse clinical outcomes and greater pathological damage compared to obese NAFLD patients [42,43]. The most widely adopted definition identifies hepatic steatosis with a BMI <25 kg/m^2 (or <23 kg/m^2 in Asians) in the absence of significant alcohol intake [44]. In our study, we elucidated that *AKK* abundance contributed to obese NAFLD but not to lean NAFLD, suggesting a

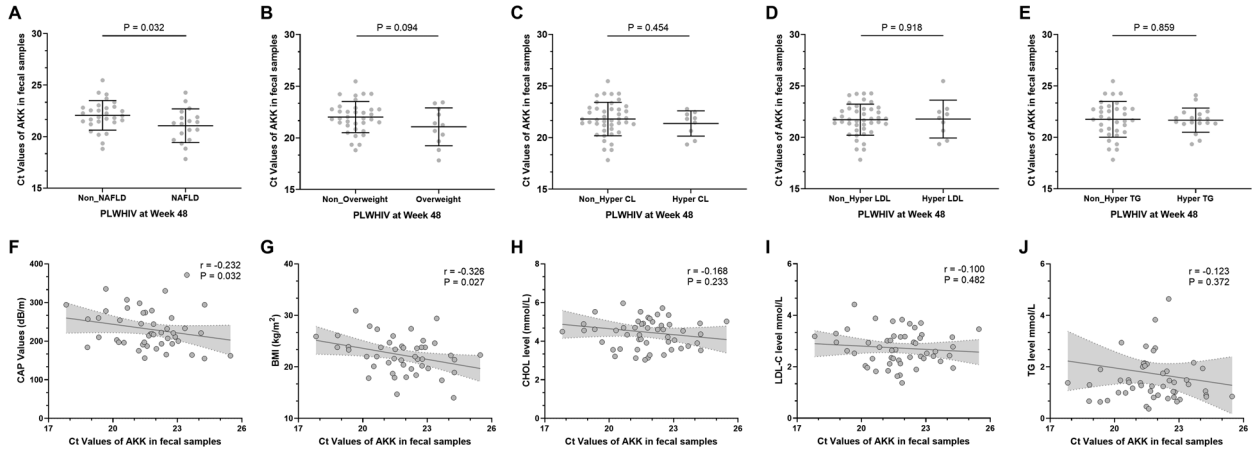

Figure 6. Relationship between abundance of AKK and metabolic indicators at week 48. (A) Abundance of AKK in fecal samples of PLWHIV of non-NAFLD group and NAFLD group at week 48. (B) Abundance of AKK in fecal samples of PLWHIV of non-overweight group and overweight group at week 48. (C) Abundance of AKK in fecal samples of PLWHIV of non-hypercholesterolemia group and hypercholesterolemia group at week 48. (D) Abundance of AKK in fecal samples of PLWHIV of non-hyper LDL-C group and hyper LDL-C group at week 48. (E) Abundance of AKK in fecal samples of PLWHIV of non-hypertriglyceridemia group and hypertriglyceridemia group at week 48. (F) Pearson correlation analysis between CAP values and Ct values of gut AKK abundance among PLWHIV at week 48. (G) Pearson correlation analysis between BMI and Ct values of gut AKK abundance among PLWHIV at week 48. (H) Pearson correlation analysis between CHOL levels and Ct values of gut AKK abundance among PLWHIV at week 48. (I) Pearson correlation analysis between LDL-C levels and Ct values of gut AKK abundance among PLWHIV at week 48. (J) Pearson correlation analysis between TG levels and Ct values of gut AKK abundance among PLWHIV at week 48. *Abbreviations:* AIDS: Acquired immunodeficiency syndrome; AKK: *Akkermansia muciniphila*; ALB: Albumin; ALT: Alanine transaminase; BMI: Body mass index; CL: Cholesterol; CR: Creatinine; CRP: C-reactive protein; INSTI: Integrase strand transfer inhibitor; LDL-C: Low density lipoprotein-cholesterol; NAFLD: Non-alcoholic fatty liver disease; TG: Triglycerides; UA: Uric acid.

divergence in the pathogenesis of these two NAFLD phenotypes among PLWHIV. Previous research has indicated that lean NAFLD individuals present a more favorable metabolic profile, characterized by a lower waist-to-hip ratio, a lower incidence of diabetes, higher serum high-density lipoprotein, and lower serum triglycerides than their obese NAFLD counterparts [30]. This observation led researchers to propose that lean NAFLD patients may possess an obesity-resistant phenotype [45]. Additionally, investigations revealed that the lean NAFLD group exhibited a distinctive gut microbiota profile with an increased abundance of the *Clostridium* and *Ruminococcaceae* genus, both involved in bile acid (BA) formation [46]. Given the unique context of PLWHIV, we hypothesize that HIV infection may induce a characteristic reduction in *AKK*, playing a significant role in the subsequent onset of NAFLD.

Given the pivotal role that *AKK* plays in maintaining intestinal integrity [24,47], and considering established evidence on the correlation between intestinal permeability and the development of NAFLD [48,49], it is plausible to hypothesize a cascade effect following HIV infection. Upon the occurrence of HIV infection, the virus has the capacity to target intestinal lymphoid tissue, thereby disrupting intestinal integrity [16]. Subsequently, distinctive variations in gut microbiota, including a reduction in *AKK*, ensue [15], leading to the manifestation of 'leaky gut syndrome'. This phenomenon involves microbial translocation, wherein bacterial products traverse from the gut into the bloodstream, resulting in chronic immune activation and sustained inflammation [50]. These processes may contribute to the development of NAFLD in the context of HIV infection [49]. This hypothesis requires further evidence for confirmation.

A study tracking the dynamics of bacterial clusters in PLWHIV following acute HIV infection observed a continual decrease in the abundance of *AKK* after 6 months. Notably, the introduction of ART was associated with an even lower abundance of *AKK* [15]. Consequently, we investigated whether the abundance of *AKK* could serve as a predictor of NAFLD among PLWHIV at week 48 after the initiation of ART and confirmed this hypothesis. However, we did not consistently track dynamic changes in *AKK* abundance following the administration of ART.

Acknowledging the inherent limitations of our study is imperative. Firstly, we were unable to ascertain the daily dietary status of our participants, which may

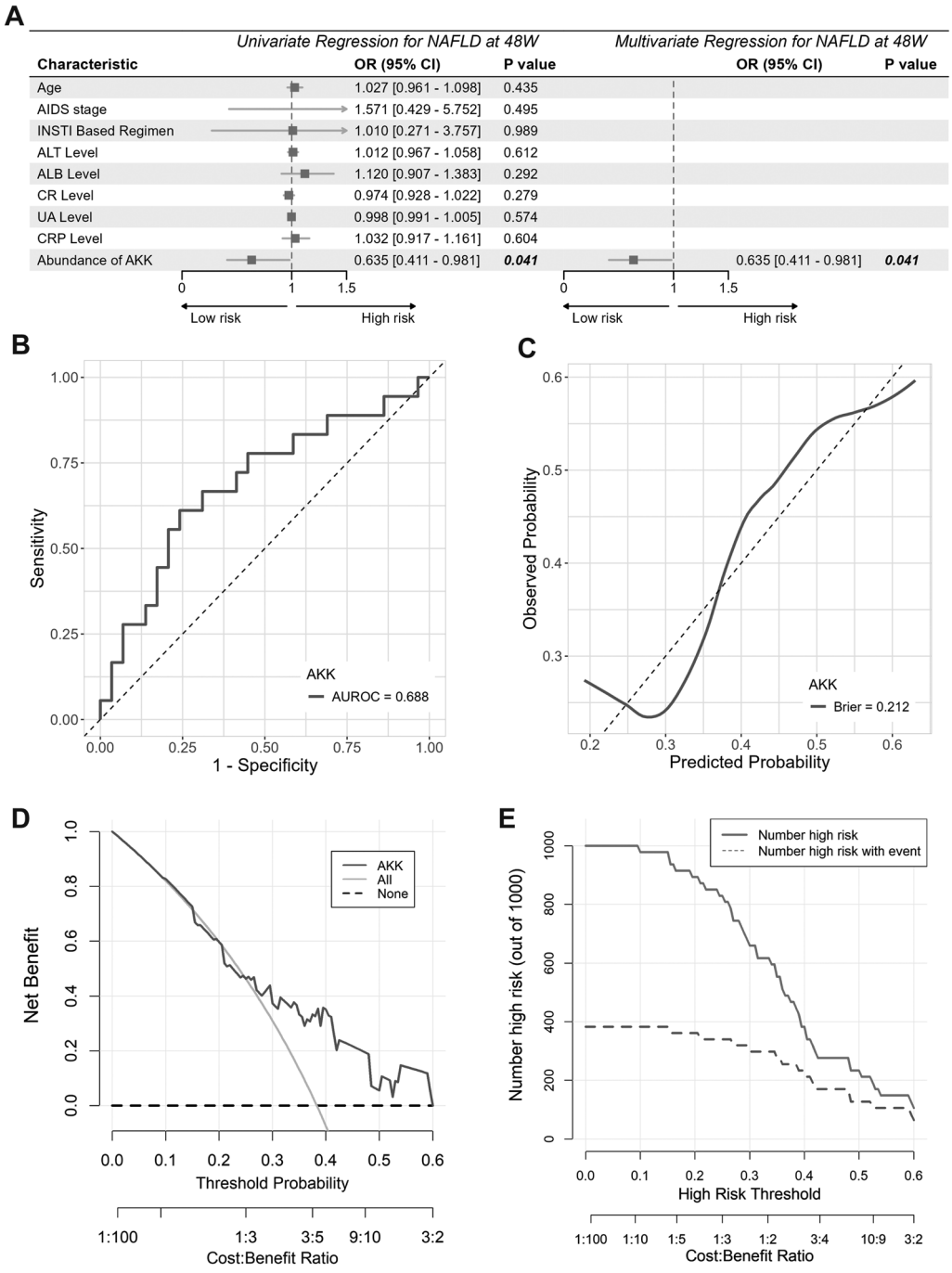

Figure 7. Abundance of AKK as a predicator for NAFLD at week 48. (A) Univariate and multivariate analysis for NAFLD among PLWHIV at week 48. (B) The ROC curve of abundance of AKK to predict NAFLD among PLWHIV at week 48. (C) The calibration curve of AKK abundance. (D) The decision curve analysis (DCA) of AKK abundance. (E) The clinical impact curve (CIC) of AKK abundance. *Abbreviations:* AIDS: Acquired immunodeficiency syndrome; AKK: *Akkermansia muciniphila*; ALB: Albumin; ALT: Alanine transaminase; CR: Creatinine; CRP: C-reactive protein; INSTI: Integrase strand transfer inhibitor; NAFLD: Non-alcoholic fatty liver disease; UA: Uric acid.

have influenced the results of fecal *AKK* abundance. Secondly, we did not adjust for sexual orientation, a factor that could impact the results, as previously reported [51]. Thirdly, we opted for a non-invasive method to assess liver steatosis instead of conducting a liver biopsy for pathological evidence.

5. Conclusions

The abundance of *AKK* was characteristically declined in PLWHIV with metabolic disorders and was associated with overweight and NAFLD, specifically obese-NAFLD, among PLWHIV. Furthermore, it can predict NAFLD after 48 weeks of HAART.

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Ethical approval

The study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Nanfang Hospital (NFEC-2021-448). All patient provided written informed consent, agreed to follow the protocol, and take specimens, and was willing to anonymously publish details of the medical record.

Author contributions

Jie Peng, Shaohang Cai, and Jian Wu conceived and designed the study. Zhe Qian, Suling Chen, and Xiaoyang Liao analyzed and interpreted the data, drafted, and finalized the manuscript. Yuyuan Xu, Jingfang Xie, Huiqun Zhong, Lang Ou, Xiang Zuo, and Xuwen Xu participated in the recruitment of participants and data collection. All the authors reviewed the final version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The raw metagenomic sequencing data are available in the SRA database under BioProject ID PRJNA1167913: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1167913. Other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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