Open Forum Infectious Diseases

MAJOR ARTICLE

HIV-Infected Individuals on ART With Impaired Immune Recovery Have Altered Plasma Metabolite Profiles

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Background. Multiple host factors may influence immune reconstitution in HIV-infected people after the initiation of suppressive antiretroviral therapy (ART). Aberrant metabolic pathways have been reported in people with HIV (PWH) on ART. We hypothesized that alterations in plasma metabolites were associated with immune recovery following ART.

Methods. In this cross-sectional study, the plasma metabolomic profiles of PWH on ART were evaluated. PWH of slow and fast immune recovery were classified by increase in CD4 T cells following 2 years of ART. Targeted plasma metabolite profiling by liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry to determine metabolite signatures for HIV recovery identified >200 metabolites.

Results. Notably, indole-3-propionic acid was downregulated during HIV, possibly reflecting impaired gastrointestinal epithelium homeostasis. The most important metabolite discriminating between the PWH with fast and slow immune recovery was cysteine. Upregulated cysteine and cysteine pathways may contribute to redox-balance maintenance and T-cell function in PWH with fast immune recovery. Additionally, serine and glycine metabolism and bile acid biosynthesis were the most perturbed metabolic pathways in PWH.

Conclusions. These results provide a starting point for developing biomarker candidates for immune recovery in PWH on ART and provide insight into the interplay of metabolism and immune response in HIV infection.

Keywords. antiretroviral therapy; HIV; immune recovery; metabolomics.

HIV is a major global health issue, with almost 37 million people living with HIV and approximately a million HIV/AIDS-related deaths occurring in 2017 [1]. With no cure in sight, we are heavily reliant on preventative measures and antiretroviral therapy (ART) to lessen the burden of HIV and decrease the number of AIDS-related deaths.

Some people with HIV (PWH) respond better than others to ART. Individuals with poor immunological recovery of CD4 T cells early on during treatment have been shown to have increased immune depletion, as well as higher risk of both AIDS-related and non-AIDS-related conditions [2, 3], independent of ART-mediated viral suppression [4, 5].

Microbial translocation across the intestinal barrier is a significant driver of inflammation in PWH and persists even with suppressive ART [6]. Immune and inflammatory responses within the intestinal mucosa are characterized by shifts in tissue metabolism [7]. The gut microbiota varies between HIV-infected ART-suppressed and HIV-uninfected people [8], and between PWH immunological responders and nonresponders [9]. Recent studies have reported abnormalities involving several metabolic pathways in PWH on ART [10, 11]. In addition to bacteria, the enteric microbiome contains viruses [12], and low CD4 counts have been associated with increased levels of adenovirus-derived nucleic acid sequences in fecal samples independent of ART treatment [13]. The mechanisms associated with slow immune recovery after the initiation of ART and virus control are poorly understood, and a large body of evidence has been dedicated to understanding this phenomenon from an immunological perspective. Metabolomics, the large-scale study of small molecules, intermediates, and products of metabolism, is a developing diagnostic tool that has been useful in the discovery of novel biomarkers in a number of health issues including cancer and cardiovascular disease [14, 15]. The plasma metabolome reflects the underlying biochemical activity of the host and will reflect responses to disease [16]. Host plasma metabolites also predict gut microbiome diversity in healthy adults [17].

To this end, we examined a large cohort of adult PWH who received combination ART as their first antiretroviral regimen and achieved viral load suppression within 6–12 months of treatment initiation. The cohort consisted

Received 29 January 2021; editorial decision 27 May 2021; accepted 31 May 2021.

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Open Forum Infectious Diseases®2021

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DOI: 10.1093/ofid/ofab288

of 2 groups of HIV-infected individuals, characterized by slow and fast immune recovery based on the increase in CD4 T-cell numbers following 2 years of suppressive ART, as well as a group of age-, sex-, and ethnically matched HIV-negative individuals.

The aim of this retrospective cross-sectional study was to identify alterations in plasma metabolites in PWH and to see if differences in plasma metabolome signatures were associated with the diverse profiles of immune recovery following ART initiation. Indole-3-propionate (IPA), a deamination product of tryptophan produced by gut microbiota, was the most important metabolite in discriminating between HIV-positive and HIV-negative individuals. The most important metabolite discriminating between the 2 groups of PWH was cysteine. Decreased plasma levels of cysteine in individuals with slow CD4 reconstitution suggest an association with oxidative stress and impaired immune recovery. In addition, metabolite set enrichment analysis of plasma indicated perturbation of several metabolic pathways in HIV-infected individuals compared with HIV-negative controls. Bile acid biosynthesis and glycine and serine metabolism were among the top de-regulated pathways in HIV-positive individuals.

METHODS

Study Population

Individuals were selected for the study from the IL7R SNPS and Malaysian HIV and Aging (MHIVA) cohorts in Malaysia [18]. All individuals were aged >18 years, had received combination antiretroviral therapy as their first ARV regimen, and had achieved suppressed viral load (HIV RNA <50 copies/mL) within 6–12 months of initiating ART. There were a total of 563 people recruited to these cohorts, and individuals were selected for this study if they displayed extreme phenotypes of immune reconstitution post-ART; that is, individuals with impaired/slow CD4 T-cell recovery were defined as those with a rise in CD4 T cells <50 cells/year in the first 2 years following suppressive ART, while efficient/fast recovery was defined as an increase in CD4 T-cell counts of >200 cells/year in the first 2 years following ART initiation. The initial study population included 75 individuals with fast immune recovery, 66 with slow immune recovery, and 70 HIV-negative control individuals. All samples were collected postfasting in EDTA collection tubes. Ten samples (2 with fast and 8 with slow immune recovery) were excluded from analysis due to low CD4 T-cell counts at the time of sample collection (range, 0–85 CD4 T cells/μL). Another 10 (2 fast and 8 with slow immune recovery) samples were excluded from analysis due to detection of trimethoprim and sulfamethoxazole (TMP-SMZ) in samples, which might influence the metabolome [19]. The HIV-negative population was matched for age, sex (10 women and 60 men), and ethnicity (51 Chinese, 12 Malay, and 7 Indian).

Patient Consent

Written informed consent was obtained from all study participants. The study protocol was approved by the University of Malaya Medical Center review board (IL7R: MEC Ref. No. 896.32; MHIVA: MEC 20151–937).

Metabolic Profiling

Metabolic profiling with gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) was performed at the Sweden Metabolomics Center, Umeå, Sweden (SMC). Detailed information about sample preparation, mass spectrometry, data processing, and performance of the standards and data sets is available in the Supplementary Data (Supplementary Figures 1–3).

Statistical Analysis

A combination of univariate and multivariate bioinformatic approaches was performed using the R-script-based online tool MetaboAnalyst 4.0 [20]. The Mann-Whitney test and chi-square test were used to compare differences in patient demographics for continuous and categorical parameters, respectively. The Kruskal-Wallis test with a post hoc Dunn's test was used for univariate statistics of single metabolites. Correlation was evaluated by Spearman's rank test.

Data Normalization and Transformation

The data generated were expressed as relative response ratios based on peak intensity. To compensate for batch analysis, samples were normalized by an injection standard (GC-MS) or internal standard (LC-MS). Normalized values were log-transformed and pareto-scaled before multivariate analysis in MetaboAnalyst 4.0. Differences between disease groups were further visualized by component partial least-squares discriminant analysis (PLS-DA) models for each disease group comparison; HIV fast (F) vs controls (C), and HIV slow (S) vs C, for GC-MS data and LC-MS data, respectively. The quality of the PLS-DA model was evaluated by Q2, R2X, R2Y, and permutation test of separation distance (Supplementary Tables 2–3).

To investigate the biological relevance of the metabolomic differences between groups, quantitative metabolite set enrichment analysis (MSEA) was carried out. The analysis was performed using normalized values of intensity of putatively resolved metabolites in plasma using Human Metabolome Database (HMDB) identification. Enrichment was evaluated using the Small Molecule Pathway Database in MetaboAnalyst 4.0.

RESULTS

Demographics of Study Population

Among PWH, there were no differences in the following parameters between groups with fast and slow immune recovery: age at initiation of ART, baseline CD4 T-cell count, sex, co-infection

with hepatitis B or hepatitis C, risk category for HIV transmission, or duration of suppressive ART (Table 1). In the control group, hepatitis B antigen was detected in 1 individual, and none of the controls had positive hepatitis C serology (data not shown). In the group with slow immune recovery profiles, there was a smaller proportion of individuals of Indian ethnicity and, as expected, lower current CD4 T-cell counts at the time of sample collection.

Plasma Metabolomic Pattern of Cohorts

Mass spectrometry of the plasma samples led to the generation of 3 data sets that were processed separately: LC-MS negative, LC-MS positive, and GC-MS. A targeted processing approach of LC-MS data identified 180 putative metabolites (134 positive and 46 negative). Targeted processing of the GC-MS data set identified 97 putative metabolites.

Plasma Metabolite Profiling of HIV-Infected Individuals

Pair-wise analysis between HIV-negative controls and the 2 groups of PWH was performed to identify significantly dysregulated metabolites. As a first step in data analysis, the metabolic profiles of plasma samples from the control group were compared with those of PWH with fast and slow immune

Table 1. Demographics and Clinical Characteristics

	Efficient/Fast (n = 71)	Impaired/Poor (n = 50)	
	Median (IQR)	Median (IQR)	*P* Value[a]
Age at cART initiation, y	36 (32–42)	37 (31–43)	.927
Time to cART initiation, mo	1.9 (0.9–14)	1.9 (0.9–12)	.770
Duration on suppressive cART, y	6 (4–11)	7 (5–11)	.086
Baseline CD4 T-cell count, cells/μL	107 (30–246)	165 (40–336)	.317
Current CD4, cells/μL	751 (578–877)	428 (264–537)	<.001
Baseline CD4 T-cell count, cells/μL	107 (30–246)	165 (40–336)	.317
Baseline CD4 T-cell counts			.803[b]
<200 cells/μL	47 (60)	32 (40)	
>200 cells/μL	24 (57)	18 (43)	
Sex, No. (%)			.174[b]
Male	62 (61)	39 (39)	
Female	9 (45)	11 (55)	
Ethnicity, No. (%)			.029[b]
Chinese	45 (51)	43 (49)	
Indian	10 (91)	1 (9)	
Malay	15 (75)	5 (25)	
Others	1 (50)	1 (50)	
Hepatitis C Ab, No. (%)			.243[b]
Yes	2 (67)	1 (33)	
No	67 (60)	44 (40)	
Not recorded	2 (29)	5 (71)	
Hepatitis B Ag, No. (%)			.085[b]
Yes	2 (29)	5 (71)	
No	68 (62)	42 (38)	
Not recorded	1 (25)	3 (75)	
Receiving bactrim at cART initiation,[c] No. (%)			.939[b]
Yes	38 (55)	31 (45)	
No	13 (54)	11 (46)	
HIV transmission risk category, No. (%)			.150[b]
MSM	20 (69)	9 (31)	
IDU	2 (67)	1 (33)	
Heterosexual	33 (49)	34 (51)	
Others	15 (71)	6 (29)	
History of AIDS-defining illness at cART initiation, No. (%)			.262[b]
Yes	40 (64)	23 (36)	
No	31 (53)	27 (47)	
Baseline cART regimen, No. (%)			.386[b]
NNRTI-based	69 (59.5)	47 (40.5)	
PI-based	2 (40)	3 (60)	

Abbreviations: cART, combination antiretroviral therapy; IDU, injection drug use; IQR, interquartile range; MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Mann-Whitney U test.
[b]chi-square test.

recovery, respectively. PLS-DA analysis showed a clear discrimination of metabolomic plasma profiles of HIV-infected individuals with slow or fast immune recovery, compared with the profiles of HIV-negative controls. The explained variance of component 1 and component 2 varied between 7.8% and 12% for GC-MS (Figure 1A–B) and between 11% and 19% for LC-MS (Figure 2A–D). The reliability of the PLS-DA models was confirmed by 10-fold cross-validation, and the best discrimination between groups was achieved using 5–7 components (Supplementary Table 2). R^2 and Q^2 values close to 1 indicated good performance of the model. In addition, permutation tests (1000 permutations) by separation distance generated P values <.001, indicating significant differences between groups compared, that is, differences that are unlikely to be attributable to chance or sampling bias.

Metabolites Differentiating Between HIV-Infected Individuals and HIV-Negative Controls

Variable importance in projection (VIP) scores were used to identify the metabolites with the highest contribution to the separation between groups (Supplementary Figure 4A–C). Indole-3-propionate (IPA) was one of the top metabolites discriminating between the HIV groups and the HIV-negative control group. The mean intensities of IPA and tryptophan in the plasma of PWH were lower than in the plasma of HIV-negative controls (Supplementary Figure 4). Kynurenic acid (kynurenate) and tryptophan betaine (lenticin) were other important factors that discriminated the HIV group with slow immune recovery from the HIV-negative group (Supplementary Figure 4B). Univariate analysis of tryptophan metabolites showed decreased levels of tryptophan and its bacterial metabolite IPA as well as the tryptophan 2,3-dioxygenase (TDO)/indoleamine-2,3-dioxygenase 1 (IDO-1) metabolites kynurenine and kynurenate (Figure 3A). Increased levels of fructose were also characteristic for HIV-infected groups (Supplementary Figure 4). Bile acids and lipid compounds contributed to the separation of groups of metabolites identified by LC-MS in negative mode (Supplementary Figure 4C). In all data sets, 44 unique metabolites had a VIP score ≥1.6 (Supplementary Table 1). All metabolites with VIP score ≥1.6 were significantly different between groups, with a corrected P <.02 (Supplementary Table 1). Among the metabolites, the most common chemical subclass was amino acid (Supplementary Table 1). Of the top discriminating metabolites, 13 were unique for separating the slow immune recovery HIV group from the HIV-negative control group, 11 were unique for separating the fast immune recovery group from HIV-negative controls, and 20 were shared between HIV groups (Supplementary Figure 4D).

Plasma Levels of Cysteine Correlated With HIV Immune Recovery

When the 2 PWH groups were compared with each other, the PLS-DA model did not discriminate the 2 groups (Supplementary Figure 5). Instead, univariate analysis was performed to show the differences in cysteine levels between groups (Figure 3B). There was a moderate correlation (R_s = 0.3; P = .005) between plasma

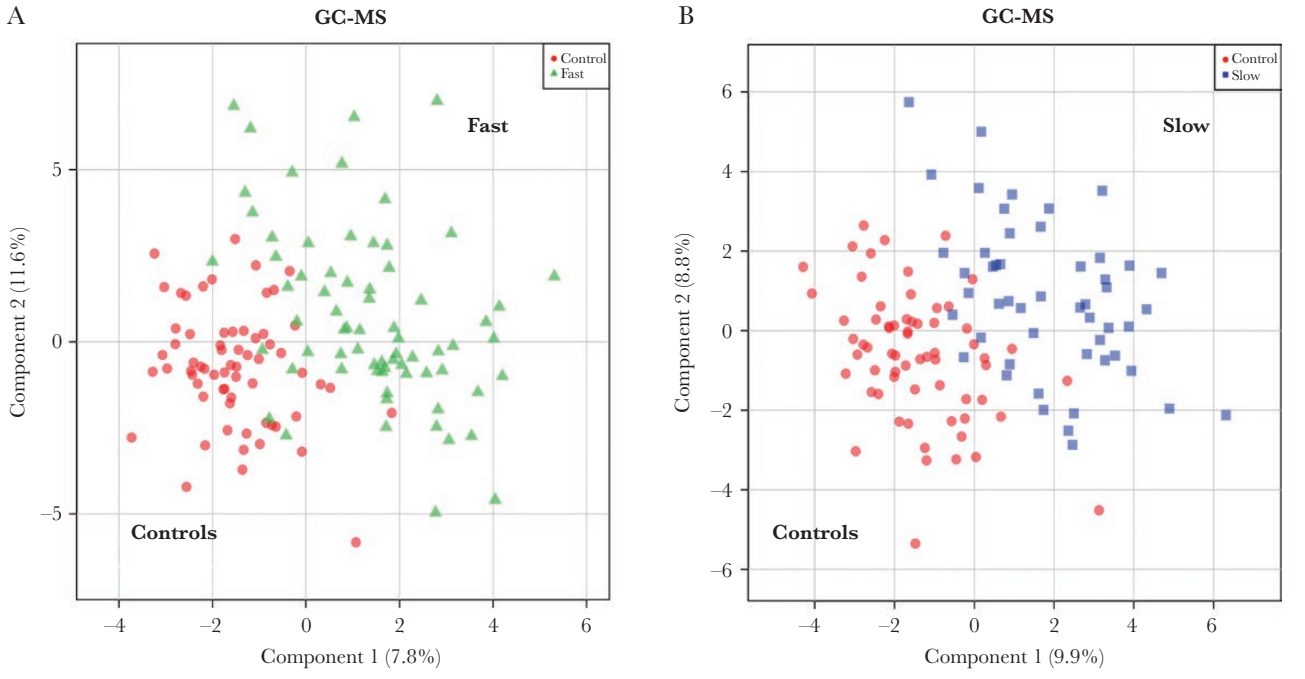

Figure 1. Partial least-squares discriminant analysis (PLS-DA) of gas chromatography–mass spectrometry (GC-MS) plasma metabolites. GC-MS data, comparing the plasma metabolites of HIV-positive individuals with fast immune recovery (fast, n = 70) and HIV-negative controls (control, n = 65) (A) and HIV-positive individuals with slow immune recovery (slow, n = 50) and HIV-negative controls (B).

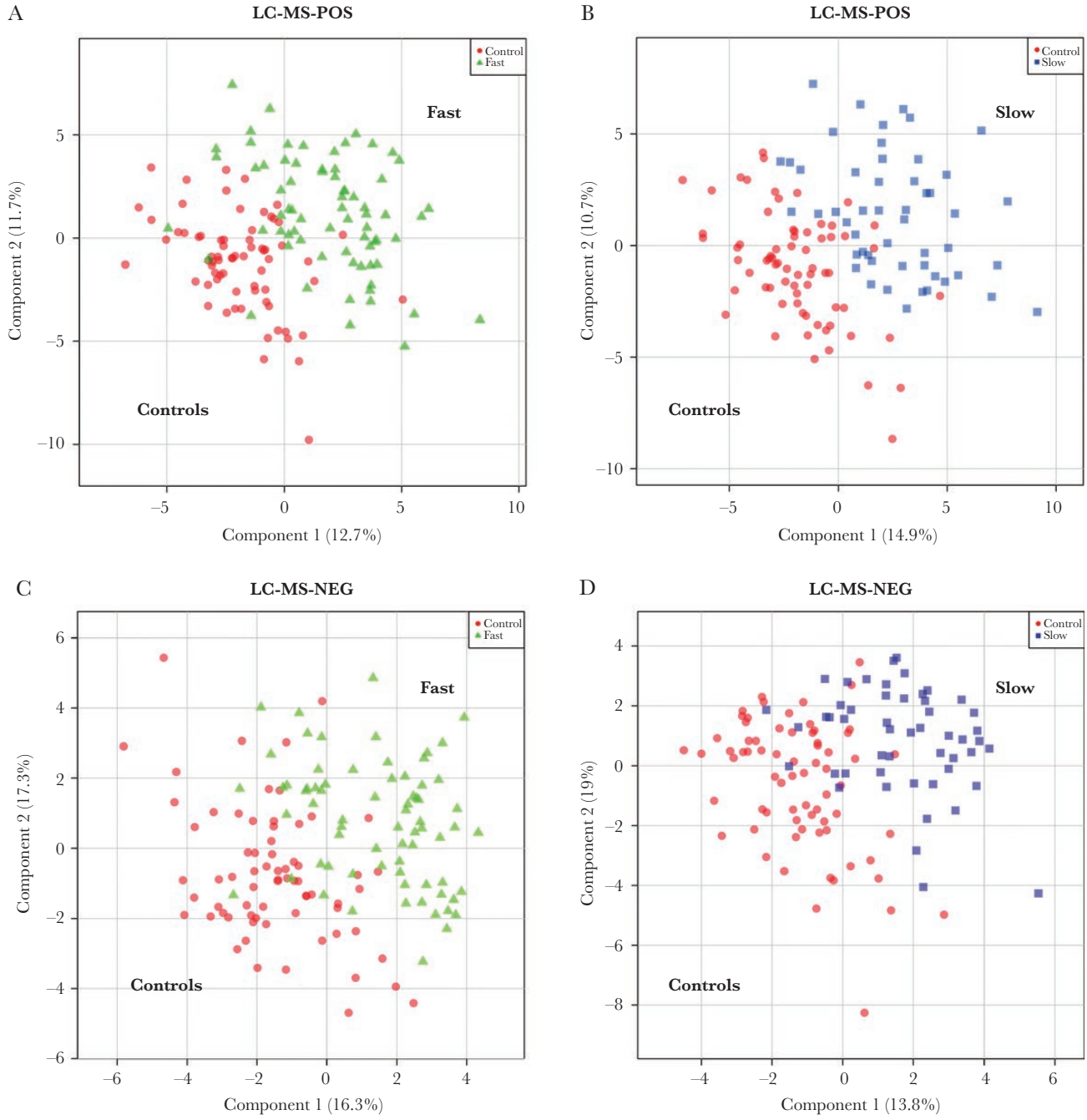

Figure 2. Partial least-squares discriminant analysis (PLS-DA) of liquid chromatography–mass spectrometry (LC-MS) plasma metabolites. LC-MS-positive mode data, comparing the plasma metabolites of HIV-positive individuals with fast immune recovery (fast, n = 71) and HIV-negative controls (control, n = 70) (A) and HIV-positive individuals with slow immune recovery (slow; n = 50) and HIV-negative controls (B). C and D, LC-MS-negative mode data.

intensities of cysteine and number CD4 cells at sample collection in the HIV-positive populations (Figure 3C). The relative plasma level of cysteine was 1.51 times higher ($P < .0001$) in individuals with fast immune recovery (Figure 3B).

Altered Glycine and Serine Metabolism Was Associated With HIV Infection

We next sought to identify differential biologically meaningful metabolic pathways relating to immune reconstitution after initiation of ART. Quantitative metabolite set enrichment analysis was performed on normalized LC-MS and GC-MS data sets. In total, 80 metabolites were included in the enrichment analysis of the GC-MS data sets (Figure 4). Enrichment analysis revealed that glycine and serine metabolism was one of the most significantly affected pathways in both HIV groups when compared with HIV-negative controls (Figure 4A–B). Glycine serine metabolism was the pathway that hit the highest number

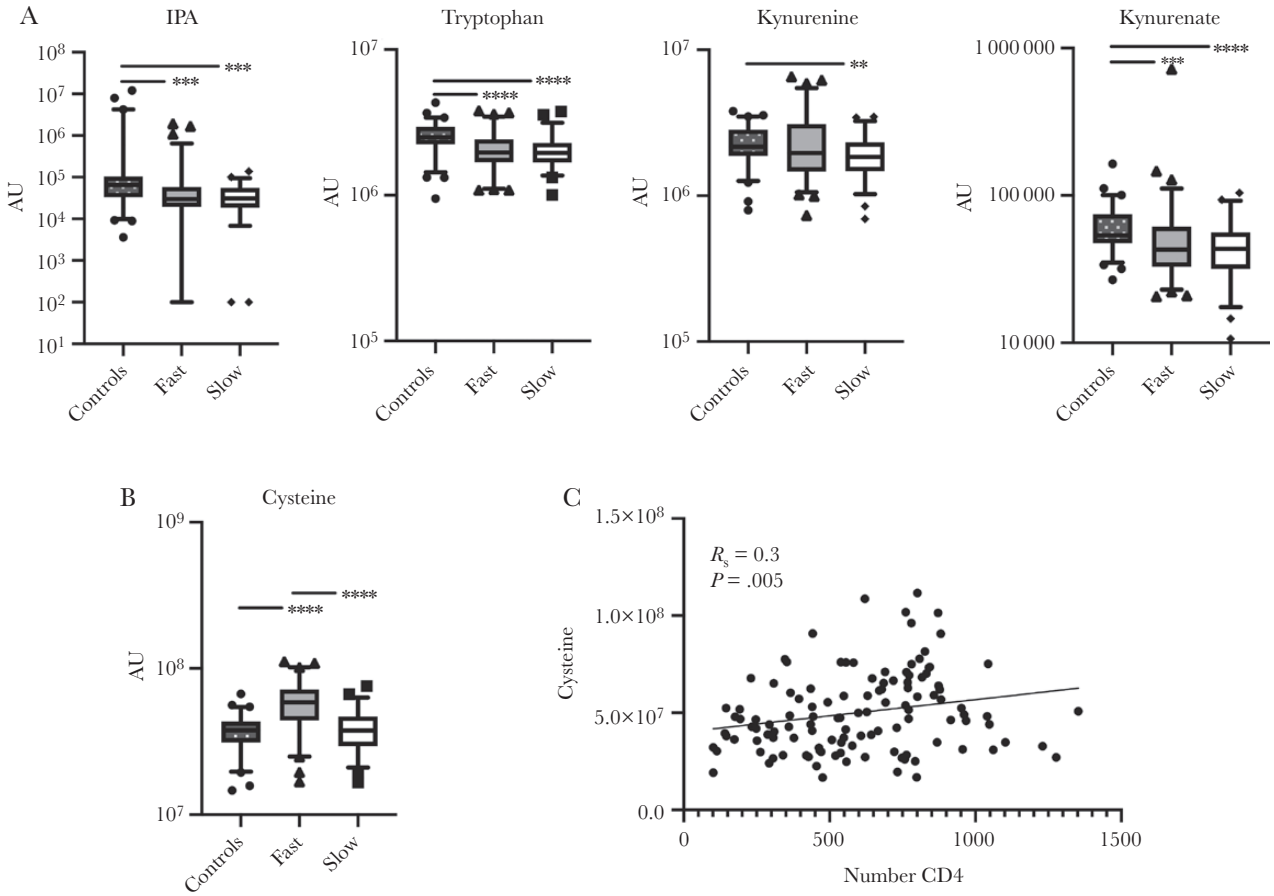

Figure 3. Plasma levels of single metabolites and CD4 T-cell numbers. Univariate statistical analysis of plasma indole-3-propionic acid (IPA), tryptophan, kynurenine, and kynurenate (A). Cysteine levels are presented as arbitrary units (AU) (B). B, Spearman correlation of cysteine plasma intensity and CD4 number in HIV-positive individuals. Medians and 5th–95th percentiles are shown. $^{***}P < .001$, Kruskal-Wallis test with Dunn's post-test. Fast n = 70, slow n = 50, and controls n = 65.

of metabolites (n = 9) in the data set (Supplementary Table 4). Fold enrichment was 16.7, Holm-corrected $P = 5.1*10^{-15}$(F vs C), and 12.3, Holm-corrected $P = 3.2*10^{-9}$(S vs C), respectively. Other affected metabolic pathways, including metabolites other than glycine serine metabolism, were Warburg effect, Holm-corrected $P = 4.5*10^{-5}$ (F vs C) and $P = .004$ (S vs C), and urea cycle, Holm-corrected $P = 7.6*10^{-5}$ (F vs C) and $P = 1.3*10^{-7}$ (S vs C) (Supplementary Table 4). The differences were most pronounced between HIV-negative controls and the group of HIV-positive individuals with slow immune recovery.

Enrichment of Metabolites Associated With Bile Acid Biosynthesis Was Associated With Fast Immune Recovery

Enrichment analysis of the LC-MS data set included 145 mapped metabolites with an HMDB pathway assignment. The analysis revealed that the bile acid biosynthesis pathway hit 10 metabolites in the data sets. It was the only affected pathway that hit more than 3 metabolites (Supplementary Table 5). Bile acid biosynthesis was significantly enriched in the plasma of PWH with fast immune recovery compared with HIV-negative controls. The fold enrichment was 4.7, and Holm-corrected $P = .008$ (Figure 5A). The bile acid biosynthesis pathway was not significantly enriched in PWH with slow immune recovery (Figure 5B). Other significantly enriched pathways (Holm-corrected $P < .05$), that is, phenylalanine and tyrosine metabolism, beta-alanine metabolism, and pyrimidine metabolism, only hit 2 metabolites in the LC-MS data sets. The differences were most pronounced between HIV-negative controls and the group of PWH with fast immune recovery (Supplementary Table 5). Univariate statistics showed decreased plasma levels of the primary bile acid chenodeoxycholic acid and its derivatives in HIV-infected individuals (Supplementary Figure 6A); changes were more pronounced in the fast immune recovery group. There were also differences in plasma levels of cholic acid derivatives between groups (Supplementary Figure 6B).

DISCUSSION

In the present study, we have investigated the plasma metabolomic profiles of HIV-infected individuals who demonstrated a diverse profile of fast and slow reconstitution of CD4 T cells following ART, compared with demographically matched

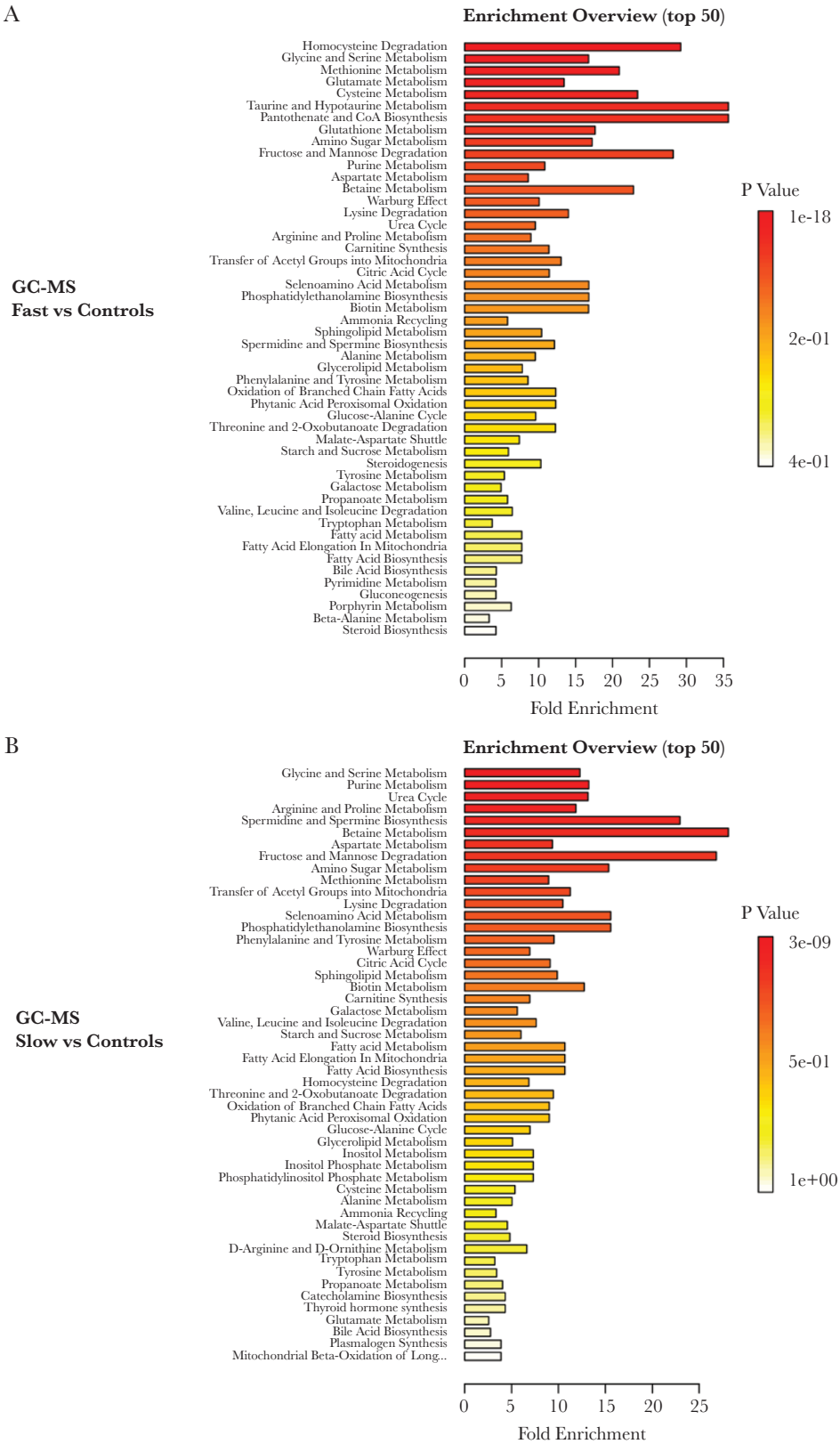

Figure 4. Metabolite set enrichment analysis gas chromatography–mass spectrometry. Summary plot of over-representation analysis of plasma metabolites in HIV-positive individuals with fast immune recovery (A) and slow immune recovery (B) compared with HIV-negative controls.

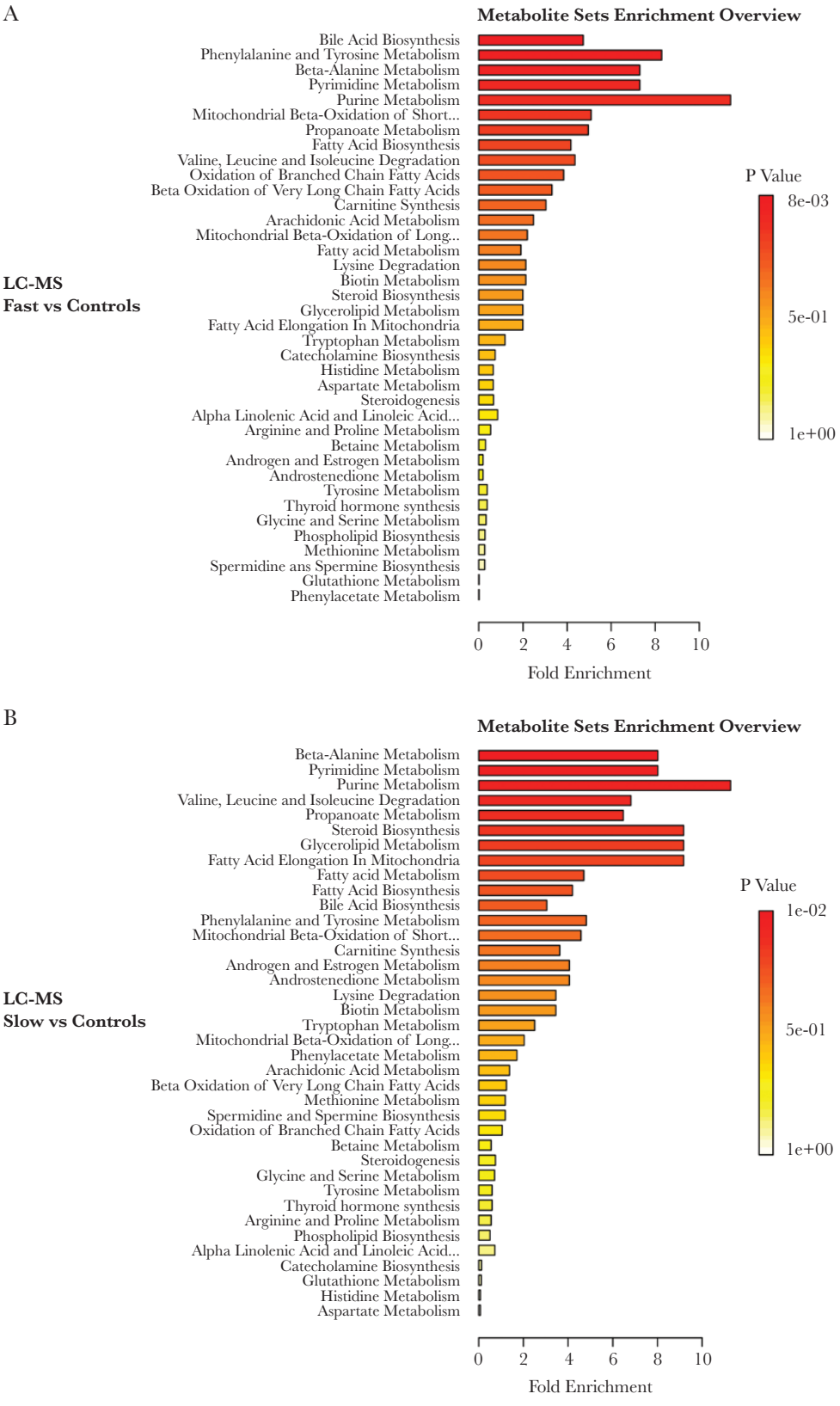

Figure 5. Metabolite set enrichment analysis liquid chromatography–mass spectrometry. Summary plot of over-representation analysis of plasma metabolites in HIV-positive individuals with fast immune recovery (A) and slow immune recovery (B) compared with HIV-negative controls.

HIV-negative individuals. Using a targeted metabolomic processing approach and applying bioinformatic analysis, we found various discriminating factors between the 2 HIV groups and between the HIV-positive groups and HIV-negative controls. Our data analysis identified 180 putative metabolites (134 positive and 46 negative) from LC-MS and 97 putative metabolites through GC-MS.

An array of factors can influence ART-induced immune recovery in HIV-infected individuals. HIV infection and ART are associated with perturbation of lipid profiles [21–23]. In addition to low levels of HIV replication, the antiviral therapy itself can induce alteration in the HIV-infected individuals, as shown for protease inhibitor–based ART, which can cause lipid alterations due to inflammation, hepatic function, and microbial translocation [24]. In our study, the differences in metabolic profiles seen between the fast and slow immune recovery groups should be due to biological differences and not metabolic changes given that the antiretroviral drugs received were similar between the groups. This is supported by the comparable plasma lipidomic profile induced by darunavir-based or integrase inhibitor–based antiretroviral therapy [25]. Host metabolic factors have been associated with poor CD4 recovery in HIV. It has been suggested that adipose tissue may affect the recovery of peripheral CD4 T cells and that hyperactivation of glycolysis in CD4 T cells may drive CD4 T-cell depletion in HIV infection [26, 27], changes that are likely to be reflected in the plasma metabolite profiles.

One of the metabolites separating PWH from the HIV-negative controls was IPA, a strong antioxidant microbial metabolite [28], which is produced when tryptophan is deaminated [29]. IPA is anti-inflammatory and important for intestinal barrier integrity and immune cell function [30]. Disruption of the gastrointestinal barrier, that is, dysbiosis, is a hallmark of many intestinal and chronic inflammatory diseases, including HIV infection [31]. In addition, decreased plasma levels of IPA have been reported to correlate with reduced gut microbial diversity [17]. Thus, reductions in circulating IPA may reflect changes in gut microbial activity, dysbiosis, and persistent low-grade inflammation in ART-treated HIV-infected individuals, as previously reported [32–34].

The decreased plasma levels of tryptophan found in both HIV groups are unlikely to be attributed to increased TDO/IDO-1 activity, as plasma kynurenine and kynurenate also were decreased in the HIV groups. Differences in uptake of tryptophan in the small intestine could explain the differences. Dietary preferences should be of minor importance in this study, given the relatively large groups and that controls were matched for ethnicity. Studies on germ-free animals have shown that the gut microbiota directly regulates circulating tryptophan [35], and altering the gut microbiota through the administration of probiotics in human supplementation trials has also influenced circulating tryptophan levels [36]. Hence, alterations in gut microbial activity may have contributed to the changes in plasma levels of tryptophan and its metabolites seen in HIV-positive individuals.

We observed elevated levels of cysteine in the plasma of the fast immune recovery HIV group, compared with that of the slow immune recovery HIV group. Levels of cysteine were also elevated in the fast immune recovery HIV group compared with the noninfected controls. Moreover, several pathways—homocysteine degradation, cysteine metabolism, and taurine and hypotaurine metabolism—were more enriched in the fast immune recovery group. There was also a positive correlation in CD4 T-cell numbers and cysteine levels. The correlation of cysteine with CD4 T-cell numbers is in contrast to previous findings in HIV-infected youths [37] and could be due to a different composition of ethnicities and/or age of the cohorts. Cysteine provides the redox-active thiol group of glutathione, which is one of the most important endogenous antioxidants [38]. Signs of oxidative stress have been observed in individuals with HIV infection [39, 40]. T-cell proliferation drives the production of reactive oxygen species and is cysteine dependent [41]. It can be hypothesized that elevated plasma levels of cysteine may contribute to T-cell proliferation and improved antiviral T-cell responses by facilitating glutathione synthesis. In vitro studies recently showed that N-acetylcysteine may regulate antitumor immunity [42]. All these pathways may collectively improve T-cell function and favor efficient immune reconstitution following ART.

Applying MSEA to annotated compounds revealed statistically significant alterations in several metabolic pathways when the 2 groups of PWH were compared with noninfected people. One of the most pronounced changes associated with HIV was detected in glycine and serine metabolism. The conversion of serine to glycine is catalyzed by serine hydroxymethyltransferase. Glycine, in turn, refuels one-carbon metabolism, which directly controls the levels of methionine, serine, and glycine [43]. The levels of all these amino acids were decreased in HIV-positive individuals with fast immune recovery. One-carbon metabolism is a complex process that also indirectly controls cysteine levels and plays an important role in the maintenance of cellular redox balance [44]. Hence, altered serine and glycine metabolism may contribute to efficient immune recovery by balancing inflammation-induced oxidative stress in HIV-infected individuals.

Bile acids are the final products of the catabolism of cholesterol and facilitate the absorption of dietary fat. Bile acid deconjugation and the conversion of primary bile acids to secondary bile acids are catalyzed by bacterial enzymes [45]. Among metabolites detected by LC-MS, bile acid biosynthesis was the most enriched pathway in the fast HIV infection group. In contrast, this pathway was not significantly enriched in the slow HIV infection group. Bile acid

metabolites function as biological detergents, and bile acid tolerance is an important property of gut colonization bacteria [45]. The interplay between bile acid metabolism and the gut microbiota is complex. Accumulation of toxic bile acids may cause inflammation [46], but bile acids may also prevent epithelial deterioration and bacterial translocation by activation of the nuclear farnesoid X receptor [47]. Targeting the bile acid–microbiota axis may be a potential approach to improve immune function in HIV.

Our study has several limitations. Most importantly, information about how longitudinal changes in metabolites reflect immune reconstitution during ART suppression is lacking due to the retrospective cross-sectional design of the study. In addition, the diet and nutritional status of the cohorts could influence the metabolic profiles of the cohorts. On the other hand, the large size of the cohorts and the similarities in age, gender, and ethnicity may compensate for individual differences in nutritional intake. The HIV-negative control group was not matched for sexual behavior, which is a weakness as differences in the gut microbiome have been observed in a study of men who have sex with men (MSM) independent of HIV status [48]. However, in one of our previous studies, being a man who has sex with men was not found to have an independent influence on the gut microbiota [9], and it was recently reported that HIV-associated gut microbiota features correlate with disease progression and immune activation rather than sexual preferences [49]. Also, MSM are subjected to punitive actions in Maylasia, and disclosures of sexual preferences are in general highly unreliable. The strength of the study is the selection of participants with extreme profiles of immune recovery following comparable baseline demographics and HIV-related characteristics at ART initiation. This approach allowed us to identify important host-driven metabolic factors that could potentially modulate immune reconstitution.

In conclusion, we present a view of the plasma metabolic changes related to efficient and slow, that is, poor, immune recovery following suppressive ART in a large cohort of HIV-infected individuals. Decreased plasma levels of the bacterial metabolite IPA and changes in bile acid composition in PWH may reflect microbial dysbiosis. We also show that efficient immune recovery is associated with elevated levels of plasma cysteine that may be the result of altered serine and glycine metabolism, pointing toward a role for redox balance in CD4 T-cell reconstitution in HIV infection. This study highlights several biomarkers and metabolic pathways that could facilitate the understanding and monitoring of immune recovery after initiation of ART in HIV-infected individuals.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Swedish Metabolomics Centre, Umeå, Sweden (www.swedishmetabolomicscentre.se) is acknowledged for metabolic profiling by GC-MS and LC-MS.

Financial support. This work has received financial support from The Swedish Research Council project grant 01091 (M.L.); ALF grants, Region Östergötland, Sweden (M.L. and S.N.); and Linköping University Hospital Research Fund (M.L.). The establishment of the cohort was supported by the High Impact Research Grant to the Centre of Excellence for Research in AIDS (CERIA) University of Malaya, H-20001-E000001 (R.R. and A.K.).

Potential conflicts of interest. None of the authors have any conflicts of interest to declare. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. World Health Organization. Global Health Observatory (GHO) data: HIV/AIDS WHO. **2019**. Available at: https://www.who.int/gho/hiv/en/. Accessed 30 January 2019.
2. Baker JV, Peng G, Rapkin J, et al; Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA). Poor initial CD4+ recovery with antiretroviral therapy prolongs immune depletion and increases risk for AIDS and non-AIDS diseases. J Acquir Immune Defic Syndr **2008**; 48:541–6.
3. Raffi F, Le Moing V, Assuied A, et al. Failure to achieve immunological recovery in HIV-infected patients with clinical and virological success after 10 years of combined ART: role of treatment course. J Antimicrob Chemother **2017**; 72:240–245.
4. Brown TT, Glesby MJ. Management of the metabolic effects of HIV and HIV drugs. Nat Rev Endocrinol **2011**; 8:11–21.
5. Takuva S, Maskew M, Brennan AT, et al. Poor CD4 recovery and risk of subsequent progression to AIDS or death despite viral suppression in a South African cohort. J Int AIDS Soc **2014**; 17:18651.
6. Godfrey C, Bremer A, Alba D, et al. Obesity and fat metabolism in human immunodeficiency virus-infected individuals: immunopathogenic mechanisms and clinical implications. J Infect Dis **2019**; 220:420–31.
7. Taylor CT, Colgan SP. Regulation of immunity and inflammation by hypoxia in immunological niches. Nat Rev Immunol **2017**; 17:774–85.
8. Gootenberg DB, Paer JM, Luevano JM, Kwon DS. HIV-associated changes in the enteric microbial community: potential role in loss of homeostasis and development of systemic inflammation. Curr Opin Infect Dis **2017**; 30:31–43.
9. Lee SC, Chua LL, Yap SH, et al. Enrichment of gut-derived fusobacterium is associated with suboptimal immune recovery in HIV-infected individuals. Sci Rep **2018**; 8:14277.
10. Babu H, Sperk M, Ambikan AT, et al. Plasma metabolic signature and abnormalities in HIV-infected individuals on long-term successful antiretroviral therapy. Metabolites **2019**; 9:210.
11. Peltenburg NC, Schoeman JC, Hou J, et al. Persistent metabolic changes in HIV-infected patients during the first year of combination antiretroviral therapy. Sci Rep **2018**; 8:16947.
12. Norman JM, Handley SA, Virgin HW. Kingdom-agnostic metagenomics and the importance of complete characterization of enteric microbial communities. Gastroenterology **2014**; 146:1459–69.
13. Monaco CL, Gootenberg DB, Zhao G, et al. Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host Microbe **2016**; 19:311–22.
14. Shah SH, Kraus WE, Newgard CB. Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. Circulation **2012**; 126:1110–20.
15. Zhang L, Zheng J, Ahmed R, et al. A high-performing plasma metabolite panel for early-stage lung cancer detection. Cancers **2020**; 12:622.
16. Holmes E, Wilson ID, Nicholson JK. Metabolic phenotyping in health and disease. Cell **2008**; 134:714–7.
17. Wilmanski T, Rappaport N, Earls JC, et al. Blood metabolome predicts gut microbiome α-diversity in humans. Nat Biotechnol **2019**; 37:1217–28.
18. Rajasuriar R, Palmer C, Abdel-Mohsen M, Kamaruzzaman SB. Integrative biomarkers of biologic aging in HIV. AIDS **2019**; 33:345–7.
19. Yang JJ, Huang CH, Liu CE, et al. Multicenter study of trimethoprim/sulfamethoxazole-related hepatotoxicity: incidence and associated factors among

HIV-infected patients treated for *Pneumocystis jirovecii* pneumonia. PLoS One **2014**; 9:e106141.
20. Clarke CJ, Haselden JN. Metabolic profiling as a tool for understanding mechanisms of toxicity. Toxicol Pathol **2008**; 36:140–7.
21. Funderburg NT, Mehta NN. Lipid abnormalities and inflammation in HIV inflection. Curr HIV/AIDS Rep **2016**; 13:218–25.
22. Lo J. Dyslipidemia and lipid management in HIV-infected patients. Curr Opin Endocrinol Diabetes Obes **2011**; 18:144–7.
23. Waheed AA, Freed EO. The role of lipids in retrovirus replication. Viruses **2010**; 2:1146–80.
24. Cassol E, Misra V, Holman A, et al. Plasma metabolomics identifies lipid abnormalities linked to markers of inflammation, microbial translocation, and hepatic function in HIV patients receiving protease inhibitors. BMC Infect Dis **2013**; 13:203.
25. Mena A, Clavero E, Díaz-Díaz JL, Castro A. Similar plasma lipidomic profile in people living with HIV treated with a darunavir-based or an integrase inhibitor-based antiretroviral therapy. Sci Rep **2019**; 9:17184.
26. Palmer CS, Ostrowski M, Gouillou M, et al. Increased glucose metabolic activity is associated with CD4+ T-cell activation and depletion during chronic HIV infection. AIDS **2014**; 28:297–309.
27. Yang X, Su B, Zhang X, et al. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: challenges of immunological non-responders. J Leukoc Biol **2020**; 107:597–612.
28. Andrisic L, Dudzik D, Barbas C, et al. Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer. Redox Biol **2018**; 14:47–58.
29. Gao J, Xu K, Liu H, et al. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Front Cell Infect Microbiol **2018**; 8:13.
30. Wlodarska M, Luo C, Kolde R, et al. Indoleacrylic acid produced by commensal *Peptostreptococcus* species suppresses inflammation. Cell Host Microbe **2017**; 22:25–37.e6.
31. Chung CY, Alden SL, Funderburg NT, et al. Progressive proximal-to-distal reduction in expression of the tight junction complex in colonic epithelium of virally-suppressed HIV+ individuals. PLoS Pathog **2014**; 10:e1004198.
32. Rajasuriar R, Booth D, Solomon A, et al. Biological determinants of immune reconstitution in HIV-infected patients receiving antiretroviral therapy: the role of interleukin 7 and interleukin 7 receptor α and microbial translocation. J Infect Dis **2010**; 202:1254–64.
33. Rajasuriar R, Wright E, Lewin SR. Impact of antiretroviral therapy (ART) timing on chronic immune activation/inflammation and end-organ damage. Curr Opin HIV AIDS **2015**; 10:35–42.
34. Yap SH, Abdullah NK, McStea M, et al. HIV/human herpesvirus co-infections: impact on tryptophan-kynurenine pathway and immune reconstitution. PLoS One **2017**; 12:e0186000.
35. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A **2009**; 106:3698–703.
36. Strasser B, Geiger D, Schauer M, et al. Probiotic supplements beneficially affect tryptophan-kynurenine metabolism and reduce the incidence of upper respiratory tract infections in trained athletes: a randomized, double-blinded, placebo-controlled trial. Nutrients **2016**; 8:752.
37. Ziegler TR, Judd SE, Ruff JH, et al. Amino acid concentrations in HIV-infected youth compared to healthy controls and associations with CD4 counts and inflammation. AIDS Res Hum Retroviruses **2017**; 33:681–9.
38. Zhang H, Forman HJ. Glutathione synthesis and its role in redox signaling. Semin Cell Dev Biol **2012**; 23:722–8.
39. Buhl R, Jaffe HA, Holroyd KJ, et al. Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. Lancet **1989**; 2:1294–8.
40. Watson WH, Ritzenthaler JD, Peyrani P, et al. Plasma cysteine/cystine and glutathione/glutathione disulfide redox potentials in HIV and COPD patients. Free Radic Biol Med **2019**; 143:55–61.
41. Levring TB, Hansen AK, Nielsen BL, et al. Activated human CD4+ T cells express transporters for both cysteine and cystine. Sci Rep **2012**; 2:266.
42. Pilipow K, Scamardella E, Puccio S, et al. Antioxidant metabolism regulates CD8+ T memory stem cell formation and antitumor immunity. JCI Insight **2018**; 3:e122299.
43. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. Cell Metab **2017**; 25:27–42.
44. Amelio I, Cutruzzolá F, Antonov A, et al. Serine and glycine metabolism in cancer. Trends Biochem Sci **2014**; 39:191–8.
45. Winston JA, Theriot CM. Diversification of host bile acids by members of the gut microbiota. Gut Microbes **2020**; 11:158–71.
46. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nat Rev Gastroenterol Hepatol **2018**; 15:111–28.
47. Inagaki T, Moschetta A, Lee YK, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A **2006**; 103:3920–5.
48. Noguera-Julian M, Rocafort M, Guillén Y, et al. Gut microbiota linked to sexual preference and HIV infection. EBioMedicine **2016**; 5:135–46.
49. Vujkovic-Cvijin I, Sortino O, Verheij E, et al. HIV-associated gut dysbiosis is independent of sexual practice and correlates with noncommunicable diseases. Nat Commun **2020**; 11:2448.