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Gut Microbiota Alterations after Switching from a Protease Inhibitor or Efavirenz to Raltegravir in a Randomized, Controlled Study

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Running title: effect of ART agent switch on gut microbiota

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A.M.H. is currently employed by Orion Pharma, but the work reported in this paper was completed before the start of the employment.

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ABSTRACT

Objective: To study gut microbiota before and 24 weeks after a single antiretroviral agent switch.

Design: HIV-positive subjects with efavirenz (EFV) or a protease inhibitor (PI) based antiretroviral therapy (ART) were randomized to switch EFV or PI to raltegravir (RAL group, n=19) or to continue unchanged ART (EFV/PI group, n=22). Age and weight matched HIV-negative subjects (n=10) were included for comparison.

Methods: Microbiota was analyzed using 16S rRNA sequencing. Serum intestinal fatty acid binding protein (I-FABP) and serum lipopolysaccharide-binding protein (LBP) were measured as gut permeability markers. Three-day food diaries were collected.

Results: At week 24, microbiota diversity (Chao1 index) was higher in RAL than EFV/PI group (p=0.014), and RAL group did not differ from HIV-negative subjects. In subgroup analysis switching from EFV (p=0.043), but not from a PI to RAL increased Chao1. At week 24, RAL and EFV/PI group differed in the relative abundance of *Prevotella 9* (higher in RAL, p=0.01), *Phascolarctobacterium* and *Bacteroides* (lower in RAL, p=0.01 and p=0.03).

Dietary intakes did not change during the study and do not explain microbiota differences. Also, I-FABP and LBP remained unchanged.

Conclusions: Here we demonstrate that a single ART agent switch caused microbiota alterations, most importantly, an increase in diversity with EFV to RAL switch. Previously we reported weight gain, yet reduced inflammation in this cohort. The observed microbiota differences between RAL and EFV/PI groups may be associated with reduced inflammation and/or increase in weight. Further studies are needed to evaluate inflammatory and metabolic capacity of microbiota with ART switches.

INTRODUCTION

Human gastrointestinal tract is inhabited by a diverse collection of microbes, which are collectively known as the gut microbiota. The interplay between these microbes and the host is complex and a shift in their homeostasis due to various triggers, including HIV infection or dietary habits, may lead to unfavourable health outcomes [1, 2].

Gut microbiota dysbiosis is characterized by low bacterial diversity as well as compositional alterations.. The disruption of gut barrier function is frequently associated with dysbiosis, allowing translocation of microbial products into the systemic circulation, thus leading to low grade systemic inflammation [3]. This may eventually facilitate the development of metabolic and cardiovascular comorbidities [2, 4]. Dysbiotic microbiota can also affect the body weight, by for instance altering the gut satiety hormone secretion as well as energy harvest and fat storage [5]. In obesity, the altered microbiota is shown to facilitate energy harvest not only for further microbial growth but also to the host [5].

HIV-infection and antiretroviral therapy (ART) have been associated with an increased prevalence of several metabolic abnormalities and systemic inflammation [6]. However, the potential role of gut microbiota on these complications is incompletely known. HIV-infection itself has been associated with dysbiotic changes at an early stage of the infection [7] and ART initiation in ART-naïve subjects does not fully restore these alterations [1].

During recent years, the use of integrase inhibitors (INSTI) has been associated with increased weight gain [8]. The effect of ART, especially INSTIs, on gut microbiota is known poorly. In a cross-sectional study,, people living with HIV (PLWH), and receiving INSTI raltegravir (RAL) based ART regimen had microbiota diversity similar to that of HIV-negative subjects as opposed to those treated with a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) based ART regimen who had reduced gut microbiota diversity [9]. Yet, no study has evaluated the effect of an ART switch on the gut microbiota among virologically suppressed patients.

Previously, we reported weight gain and increase in body fat and subcutaneous adipose tissue volume when switching from efavirenz (EFV) or a PI to RAL in PLWH with good

virological response [10]. Regardless of weight gain, liver fat content and visceral adipose tissue volume remained unchanged, while circulating lipids and inflammatory markers improved in subjects who switched to RAL as compared to those who continued unchanged ART [10].

In this study, we hypothesized that switching from EFV or a PI to RAL leads to changes in gut microbiota diversity and composition. We also determined the effect of this ART switch on the markers of intestinal permeability and microbial translocation into the bloodstream. Furthermore, we compared the gut microbiota of PLWH with that of HIV-negative subjects with similar demographics including overweight or obesity.

METHODS

Study subjects

Adult HIV-positive subjects with ongoing EFV- or a PI-based ART regimen and with body mass index (BMI) >25kg/m^2 together with at least one metabolic syndrome component or with radiologically confirmed fatty liver were invited to participate in a randomized, controlled study to evaluate the effect of switching EFV or a PI to RAL on liver fat, body composition and on gut microbiota. The changes in liver fat and body composition were reported previously [10]. Briefly, 45 participants stratified by gender and ART class, were randomized 1:1 to switch EFV or a PI to RAL 1200 mg once daily with no other changes in the ART (RAL group), or to continue the unchanged EFV- or PI -containing regimen (EFV/PI group); patient disposition is shown in Supplementary Figure 1, http://links.lww.com/QAD/C701. Eventually, 41 subjects participated in the microbiota study. We additionally included ten HIV-negative subjects matched for BMI and age for the comparison of gut microbiota with HIV-positive subjects.

Assessments

For the HIV-positive subjects, all study assessments including laboratory and imaging studies were performed at baseline and at week 24. Three-day food diaries were collected at baseline and at week 24 for the HIV-positive subjects and at one timepoint for HIV-negative controls. A registered dietitian analyzed the food intakes with Aivodiet nutrient calculation program (v. 2.2.0.1., AivoFinland Oy, Finland).

Laboratory analysis of the HIV-positive subjects included serum vitamin D concentration and fecal calprotectin using standard methods. Serum intestinal fatty acid binding protein (I-FABP) and serum lipopolysaccharide binding protein (LBP) were measured by enzyme-linked immunosorbent assay (Human FABP2/I-FABP, Quantikine ELISA, R&D systems, USA and Invitrogen Human LBP ELISA, Thermo Fisher, USA).

Stool sample Collection and Processing

Study subjects collected stool into standardized samplingtubes, which were frozen at -80 C within 24 hours of collection. The history of antibiotic use during the 3 months preceding stool sample collection was recorded. Eventually, 41 HIV-positive subjects gave a sample at baseline and 37 HIV-positive subjects at week 24. HIV-negative subjects gave a sample only at one timepoint.

DNA extraction and sequencing

Fecal DNA was extracted as described previously [11].. DNA concentrations were measured with Quant-iT™ dsDNA Assay High Sensitivity (HS) kit (Invitrogen, Thermo Fisher Scientific, USA). Microbiota profiling was conducted by the 16S rRNA gene amplicon sequencing (MiSeq, Illumina, USA) with primers targeting V3-V4 hypervariable region [12]. Phusion High-Fidelity PCR Master Mix with HF Buffer (Thermo Fisher Scientific) and primers S-D-Bact-0341-b-S-17 5‘-CCTACGGGNGGCWGCAG-3’and reverse S-D-Bact-0785-a-A-21 5‘-GACTACHVGGGTATCTAATCC-3’ were used for PCR with the following program: 98 °C for 1 minute, 40 cycles of 98 °C for 10 seconds, 64 °C for 40 seconds and 72 °C for 40 seconds, 72 °C for 10 min. Nine samples that didn´t work in the one-step PCR were run with 2-step program, where 16S primers were used in the first PCR (45 cycles) and index primers in the second PCR (25 cycles) with the PCR products from the first step as a template. After PCR purification, and mixing into a library pool, sequencing was done in the Finnish Institute for Molecular Medicine, Univ. Helsinki, Finland.

The 16S rRNA gene sequences were quality-filtered and clustered to operational taxonomic units (OTUs) at the 97% similarity using CLC Microbial Genomics Package (Qiagen, Hilden, Germany). The rRNA gene sequences were classified using SILVA SSU Ref database (v132, 99%).

Statistical analysis

The statistical analyses were performed with CLC Microbial Genomics Package and IBM SPSS Statistics v26 for Windows (SPSS, Chicago, IL, USA). The taxonomic differences between the groups were analyzed with ANOVA-like comparison in CLC Microbial Genomics Package, followed by Benjamini-Hochberg correction for multiple testing. The taxonomic differences between the time points were analyzed with Wilcoxon signed rank test in SPSS, followed by Benjamini-Hochberg correction for multiple testing. In group and time point comparisons, the statistical significance was set at $p < 0.05$ after the multiple testing corrections.

To estimate alpha-diversity, Chao1 and Shannon indices were determined. Chao1 is an estimator based on abundance indicating the number of species living in a habitat i.e. species richness. Shannon index takes into account both the species richness and their relative

abundance (evenness). Bray Curtis distance was used to determine inter-individual species diversity of the gut microbiota.

Ethical considerations

The study was approved by the ethics committee of Helsinki University Hospital and the ART switch part also by the Finnish Medicines Agency. The switch study is registered at the European Clinical Trials Database (EudraCT 2017-003430-85) and in ClinicalTrials.gov (NCT03374358). The overall study was conducted in compliance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All study subjects provided a written informed consent.

RESULTS

A total of 41 HIV-positive and ten HIV-negative subjects participated in the microbiota study. The study groups did not differ significantly with respect to baseline clinical characteristics (Table 1).

All HIV-positive participants had plasma HIV-1 RNA less than 50 copies per mL at baseline. CD4 counts were also similar between the groups (Table 1). At week 24, the median CD4 count was 719 (538-848) cells/μL in RAL group and 793 (572-1020) cells/μL in EFV/PI group, p=0.4.

None of the subjects had consumed antibiotics within three months before stool sample collections. There were no changes in diet, *i.e.*, in caloric intake, or in carbohydrate, protein, fat or fibre intake between baseline and week 24 visits in HIV-positive group and the caloric and main nutrient intakes did not differ between HIV-positive and HIV-negative subjects (Supplementary table 1, http://links.lww.com/QAD/C704).

Gut microbiota at baseline

When all HIV-positive participants were compared to HIV-negative subjects, no differences were found in Bray Curtis distance (*i.e.*, inter-individual species diversity of the gut microbiota) or alpha-diversity measures (Chao1 *i.e.*, species richness and Shannon *i.e.*, species diversity), or in gut microbiota composition (data not shown). However, according to Bray Curtis distance and PERMANOVA analysis, EFV (p=0.00003) and PI (p=0.01236) groups differed at baseline from HIV-negative subjects significantly but not from each other (Figure 1). Concerning alpha-diversity measures, there were no differences in Chao1 or Shannon indices between the PI and EFV groups, or PI and HIV- negative groups. However, EFV group had lower Chao1, but not Shannon index than HIV-negative group (Figure 1). Regarding the gut microbiota composition, PI and EFV groups differed from each other and from HIV-negative group in the relative abundance of several microbial genera (Table 2). The average composition of the gut microbiota at phylum, family and genus level are shown in Supplementary figure 2, http://links.lww.com/QAD/C702.

Among HIV-positive subjects, we also compared the gut microbiota between men who have sex with men (MSM) and heterosexual participants at baseline. There were no differences in the gut microbiota diversity (Chao1 or Shannon) or Bray-Curtis distance measures between these groups (data not shown). However, there were some differences in the relative abundance of specific bacterial taxa between MSM and heterosexual PLWH (Supplementary figure 3, http://links.lww.com/QAD/C702). As MSM subjects were evenly distributed among the study groups (n=5 in EFV to RAL, n=5 in EFV control, n=7 in PI to RAL and n=4 in PI control, p=0.3), further analysis were carried out without considering sexual orientation as a confounding factor.

Gut microbiota changes associated with ART switch

There were no significant differences at baseline in gut microbiota diversity or composition between those randomized to RAL (RAL group) and those continuing the current PI or EFV based ART (EFV/PI group) (data not shown). At week 24, RAL group had higher Chao1 index (p=0.014) than EFV/PI group (Figure 2).

Since EFV and PI groups differed in gut microbiota at baseline, we analyzed these groups separately. In the group that switched EFV to RAL, the alpha-diversity indices Chao1 (p=0.043) and Shannon (p=0.043) increased significantly, but no changes occurred in EFV control group. There were no significant changes among those who either switched PI to RAL or continued with PI based ART. In summary, the switch from EFV or a PI to RAL resulted in an increase in bacterial diversity in the gut microbiota, which could be attributed to the switch from EFV to RAL (Figure 2).

We also compared the alpha-diversity indices between HIV-positive and HIV-negative groups at week 24 (Figure 2). Those who had switched from EFV to RAL had similar alpha-diversity indices as HIV-negative subjects (p=0.775), whereas EFV control group had lower Chao1 index than HIV-negative subjects (P=0.037). The group switching from a PI to RAL or PI control group did not differ from HIV-negative in alpha-diversity indices.

Between RAL and EFV/PI groups, there were modest differences in the relative abundance of three microbial genera at week 24 (Figure 3). RAL group had slightly higher relative abundance of *Prevotella* 9 than the control group (p=0.01), as well as lower abundance of *Phascolarctobacterium* (p=0.01) and *Bacteroides* (p=0.03). Concerning the subgroups with ART switch from EFV to RAL and from a PI to RAL, there were no significant within group longitudinal microbiota compositional changes in *Prevotella* 9, *Phascolarctobacterium or Bacteroides* (data not shown). Thus, the subtle changes in taxonomic composition in conjunction to switches in medication couldn´t be attributed specifically to either of the subgroups. Compositional changes did not correlate with the changes in body weight, nor with changes in inflammatory markers (data not shown).

Gut barrier biomarkers

The median (IQR) concentration of the gut wall barrier biomarker I-FABP2 was significantly higher in EFV group compared to PI group at baseline (3.19 (2.39-4.34) vs 1.55 (1.22-2.35), $p<0.001$), whereas there were no differences in serum LPS, fecal calprotectin or in serum vitamin D concentrations between these groups. There were no significant differences in these variables between RAL and EFV/PI groups at baseline, week 24 or longitudinally within the groups (Table 1).

DISCUSSION

Here we demonstrated for the first time in a randomized, longitudinal study setting that a single ART agent switch can alter the gut microbiota diversity and composition in stable ART experienced PLWH. Also, our results support the hypothesis that ART induced alterations in the gut microbiota may play a role in metabolic and inflammatory changes in PLWH.

At baseline, we didn´t find differences in microbial alpha-diversity indices or Bray Curtis distance between all HIV-positive and HIV-negative subjects. However, when looking at EFV and PI groups separately, the Bray Curtis distances of these sub-groups differed from HIV-negative subjects. Also, EFV group had lower Chao1 index than HIV-negative subjects, while there were no differences between the PI and HIV-negative groups. Our results contradict the earlier findings of lower alpha-diversity among PLWH than HIV-negative subjects regardless of the ART regimen used [13], which may be explained by differences in the baseline and geographical characteristics. In a recent meta-analysis, HIV-infection was associated with decreased alpha-diversity only in men who have sex with women (MSW) and to a lesser degree in women [14]. However, we didn´t detect differences in alpha-diversity indices between MSM and heterosexual PLWH.

Several gut microbiota compositional differences were also observed between study groups at baseline. *Phascolarctobacterium* was more abundant in PI than EFV and HIV-negative groups, while *Fusobacterium*, *Acidaminococcus* and *Megasphera* were more abundant in EFV than PI and HIV-negative groups. *Ruminococcaceae* UCG-014 was more abundant in EFV than in PI group.

Previously, abundance of *Ruminococcus* has been linked to HIV infection [15, 16] but not to any specific ART agent. Opposite finding has also been reported with a depletion of *Ruminococcus* in rectal mucosal samples from untreated HIV-positive men [17]. *Ruminococcus* may have proinflammatory properties [18]. *Acidaminococcus* and *Megasphera* are also opportunistic pathogens, which have been linked to atherosclerotic conditions [19, 20].

At week 24, RAL group had higher alpha-diversity than EFV/PI group, which could be attributed specifically to the change from EFV to RAL. This ART switch led to a comparable

alpha-diversity to that of HIV-negative subjects. This finding is in line with the previous findings describing similar alpha-diversity between PLWH using RAL-based regimen and HIV-negative controls in across-sectional study [9]. The increase of bacterial alpha-diversity following the switch from EFV to RAL can be considered a positive outcome. Higher bacterial diversity has been associated with good health and decreased diversity with obesity and metabolic disorders.[21].

We also evaluated whether a skewed distribution of HIV-related variables or metabolic comorbidities between the study groups might have affected our results. Among HIV-specific factors, e.g low CD4 count has been associated with reduced microbial diversity [22, 23]. However, the study groups were comparable regarding all HIV-specific variables (CD4 count, time since diagnosis of HIV, duration of ART, distribution of different PIs). Metabolic co-morbidities such as diabetes or NAFLD are associated with dysbiotic findings in gut microbiota in general population including perturbation in microbiota composition, increase in gut permeability and facilitation of the passage of inflammatory factors to the blood [21, 24], yet data among PLWH are limited and conflicting [25, 26]. In any case, our study groups had similar prevalences of metabolic comorbidities, so the observed differences in gut microbiota could not be explained by these confounding factors.

To our knowledge, no earlier longitudinal studies have evaluated the role of ART switch on gut microbiota diversity in PLWH. However, there are few previous longitudinal studies among treatment-naïve PLWH starting their first ART regimen. Recently, a reduction in alpha-diversity in two PLWH who started a NNRTI-based regimen as compared to two PLWH who started a PI-based regimen was shown [27]. Similarly, a reduction in alpha-diversity was reported in 19 naïve PLWH after starting a NNRTI or a PI-based ART [22]. In these studies, however, the observed microbiota changes may be confounded by the concomitant reduction in HIV viral load and diet intake wasn´t reported.

We also detected microbial compositional differences at week 24. RAL group had slightly higher relative abundance of *Prevotella 9* as well as lower abundance of *Bacteroides* and *Phascolarcobacterium* than EFV/PI group. The relevance of these differences to health are not straightforward to interpret. *Prevotellaceae* has been associated with HIV-infection [28], yet more recently only to MSM independent of HIV status [29, 30]. On the other hand, its high abundance is also characteristic of healthy individuals consuming plant-rich diet [31]. *Bacteroides* spp. are considered to play a role in promoting T-regulatory cell function [32] and reinforcing epithelial barrier function [33]. Moreover, reduced abundance of Bacteroidetes has also been linked with HIV-infection and obesity [28]. *Phascolarctobacterium* can produce short-chain fatty acids, mainly propionate which can inhibit inflammatory processes [34, 35]. Increased abundance of *Phascolarctobacterium* was associated with success in weight loss [36] but also with type 2 diabetes [37] and a reduction in *Phascolarcobacterium* was associated with inflammatory bowel diseases [38]. Thus, some of the observed changes may seem unfavorable rather than beneficial, but overall microbiota confers its health effects as concerted action of all its functionalities. Further studies are needed to both confirm the changes related to the medication change and to decipher

potential changes in the inflammatory and metabolic capacity of the microbiota as a community.

The gut permeability biomarker I-FABP2 was higher in EFV group compared to the PI group at baseline, but it did not change after switching EFV to RAL. Although we detected alterations in microbiota, we didn´t find any significant changes in gut permeability biomarkers after the ART switch. Previously, one study reported increased I-FABP2 with PI compared to EFV based regimen or HIV-negative group [13], while another study showed a non-significant increase in I-FABP2 after a switch from EFV to INSTI [39]. Alsoan increase in I-FABP2 but no change in LBP was reported in naïve PLWH, who started either a PI or RAL based regimen, however, the increase in I-FABP2 levels didn´t differ between the treatment groups [40].

Recent data suggest that vitamin D may influence gut microbiota [41]. EFV has been associated with low vitamin D concentrations [42]. We therefore also investigated vitamin D in our study but didn´t observe significant differences between the groups or longitudinally.

The main clinical findings of the present study [10] were somewhat controversial including weight gain but improved inflammatory parameters in RAL as compared to EFV/PI group. One may hypothesize that an increased gut microbiota diversity in RAL group could contribute to the improvement in the inflammatory markers. On the other hand, the gut microbiota compositional changes detected in RAL group have been previously linked to weight gain [36]. However, no direct correlations between microbial findings and clinical outcomes were found possibly due to small study size.

The measured confounding factors, *i.e*., diet and use of antibiotics didn´t explain the alterations in microbiota after the ART switch. A direct effect of antiretrovirals on gut microbiota has been studied *in vitro*, showing that EFV and zidovudine had antimicrobial activity against *Bacteroides fragilis* and *Prevotella* spp. [27]. EFV also inhibited the growth of *Enterococcus faecalis* [27]. It may also be assumed that antiretrovirals could affect the dynamics of gut phageome and consequently, induce changes in the bacterial population. Phageome is considered to participate in the regulation of complex bacterial networks, but understanding its role is still incomplete [43], and the effect of different (antiviral) drugs on phageome is yet to be studied.

The main limitation as with all switch studies is the difficulty to determine to what extent the observed effects are directly caused by the introduction of a new ART agent versus by the withdrawal of the previous agent. In addition, the number of participants was limited especially regarding the subgroup analyses.

Furthermore, our microbiota profiling was limited to the bacterial population and thus, we didn´t address possible changes in the archaeal, yeast, fungal, prokaryotic and viral populations.. Nevertheless, the randomized, controlled and longitudinal design with dietary

records as well as the inclusion of HIV-negative age and BMI-matched comparison group are clear strengths of our study.

In conclusion, our results support the hypothesis that different ART components have an impact on the gut microbiota diversity and composition. EFV based ART regimen was associated with lower alpha-diversity than PI based ART, but switching EFV to RAL restored bacterial diversity to the same level as in HIV-negative subjects. Further studies are needed to explore whether the improved diversity could explain the beneficial clinical effects of this ART switch and whether the observed microbiota compositional changes may be linked with increased weight gain with INSTIs.

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Author statement and guarantors of the article are J.P.S. and A.M.H.

A.M.H. designed the study, collected data, analysed data and wrote the manuscript.

S.P. analysed data, generated figures and reviewed the manuscript.

R.S. designed the study and reviewed the manuscript.

A.K.H. analysed data and wrote the manuscript.

P.A. and K.H.P. designed the study and reviewed the manuscript.

J.P.S. designed the study, wrote and reviewed the manuscript.

REFERENCES

1. Bandera A, De Benedetto I, Bozzi G, Gori A. **Altered gut microbiome composition in HIV infection: causes, effects and potential intervention**. *Curr Opin HIV AIDS* 2018; **13**:73-80.

2. Cani PD. **Human gut microbiome: hopes, threats and promises**. *Gut* 2018; **67**:1716-1725.

3. Paone P, Cani PD. **Mucus barrier, mucins and gut microbiota: the expected slimy partners?** *Gut* 2020; **69**:2232-2243.

4. Kasselman LJ, Vernice NA, DeLeon J, Reiss AB. **The gut microbiome and elevated cardiovascular risk in obesity and autoimmunity**. *Atherosclerosis* 2018; **271**:203-213.

5. Gomes AC, Hoffmann C, Mota JF. **The human gut microbiota: Metabolism and perspective in obesity**. *Gut Microbes* 2018; **9**:308-325.

6. Bourgi K, Wanjalla C, Koethe JR. **Inflammation and Metabolic Complications in HIV**. *Curr HIV/AIDS Rep* 2018; **15**:371-381.

7. Zevin AS, McKinnon L, Burgener A, Klatt NR. **Microbial translocation and microbiome dysbiosis in HIV-associated immune activation**. *Curr Opin HIV AIDS* 2016; **11**:182-190.

8. Bailin SS, Gabriel CL, Wanjalla CN, Koethe JR. **Obesity and Weight Gain in Persons with HIV**. *Curr HIV/AIDS Rep* 2020; **17**:138-150.

9. Villanueva-Millán MJ, Pérez-Matute P, Recio-Fernández E, Lezana Rosales JM, Oteo JA. **Differential effects of antiretrovirals on microbial translocation and gut microbiota composition of HIV-infected patients**. *J Int AIDS Soc* 2017; **20**:21526.

10. Hanttu A, Vuoti S, Kivelä P, Arkkila P, Lundbom N, Hakkarainen A, et al. **Liver Fat, Adipose Tissue, and Body Composition Changes After Switching from a Protease Inhibitor or Efavirenz to Raltegravir**. *AIDS Patient Care STDS* 2021; **35**:335-341.

11. Salonen A, Nikkilä J, Jalanka-Tuovinen J, Immonen O, Rajilić-Stojanović M, Kekkonen RA, et al. **Comparative analysis of fecal DNA extraction methods with phylogenetic microarray: effective recovery of bacterial and archaeal DNA using mechanical cell lysis**. *J Microbiol Methods* 2010; **81**:127-134.

12. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. **Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies**. *Nucleic Acids Res* 2013; **41**:e1.

13. Pinto-Cardoso S, Lozupone C, Briceño O, Alva-Hernández S, Téllez N, Adriana A, et al. **Fecal Bacterial Communities in treated HIV infected individuals on two antiretroviral regimens**. *Sci Rep* 2017; **7**:43741.

14. Tuddenham SA, Koay WLA, Zhao N, White JR, Ghanem KG, Sears CL. **The Impact of Human Immunodeficiency Virus Infection on Gut Microbiota α-Diversity: An Individual-level Meta-analysis**. *Clin Infect Dis* 2020; **70**:615-627.

15. Wang Z, Usyk M, Sollecito CC, Qiu Y, Williams-Nguyen J, Hua S, et al. **Altered Gut Microbiota and Host Metabolite Profiles in Women With Human Immunodeficiency Virus**. *Clin Infect Dis* 2020; **71**:2345-2353.

16. Zhou Y, Ou Z, Tang X, Zhou Y, Xu H, Wang X, et al. **Alterations in the gut microbiota of patients with acquired immune deficiency syndrome**. *J Cell Mol Med* 2018; **22**:2263-2271.

17. McHardy IH, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, et al. **HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota**. *Microbiome* 2013; **1**:26.

18. Hills RD, Jr., Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. **Gut Microbiome: Profound Implications for Diet and Disease**. *Nutrients* 2019; **11**.

19. Han Y, Gong Z, Sun G, Xu J, Qi C, Sun W, et al. **Dysbiosis of Gut Microbiota in Patients With Acute Myocardial Infarction**. *Front Microbiol* 2021; **12**:680101.

20. Yin J, Liao SX, He Y, Wang S, Xia GH, Liu FT, et al. **Dysbiosis of Gut Microbiota With Reduced Trimethylamine-N-Oxide Level in Patients With Large-Artery Atherosclerotic Stroke or Transient Ischemic Attack**. *J Am Heart Assoc* 2015; **4**.

21. Aron-Wisnewsky J, Warmbrunn MV, Nieuwdorp M, Clément K. **Metabolism and Metabolic Disorders and the Microbiome: The Intestinal Microbiota Associated With Obesity, Lipid Metabolism, and Metabolic Health-Pathophysiology and Therapeutic Strategies**. *Gastroenterology* 2021; **160**:573-599.

22. Nowak P, Troseid M, Avershina E, Barqasho B, Neogi U, Holm K, et al. **Gut microbiota diversity predicts immune status in HIV-1 infection**. *Aids* 2015; **29**:2409-2418.

23. Lu D, Zhang JB, Wang YX, Geng ST, Zhang Z, Xu Y, et al. **Association between CD4(+) T cell counts and gut microbiota and serum cytokines levels in HIV-infected immunological non-responders**. *BMC Infect Dis* 2021; **21**:742.

24. Safari Z, Gérard P. **The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD)**. *Cell Mol Life Sci* 2019; **76**:1541-1558.

25. Maurice JB, Garvey L, Tsochatzis EA, Wiltshire M, Cooke G, Guppy N, et al. **Monocyte-macrophage activation is associated with nonalcoholic fatty liver disease and liver fibrosis in HIV monoinfection independently of the gut microbiome and bacterial translocation**. *Aids* 2019; **33**:805-814.

26. Yanavich C, Perazzo H, Li F, Tobin N, Lee D, Zabih S, et al. **A pilot study of microbial signatures of liver disease in those with HIV mono-infection in Rio de Janeiro, Brazil**. *Aids* 2022; **36**:49-58.

27. Ray S, Narayanan A, Giske CG, Neogi U, Sonnerborg A, Nowak P. **Altered Gut Microbiome under Antiretroviral Therapy: Impact of Efavirenz and Zidovudine**. *ACS Infect Dis* 2021; **7**:1104-1115.

28. 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.

29. Noguera-Julian M, Rocafort M, Guillén Y, Rivera J, Casadellà M, Nowak P, et al. **Gut Microbiota Linked to Sexual Preference and HIV Infection**. *EBioMedicine* 2016; **5**:135-146.

30. Armstrong AJS, Shaffer M, Nusbacher NM, Griesmer C, Fiorillo S, Schneider JM, et al. **An exploration of Prevotella-rich microbiomes in HIV and men who have sex with men**. *Microbiome* 2018; **6**:198.

31. Tett A, Pasolli E, Masetti G, Ercolini D, Segata N. **Prevotella diversity, niches and interactions with the human host**. *Nat Rev Microbiol* 2021; **19**:585-599.

32. Zafar H, Saier MH, Jr. **Gut Bacteroides species in health and disease**. *Gut Microbes* 2021; **13**:1-20.

33. Hiippala K, Kainulainen V, Suutarinen M, Heini T, Bowers JR, Jasso-Selles D, et al. **Isolation of Anti-Inflammatory and Epithelium Reinforcing Bacteroides and Parabacteroides Spp. from A Healthy Fecal Donor**. *Nutrients* 2020; **12**.

34. Wu F, Guo X, Zhang J, Zhang M, Ou Z, Peng Y. **Phascolarctobacterium faecium abundant colonization in human gastrointestinal tract**. *Exp Ther Med* 2017; **14**:3122-3126.

35. Hosseini E, Grootaert C, Verstraete W, Van de Wiele T. **Propionate as a health-promoting microbial metabolite in the human gut**. *Nutr Rev* 2011; **69**:245-258.

36. Muñiz Pedrogo DA, Jensen MD, Van Dyke CT, Murray JA, Woods JA, Chen J, et al. **Gut Microbial Carbohydrate Metabolism Hinders Weight Loss in Overweight Adults**

Undergoing Lifestyle Intervention With a Volumetric Diet. *Mayo Clin Proc* 2018; **93**:1104-1110.

37. Wang TY, Zhang XQ, Chen AL, Zhang J, Lv BH, Ma MH, et al. **A comparative study of microbial community and functions of type 2 diabetes mellitus patients with obesity and healthy people**. *Appl Microbiol Biotechnol* 2020; **104**:7143-7153.

38. Bajer L, Kverka M, Kostovcik M, Macinga P, Dvorak J, Stehlikova Z, et al. **Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis**. *World J Gastroenterol* 2017; **23**:4548-4558.

39. Asundi A, Robles Y, Starr T, Landay A, Kinslow J, Ladner J, et al. **Immunological and Neurometabolite Changes Associated With Switch From Efavirenz to an Integrase Inhibitor**. *J Acquir Immune Defic Syndr* 2019; **81**:585-593.

40. El Kamari V, Moser C, Hileman CO, Currier JS, Brown TT, Johnston L, et al. **Lower Pretreatment Gut Integrity Is Independently Associated With Fat Gain on Antiretroviral Therapy**. *Clin Infect Dis* 2019; **68**:1394-1401.

41. Tangestani H, Boroujeni HK, Djafarian K, Emamat H, Shab-Bidar S. **Vitamin D and The Gut Microbiota: a Narrative Literature Review**. *Clin Nutr Res* 2021; **10**:181-191.

42. Welz T, Childs K, Ibrahim F, Poulton M, Taylor CB, Moniz CF, et al. **Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase**. *Aids* 2010; **24**:1923-1928.

43. Shkoporov AN, Hill C. **Bacteriophages of the Human Gut: The "Known Unknown" of the Microbiome**. *Cell Host Microbe* 2019; **25**:195-209.

Figure 1. A) Bray Curtis distance at baseline, *i.e.*, inter-individual species diversity of the gut microbiota. PCo, principal component. B) Chao1, a measure of alpha-diversity indicating species richness; and Shannon index *i.e.,* species diversity at baseline. EFV, efavirenz ; PI, protease inhibitor; and HIV-neg, HIV-negative groups.

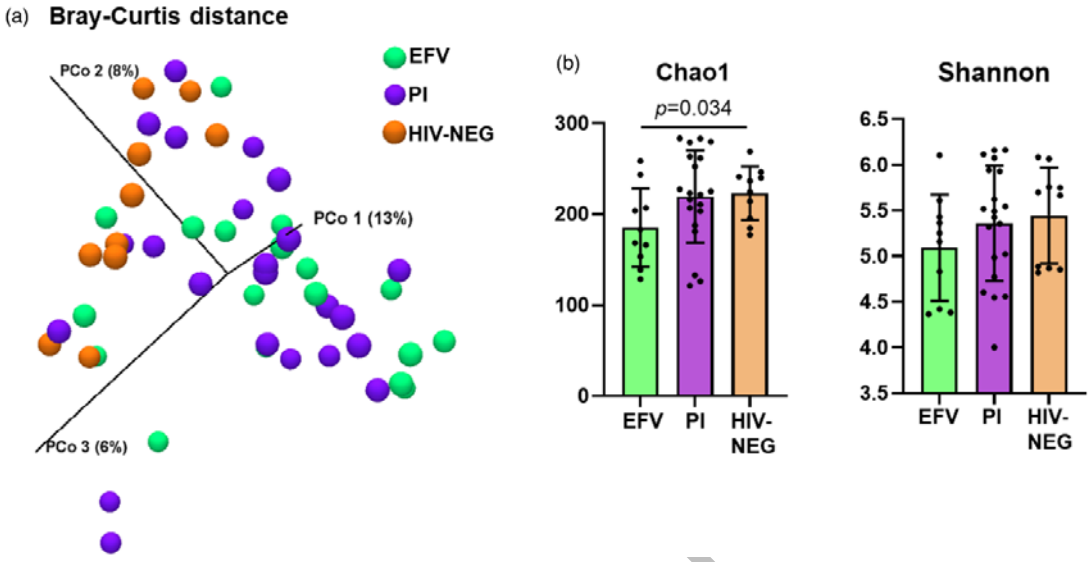

Figure 2. The gut microbiota alpha-diversity measures Chao1 and Shannon A) at week 24 (W 24) in efavirenz/protease inhibitor (EFV/PI) control (CTRL), raltegravir (RAL) and HIV-negative (HIV-neg) group. RAL-group comprise subjects who switched EFV or a PI to RAL; EFV/PI control group remained on unchanged EFV or a PI containing antiretroviral therapy (ART). B) at baseline (BL) and 24 weeks (W 24) after the switch. EFV RAL: subjects who switched from EFV to RAL; EFV CTRL: subjects who continued unchanged EFV-containing regimen C) at BL and 24 weeks (W 24) after the switch. PI RAL: subjects who switched from a PI to RAL; PI CTRL: subjects who continued unchanged PI-containing regimen

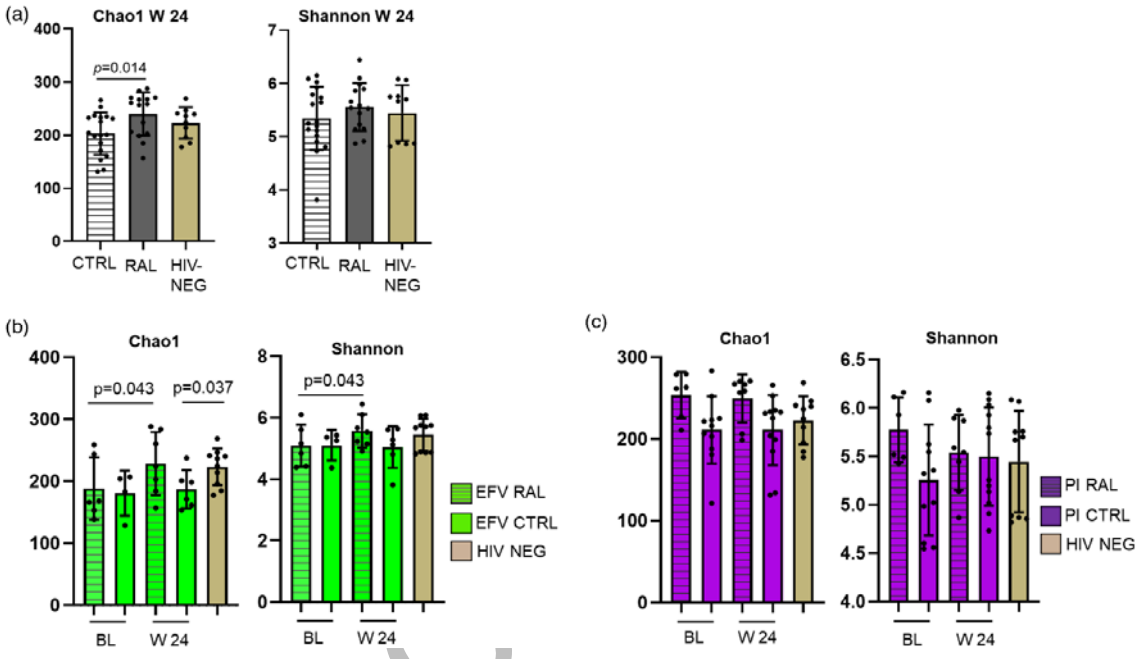

Figure 3. Microbial taxa, which abundance were different between the arm with no change in ART regimen (CTRL) and raltegravir (RAL) groups at week 24 (W 24). RAL group comprise subjects who switched efavirenz or a protease inhibitor to RAL; control group (CTRL) remained on unchanged EFV or a PI containing antiretroviral therapy.

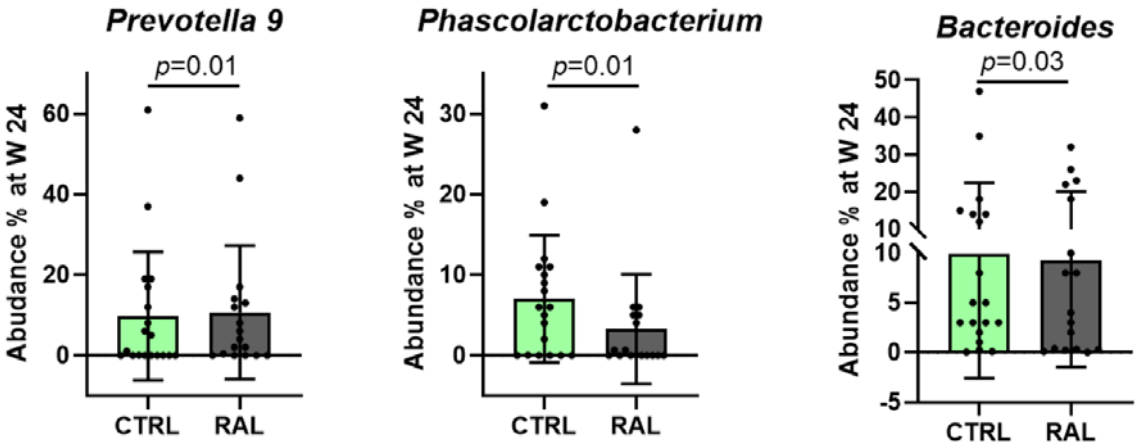

Table 1. Baseline characteristics of PLWH and HIV negative subjects, and the effects of switching EFV or a PI to RAL (RAL group) versus continuing unchanged EFV or PI containing regimen (EFV/PI group) among PLWH

	RAL group (n=19)			**EFV/PI group (n=22)**				**HIV-neg (n=10)**	
Variable	Baseline	24 weeks	*P*-value*	Baseline	24 weeks	*P*-value*	*P*-value##		*p*-value†
Age (years)	49 (43-55)			51 (43-63)				41 (38-42)	0.1
Male (*n*, %)	16 (84)			17 (77)				5 (50)	0.1
MSM (*n*, %)	12 (63)			9 (41)					0.2
CD4+ T-cell count (cells/μL)	685 (514-823)			803 (633-966)					0.2
$CD4^+$ nadir (cells/μL)	204 (71-291)			251 (103-300)					1.0
Time since HIV diagnosis (months)	132 (93-156)			160 (95-204)					0.3
Duration of ART (months)	111 (55-134)			113 (82-169)					0.4
TDF (*n*, %)	9 (47)			9 (41)					1.0
EFV (*n*, %)	8 (42)			9 (41)					1.0
PI (n, %)	11 (58)			13 (59)					1.0
DRV (n)	7			8					
ATV (n)	3			3					

fAPV (n)	1			1					
LPV (n)	0			1					
BMI (kg/m^2)	27.2 (24.2-30.7)	28.9 (25.9-31.1)	0.015	28.8 (27.0-31.9)	29.1 (27.0-32.0)	0.6	0.3	28.0 (26.7-34.7)	0.4
Diabetes (n, %)	0 (0)			1 (5)				1 (10)	0.5
NAFLD (n, %)	6 (32)			8 (38)				1 (10)	0.2
Dyslipidemia (n, %)	3 (16)			6 (29)				4 (40)	0.7
Hypertension (n, %)	7 (37)			9 (43)				3 (30)	0.7
S-I-FABP2 (ng/mL)	2.6 (1.5-3.8)	2.6 (1.6-3.9)	0.9	1.9 (1.4-4.0)	2.0 (1.5-4.5)	0.8	0.9		
S-LBP (mg/mL)	12.5 (10.0-16.7)	12.5 (9.7-16.3)	0.9	14.1 (10.5-15.6)	13.3 (10.6-16.4)	0.5	0.6		
S-D-25 (nmol/L)	45 (37-79)	56 (42-84)	0.2	59 (44-72)	64 (43-83)	0.4	0.7		
F-calpro (mg/g)	13 (6-28)	19 (9-46)	0.1	17 (11-29)	20 (11-35)	0.8	0.5		

PLWH, people living with HIV; RAL, raltegravir; EFV, efavirenz; PI, protease inhibitor; HIV-neg, HIV-negative; MSM, men who have sex with men; ART, antiretroviral therapy; TDF, tenofovir disoproxil fumarate; DRV, darunavir; ATV, atazanavir; fAPV, fosamprenavir; LPV, lopinavir; BMI, Body-mass-index; NAFLD, non-alcoholic fatty liver disease diagnosed by magnetic resonance spectroscopy; S-I-FABP2, serum intestinal fatty acid binding protein; S-LBP, serum lipopolysaccharide binding protein; S-D-25, serum D-vitamin; F-calpro, fecal calprotectin. Data are shown as median (IQR). *p-value for the change between baseline and 24 weeks within RAL and Control groups. ##p-value for the comparison of changes in RAL versus Control group. . †p-value for the comparison of baseline characteristics between RAL, Control and HIV neg groups (age, gender, BMI, diabetes, NAFLD, dyslipidemia, hypertension), or between RAL and Control groups (MSM, TDF, EFV, CD4+ lymphocyte count, CD4 nadir, time since diagnosis, duration of ART).

Table 2. Differences in the gut microbiota abundances between the study groups at baseline.

Comparison	Fold difference	p-value (FDR)
Efavirenz vs HIV negative		
Megasphera	89.23	0.00654
Acidaminococcus	62.51	0.00971
Acidaminococcus	83.17	0.02000
Fusobacterium	50.34	0.02000
Protease inhibitor vs HIV negative		
Phascolarctobacterium	130.71	0.00318
Protease inhibitor vs Efavirenz		
Phascolarctobacterium	155.56	0.000443
Phascolarctobacterium	33.14	0.02000
Fusobacterium	-55.63	0.000443
Fusobacterium	-13.01	0.03000
Fusobacterium	-11.04	0.05000
Acidaminococcus	-14.22	0.00138
Acidaminococcus	-12.34	0.04000
Acidaminococcus	-11.69	0.05000
Megasphera	-11.29	0.00937
Ruminococcaceae UCG-014	-16.38	0.01000
Ruminococcaceae UCG-014	-15.68	0.02000

FDR false discovery rate Benjamin-Hochberg for multiple comparison