

CHARACTERIZATION OF PERIPHERAL MICROBIAL PRODUCTS ASSOCIATED WITH HIV INFECTION AND CANCER

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Abstract

- Introduction:** People with HIV (PWH) experience persistent inflammation and have an elevated risk of cancer, even with successful antiretroviral therapy. Microbial dysbiosis can promote subsequent inflammation by upregulating TGF- β and the translocation of microbial products into the bloodstream, thus increasing cancer risk. This study aimed to quantify levels of TGF- β and microbial products (lipopolysaccharide [LPS], and short-chain fatty acids [SCFA]) in blood plasma of PWH and without HIV (PWOH) to determine the relationship of all these factors in Puerto Ricans PWH.
- Methods:** Blood samples, sociodemographic, and clinical data from 80 adults (50 PWH and 30 PWOH) were collected. PWH were virally suppressed with a median CD4 count of 694 cells/ μ L. We measured TGF- β , LPS, and soluble CD14 (sCD14) using immunoassays. SCFA (acetate, butyrate, and propionate) were measured using GC-MS. Differences in the median levels of all variables were evaluated using Mann-Whitney test. Association of inflammation with peripheral microbial products was assessed by univariate and multivariate fixed-effects regression analyses. Statistical analyses were performed in R-statistical software.
- Results:** PWH had significantly higher levels of TGF- β ($p=0.017$), higher levels of LPS ($p<0.001$), higher levels of sCD14 ($p=0.007$), and significantly lower levels of butyrate ($p<0.001$). Higher levels of TGF- β were associated with higher levels of LPS ($\rho=0.38$, $p=0.017$) and higher levels of sCD14 ($\rho=0.27$, $p=0.016$) these relationships remain significant after adjusting for HIV status ($p<0.001$).
- Conclusion:** Our findings suggest that LPS in blood plasma may contribute to cancer risk in Puerto Rican PWH and may represent potential novel biomarkers for cancer prevention.

Introduction

- People with HIV (PWH) are at greater risk of cancer compared to people without HIV (PWOH), even with antiviral therapy, suggesting that there are other factors that contribute to cancer risks. (Grulich AE, *et al.* 2007)
- Puerto Rico (PR) is among the top 10 in terms of HIV prevalence and cumulative AIDS cases among the US. (Lechner, M., *et al.* 2022)
- Oral microbial dysbiosis can contribute to persistent inflammation, which can result in disruption of the epithelial barrier and translocation of microbial products such as lipopolysaccharides (LPS) and other oncometabolites into the bloodstream. (Bao, H.-D., *et al.* 2018).
- Microbial products can travel systemically to distant sites and facilitate oncogenesis, by upregulation of TGF- β expression, which is associated with microbial induced cancers. (Bao, H.-D., *et al.* 2018)

Methods

Figure 1 - Methods flowchart

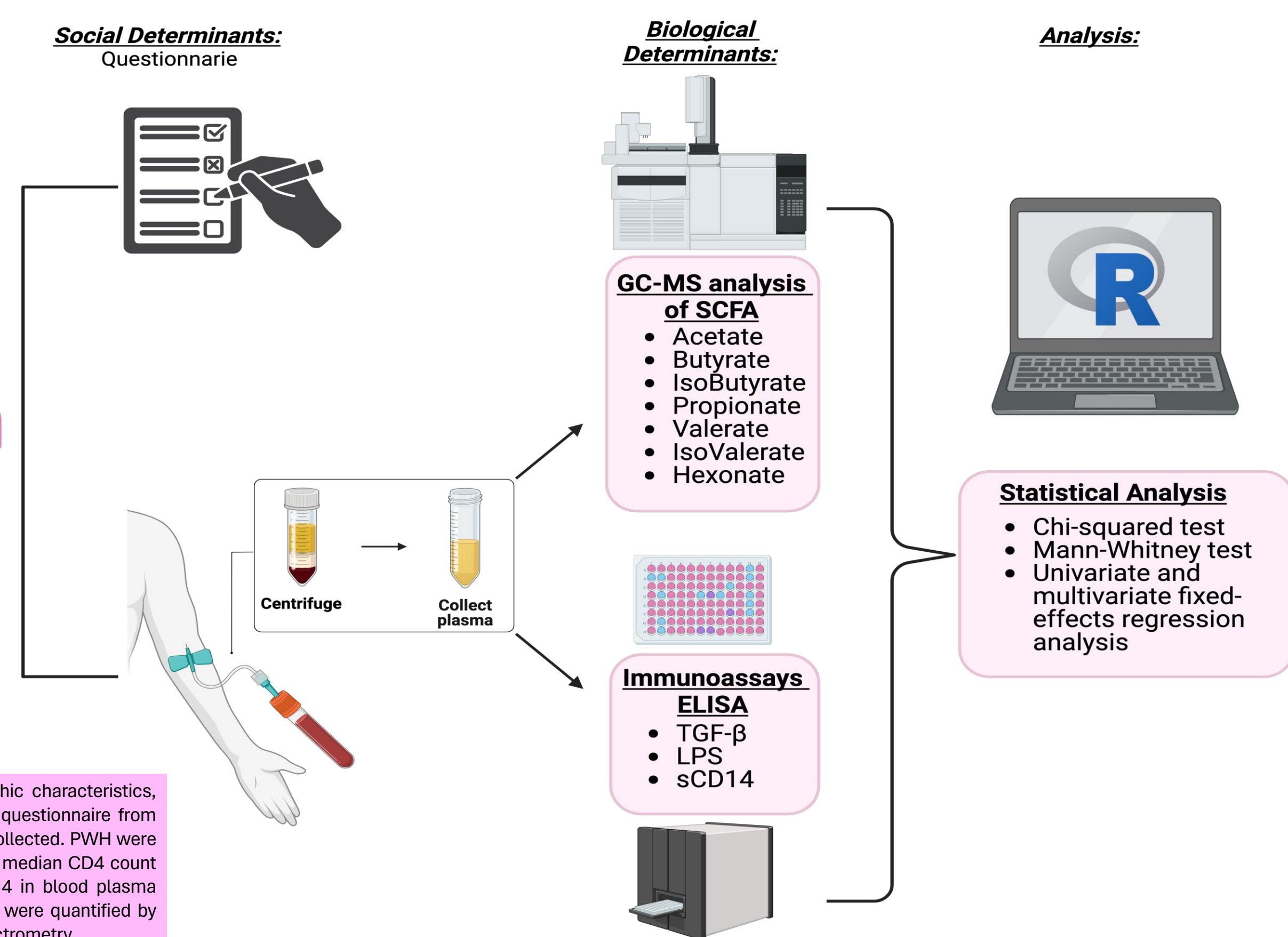


Figure 1: Blood samples, sociodemographic characteristics, lifestyle variables were collected through questionnaire from 80 adults (50 PWH and 30 PWOH) were collected. PWH were virally suppressed (<50 copies/mL) with a median CD4 count of 694.0 cells/ μ L. TGF- β , LPS, and sCD14 in blood plasma were measured by ELISA. Levels of SCFA were quantified by gas chromatography followed by mass spectrometry.

Results

Table 1 – Sociodemographic Characteristics

Variables	Total N	PWH n=50	PWOH n=30	p-value*
Biological Sex				<< 0.001
Male	43	36	7	
Female	37	14	23	
Age (years)				<< 0.001
Average \pm sd	44 \pm 13	47 \pm 11	37 \pm 13	
CD4 Absolute Count				
Average \pm sd		694 \pm 310		
Income Level				0.54
<\$20,000	26	18	8	
>\$20,000	37	23	14	
Marital Status				0.38
Single	66	45	21	
Married	12	4	8	

Table 1: Demographic and clinical characteristics of study participants: PWH were mostly male and significantly older than PWOH. There were no association between HIV status and income level nor marital status. *Represents the p-value of a chi-squared test or Mann-Whitney.

Figure 2 – TGF- β Levels in blood plasma associated HIV Status

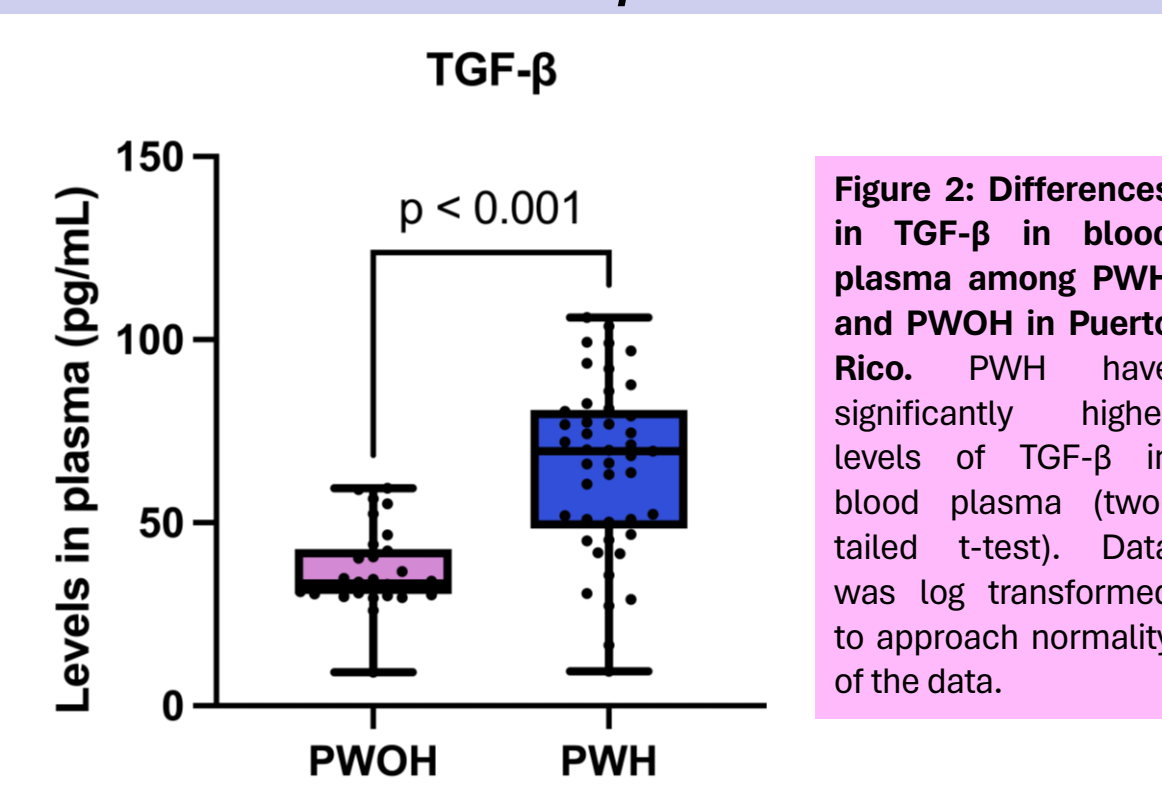


Figure 2: Differences in TGF- β in blood plasma among PWH and PWOH in Puerto Rico. PWH have significantly higher levels of TGF- β in blood plasma (two-tailed t-test). Data was log transformed to approach normality of the data.

Figure 3 – LPS and sCD14 Levels in blood plasma associated HIV Status

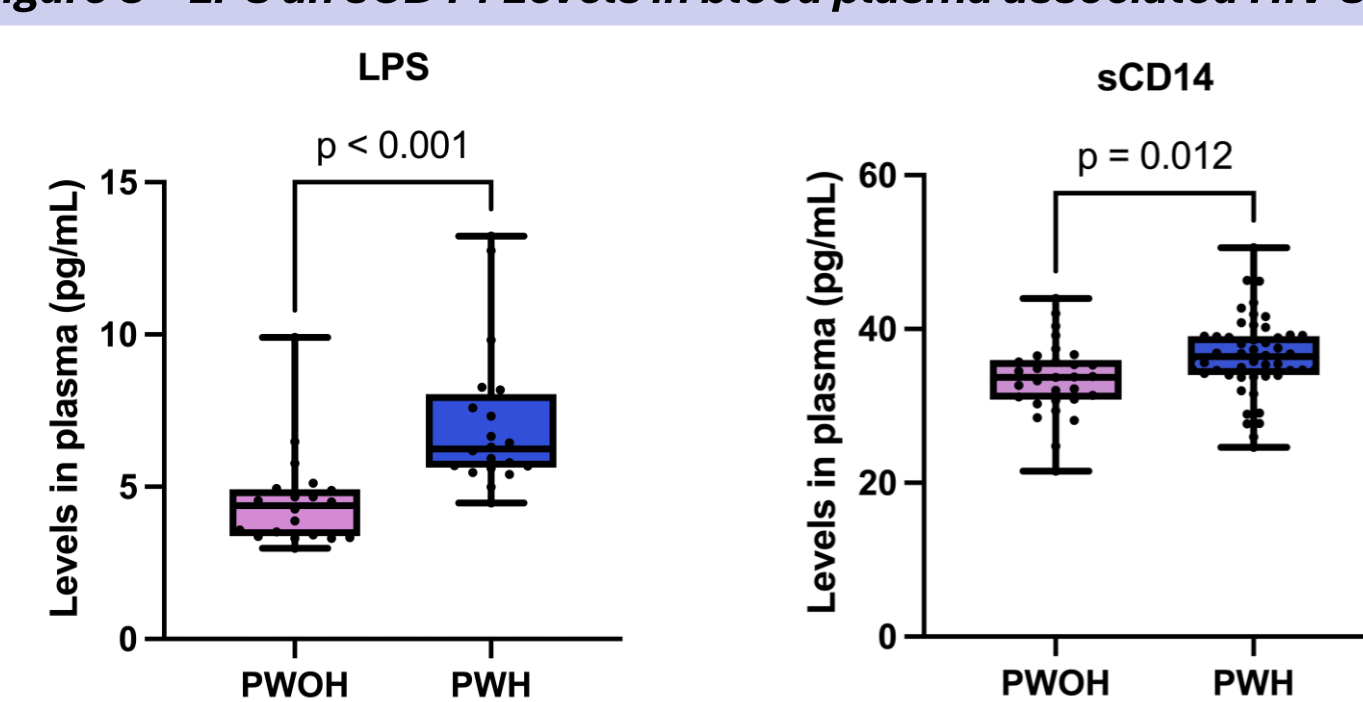


Figure 3: Differences in marker of microbial translocation in blood plasma among PWH and PWOH in Puerto Rico. PWH have statistical higher levels of (a) LPS and (b) sCD14 in blood plasma (two-tailed t-test). Data was square root and log transformed for LPS and sCD14, respectively to approach normality of the data.

Table 2 – Correlations of TGF- β with markers of microbial translocation and SCFA

Variables	Rho	p-value*
Markers of Microbial Translocation		
sCD14	0.28	0.015
LPS	0.38	0.017*
Microbial Products		
SCFA's		
Butyrate	-0.16	0.176
Iso-butyrate	-0.23	0.046
Acetate	-0.15	0.202
Propionate	-0.13	0.273
Valerate	0.15	0.195
Iso-valerate	0.06	0.635
Hexanoate	-0.04	0.709

Table 2: Correlation TGF- β with Inflammatory markers and Microbial products. Higher levels of TGF- β were associated with higher levels of LPS and higher levels of sCD14, also were associated with lower levels of iso-butyrate. *This relationship remained significant after adjusting for HIV status, biological sex and age.

Figure 4 – plasma associated HIV Status– Levels of SCFA in blood

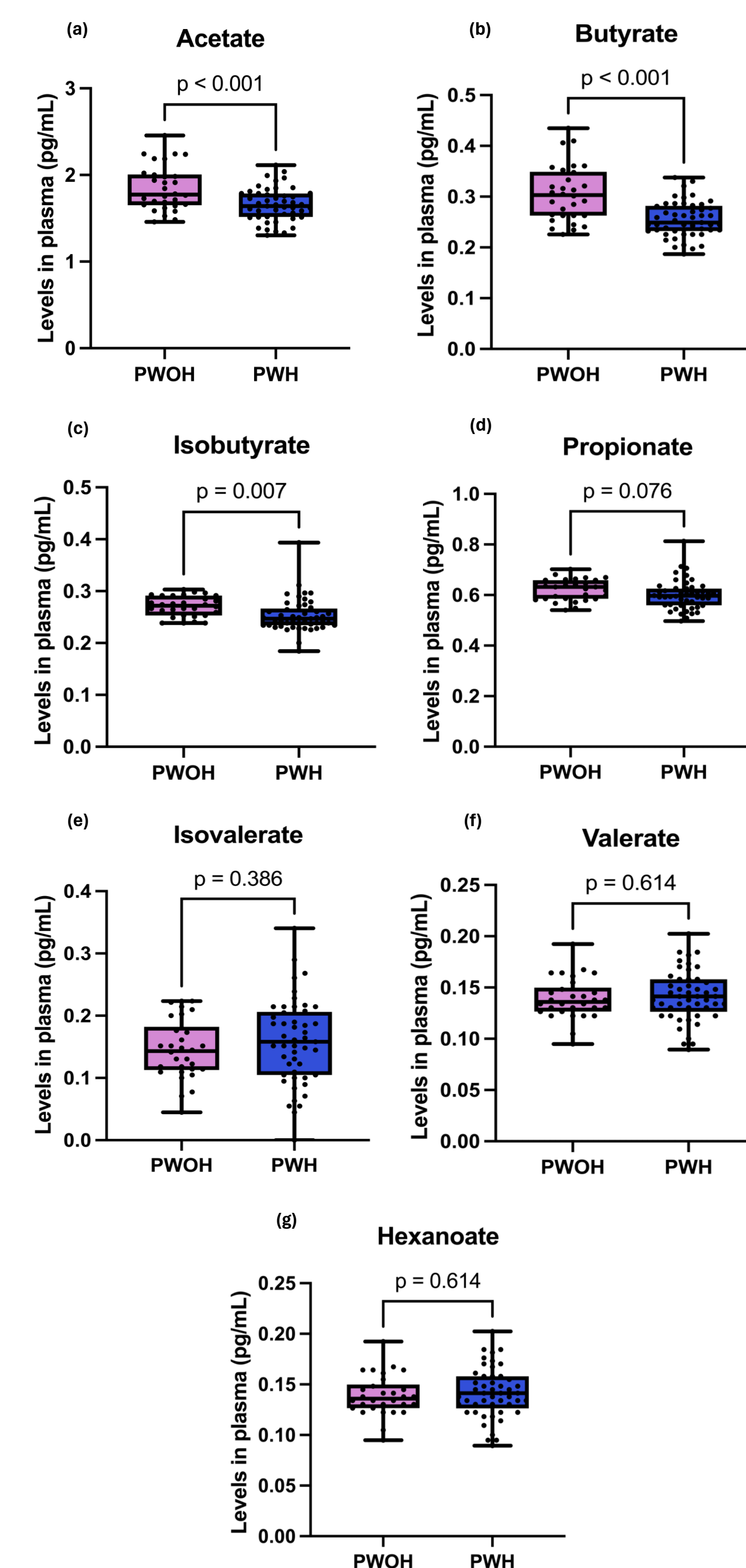


Figure 4: Differences in the levels of SCFA in blood plasma among PWH and PWOH in Puerto Rico. PWH had significantly lower levels of (a) acetate, (b) butyrate, and (c) iso-butyrate levels in blood plasma. There were no significant differences between study groups in the levels of (d) propionate, (e) iso-valerate, (f) valerate, and (g) hexanoate (two-tailed t-test). Data was square root transformed to approach normality of the data.

Conclusion

- PWH had more systemic inflammation and more microbial translocation.
- The increased levels of LPS can contribute to chronic inflammation and failure of immune reconstitution in PWH. (Luo, Z., *et al.* 2022)
- Higher levels of TGF- β has been related to HIV disease progression and is a marker of microbial induced oncogenesis. (Lutz, M., *et al.* 1993)
- In addition, lower levels of SCFA may reflect gut microbial dysbiosis, poor gut integrity, and impaired SCFA production by the gut microbiome.
- Our findings suggest that LPS in blood plasma may contribute to higher cancer risk in Puerto Rican PWH and may represent a potential novel biomarker for cancer prevention.

Acknowledgements

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Future Work

- Quantify other markers of inflammation: IL-6, IL-8, IL-1 β , IL-2, TNF- α , and IFN- γ .
- T-cells and B-cells immunophenotyping for subsets of cells, proliferation, activation, and exhaustion.
- Quantity markers of gut integrity in blood plasma: FABP, Citrulline, and Claudin.