6-30-2021

The Effect of the TyG Index on Liver Steatosis, Immune Activation, Oxidative Stress, Liver Fibrosis Pathways and Liver Fibrosis in the Miami Adult Studies on HIV (MASH) Cohort

Colby S. Teeman
Florida International University, cteem001@fiu.edu

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

THE EFFECT OF THE TYG INDEX ON LIVER STEATOSIS, IMMUNE ACTIVATION, OXIDATIVE STRESS, LIVER FIBROSIS PATHWAYS AND LIVER FIBROSIS IN THE MIAMI ADULT STUDIES ON HIV (MASH) COHORT

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

DIETETICS AND NUTRITION

by

Colby S. Teeman

2021

To: Dean Tomás R. Guilarte
R.Stempel College of Public Health and Social Work

This dissertation, written by Colby S. Teeman, and entitled The Effect of the TyG Index on Liver Steatosis, Immune Activation, Oxidative Stress, Liver Fibrosis Pathways and Liver Fibrosis in the Miami Adult Studies on HIV (MASH) Cohort, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

_____
Tan Li

_____
Evelyn Enrione

_____
Adriana Campa

_____
Marianna Baum, Major Professor

Date of Defense: June 30, 2021

The dissertation of Colby S. Teeman is approved.

_____
Dean Tomás R. Guilarte
R.Stempel College of Public Health and Social Work

_____
Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2021

DEDICATION

This dissertation is dedicated to people all around the world living with the HIV virus. The social stigma, lifestyle challenges, and physical complications these individuals overcome on a daily basis is inspiring. Furthermore, I dedicate this dissertation to all of those who I have kept in my inner circle throughout all of these years; Mom, Dad, Jayce, Tenaya, Nicholas, Ashli, Jon, Nathan, Sean, Alex, Dee and Santi, having this support system around me over all of these years has made this possible. Lastly, I dedicate this dissertation to my fiancé Nicole, you continue to challenge me, inspire me, and believe in me no matter how many times I come up short. I love you with all my heart.

ACKNOWLEDGMENTS

Five years ago, I came to Miami after living in small Midwest towns my entire life. The entire city, the speed of driving, the language, the culture, and the university all represented great change for me. I came to the department of Dietetics and Nutrition with a very brief publication record that gave me a false sense of confidence among my peers within the department. After choosing to join Dr. Baum's laboratory and deciding study HIV for the duration of my PhD I soon realized how misguided my confidence was. Countless individuals have contributed both directly and indirectly to making this work possible.

First and foremost, I would like to thank my parents Johnny and Teresa Teeman, throughout my now 11-year academic journey across three different states you have been there every step of the way. Moving in and out of apartments, supportive phone calls, long weekend visits, and financial support, whatever I needed at the time you never hesitated to provide the exact means of support that I needed.

My committee members Dr. Campa, Dr. Enrione, and Dr. Li, each of you has helped mold this work and provided invaluable guidance towards my professional development as a scientist. Dr. Campa, your experience working in the field of HIV provided tremendous insight into study design and procedures. Dr. Enrione, by asking me to re-visit many sections of my dissertation you forced me to dig so much deeper into the background literature than I previously viewed as acceptable. Dr. Li, you have remained patient with me as I have navigated my way through the world of statistics even as I continued to want the easy and

quick answer you always forced me to think critically and have a complete understanding of every statistical concept I used.

Thank you to my fiancé Nicole, as our relationship has grown alongside both of our academic careers you have set the daily example of how to study, focus, and take great pride in the academic work we produce. Our daily question and answer sessions, the countless questions that I never had an answer for but you forced me to find one. The dream that we share for our future life together provides the daily motivation I need to complete this work.

To the guy who has helped maintain my sanity throughout this entire process my good friend Alex Tur. All of the early morning gym sessions, Friday beers, and weekend cookouts have helped me maintain a work-life balance that I desperately needed on many occasions over the past 4+ years.

Thank you to each of the laboratory mentors I have had throughout this dissertation process, Dr. Mukesh Mudgal, Dr. Yongjun Huang, and Leo Acuna. When I started working in Dr. Baum's laboratory, I had zero experience working in a wet lab and each of you have played an integral role in helping develop my lab skills from a true beginner to someone who felt comfortable with every single laboratory technique we can perform in the lab.

Thank you to my closest peer mentors Dr. Jacky Hernandez and Dr. Javier Tamargo. We have advanced through this program together and faced many of the same challenges. It has been a true pleasure to collaborate with each of you on this project and learn how to be a researcher alongside you. Watching each of you complete your doctoral work before me has provided me

with a road map for success and tremendous mentorship and for that I am very grateful.

Lastly, thank you to Dr. Baum for providing me the opportunity to make all this work happen. You have consistently challenged me, taught me, and guided me as a researcher all at the same time. You have always been there to push me harder when I need to get work done, guide me as a writer, and direct my research for the betterment of myself and the group as a whole.

ABSTRACT OF THE DISSERTATION

THE EFFECT OF THE TYG INDEX ON LIVER STEATOSIS, IMMUNE ACTIVATION, OXIDATIVE STRESS, LIVER FIBROSIS PATHWAYS AND LIVER FIBROSIS IN THE MIAMI ADULT STUDIES ON HIV (MASH) COHORT

by

Colby S. Teeman

Florida International University, 2021

Miami, Florida

Professor Marianna Baum, Major Professor

The purpose of this study was to establish the Triglyceride-Glucose (TyG) Index Ln (fasting TG x fasting glucose/2) as a predictor of liver steatosis in People Living with HIV (PLWH) and determine the effect of increased TyG Index on biomarkers of immune activation, inflammation, oxidative stress, apoptosis, and liver fibrosis. Four-hundred and eighty participants were selected from the Miami Adult Studies on HIV (MASH) cohort, two-hundred and eleven were PLWH, and two-hundred and sixty-nine were uninfected controls. Biomarkers were analyzed from blood samples collected at the FIU Borinquen Clinic. Primary research outcomes were analyzed using multiple linear and logistic regression, pairwise analyses, and ROC curves.

The TyG Index was determined to be a good predictor of liver steatosis among PLWH and uninfected controls (AUC=0.738 and AUC=0.702), respectively. Participants in the High TyG Risk category were 4.638 times more likely to have liver steatosis than those in the Low TyG Risk category [95% CI:(2.075, 10.368)]. Greater TyG Index was associated with higher immune

activation markers Ln sCD14 (β=0.080, P=0.050) and Ln sCD163 (β=0.164, P=0.008). Linear regression analysis found HIV infection to be associated with higher levels sCD27 (β=0.181, P=0.005), and liver fibrosis pathway biomarkers Ln TGF- β (β=0.915, P<0.001) and Ln TIMP-1 (β = 0.118, P=0.034). Dietary saturated fat intake was associated with increased hepatic apoptosis (β = 3.26, P=0.050). Linear regression analysis indicated HIV infection was associated with decreased Free GSH (β = -95.24, P=0.003) and decreased Total GSH (β= -93.60, P=0.003), indicating higher oxidative stress. Logistic regression analysis adjusted for Age, Sex, BMI, HIV Infection, and Cocaine Use, showed greater TyG Index was associated with greater likelihood of liver fibrosis in PLWH only [OR= 1.783 (1.114, 2.855), P=0.016] and all MASH Cohort participants [OR=1.244 (0.914, 1.692), P=0.046].

These data indicate a consistent relationship between increased TyG Index and biological pathways that lead to liver fibrosis. As liver disease becomes a more prominent concern among PLWH, it is crucial for health care professionals to address markers of metabolic health, such as the TyG Index, as a means to effectively manage liver steatosis and avoid the development of liver fibrosis.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

CHAPTER V

LIST OF FIGURES

FIGURE PAGE

ABBREVIATIONS AND ACRONYMS

8-oxodG 7,8-dihydroxy-8-oxo-2’-deoxyguanosine

ACC2 acetyl-CoA carboxylase-2

ALD Alcoholic liver disease

ANCOVA Analysis of covariance

ART Antiretroviral Therapy

ATP Adenosine triphosphate

AUC Area under the ROC Curve

AUDIT Alcohol Use Disorders Identification Test

BID BH3-interacting domain

BMI Body mass index

CCR5 C-C chemokine receptor type five

CD4+ T cells Cluster Differentiation T helper cells

ChREBP carbohydrate responsive element-binding protein

CPT1 Carnitine palmityltransferase-1

CTGF Connective tissue growth factor

CXCR4 C-X-C Motif Chemokine Receptor 4

CYP2E1 Cytochrome P450 Family 2 Subfamily E Member 1

DAMP Danger-associated molecular pattern

DBP Diastolic blood pressure

DISC Death inducing signaling complex

DNA Deoxyribonucleic acid

DNL De novo lipogenesis

ECM Extracellular matrix

ER Endoplasmic reticulum

FFA Free fatty acid

GK Glycogen kinase

GPx Glutathione peroxidase

GR Glutathione reductase

GSH Glutathione

GSS Glutathione synthase

GSSG Oxidized glutathione

HBV Hepatitis B virus

HDL High density lipoprotein

HIV Human Immunodeficiency Virus

HNE 4-hydroxy-2-nonenal

HOMA-IR Homeostatic Model Assessment of Insulin Resistance

HSC Hepatic Stellate Cell

HSL Hormone sensitive lipase

IL-6 Interleukin 6

IR Insulin resistance

JNK Jun N-terminal kinase

LPS Lipopolysaccharide

LXR liver X receptor

MASH Miami Adult Studies in HIV

MCP monocyte chemoattractant protein

MDA malondialdehyde

MMP Matrix metalloproteinase

MRE Magnetic Resonance Elastography

NAFLD Non-alcoholic fatty liver disease

NASH Non-alcoholic steatohepatitis

NCEP-ATP National Cholesterol Education Program Adult Treatment Panel

NRTI Nucleoside reverse transcriptase inhibitor

OR Odds Ratio

OS Oxidative stress

PI Protease inhibitor

PLWH People Living with HIV

PDFF Protein Density Fat Fraction

PUFA Polyunsaturated fatty acid

ROC Receiver operator characteristic

ROS Reactive oxygen species

SBP Systolic blood pressure

SCFA Short chain fatty acid

SREBP1-c Sterol regulatory element-binding protein-1c

SUD Substance use disorder

T2DM Type two diabetes

TG Triglycerides

TGF-β Transforming growth factor beta

TIMP tissue inhibitor of metalloproteinase

TLR Toll-like receptor

TNF-α Tumor necrosis factor alpha

TyG Triglyceride-glucose

VL Viral load

VLDL Very low-density lipoprotein

WC Waist circumference

THE EFFECT OF THE TYG INDEX ON LIVER STEATOSIS, IMMUNE ACTIVATION, OXIDATIVE STRESS, LIVER FIBROSIS PATHWAYS AND LIVER FIBROSIS IN THE MIAMI ADULT STUDIES ON HIV (MASH) COHORT

Chapter I: INTRODUCTION

STATEMENT OF PROBLEM

There are greater than one-million people living in the United States with HIV infection and nearly forty-million around the world.[1] In recent decades, antiretroviral therapy (ART) has increased the rate of controlled HIV viral load (VL) and helped people living with HIV (PLWH) maintain higher $CD4^+$ cell counts, thus resulting in increased life expectancy of PLWH.[2] Lower HIV VL and higher $CD4^+$ cell counts have resulted in a decrease in AIDS related events.[3] Reduced AIDS mortality and chronic exposure to ART have led to an increase in chronic diseases commonly seen in the general population; including cardiovascular disease, diabetes, hyperlipidemia, hypertension, and obesity.[4] Of particular interest to this work is the relationship between HIV and liver disease, PLWH are 3.7x more likely to die of liver disease than the general population.[5] Non-alcoholic fatty liver disease (NAFLD), also known as liver steatosis, is the most common liver disease in the world.[6] Between 5-10% of individuals with NAFLD will develop non-alcoholic steatohepatitis (NASH) and 38% of these individuals will develop liver fibrosis.[7] The prevalence of NAFLD in PLWH has been reported at 35% around the world,[8] compared to 25% in the general population.[9]

Insulin resistance (IR) has been previously shown to increase the risk of NAFLD and liver fibrosis.[10,11] IR increases hepatic de novo lipogenesis[12] and adipose tissue dysfunction, promoting the release of pro-inflammatory adipokines and cytokines,[13] and leading to chronic hepatic inflammation. IR is likely related to immune activation, which remains increased in PLWH even after successful

ART.[14] It has been previously shown that PLWH with NAFLD have nearly twice the likelihood of developing NASH compared to uninfected individuals.[15]

Among PLWH, ART use has been hypothesized as a major factor in the development of NAFLD, possibly related to increased IR. Nucleoside reverse transcriptase inhibitors (NRTIs) have traditionally been considered the most harmful classification of ART drugs related the development of NAFLD, likely by increasing IR and dyslipidemia.[16-18] Older NRTIs may decrease lipid oxidative phosphorylation, increase reactive oxygen species (ROS) and lead to an accumulation of lipids in the liver.[19] Newer NRTIs have been shown to have more favorable impacts on metabolic markers.[20-21] Protease inhibitors (PIs) also appear to be detrimental to metabolic health by increasing hyperglycemia, hyperinsulinemia and impaired secretion of insulin from beta cells.[22-23]

Increased microbial translocation may also contribute to NAFLD development and related inflammatory processes. Visceral adipose tissue (VAT) and Kupffer cells in the liver respond to activated Toll Like Receptor-4 (TLR-4) and increase transcription of TNF-α and IL-6, which can both contribute to IR.[24,25] In PLWH, individuals who start ART as early as the first 2-3 weeks after HIV infection continue to have increased levels of plasma markers of microbial translocation.[26] Microbial translocation promotes liver fibrosis through activation of Kupffer cells and Hepatic Stellate Cells (HSCs).[27] Activated HSCs are associated with higher levels of tissue inhibitor of metalloproteinases (TIMPs). Increased TIMP promotes liver fibrosis by inhibiting the breakdown of the extracellular matrix (ECM).[28,29]

The progression from NAFLD to NASH is likely facilitated by HSC activation and proliferation. Increased insulin and glucose, hallmarks of NAFLD increase HSC activation and connective tissue growth factor (CTGF), both promoters of liver fibrosis.[30] The activation of HSCs is also increased in settings of liver apoptosis and elevated ROS.[31] Increased ROS damages mitochondrial membranes and ultimately leads diminished mitochondrial function[32,33] and increased hepatic apoptosis.[34,35] Furthermore, individuals with NASH have reduced antioxidant capacity which corresponds to NASH severity.[36,37] Apoptosis related to NASH can be initiated FFA accumulation,[38,39] and exposure to inflammatory cytokines.[40,41] Viral HIV envelope proteins contribute to hepatocyte apoptosis both directly and through inflammatory pathways.[42,43]

Significance of the Study

Due to the high level of liver disease mortality among PLWH, it is imperative to understand the interaction between multiple physiological and lifestyle factors that may lead to liver disease in this population. Poor metabolic health has been previously associated with greater liver fibrosis development in PLWH[11]; however, to the best of our knowledge there are no studies that have looked directly at the effect of insulin resistance and liver steatosis on immune activation, liver fibrosis pathways and the likelihood of liver fibrosis in PLWH. This work fills in important gap in the current research literature and builds upon previous findings from the MASH Cohort. Previously published literature from the MASH Cohort has focused on the effect of anti-oxidant nutrients,[44] and different aspects of social and lifestyle including alcohol abuse, cocaine abuse, and food

insecurity.[45-47] As advances in ART continue to increase the quality of life in PLWH it is vital to focus on the role of metabolic health as a determinant of liver disease outcomes in the MASH Cohort which is now composed of individuals with high rates of ART adherence and controlled HIV Viral Load.

Innovation

The TyG Index is a marker of insulin resistance,[48] that has been shown to be associated with steatosis.[49] It is calculated from the following equation.

$$Ln\left[\frac{fasting\ TG\ \left(\frac{mg}{dL}\right) * fasting\ glucose\ \left(\frac{mg}{dL}\right)}{2}\right]$$

Unfortunately, the gold standard for detecting steatosis is liver biopsy or MRI-PDFF scans, both of which can be invasive, expensive, and not for practical use among larger populations. The TyG Index only requires values from a much easier to obtain blood draw that occurs at routine health screenings. Analyses throughout this work show similar findings between TyG Index and liver steatosis for biomarker and liver disease outcomes, reflecting the possibly of using the TyG Index in PLWH as a proxy for steatosis risk. Furthermore, these analyses were performed in a manner to detect possible cumulative effects from the HIV virus and steatosis risk, while controlling for cocaine use.

AIM FOR CHAPTER III: THE TRIGLYCERIDE-GLUCOSE (TYG) INDEX AS A PREDICTOR OF LIVER STEATOSIS IN THE MIAMI ADULT STUDES ON HIV (MASH) COHORT

Specific Aim 1: Determine if the TyG Index is a predictor of NAFLD in the MASH Cohort.

Hypothesis 1a: The TyG Index will be a predictor of liver steatosis in both uninfected controls and PLWH when controlling for Age, Sex, BMI, Cocaine Use, and HIV Status.

Hypothesis 1b: The TyG Index will be a better predictor of liver steatosis in PLWH compared to uninfected controls.

AIM FOR CHAPTER IV: THE TYG INDEX AND LIVER STEATOSIS ARE ASSOCIATED WITH GREATER LEVELS OF IMMUNE ACTIVATION AND LIVER FIBROSIS BIOMARKERS IN THE MIAMI ADULT STUDIES ON HIV (MASH) COHORT

Specific Aim 2: Determine if the TyG Index is an independent predictor of biomarkers of immune activation and liver fibrosis.

Hypothesis 2a: Higher TyG Index values will be associated with increased levels of immune activation and liver fibrosis.

Hypothesis 2b: Cocaine use and HIV infection will be significant predictors of immune activation, and liver fibrosis.

Specific Aim 3: Determine if there is a cumulative effect between HIV infection and liver steatosis on biomarkers of immune activation and liver fibrosis.

Hypothesis 3a: Study groups with HIV infection and liver steatosis will have higher mean levels of immune activation and liver inflammation biomarkers compared to uninfected controls and participants without liver steatosis.

AIM FOR CHAPTER V: THE TYG INDEX IS ASSOCIATED WITH GREATER LIVER STIFFNESS AND LIVER FIBROSIS IN PEOPLE LIVING WITH HIV (PLWH)

Specific Aim 4: Determine if there is an association between the TyG Index and oxidative stress, apoptosis and liver fibrosis in the MASH Cohort.

Hypotheses 4a: Higher TyG Index values will be significantly associated with increased oxidative stress, apoptosis, and liver fibrosis.

Hypothesis 4b: Cocaine use and HIV infection will be significant predictors of oxidative stress, apoptosis, and liver fibrosis.

Specific Aim 5: Determine if there is a cumulative effect between HIV infection and liver steatosis on mean levels of oxidative stress, apoptosis, and liver stiffness.

Hypothesis 5a: Study groups with HIV infection and liver steatosis will have higher mean levels of oxidative stress, apoptosis, and liver stiffness compared to uninfected controls and participants without liver steatosis.

Sample Size

This study selected 480 participants (211 PLWH, 269 uninfected controls) from the Miami Adult Studies in HIV (MASH) Cohort who were recruited to the FIU-Borinquen Research Clinic for participation in the parent study.

STATISTICAL ANALYSIS

Table 1. Summary of the primary hypothesis, independent variables, dependent variables and statistical tests performed for each chapter.

Hypotheses	Primary Independent Variables	Primary Dependent Variables	Statistical Analyses
Chapter III Hypothesis 1a	TyG Index	Liver Steatosis	T-Test, Chi-Square, Multiple Logistic Regression, ROC Curve, Youden Index
Chapter III Hypothesis 1b	TyG Index	Liver Steatosis	T-Test, DeLong ROC Comparisons Test
Chapter IV Hypothesis 2a	TyG Index	sCD14, sCD27, sCD163, TGF-β, TIMP-1	T-Test, Pearson Correlation, Multiple Linear Regression
Chapter IV Hypothesis 2b	Cocaine Use, HIV Infection	sCD14, sCD27, sCD163, TGF-β, TIMP-1	Pearson Correlation, Multiple Linear Regression
Chapter IV Hypothesis 3a	HIV Infection, Liver Steatosis	sCD27, sCD163, TGF-β, TIMP-1	Pairwise Comparisons, One-Way ANOVA
Chapter V Hypothesis 4a	TyG Index	Free GSH, Apoptosis, Liver Fibrosis	T-Test, Multiple Linear Regression
Chapter V Hypothesis 4b	Cocaine Use, HIV Infection	Free GSH, Apoptosis, Liver Fibrosis	Pearson Correlation, Multiple Linear Regression
Chapter V Hypothesis 5a	HIV Infection, Liver Steatosis	Free GSH, Apoptosis, Liver Fibrosis	Pairwise Comparisons, One-Way ANOVA

All Multiple linear regression analysis with TyG Index as the primary independent variable will control for Age, Sex, BMI, Cocaine Use, and HIV status/ HIV VL.

References

1. UN Joint Programme on HIV/AIDS. 15 Years, 15 Lesson of hope from the AIDS response.

2. Bhaskaran K, Hamouda O, Sannes M, et al. Changes in the Risk of Death After HIV Seroconversion Compared With Mortality in the General Population. JAMA. 2008;300(1):51-59. Doi: 10.1001/jama.300.1.51

3. Adih WK, Selik RM, Hall HI et al.. Associations and Trends in Cause-Specific Rates of Death Among Persons Reported with HIV Infection, 23 U.S. Jurisdictions, Through 2011. Open AIDS J. 2016. doi:10.2174/1874613601610010144

4. Weber R, Sabin CA, Friis-Moller N, et al. Liver-Related Deaths in Persons Infected With the Human Immunodeficiency Virus: The D:A:D Study. Arch Intern Med. 2006;166:1632-1641. doi:10.1001/archinte.166.15.1632

5. Croxford S, Kitching A, Desai S, et al. Mortality and causes of death in people diagnosed with HIV in the era of highly active antiretroviral therapy compared with the general population: an analysis of a national observational cohort. Lancet Public Health. 2017;2(1):e35-e46. doi:10.1016/S2468-2667(16)30020-2

6. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: A review of available epidemiological data. J Hepatol. 2013;58(3):593-608. doi:10.1016/j.jhep.2012.12.005

7. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038-1048. doi:10.1016/j.metabol.2015.12.012

8. Maurice JB, Patel A, Scott AJ, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. AIDS. 2017;31(11):1621-1632. doi:10.1097/QAD.0000000000001504

9. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. doi:10.1002/hep.28431

10. Blanco F, Barreiro P, Ryan P, et al. Risk factors for advanced liver fibrosis in HIV-infected individuals: Role of antiretroviral drugs and insulin resistance. J Viral Hepat. 2011;18(1):11-16. doi:10.1111/j.1365-2893.2009.01261.x

11. Lemoine M, Lacombe K, Bastard JP, et al. Metabolic syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected patients. AIDS. 2017;31(14):1955-1964. doi:10.1097/QAD.0000000000001587

12. Bugianesi E, Moscatiello S, Ciaravella MF, Marchesini G. Insulin Resistance in Nonalcoholic Fatty Liver Disease. Curr Pharm Des. 2010;16(17):1941-1951. doi:10.2174/138161210791208875

13. Guilherme A, Virbasius J V., Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(5):367-377. doi:10.1038/nrm2391

14. Williams JC, Zhang X, Karki M, et al. Soluble CD14, CD163, and CD27 biomarkers distinguish ART-suppressed youth living with HIV from healthy controls. J Leukoc Biol. 2018;103(4):671-680. doi:10.1002/JLB.3A0717-294RR

15. Vodkin I, Valasek MA, Bettencourt R, et al. Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: A case-control study. Aliment Pharmacol Ther. 2015;41(4):368-378. doi:10.1111/apt.13052

16. Brown TT, Li X, Cole SR, et al. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. AIDS. 2005;19(13):1375-1383. doi:10.1097/01.aids.0000181011.62385.91

17. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety otenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: A 3-year randomized trial. JAMA. 2004;292(2):191-201. doi:10.1001/jama.292.2.191

18. Crane HM, Grunfeld C, Willig JH, et al. Impact of NRTIs on lipid levels among a large HIV-infected cohort initiating antiretroviral therapy in clinical care. AIDS. 2011;25(2):185-195. doi:10.1097/QAD.0b013e328341f925

19. Gardner K, Hall PA, Chinnery PF, Payne BAI. HIV Treatment and Associated Mitochondrial Pathology:Review of 25 Years of in Vitro, Animal, and Human Studies. Toxicol Pathol. 2014;42(5):811-822. doi:10.1177/0192623313503519

20. Johnson AA, Ray AS, Hanes J, et al. Toxicity of Antiviral Nucleoside Analogs and the Human Mitochondrial DNA Polymerase. J Biol Chem. 2001;276(44):40847-40857. doi:10.1074/jbc.M106743200

21. Birkus G, Hitchcock MJM, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: Comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother. 2002;46(3):716-723. doi:10.1128/AAC.46.3.716-723.2002

22. Schütt M, Zhou J, Meier M, Klein HH. Long-term effects of HIV-1 protease inhibitors on insulin secretion and insulin signaling in INS-1 beta cells. J Endocrinol. 2004;183(3):445-454. doi:10.1677/joe.1.05620

23. Behrens G, Dejam A, Schmidt H, et al. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. AIDS. 1999;13(10):E63-70. doi:10.1097/00002030-199907090-00001

24. Larter CZ, Farrell GC. Insulin resistance, adiponectin, cytokines in NASH: Which is the best target to treat? J Hepatol. 2006;44(2):253-261. doi:10.1016/j.jhep.2005.11.030

25. Johnston AM, Pirola L, Van Obberghen E. Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling. FEBS Lett. 2003;546(1):32-36. doi:10.1016/S0014-5793(03)00438-1

26. Utay N, Ananworanich J, Slike B, et al. Inflammation persists despite early initiation of ART in acute HIV infection [abstract 47]. In: Program and abstracts of the 2015 Conference on Retroviruses and Opportunistic Infections, Seattle, Washington, 23–26 February 2015.

27. Joshi D, O'Grady J, Dieterich D, et al. Increasing burden of liver disease in patients with HIV infection. Lancet. 2011; 377:1198-209. doi: 10.1016/S0140-6735(10)62001-6.

28. Robert S, Gicquel T, Bodin A, et al. Characterization of the MMP/TIMP imbalance and collagen production induced by IL-1 β or TNF-α release from human hepatic stellate cells. PLoS One. 2016;11(4):e0153118. doi:10.1371/journal.pone.0153118

29. Roderfeld M. Matrix metalloproteinase functions in hepatic injury and fibrosis. Matrix Biol. 2018;68-69:452-462. doi:10.1016/j.matbio.2017.11.011

30. Paradis V, Perlemuter G, Bonvoust F, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: A potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001;34(4 I):738-744. doi:10.1053/jhep.2001.28055

31. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2011;6:425-456. doi:10.1146/annurev-pathol-011110-130246

32. Pessayre D, Fromenty B, Berson A, et al. Central role of mitochondria in drug-induced liver injury. Drug Metab Rev. 2012;44(1):34-87. doi:10.3109/03602532.2011.604086

33. Fromenty B, Robin MA, Igoudjil A, et al. The ins and outs of mitochondrial dysfunction in NASH. Diabetes Metab. 2004;30(2):121-138. doi:10.1016/S1262-3636(07)70098-8

34. Begriche K, Massart J, Robin MA, et al. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. Hepatology. 2013;58(4):1497-1507. doi:10.1002/hep.26226

35. Luedde T, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: Mechanisms and clinical relevance. Gastroenterology. 2014;147(4):765-783. doi:10.1053/j.gastro.2014.07.018

36. Liu W, Baker S, Baker R, Zhu L. Antioxidant Mechanisms in Nonalcoholic Fatty Liver Disease. Curr Drug Targets. 2015;16(12):1301-1314. doi:10.2174/1389450116666150427155342

37. Videla LA, Rodrigo R, Orellana M, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci. 2004;106(3):261-268. doi:10.1042/CS20030285

38. Mota M, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism. 2016;65(8):1049-1061. doi:10.1016/j.metabol.2016.02.014

39. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. Semin Liver Dis. 2008;28(4):360-369. doi:10.1055/s-0028-1091980

40. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998;94(4):491-501. doi:10.1016/S0092-8674(00)81590-1

41. Milhas D, Cuvillier O, Therville N, et al. Caspase-10 triggers bid cleavage and caspase cascade activation in fasL-induced apoptosis. J Biol Chem. 2005;280(20):19836-19842. doi:10.1074/jbc.M414358200

42. Babu CK, Suwansrinon K, Bren GD, et al. HIV induces TRAIL sensitivity in hepatocytes. PLoS One. 2009;4(2):1-9. doi:10.1371/journal.pone.0004623

43. Forrester JE, Rhee MS, McGovern BH, et al. The association of HIV viral load with indirect markers of liver injury. J Viral Hepat. 2012;19(2):e202-e211. doi:10.1111/j.1365-2893.2011.01529.x

44. Martinez SS, Campa A, Li Y, et al. Low Plasma Zinc Is Associated with Higher Mitochondrial Oxidative Stress and Faster Liver Fibrosis Development in the Miami Adult Studies in HIV Cohort. J Nutr. 2017;147(4):556-562. doi:10.3945/jn.116.243832

45. Baum MK, Raflie C, Lai S, et al. Alcohol use accelerates HIV disease progression. AIDS Re Hum Retroviruses. 2010;26(5):511 doi:10.1089/aid.2009.0211

46. Campa A, Martinez SS, Sherman KE. Cocaine Use and Liver Disease are Associated with All- Cause Mortality in the Miami Adult Studies in HIV (MASH) Cohort. J Drug Abus. 2016;2(4):pii:27. doi:10.21767/2471-853x.100036

47. Tamargo JA, Sherman KE, Campa A, et al. Food insecurity is associated with magnetic resonance-determined nonalcoholic fatty liver and liver fibrosis in low-income, middle-aged adults with and without HIV. Am J Clin Nutr. 2021 Jan 29:nqaa362. doi: 10.1093/ajcn/nqaa362. Epub ahead of print. PMID: 33515016.

48. Tang A, Tan J, Sun M, et al. Nonalcoholic Fatty Liver Disease: MR Imaging of Liver Proton Density Fat Fraction to Assess Hepatic Steatosis. Radiology. 2013;267(2):422-431. doi:10.1148/radiol.12120896

49. Venkatesh SK, Ehman RL. Magnetic resonance elastography of liver. Magn Reson Imaging Clin N Am. 2014. doi:10.1016/j.mric.2014.05.001

Chapter II: LITERATURE REVIEW

Direct effects of HIV on NAFLD and Liver Disease Progression

The rate of NAFLD in PLWH is approximately twice as higher when compared to the general population.[1,2] Liver-related disease accounts for approximately 5.2% of all deaths in PLWH.[3] It has been shown that PWLH with NAFLD have almost double the rates of NASH compared to age/sex-matched HIV negative controls.[4] Lipid accumulation alone increases Hepatic Stellate Cell (HSC) activation, and this process may be exacerbated by HIV. This was illustrated by a study that showed that the mean BMI among PLWH with NAFLD histology was 26 kg/m^2, while the mean BMI of non-infected participants with NAFLD histology was 30 kg/m^2. Higher levels of insulin and glucose, hallmarks of NAFLD, increase HSC proliferation and also increase connective tissue growth factor (CTGF), another promoter of liver fibrosis.[6]

The prevalence of liver fibrosis in PLWH ranges between 17-41%.[7,8] In PLWH, the HIV Gp120 protein receptor directly induces apoptosis and instigate liver injury initiating fibrotic pathways.[9] Liver fibrosis is the process of increased deposition of extracellular matrix (ECM) proteins into the space of Disse, which is the part of the liver between hepatocytes and liver sinusoids. The cells primarily involved with the depositing of ECM are HSCs. HSCs must become activated through release of debris from injured hepatocytes, lipid accumulation, ROS, or exposure to certain cytokines.[10] Over time, increased deposits of ECM and decline in the removal of these proteins by matrix metalloproteinases (MMPs) results in a buildup of scar tissue that replaces normal liver parenchyma tissue.[11]

NAFLD Prevalence

The prevalence of NAFLD in the general population has been estimated to be around 20 to 24 %[12,13] but may be as high as 50% according to some studies[14,15]. The disparities between these ranges may be because this problem is growing among individuals with chronic liver disease in recent years; the proportion of liver disease due to NAFLD has increased from 47% to 75% over the last 20 years.[16] This rate is even higher among individuals who are obese (between 57-98%)[12] and have type 2 diabetes.[17] Genetically, it appears that Hispanics present the highest risk of NAFLD development, followed by European ancestry, then African Americans.[18]

Mechanisms of Lipid Accumulation in NAFLD

The simplest form of NAFLD is triglyceride accumulation within the liver that is present without inflammation. When NAFLD is more severe, and is accompanied by substantial inflammation and possible fibrosis, the condition is considered NASH. It has been estimated that anywhere from 6-55% of patients with NAFLD also have NASH.[19–21] The development of NAFLD begins with an increase in insulin resistance (IR), often a consequence of obesity-related inflammation. In obese individuals, adipocyte expansion leads to adipocyte dysfunction that results in an abnormal release of adipokines, several of which are pro-inflammatory.[22] This pro-inflammatory state results in IR, which inhibits the function of the hormone sensitive lipase (HSL), an enzyme which increases the mobilization of free fatty acids (FFAs) and their transport to the liver.[23] Insulin also increases the synthesis of FFAs in the liver by enhancing the transcription

factor sterol regulatory element-binding protein-1c (SREBP1-c).[24] Along with an increase in IR, there is an increase in circulating glucose levels. Increased glucose can also upregulate the synthesis of FFAs in the liver through increased expression of carbohydrate responsive element-binding protein (ChREBP).[24] Dietary factors that can influence the accumulation of FFAs include increased dietary cholesterol and saturated fat. Both of these nutrients have been shown to upregulated SREBP-1c, whereas unsaturated fat intake appears to downregulate SREBP-1c.[25] Along with systemic IR, IR can also occur in the liver itself. Liver IR worsens FFA accumulation and can increase the production of toxic FFA metabolites such as ceramides. Systemic inflammatory markers such as TNF-a and IL-6 can also worsen hepatic IR.

Differentiating between NAFLD and NASH

Insulin resistance followed by accumulation of triglyceride in the liver alone is not a condition that is characterized as NASH. Only when NAFLD is combined with inflammation and OS is the condition considered NASH. It has been recently demonstrated that NAFLD and NASH are separate conditions that often occur simultaneously but can be independent from one another.[26] Studies have shown that NASH development is possible without previous steatosis.[27] Hepatocytes may first become stressed by inflammation or ROS and this may lead to a downstream accumulation of lipids in the liver. In this case, NASH precedes steatosis, not vice-versa. NAFLD and NASH typically develop through concurrent pathways but should be considered separate conditions with a high risk of one of the conditions preceding the diagnosis of the other.[28]

It is important for researchers to fully understand the mechanisms behind the progression of non-inflammatory NAFLD to inflammatory and possibly fibrotic and eventually cirrhotic NASH. Increased FFA oxidation, in NASH, appears to cause substantially more damage than simple triglyceride (TG) accumulation alone, which is a hallmark of NAFLD. When FFAs are oxidized in the liver toxic metabolites are formed and can result in OS, inflammation and liver injury.[29–31] TG accumulation, common in non-progressive NAFLD, is not toxic when stored in the liver and it is simply a result of increased FFA transport[32] and decreased removal from beta oxidation, VLDL export, and lipophagy.[33,34] Decreased beta oxidation occurs during early stages of fat accumulation, typically triggered by increased IR which increases the lipolysis of peripheral fat and increases FFA transport to the liver.[35,36] FFAs from lipolysis make up the majority (59%) of FFAs present in the livers of NAFLD patients.[33] The liver can also contribute to the buildup of lipids through de novo lipogenesis (DNL) via SREBP1 and ChREBP.[37] Each of these transcription factors is further upregulated under conditions of IR. Insulin also has an inhibitory effect on the CYP2E1 enzyme, the major enzyme that metabolizes both ethanol and FFAs.[38] The CYP2E1 gene expression positively correlates with liver fat[39] and is higher in patients with NASH compared to simple NAFLD.[40,41] This may be due to the production of highly reactive carbonyl compounds during CYP2E1 FFA oxidation.[38,40]

Progressing from NASH to Fibrosis

As described above, liver fibrosis is the process of accumulating ECM proteins in the space of Disse.[42] Fibrosis progression is primarily regulated by the

activation of HSCs and the resulting release of chemical mediators that lead to deposition of the ECM.[43,44] The ECM is regulated by the balance between pro-fibrogenic tissue inhibitors of metalloproteinases (TIMPs) and the anti-fibrogenic matrix metalloproteinases (MMPs). Levels of TIMPs have been shown to progressively increase as liver fibrosis advances. The increase in TIMP-1 has been associated with the activation of HSCs that is the result of increased pro-inflammatory cytokines that were mentioned above.[45] MMPs are a group of zinc-dependent enzymes that regulate the ECM through inhibition of TIMPs.[46] The upregulation of TIMPs may be a contributor to the development of fibrosis by inhibiting the breakdown of the matrix.[47] The individual pathways outlining the roles of HIV, OS, apoptosis, and immune activation and inflammation in pathology of fibrosis are described in their respective sections below.

Role of HIV in Liver Fibrosis

ART blocks uncontrolled viral replication of HIV, but low levels of replication occur even when ART is considered successful.[48,49] The HIV virus does not replicate inside of hepatocytes, but hepatocytes do have the CXCR4 and CCR5 co-receptors required for HIV binding.[50] When HIV activates the CXCR4 and CCR5 receptors increase pro-collagen alpha-1, one of the components of type I collagen found in the ECM, a sign of liver fibrosis.[51] HIV can infect HSCs and can directly stimulate collagen I expression and monocyte chemoattractant protein (MCP-1) in these cells. The HIV envelope protein Gp 120 can also activate TIMP-1.[52] The increased expression of TIMP-1 and MCP-1 is heavily involved in the recruitment of leukocytes and fibrosis.

Oxidative Stress in NASH and Fibrosis

Oxidative stress can be defined as an imbalance between the antioxidant defenses of the body and the production of reactive oxygen species (ROS). ROS are oxygen molecules that easily react with and damage biological molecules. Oxidative stress can be measured via different methodologies to reflect ROS damage in different tissues in the body. Systemic oxidative stress can be measured in blood using oxidized glutathione, lipid peroxidation can be measured by malondialdehyde (MDA) or 4-hydroxy-2-nonenal (HNE), and 7,8-dihydroxy-8-oxo-2'-deoxyguanosine (8-oxodG) measures oxidized DNA damage.[53]

It has been previously shown that individuals with NASH have higher levels of OS than those with simple steatosis.[54] The most prominent source of OS is from increased FFA oxidation in the mitochondria.[55,56] This primarily occurs in the electron transport chain at complexes I and II, electrons escape these complexes and interact with oxygen molecules and form ROS.[57] ROS activates the Jun N-terminal kinase (JNK) pathway that results in mitochondrial damage within the mitochondrial membrane.[58] Mitochondrial damage leaves the electron transport chain susceptible to further ROS production and the vicious cycle continues until mitochondrial function is diminished.[57,59] Diminished mitochondrial function decreases ATP production and can also result in cell death linking OS to apoptosis (see apoptosis section).[60,61] OS can also have an effect on DNA replication; individuals with diagnosed NASH have been shown to have lower levels of mitochondrial DNA than individuals with simple NAFLD.[62] One common

marker of mitochondrial OS is 8-oxo-dG and this has been shown to be increased in individuals with NASH.[63]

In addition to an increase in ROS production in NAFLD and NASH, there also appears to be a decrease in antioxidant capacity. Increased ROS production consumes the antioxidant molecules glutathione and coenzyme Q10. Also, antioxidant enzymes appear to be inhibited by oxidation. Decreased antioxidant enzyme capacity correlates with severity of NASH.[64,65]

HIV and Oxidative Stress

The HIV virus induces oxidative stress by several mechanisms; HIV envelope proteins Gp120, Tat, Nef, Vpr, RT each have connections to oxidative pathways, but the Gp120 protein likely has the most prominent effect on the liver. Gp120 binds to the chemokine receptors CCR5 and CXCR4 on the HIV virus. When Gp120 exposure to these receptors increases, expression of proinflammatory cytokines including TGF-ß1, MCP-1, IL-6, and TIMP-1 are increased. TGF-ß1 promotes hepatic fibrosis and the increase in MCP-1 also results in higher levels of hepatic inflammation and risk for developing fibrosis.[46]

In addition to promoting pro-fibrogenic pathways through increased oxidative stress, the HIV virus also decreases antioxidant defense systems. Studies have shown that the Gp120 and Tat envelope proteins lead to the downregulation of glutathione synthase (GSS), glutathione reductase (GR) and glutathione peroxidases (GPx). The result of this down regulation is a decrease in the total glutathione content and an increase in the oxidized glutathione (GSSG) to total glutathione (GSH) ratio.[66,67] The effect of Tat on reduced

glutathione production is most likely stronger than the effect of Gp120.[66] Furthermore, Vpr is another protein on the surface of the virus that may reduce GSH levels by decreasing ATP synthesis in mitochondria. Decreased ATP synthesis reduces the levels of GSH because two molecules of ATP are required for the synthesis of every glutathione molecule.[68,69]

Oxidative stress has been shown to facilitate disease progression and liver disease-related mortality. Increased oxidative stress can lower $CD4^+$ counts and damage DNA within $CD4^+$ cells.[70–72] Also, evidence shows OS may stimulate HIV replication,[73] play a role in in accelerated aging, and contribute to the development of chronic diseases.[74]

Two markers of OS that are of particularly relevant to this review are MDA and HNE. Each of these markers are secondary aldehydes that are formed during lipid peroxidation and can cause liver damage. HNE is likely the most toxic [75]and significantly correlates with the grade of inflammation and fibrosis.[76] HNE adducts are aldehyde metabolites of lipid peroxidation and have been shown to increase during NASH progression. [77]An important inflammatory pathway in the progression of NAFLD are the Toll-like receptor (TLR) pathways. Specifically, TLR-7 is a pathway that may reduce lipid accumulation within hepatocyte and reduce the risk of NAFLD. Increased levels HNE and MDA seen in HIV [70,71]have been shown to inhibit TLR-7 and worsen NAFLD progression.[78] HNE can also activate the c-Jun NH_2-terminal kinase (JNK) pathway, which is a regulator of metabolic pathways that contribute to NAFLD and liver injury. This supports the

biomarker of OS.[79]

Mitochondrial Dysfunction in NASH and Liver Fibrosis

NAFLD develops due to the rate of beta oxidation lagging behind FFA accumulation from increased FFA uptake and synthesis. The initial decrease in beta oxidation of FFAs lead to the production of ketone bodies. In the previous sections it was mentioned that IR increased expression of SREBP-1. SREBP-1c induces an increase in acetyl-CoA carboxylase-2 (ACC2) which increases malonyl-CoA and results in decreased expression of carnitine palmityltransferase-1 (CPT1) inside of the mitochondria. The final result of decreased CPT1 is decreased beta oxidation of fatty acids and lipid accumulation.

As lipid accumulation progresses, FFA oxidation begins to increase as a compensatory mechanism. This is accomplished through increased activity of the PPAR-alpha gene. This gene enhances the activity of CPT-I and CPT-I loses affinity for malonyl-CoA,[55,80,81] resulting in an increased in FFA beta-oxidation. Increased oxidation of FFAs however, results in increased ROS production and possibly toxic metabolites, including oxidized cardiolipin[82,83] and ceramides.[84] Lastly, increased ROS produced from oxidation of FFAs result in damage to the electron transport chain of mitochondria directly, and damage to mitochondrial DNA.[59] ROS also results in elevations in mitochondrial $Ca2^{+}$ which produces more ROS. $Ca2^{+}$ increases the delivery of electrons to the electron transport chain but blocks the protein complex within the chain which increases electron leakage from the chain and promotes ROS production[85] Much of this $Ca2^{+}$ comes

from a stressed endoplasmic reticulum (ER).[86] Mitochondrial damage from ROS over time will eventually result in decreased beta oxidation of FFAs, which will make NAFLD even worse, increase OS and inflammation, and trigger fibrosis pathways.

HIV and Mitochondrial Dysfunction

HIV itself can deplete mitochondrial DNA in $CD8^+$ cells, B cells, and $CD4^+$ cells. Mitochondrial dysfunction is another factor that can induce apoptosis, lipid accumulation and eventual fibrosis.[87,88] Accumulation of lipids occurs due to a decrease in beta-oxidation of fatty acids, the lipids accumulate and can lead to liver fibrosis development[60,89] Adipose tissue in PLWH actually has an increase in mitochondrial content. This could be a compensatory mechanism or simply alter lipid metabolism and promote liver fat accumulation. Additionally, it has been previously demonstrated that ART may also induce mitochondrial toxicity (see ART section below).[87,89]

Apoptosis, NASH, and Fibrosis

Apoptosis is one of the most distinguishable features that separates individuals with NASH from NAFLD.[90] Greater apoptosis correlates with greater degree of liver injury in NASH.[91] One of the major proposed mechanisms initiating apoptic pathways is excess lipid accumulation, mainly FFAs. All four apoptosis pathways, intrinsic, extrinsic, ER stress, and lysosomal, can be initiated by FFA accumulation.[30,84] The intrinsic or mitochondrial pathway is initiated by cellular stress that results in increased mitochondrial membrane permeability and release of pro-apoptic proteins into the cytosol .[92] The source of

cellular stress in this pathway is increased mitochondrial OS.[93] Dietary saturated FFAs can activate the pro-apoptic proteins Bax and PUMA via the JNK activation pathway.[94–96] In addition to mitochondrial stress, endoplasmic reticulum (ER) stress can also activate apoptic pathways from saturated FFAs through JNK signaling as well.[97] This occurs due to disruption of Ca2+ release from the ER.[98]

Extrinsic apoptosis can be trigged via pro-inflammatory cytokines released during the inflammatory processes discussed previously. This pathway is initiated when cell plasma membranes activate their cytokine receptors to pro-inflammatory cytokine ligands, mainly the TNF-R, and TRAILR and Fas receptors. These receptors trigger apoptosis through the formation of the death inducing signaling complex (DISC) and subsequent activation of the caspase-2 mediated pathway that cleaves the BH3-interacting domain death agonist (BID) protein. BID recruits Bax to the mitochondrial and this event then triggers intrinsic apoptosis.[99,100] It appears that the most important apoptic pathway in NASH are the Fas/FasL and the TRAILR/TRAIL pathways, likely due to FFAs initiation. Cell lines treated with these ligands show increased susceptibility to apoptosis.[100,101]

The lysosomal pathway induces apoptosis by releasing cathepsin B into the cytosol.[102,103] This pathway links to the intrinsic pathway by activating pro-caspase 2 and inducing increased mitochondrial membrane permeability.[104]

HIV and Apoptosis

HIV likely induces apoptosis via the death receptor pathway through its envelope protein Gp120. Gp120 interacts with the chemokine receptors CCR4 and CCR5 on several different types of cells, including hepatocytes and $CD4^+$

lymphocytes which triggers apoptosis [105] Gp120 induces hepatocyte apoptosis by increasing the expression of TRAIL receptor.[106] Higher HIV VL could lead to more Gp120 exposure to hepatocytes and therefore leading to greater rates of apoptosis in inflammation.[107]

Gp120 within infected hepatocytes leads to apoptosis of those cells; however, apoptosis can spread to non-infected $CD4^+$ cells and lead to their long-term depletion. HIV infected cells may induce apoptosis in non-infected $CD4^+$ cells by a process called “bystander killing”.[108] This can be accomplished in multiple ways. The infected cell and a healthy $CD4^+$ cell may partially fuse and produce an interaction between HIV viral proteins and receptors on the noninfected cell and exchange lipid membranes. This partial fusion and interaction triggers cell death in the uninfected cell.[109] Infected cells can also go through the process of complete cellular and nuclear fusion which will eventually lead to cell death of the fused cell later on in the cell cycle.[110] Lastly, when infected cells begin going through the process of apoptosis, they can fuse with noninfected $CD4^+$ cells and apoptosis is induced in both cells.[111]

Microbial Translocation and Inflammation in NASH

Common markers of increased microbial translocation are LPS, LPS binding protein, and sCD14. sCD14 has been shown to be increased in PLWH and correlated with liver fibrosis. LPS activates toll-like receptor 4 (TLR-4), TLR-4 actives Kupffer cells in the liver which produce pro-inflammatory cytokines that can activate HSCs or recruit other immune cells.[112,113] Kupffer cells play a major role in the overall inflammatory response in the liver and attempt to repair liver

damage.[114] HIV may deplete Kupffer cells which may reduce HSC activation, but overall inflammatory effect remains due a decreased ability to clear microbial translocation products from the liver.[115,116] Kupffer cells have been shown to release TGF-β in response to TLR-4 from LPS indicating a direct fibrotic response.[117] In addition to the activation of Kupffer cells and HSCs, LPS can also promote hepatocyte cell death through the systemic immune response and increased production of proinflammatory cytokines.[118,119] Increased microbial translocation products as a result of dysbiosis or gut endothelial damage may also contribute to NAFLD development. The primary microbial translocation product of concern is LPS, which activates TLR-4 and can enhance the production of ceramide synthesis, a toxic metabolite. Visceral adipose tissue and Kupffer cells respond to TLR-4 with increased production of TNF-α and IL-6 which can both contribute to IR.[120,121] Inflammation in NASH can be traced back to inflammatory cytokines from adipose tissue, the gut, or the liver itself.[122] Obesity leads to increased adipocyte size that results in dysregulation of adipocytes which Dysregulated adipocytes increase the production of pro-inflammatory cytokines that results in IR.[123,124] Obesity activates macrophages in adipose tissue to switch to a pro-inflammatory M1 state that can also contribute to inflammatory cytokine production.[125]

FFAs are recognized as danger-associated molecular patterns in the liver (DAMPs). DAMPs can activate the NALP3 inflammasome that activates pro-caspase-1 and increases pro-inflammatory cytokine production.[126] DAMPs can also derive from dying hepatocytes and activate inflammatory pathways through

TLR. FFAs also enhance expression of TNF-α through the NF-kB pathway.[102] In addition to TNF-α, NF-kB also upregulates TGF-B, IL-6, and IL-8, which are all primary promoters of apoptosis and fibrogenesis driving the progression of NAFLD to NASH.[127]

HIV and Microbial Translocation

Previous studies have found reduced integrity of the gut mucosa lower in PLWH due to damage of enterocytes.[128] Enterocyte damage allows microbial products typically found in the GI tract, mainly lipopolysaccharide (LPS), to leak from the intestine and travel to the liver via the portal vein.[128] The disruption of the mucosal layer is likely due to a depletion of $CD4^+$ and CD22 cells in the intestine.[129–132] Higher levels of LPS in circulation increase production of inflammatory cytokines by gut enterocytes leads to epithelial apoptosis. Increased endothelial apoptosis is associated with the disruption tight junctions between enterocytes and loss of gut integrity which further perpetuates higher levels of LPS in circulation. [130,132,133]

ART and Metabolic Health

One of the main concerns in the development of NAFLD among PLWH is the detrimental effect of ART on blood lipids. The most harmful classification of ART drugs related to the development of NAFLD are nucleoside reverse transcriptase inhibitors (NRTIs). Older NRTI drugs were associated with insulin resistance and dyslipidemia, likely due to mitochondrial toxicity from the drugs.[134–136] These drugs can impair mitochondrial DNA replication which results in decreased oxidative phosphorylation of lipids, increased ROS, and an

accumulation of liver fat.[137] Early generation NRTIs that had the worst impact on metabolic outcomes were stavudine, didanosine, and zalcitabine. Modern NRTIs with much more favorable impacts on metabolic outcomes include tenofovir, abacavir, lamivudine, and emtricitabine.[138,139] Protease inhibitors (PIs) are the second classification of ART drug with strong links to poor metabolic health. It appears the main mechanism that PIs acts as a detriment to metabolic health is through insulin resistance. PIs have been associated with hyperglycemia, hyperinsulinemia, and impaired secretion of insulin from beta cells.[140,141] Older PIs with the worst effects on insulin resistance are indinavir and ritonavir.[142] Current protease inhibitors atazanavir and darunavir have more favorable profiles metabolic profiles.[143] Protease inhibitors may increase adipocyte size due to inhibition of GLUT-4 activity.[144] Enlarged adipocytes are insulin resistant and these cells secrete less adiponectin, which increases body fat worsening liver fat and fibrosis.[145]

HIV and Nutrition

The role of nutrition in PLWH has changed drastically over the last few decades. When the HIV virus was first identified, one of the most distinguishable features of the disease was the muscle wasting that was very visible in individuals who were in advanced stages of the disease. However, advances in ART have completely changed the nutritional outlook for the majority of PLWH, instead of eating to avoid muscle wasting these individuals are managing obesity, elevated blood lipids, and type two diabetes (T2DM).[146,147] Therefore, for PLWH one of the most important considerations to their dietary

recommendations are their HIV viral load (VL), ART use and adherence, and their current BMI. Individuals who present classic symptoms of muscle wasting such as low BMI or recent decrease in BMI will be treated very differently than those who have a well-controlled disease and have a healthy or high BMI.

Areas of focus for individuals displaying signs of muscle wasting include reversing weight loss and a decrease in muscle mass, fluid accumulation, and correcting insufficient energy intake.[148] PLWH who appear to be more at risk for over-nutrition should focus on blood lipids, insulin and glucose, and blood pressure. Nutrition related health concerns that all PLWH should pay close attention to include iron and B12 related anemia,[149] gut health, diarrhea and bone density.[150,151] The focus of this review will remain on the role of nutrition in the development of NAFLD and NASH in PLWH.

Additionally, because HIV is an immune deficiency virus, the immune system needs optimal nutrition support. It has been previously shown that B vitamins and the antioxidant vitamins C and E influence the function of the immune system.[152] Often in PLWH micronutrient deficiencies can precede HIV disease progression.[153, 154] Supplementation of micronutrients in PLWH has reduced the risk immunological decline HIV morbidity.[155] Also zinc intake has also been associated with immune function in this population and may promote a health gut mucosa and reduce diarrhea.[156] Zinc also works as an antioxidant in the body and is associated with OS when deficient.[157] Through this pathway zinc has been shown to be associated with liver fibrosis progression.[158] Optimal zinc intake is important in PLWH due to over 50% of the PLWH population having low

zinc concentrations.[153,159,160]

Dietary Factors in the Development of NAFLD and NASH in PLWH

As discussed above, the initial factor that typically initiates the cascade of events that lead to lipid accumulation in the liver is obesity. Obesity in turn increases susceptibility to inflammation and insulin resistance resulting in liver fat accumulation. Current recommendations for individuals with NAFLD, whether or not they are living with HIV, begins with weight loss. The current weight loss recommendation to reduce liver fat is 5-10% of body weight.[161] Weight loss in PLWH is complicated and should be aided with outpatient nutritional counseling. Patients should plan on making long-term changes and not short-term weight-loss goals.[162] Weight loss interventions should include the entire healthcare team including social workers and community service organizations that may help with potential food insecurity issues.

Cholesterol from the diet can induce SREBP-1c activation through the liver X receptor (LXR) transcription factor. LXR also activates ChREBP, stearoyl-CoA desaturate, and FFA synthase. Saturated fatty acids may also modulate SREBP-1c activation. It has been shown that dietary saturated fatty acids can upregulate the SREBP-1c gene and unsaturated fatty acids downregulate this gene.[25] Fructose intake also activates both SREBP-1c and ChREBP as well. SREBP-1c is activated through the buildup of advanced glycation end products associated with chronic fructose intake and ChREBP is activated through fructose intermediate products and activation of glycogen kinase (GK).[163,164]

Diet and Microbial Translocation

It is known that the microbiome plays a major role in microbial translocation and dietary composition is heavily involved. A Western style diet made of high fat and high sugar content along with low fiber intake is associated with the development of obesity.[165] This type of diet has been previously associated with reduced microbial diversity in the gut.[165] This link may be related to the development of NAFLD through the gut-liver axis.[166,167] A healthy and diverse gut microbiome produces beneficial compounds such as short-chain fatty acids (SCFAs) whereas dietary components of a Western diet such as fructose, saturated fat, and cholesterol may alter the microbiome in a manner that allows increased gut permeability and translocation of harmful microbial compounds to the liver via the portal vein.[168] In PLWH, the HIV virus itself causes damage to the gut mucosa layer and exacerbates the translocation of microbial products to the portal circulation. The result of increased microbial translocation due to dietary or viral factors results in increased LPS transport, immune cell activation and inflammation through the TLR-4 pathway occurs as discussed in above sections. One of the major consequences of the TLR-4 pathway activation is an increase in OS that can result in greater hepatocyte apoptosis and activation of the NF-kB pathway. Additionally, saturated FFAs activate or amplify signaling through TLR4 in a synergistic manner with LPS.[169] Dietary components related to downregulation of OS and inflammation include intake of antioxidant micronutrients and polyunsaturated fatty acids (PUFAs).

Dietary and microbial factors within the gut also play a major role in the development of hepatic inflammation.[170] Specific nutritional components that are thought to be contributors include saturated fat and fructose; these components increase intestinal permeability to gut microbial products such as LPS.[171] Fructose can increase the presence of certain bacterial species in the gut that promote bacterial overgrowth and increase LPS.[168,172] Gut barrier dysfunction in patients with NAFLD has been previously demonstrated and led to increased LPS leakage through the intestinal membrane.[171,173] Through this pathway high fructose intake is able to activate Kupffer cells and HSCs in the liver and initiate pro-inflammatory and fibrotic pathways.[174–176]

Substance Abuse, NAFLD and NASH

Among individuals enrolled in HIV care, the prevalence of substance abuse disorders (SUD) is estimated to be approximately 48% overall. This rate varies from 21 to 71% depending on the specific site.[177] It is important to identify these individuals because SUDs have been shown to delay ART treatment and are individuals using them are less adherent to their medications. [178–180] In addition to negatively affecting their HIV treatment, different substances of abuse my directly impact HIV disease progression.[156,181,182] When multiple substances of abuse are combined, such as in the case of alcohol and cocaine, the risk of virologic failure is greatly increased.[178]

For this review, three substances of abuse will be discussed: alcohol, cocaine, and opioid use. Over 60% of PLWH use alcohol and about 15% have reported binge drinking within the last 30 days.[183] Previous studies have shown quicker liver disease progression among heavy alcohol drinkers than individuals

with NAFLD.[184] ALD has been shown to increase liver-related mortality more so than simple steatosis.[185]

The prevalence of cocaine use disorder among those in HIV care in one study was shown to be 11% and 50% in a separate cohort.[177,182] Cocaine use has been shown to greatly reduce ART adherence and ability to suppress VL.[178] Cocaine usage has also been shown to have a negative biological impact on PLWH as well. Cocaine use has been shown to increase HIV transcription and replication.[186] This leads to accelerated HIV disease progression as measured by reduced $CD4^+$ cell count and increased mortality.[182,187] Cocaine use has also been shown to have an effect on the development of fatty liver. As discussed earlier, protease inhibitor usage has been shown to be an independent risk factor increased hepatic triglyceride content.[188] Interestingly, in participants who had never used cocaine, there was no association between duration of PI use and hepatic triglycerides. However, when participants had used cocaine, this relationship was significant. The authors hypothesized that cocaine use may exaggerate the toxicity pathways that define the relationship between PIs and steatosis development.[188]

Summary

PLWH are much more likely to develop liver disease than individuals without HIV. This population presents unique challenges to the liver in the form of increased microbial translocation, inflammation, and oxidative stress. Additionally, PLWH are more prone to potentially damaging behaviors such as drug and alcohol abuse than advance liver disease. It is imperative to study how

HIV, metabolic disease, and substance abuse interactions may affect the development of liver disease. Identifying predictors of liver disease in this population through dietary habits, substance use habits, biological biomarkers, or MRE technology can provide insight for health care providers to optimize their care of PLWH and reduce their risk of developing liver disease.

References

1. Seth A, Sherman KE. Fatty liver disease in persons with HIV infection. Top Antivir Med. 2019; May;27(2):75-82. PMID: 31136997; PMCID: PMC6550355.

2. Maurice JB, Patel A, Scott AJ, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. AIDS. 2017;31(11):1621-1632. doi:10.1097/QAD.0000000000001504

3. Croxford S, Kitching A, Desai S, et al. Mortality and causes of death in people diagnosed with HIV in the era of highly active antiretroviral therapy compared with the general population: an analysis of a national observational cohort. Lancet Public Health. 2017;2(1):e35-e46. doi:10.1016/S2468-2667(16)30020-2

4. Vodkin I, Valasek MA, Bettencourt R, et al. Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: A case-control study. Aliment Pharmacol Ther. 2015;41(4):368-378. doi:10.1111/apt.13052

5. Mohammed SS, Aghdassi E, Salit IE, et al. HIV-positive patients with nonalcoholic fatty liver disease have a lower body mass index and are more physically active than HIV-negative patients. J Acquir Immune Defic Syndr. 2007;45(4):432-438. doi:10.1097/QAI.0b013e318074efe3

6. Paradis V, Perlemuter G, Bonvoust F, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: A potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001;34(4 I):738-744. doi:10.1053/jhep.2001.28055

7. Han SH, Kim SU, Kim CO, et al. Abnormal Liver Stiffness Assessed Using Transient Elastography (Fibroscan®) in HIV-Infected Patients without HBV/HCV Coinfection Receiving Combined Antiretroviral Treatment. PLoS One. 2013;8(1). doi:10.1371/journal.pone.0052720

8. Redd AD, Wendel SK, Grabowski MK, et al. Liver stiffness is associated with monocyte activation in HIV-infected ugandans without viral hepatitis. AIDS Res Hum Retroviruses. 2013;29(7):1026-1030. doi:10.1089/aid.2013.0004

9. Blackard JT, Sherman KE. HCV/HIV co-infection: Time to re-evaluate the role of HIV in the liver? J Viral Hepat. 2008;15(5):323-330. doi:10.1111/j.1365-2893.2008.00970.x

10. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2011;6:425-456. doi:10.1146/annurev-pathol-011110-130246

11. Singh S, Allen AM, Wang Z, et al. Fibrosis Progression in Nonalcoholic Fatty Liver versus Nonalcoholic Steatohepatitis: A Systematic Review and Meta- analysis of Paired-Biopsy Studies. Clin Gastroenterol Hepatol. 2015;13(4):643-654. doi:10.1016/j.cgh.2014.04.014.Fibrosis

12. Vernon G, Baranova A, Younossi ZM. Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34(3):274-285. doi:10.1111/j.1365-2036.2011.04724.x

13. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. doi:10.1002/hep.28431

14. Bedogni G, Miglioli L, Masutti F, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: The dionysos nutrition and liver study. Hepatology. 2005;42(1):44-52. doi:10.1002/hep.20734

15. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. Gastroenterology. 2011;140(1):124-131. doi:10.1053/j.gastro.2010.09.038

16. Younossi ZM, Stepanova M, Afendy M, et al. Changes in the Prevalence of the Most Common Causes of Chronic Liver Diseases in the United States From 1988 to 2008. Clin Gastroenterol Hepatol. 2011;9(6):524-530.e1. doi:10.1016/j.cgh.2011.03.020

17. Targher G, Bertolini L, Padovani R, et al. Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease in patients with type 1 diabetes. J Hepatol. 2010;53(4):713-718. doi:10.1016/j.jhep.2010.04.030

18. Wagenknecht LE, Scherzinger A, Stamm E, et al. Correlates and heritability of nonalcoholic fatty liver disease in a minority cohort. Obesity (Silver Spring). 2009;17(6):1240-1246. doi:10.1038/oby.2009.4.

19. Silverman JF, O'Brien KF, Long S, et al. Liver pathology in morbidly obese patients with and without diabetes. Am J Gastroenterol. 1990;85(10):1349-1355.

20. Matteoni CA, Younossi ZM, Gramlich T, et al. Nonalcoholic fatty liver disease: A spectrum of clinical and pathological severity. Gastroenterology. 1999;116(6):1413-1419. doi:10.1016/S0016-5085(99)70506-8

21. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: Predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121(1):91-100. doi:10.1053/gast.2001.25540

22. Baranova A, Gowder SJ, Schlauch K, et al. Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. Obes Surg. 2006;16(9):1118-1125. doi:10.1381/096089206778392149

23. Hales CN, Luzio JP, Siddle K. Hormonal control of adipose-tissue lipolysis. Biochem Soc Symp. 1978;43:97-135. doi:10.1079/pns19750044

24. Xu X, So JS, Park JG, Lee AH. Transcriptional control of hepatic lipid metabolism by SREBP and ChREBP. Semin Liver Dis. 2013;33(4):301-311. doi:10.1055/s-0033-1358523

25. Jump DB. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr Opin Lipidol. 2002;13(2):155-164. doi:10.1097/00041433-200204000-00007

26. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. Hepatology. 2010;52(5):1836-1846. doi:10.1002/hep.24001

27. Tiniakos DG, Vos MB, Brunt EM. Nonalcoholic Fatty Liver Disease: Pathology and Pathogenesis. Annu Rev Pathol Mech Dis. 2010;5(1):145-171. doi:10.1146/annurev-pathol-121808-102132

28. Yilmaz Y. Review article: Is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? Aliment Pharmacol Ther. 2012;36(9):815-823. doi:10.1111/apt.12046

29. Kasumov T, Li L, Li M, et al. Ceramide as a mediator of non-alcoholic fatty liver disease and associated atherosclerosis. PLoS One. 2015;10(5):1-26. doi:10.1371/journal.pone.0126910

30. Mota M, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism. 2016;65(8):1049-1061. doi:10.1016/j.metabol.2016.02.014

31. Chaurasia B, Summers SA. Ceramides - Lipotoxic Inducers of Metabolic Disorders. Trends Endocrinol Metab. 2015;26(10):538-550. doi:10.1016/j.tem.2015.07.006

32. Cortez-Pinto H, De Moura MC, Day CP. Non-alcoholic steatohepatitis: From cell biology to clinical practice. J Hepatol. 2006;44(1):197-208. doi:10.1016/j.jhep.2005.09.002

33. Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115(5):1343-1351. doi:10.1172/JCI23621

34. Kwanten WJ, Martinet W, Michielsen PP, Francque SM. Role of autophagy in the pathophysiology of nonalcoholic fatty liver disease: A controversial issue. World J Gastroenterol. 2014;20(23):7325-7338. doi:10.3748/wjg.v20.i23.7325

35. Tamura S, Shimomura I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. J Clin Invest. 2005;115(5):1139-1142. doi:10.1172/JCI24930

36. Marchesini G, Brizi M, Morselli-Labate AM, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med. 1999;107(5):450-455. doi:10.1016/S0002-9343(99)00271-5

37. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: Lessons from genetically engineered mice. J Clin Invest. 2008;118(3):829-838. doi:10.1172/JCI34275

38. Robertson G, Leclercq I, Farrell GC. Nonalcoholic Steatosis and Steatohepatitis. II. Cytochrome P-450 enzymes and oxidative stress. Am J Physiol Gastrointest Liver Physiol. 2001;281(5 44-5):1135-1139.

39. Chtioui H, Semela D, Ledermann M, et al. Expression and activity of the cytochrome P450 2E1 in patients with nonalcoholic steatosis and steatohepatitis. Liver Int. 2007;27(6):764-771. doi:10.1111/j.1478-3231.2007.01524.x

40. Chalasani N, Christopher Gorski J, Asghar MS, et al. Hepatic cytochrome P450 2E1 activity in nondiabetic patients with nonalcoholic steatohepatitis. Hepatology. 2003;37(3):544-550. doi:10.1053/jhep.2003.50095

41. Weltman MD, Farrell GC, Hall P, et al. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology. 1998;27(1):128-133. doi:10.1002/hep.510270121

42. Friedman SL. Hepatic stellate cells: Protean, multifunctional, and enigmatic cells of the liver. Physiol Rev. 2008;88:125-172. doi:10.1152/physrev.00013.2007

43. Friedman SL. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol. 2010;7:425-436. doi:10.1038/nrgastro.2010.97

44. Reynaert H, Thompson MG, Thomas T, Geerts A. Hepatic stellate cells: Role in microcirculation and pathophysiology of portal hypertension. Gut. 2002;50:571-581. doi:10.1136/gut.50.4.571

45. Robert S, Gicquel T, Bodin A, et al. Characterization of the MMP/TIMP imbalance and collagen production induced by IL-1 β or TNF-α release from human hepatic stellate cells. PLoS One. 2016;11(4):e0153118. doi:10.1371/journal.pone.0153118

46. Mastroianni CM, Lichtner M, Mascia C, et al. Molecular mechanisms of liver fibrosis in HIV/HCV coinfection. Int J Mol Sci. 2014;15(6):9184-9208. doi:10.3390/ijms15069184

47. Roderfeld M. Matrix metalloproteinase functions in hepatic injury and fibrosis. Matrix Biol. 2018;68-69:452-462. doi:10.1016/j.matbio.2017.11.011

48. Massanella M, Fromentin R CN. Residual inflammation and viral reservoirs: alliance against an HIV cure. Curr Opin HIV AIDS. 2016;11(2):234-241. doi:10.1016/j.physbeh.2017.03.040

49. Kiselinova M, Geretti AM, Malatinkova E, et al. HIV-1 RNA and HIV-1 DNA persistence during suppressive ART with PI-based or nevirapine-based regimens. J Antimicrob Chemother. 2015;70(12):3311-3316. doi:10.1093/jac/dkv250

50. Nomiyama H, Hieshima K, Nakayama T, et al. Human CC chemokine liver-expressed chemokine/CCL16 is a functional ligand for CCR1, CCR2 and CCR5, and constitutively expressed by hepatocytes. Int Immunol. 2001;13(8):1021-1029. doi:10.1093/intimm/13.8.1021

51. Tuyama AC, Hong F, Saiman Y, et al. Human Immunodeficiency Virus (HIV)-1 infects human hepatic stellate cells and promotes collagen I and monocyte chemoattractant protein-1 expression: Implications for the pathogenesis of HIV/hepatitis C virus-induced liver fibrosis. Hepatology. 2010;52(2):612-622. doi:10.1002/hep.23679

52. Gupta D, Rani M, Khan N, Jameel S. HIV-1 infected peripheral blood mononuclear cells modulate the fibrogenic activity of hepatic stellate cells through secreted TGF-b and JNK signaling. PLoS One. 2014;9(3):1-11. doi:10.1371/journal.pone.0091569

53. Mayne ST. Antioxidant Nutrients and Chronic Disease: Use of Biomarkers of Exposure and Oxidative Stress Status in Epidemiologic Research. J Nutr. 2018;133(3):933S-940S. doi:10.1093/jn/133.3.933s

54. Feldstein AE, Lopez R, Tamimi TAR, et al. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J Lipid Res. 2010;51(10):3046-3054. doi:10.1194/jlr.M007096

55. Begriche K, Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: Causes, consequences and possible means to prevent it. Mitochondrion. 2006;1:1-28. doi:10.1016/j.mito.2005.10.004

56. Begriche K, Massart J, Robin MA, et al. Drug-induced toxicity on mitochondria and lipid metabolism: Mechanistic diversity and deleterious consequences for the liver. J Hepatol. 2011;54(4):773-794. doi:10.1016/j.jhep.2010.11.006

57. Pessayre D, Fromenty B, Berson A, et al. Central role of mitochondria in drug-induced liver injury. Drug Metab Rev. 2012;44(1):34-87. doi:10.3109/03602532.2011.604086

58. Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of Hepatic Mitochondrial Function in Humans with Non-Alcoholic Fatty Liver Is Lost in Steatohepatitis. Cell Metab. 2015;21(5):739-746. doi:10.1016/j.cmet.2015.04.004

59. Fromenty B, Robin MA, Igoudjil A, et al. The ins and outs of mitochondrial dysfunction in NASH. Diabetes Metab. 2004;30(2):121-138. doi:10.1016/S1262-3636(07)70098-8

60. Begriche K, Massart J, Robin MA, et al. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. Hepatology. 2013;58(4):1497-1507. doi:10.1002/hep.26226

61. Luedde T, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: Mechanisms and clinical relevance. Gastroenterology. 2014;147(4):765-783. doi:10.1053/j.gastro.2014.07.018

62. Chiappini F, Barrier A, Saffroy R, et al. Exploration of global gene expression in human liver steatosis by high-density oligonucleotide microarray. Lab Investig. 2006;86(2):154-165. doi:10.1038/labinvest.3700374

63. Caldwell SH, Swerdlow RH, Khan EM, et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol. 1999;31(3):430-434. doi:10.1016/S0168-8278(99)80033-6

64. Liu W, Baker S, Baker R, Zhu L. Antioxidant Mechanisms in Nonalcoholic Fatty Liver Disease. Curr Drug Targets. 2015;16(12):1301-1314. doi:10.2174/1389450116666150427155342

65. Videla LA, Rodrigo R, Orellana M, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci. 2004;106(3):261-268. doi:10.1042/CS20030285

66. Price TO, Ercal N, Nakaoke R, Banks WA. HIV-1 viral proteins gp120 and Tat induce oxidative stress in brain endothelial cells. Brain Res. 2005;1045(1-2):57-63. doi:10.1016/j.brainres.2005.03.031

67. Richard MJ, Guiraud P, Didier C, et al. Human immunodeficiency virus type 1 tat protein impairs selenoglutathione peroxidase expression and activity by a mechanism independent of cellular selenium uptake: Consequences on cellular resistance to UV-A radiation. Arch Biochem Biophys. 2001;386(2):213-220. doi:10.1006/abbi.2000.2197

68. Monroy N, Herrero L, Carrasco L, González ME. Influence of glutathione availability on cell damage induced by human immunodeficiency virus type 1 viral protein R. Virus Res. 2016;213:116-123. doi:10.1016/j.virusres.2015.11.017

69. Mehdi K, Penninckx MJ. An Important Role for Glutathione and Y-Glutamyltranspeptidase in the Supply of Growth Requirements during Nitrogen Starvation of the Yeast Saccharomyces Cerevisiae. Vol 143.; 2019. www.microbiologyresearch.org.

70. Teto G, Kanmogne GD, Torimiro JN, et al. Lipid Peroxidation and Total Cholesterol in HAART-Naïve Patients Infected with Circulating Recombinant Forms of Human Immunodeficiency Virus Type-1 in Cameroon. PLoS One. 2013;8(6). doi:10.1371/journal.pone.0065126

71. Jareño EJ, Bosch-Morell F, Fernández-Delgado R, et al. Serum malondialdehyde in HIV-seropositive children negatively correlates with CD4+ lymphocytes count. In: BioFactors. Vol 8. Blackwell Publishing Inc.; 1998:129-132. doi:10.1002/biof.5520080121

72. Aukrust P, Luna L, Ueland T, et al. Impaired base excision repair and accumulation of oxidative base lesions in CD4+ T cells of HIV-infected patients. Blood. 2005;105(12):4730-4735. doi:10.1182/blood-2004-11-4272

73. González-Nicolás J, Resino S, Jiménez JL, et al. Tumor necrosis factor-α and nitric oxide in vertically HIV-1-infected children: Implications for pathogenesis. Eur Cytokine Netw. 2001;12(3):437-44.

74. Ivanov AV, Valuev-Elliston VT, Ivanova ON, et al. Oxidative Stress during HIV Infection: Mechanisms and Consequences. Oxid Med Cell Longev. 2016;2016:8910396. doi: 10.1155/2016/8910396.

75. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11(1):71-128. doi:10.1016/0891-5849(91)90192-6

76. Fujii H, Seki S, Kitada T, et al. In situ detection of lipid peroxidation and oxidative DNA damage in human alcoholic liver disease. J Hepatol. 2002;36:256. doi:10.1016/s0168-8278(02)80923-0

77. Serviddio G, Bellanti F, Tamborra R, et al. Uncoupling protein-2 (UCP2) induces mitochondrial proton leak and increases susceptibility of non-alcoholic steatohepatitis (NASH) liver to ischaemia-reperfusion injury. Gut. 2008;57(7):957-965. doi:10.1136/gut.2007.147496

78. Kim S, Park S, Kim B, Kwon J. Toll-like receptor 7 affects the pathogenesis of non-alcoholic fatty liver disease. Sci Rep. 2016;6:27849. doi:10.1038/srep27849

79. Singh R, Wang Y, Schattenberg JM, et al. Chronic oxidative stress sensitizes hepatocytes to death from 4-hydroxynonenal by JNK/c-Jun overactivation. Am J Physiol Liver Physiol. 2009;297(5):G907-G917. doi:10.1152/ajpgi.00151.2009

80. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183-1192. doi:10.1053/gast.2001.23256

81. Kerner J, Hoppel C. Fatty acid import into mitochondria. Biochim Biophys Acta - Mol Cell Biol Lipids. 2000;1486(1):1-17. doi:10.1016/S1388-1981(00)00044-5

82. Petrosillo G, Portincasa P, Grattagliano I, et al. Mitochondrial dysfunction in rat with nonalcoholic fatty liver. Involvement of complex I, reactive oxygen species and cardiolipin. Biochim Biophys Acta. 2007;1767(10):1260-1267. doi:10.1016/j.bbabio.2007.07.011

83. Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Oxidative stress, cardiolipin and mitochondrial dysfunction in nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20(39):14205-14218. doi:10.3748/wjg.v20.i39.14205

84. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. Semin Liver Dis. 2008;28(4):360-369. doi:10.1055/s-0028-1091980

85. Camello-Almaraz C, Gomez-Pinilla PJ, Pozo MJ, Camello PJ. Mitochondrial reactive oxygen species and Ca2+ signaling. Am J Physiol Cell Physiol. 2006;291(5):1082-1088. doi:10.1152/ajpcell.00217.2006

86. Malhotra JD, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: A vicious cycle or a double-edged sword? Antioxidants Redox Signal. 2007. doi:10.1089/ars.2007.1782

87. Maagaard A, Holberg-Petersen M, Løvgården G, et al. Distinct Mechanisms for Mitochondrial DNA Loss in T and B Lymphocytes from HIV-Infected Patients Exposed to Nucleoside Reverse-Transcriptase Inhibitors and Those Naive to Antiretroviral Treatment. J Infect Dis. 2008;198(10):1474-1481. doi:10.1086/592713

88. Douek DC, Picker LJ, Koup RA. T Cell Dynamics in HIV-1 Infection. Annu Rev Immunol. 2003;21(1):265-304. doi:10.1146/annurev.immunol.21.120601.141053

89. Pérez-Matute P, Pérez-Martínez L, Blanco JR, Oteo JA. Role of mitochondria in HIV infection and associated metabolic disorders: Focus on nonalcoholic fatty liver disease and lipodystrophy syndrome. Oxid Med Cell Longev. 2013;2013. doi:10.1155/2013/493413

90. Ulukaya E, Acilan C, Yilmaz Y. Apoptosis: Why and how does it occur in biology? Cell Biochem Funct. 2011;29(6):468-480. doi:10.1002/cbf.1774

91. Feldstein AE, Canbay A, Angulo P, et al. Hepatocyte apoptosis and Fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology. 2003;125(2):437-443. doi:10.1016/S0016-5085(03)00907-7

92. Candé C, Cecconi F, Dessen P, Kroemer G. Apoptosis-inducing factor (AIF): Key to the conserved caspase-independent pathways of cell death? J Cell Sci. 2002;115(pt24):4727-4734. doi:10.1242/jcs.00210

93. Kinnally KW, Peixoto PM, Ryu SY, Dejean LM. Is mPTP the gatekeeper for necrosis, apoptosis, or both? Biochim Biophys Acta - Mol Cell Res. 2011;1813(4):616-622. doi:10.1016/j.bbamcr.2010.09.013

94. Kakisaka K, Cazanave SC, Fingas CD, et al. Mechanisms of lysophosphatidylcholine-induced hepatocyte lipoapoptosis. Am J Physiol Gastrointest Liver Physiol. 2012;302(1):G77-G84. doi:10.1152/ajpgi.00301.2011

95. Cazanave SC, Elmi NA, Akazawa Y, et al. CHOP and AP-1 cooperatively mediate PUMA expression during lipoapoptosis. Am J Physiol Gastrointest Liver Physiol. 2010. doi:10.1152/ajpgi.00091.2010

96. Hikisz P, Kiliańska ZM. Puma, a critical mediator of cell death - one decade on from its discovery. Cell Mol Biol Lett. 2012;17(4):646-669. doi:10.2478/s11658-012-0032-5

97. Bozaykut P, Sahin A, Karademir B, Ozer NK. Endoplasmic reticulum stress related molecular mechanisms in nonalcoholic steatohepatitis. Mech Ageing Dev. 2016;(157):17-29. doi:10.1016/j.mad.2016.07.001

98. Scorrano L, Oakes SA, Opferman JT, et al. BAX and BAK regulation of endoplasmic reticulum Ca2+: A control point for apoptosis. Science 2003;300(5616):135-139. doi:10.1126/science.1081208

99. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998;94(4):491-501. doi:10.1016/S0092-8674(00)81590-1

100. Milhas D, Cuvillier O, Therville N, et al. Caspase-10 triggers bid cleavage and caspase cascade activation in fasL-induced apoptosis. J Biol Chem. 2005;280(20):19836-19842. doi:10.1074/jbc.M414358200

101. Malhi H, Barreyro FJ, Isomoto H, et al. Free fatty acids sensitise hepatocytes to TRAIL mediated cytotoxicity. Gut. 2007;56(8):1124-1131. doi:10.1136/gut.2006.118059

102. Feldstein AE, Werneburg NW, Canbay A, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-α expression via a lysosomal pathway. Hepatology. 2004;40(1):185-194. doi:10.1002/hep.20283

103. Li ZZ, Berk M, McIntyre TM, et al. The lysosomal-mitochondrial axis in free fatty acid-induced hepatic lipotoxicity. Hepatology. 2008;47(5):1495-1503. doi:10.1002/hep.22183

104. Guicciardi ME, Bronk SF, Werneburg NW, et al. Bid is upstream of lysosome-mediated caspase 2 activation in tumor necrosis factor α-induced hepatocyte apoptosis. Gastroenterology. 2005;129(1):269-284. doi:10.1053/j.gastro.2005.05.022

105. Vlahakis SR, Villasis-Keever A, Gomez TS, et al. Human Immunodeficiency Virus–Induced Apoptosis of Human Hepatocytes via CXCR4. J Infect Dis. 2003. doi:10.1086/379738

106. Babu CK, Suwansrinon K, Bren GD, et al. HIV induces TRAIL sensitivity in hepatocytes. PLoS One. 2009;4(2):1-9. doi:10.1371/journal.pone.0004623

107. Forrester JE, Rhee MS, McGovern BH, Sterling RK, Knox TA, Terrin N. The association of HIV viral load with indirect markers of liver injury. J Viral Hepat. 2012;19(2):e202-e211. doi:10.1111/j.1365-2893.2011.01529.x

108. Perfettini JL, Castedo M, Roumier T, et al. Mechanisms of apoptosis induction by the HIV-1 envelope. Cell Death Differ. 2005. doi:10.1038/sj.cdd.4401584

109. Blanco J, Barretina J, Ferri KF, et al. Cell-surface-expressed HIV-1 envelope induces the death of CD4 T cells during GP41-mediated hemifusion-like events. Virology. 2003;305(2):318-329. doi:10.1006/viro.2002.1764

110. Lifson JD, Feinberg MB, Reyes GR, et al. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. Nature. 1986;323(6090):725-728. doi:10.1038/323725a0

111. Andreau K, Perfettini JL, Castedo M, et al. Contagious apoptosis facilitated by the HIV-1 envelope: Fusion-induced cell-to-cell transmission of a lethal signal. J Cell Sci. 2004;117(23):5643-5653. doi:10.1242/jcs.01486

112. Corbitt N, Kimura S, Isse K, et al. Gut bacteria drive kupffer cell expansion via MAMP-mediated ICAM-1 induction on sinusoidal endothelium and influence preservation-reperfusion injury after orthotopic liver transplantation. Am J Pathol. 2013;182(1):180-191. doi:10.1016/j.ajpath.2012.09.010

113. Schnabl B. Interactions Between the Intestinal Microbiome and Liver Diseases. Gastroenterology. 2015;146(6):1513-1524. doi:10.1053/j.gastro.2014.01.020.

114. Dixon LJ, Barnes M, Tang H, et al. Kupffer Cells in the Liver. Compr Physiol. 2016;3(2):785-797. doi:10.1002/cphy.c120026.

115. Housset C, Boucher O, Girard PM, et al. Immunohistochemical evidence for human immunodeficiency virus-1 infection of liver kupffer cells. Hum Pathol. 1990;21(4):404-408. doi:10.1016/0046-8177(90)90202-G

116. Hufert FT, Schmitz J, Schreiber M, et al. Human kupffer cells infected with HIV-1 in vivo. J Acquir Immune Defic Syndr. 1993;6(7):772-777.

117. Seki E, De Minicis S, Österreicher CH, et al. TLR4 enhances TGF-β signaling and hepatic fibrosis. Nat Med. 2007;13(11):1324-1332. doi:10.1038/nm1663

118. Sacchi P, Cima S, Corbella M, et al. Liver fibrosis, microbial translocation and immune activation markers in HIV and HCV infections and in HIV/HCV co-infection. Dig Liver Dis. 2015;47(3):218-225. doi:10.1016/j.dld.2014.11.012

119. Szabo G. Gut-liver axis in alcoholic liver disease. Gastroenterology. 2015;148(1):30-36. doi:10.1053/j.gastro.2014.10.042

120. Larter CZ, Farrell GC. Insulin resistance, adiponectin, cytokines in NASH: Which is the best target to treat? J Hepatol. 2006;44(2):253-261. doi:10.1016/j.jhep.2005.11.030

121. Johnston AM, Pirola L, Van Obberghen E. Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling. FEBS Lett. 2003;546(1):32-36. doi:10.1016/S0014-5793(03)00438-1

122. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med. 2000;343(20):1467-1476. doi:10.1056/NEJM200011163432007

123. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab. 2007;92(3):1023-1033. doi:10.1210/jc.2006-1055

124. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) Induces Insulin Resistance in 3T3-L1 Adipocytes and Is, Like IL-8 and Tumor Necrosis Factor-α, Overexpressed in Human Fat Cells from Insulin-resistant Subjects. J Biol Chem. 2003;278(46):45777-45784. doi:10.1074/jbc.M301977200

125. Weisberg SP, Leibel RL, Anthony W, et al. Obesity is associated with macrophage accumulation in adipose tissue Find the latest version : Obesity is associated with. J Clin Invest. 2003;112(12):1796-1808. doi:10.1172/JCI200319246.

126. Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology. 2011;54(1):133-144. doi:10.1002/hep.24341

127. Hui JM, Hodge A, Farrell GC, et al. Beyond insulin resistance in NASH: TNF-α or adiponectin? Hepatology. 2004;40(1):46-54. doi:10.1002/hep.20280

128. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12(12):1365-1371. doi:10.1038/nm1511

129. Dandekar S, George MD, Bäumler AJ. Th17 cells, HIV and the gut mucosal barrier. Curr Opin HIV AIDS. 2010;5(2):173-178. doi:10.1097/COH.0b013e328335eda3

130. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med. 2004;200(6):761-770. doi:10.1084/jem.20041196

131. Kim CJ, McKinnon LR, Kovacs C, et al. Mucosal Th17 Cell Function Is Altered during HIV Infection and Is an Independent Predictor of Systemic Immune Activation. J Immunol. 2013;191(5):2164-2173. doi:10.4049/jimmunol.1300829

132. Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. Clin Microbiol Rev. 2013;26(1):2-18. doi:10.1128/CMR.00050-12

133. Nazli A, Chan O, Dobson-Belaire WN, et al. Exposure to HIV-1 Directly Impairs Mucosal Epithelial Barrier Integrity Allowing Microbial Translocation. PLoS Pathog. 2010;6(4):e1000852. doi:10.1371/journal.ppat.1000852

134. Brown TT, Li X, Cole SR, et al. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. AIDS. 2005;19(13):1375-1383. doi:10.1097/01.aids.0000181011.62385.91

135. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: A 3-year randomized trial. J Am Med Assoc. 2004;292(2):191-201. doi:10.1001/jama.292.2.191

136. Crane HM, Grunfeld C, Willig JH, et al. Impact of NRTIs on lipid levels among a large HIV-infected cohort initiating antiretroviral therapy in clinical care. AIDS. 2011;25(2):185-195. doi:10.1097/QAD.0b013e328341f925

137. Gardner K, Hall PA, Chinnery PF, Payne BAI. HIV Treatment and Associated Mitochondrial Pathology:Review of 25 Years of in Vitro, Animal, and Human Studies. Toxicol Pathol. 2014;42(5):811-822. doi:10.1177/0192623313503519

138. Johnson AA, Ray AS, Hanes J, et al. Toxicity of Antiviral Nucleoside Analogs and the Human Mitochondrial DNA Polymerase. J Biol Chem. 2001;276(44):40847-40857. doi:10.1074/jbc.M106743200

139. Birkus G, Hitchcock MJM, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: Comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother. 2002;46(3):716-723. doi:10.1128/AAC.46.3.716-723.2002

140. Schütt M, Zhou J, Meier M, Klein HH. Long-term effects of HIV-1 protease inhibitors on insulin secretion and insulin signaling in INS-1 beta cells. J Endocrinol. 2004;183(3):445-454. doi:10.1677/joe.1.05620

141. Behrens G, Dejam A, Schmidt H, et al. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. AIDS. 1999;13(10):E63-70. doi:10.1097/00002030-199907090-00001

142. Grace A. Lee MR. Effects of ritonavir and amprenavir on insulin sensitivity in healthy volunteers. AIDS. 2007;21(16):2183-2190. doi:10.1097/QAD.0b013e32826fbc54.

143. Busti AJ, Bedimo R, Margolis DM, Hardin DS. Improvement in insulin sensitivity and dyslipidemia in protease inhibitor-treated adult male patients after switch to atazanavir/ritonavir. J Investig Med. 2008;56(2):539-544. doi:10.2310/JIM.0b013e3181641b26

144. Carper MJ, Cade WT, Cam M, et al. HIV-protease inhibitors induce expression of suppressor of cytokine signaling-1 in insulin-sensitive tissues and promote insulin resistance and type 2 diabetes mellitus. Am J Physiol Endocrinol Metab. 2008;294(3):E558-567. doi:10.1152/ajpendo.00167.2007

145. Maximos M, Bril F, Portillo Sanchez P, et al. The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. Hepatology. 2015;61(1):153-160. doi:10.1002/hep.27395

146. Tate T, Willig AL, Willg JH et al. HIV infection and obesity: Where did all the wasting go? Antivir Ther. 2012;17(7):1281-1289. doi:10.1161/CIRCULATIONAHA.110.956839

147. Koethe JR, Jenkins CA, Lau B, et al. Rising Obesity Prevalence and Weight Gain among Adults Starting Antiretroviral Therapy in the United States and Canada. AIDS Res Hum Retroviruses. 2016;32(1):50-58. doi:10.1089/aid.2015.0147

148. White J V., Guenter P, Jensen G, et al. Consensus statement: Academy of nutrition and dietetics and American society for parenteral and enteral nutrition: Characteristics recommended for the identification and documentation of adult malnutrition (undernutrition). J Acad Nutr Diet. 2012;112(5):730-738. doi:10.1177/0148607112440285

149. Redig AJ, Berliner N. Pathogenesis and clinical implications of HIV-related anemia in 2013. Hematol Am Soc Hematol Educ Progr. 2013;2013:377-381. doi:10.1182/asheducation-2013.1.377

150. Irlam JH, Siegfried N, Visser ME, Rollins NC. Micronutrient supplementation for children with HIV infection. Cochrane Database Syst Rev. 2010;8(12):CD003650. doi:10.1002/14651858.CD010666

151. Warriner AH, Mugavero M, Overton ET. Bone alterations associated with HIV. Curr HIV/AIDS Rep. 2014;11(3):233-240. doi:10.1007/s11904-014-0216-x

152. WR B. Nutrition and Immunology: Principles and Practices. Totowa, NJ: Human Press; 2000.

153. Beach RS, Mantero-Atienza E, Shor-Posner G, et al. Specific nutrient abnormalities in asymptomatic HIV-1 infection. AIDS. 1992;6(7):701-708. doi:10.1097/00002030-199207000-00013

154. Baum MK, Shor-Posner G, Lu Y, et al. Micronutrients and HIV-1 disease progression. AIDS. 1995;9(9):1051-1056. doi:10.1097/00002030-199509000-00010

155. Baum, MK. Campa A, Lai Shenghan MS. Effect of Micronutrient Supplementation on Disease Progression in Asymptomatic, Antiretroviral-Naive, HIV-Infected Adults in Botswana A Randomized Clinical Trial. JAMA. 2013;310(20):2154-2163. doi:10.1016/j.physbeh.2017.03.040

156. Baum MK, Lai S, Sales S, et al. Randomized, Controlled Clinical Trial of Zinc Supplementation to Prevent Immunological Failure in HIV-Infected Adults. Clin Infect Dis. 2010;50(12):1653-1660. doi:10.1086/652864

157. Prasad AS. Impact of the discovery of human zinc deficiency on health. J Trace Elem Med Biol. 2014;28(4):357-363. doi:10.3945/an.112.003210.176

158. Martinez SS, Campa A, Li Y, et al. Low Plasma Zinc Is Associated with Higher Mitochondrial Oxidative Stress and Faster Liver Fibrosis Development in the Miami Adult Studies in HIV Cohort. J Nutr. 2017;147(4):556-562. doi:10.3945/jn.116.243832

159. Baum MK, Shor-Posner G, Zhang G, et al. HIV-1 infection in women is associated with severe nutritional deficiencies. J Acquir Immune Defic Syndr Hum Retrovirology. 1997;16(4):272-278. doi:10.1097/00042560-199712010-00008

160. Jones CY, Tang AM, Forrester JE, et al. Micronutrient levels and HIV disease status in HIV-infected patients on highly active antiretroviral therapy in the nutrition for healthy living cohort. J Acquir Immune Defic Syndr. 2006;43(4):475-482. doi:10.1097/01.qai.0000243096.27029.fe

161. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55(6):2005-2023. doi:10.1002/hep.25762

162. Rinella ME, Sanyal AJ. Management of NAFLD: A stage-based approach. Nat Rev Gastroenterol Hepatol. 2016;13(4):196-205. doi:10.1038/nrgastro.2016.3

163. Mastrocola R, Collino M, Rogazzo M, et al. Advanced glycation end products promote hepatosteatosis by interfering with SCAP-SREBP pathway in fructose-drinking mice. Am J Physiol Gastrointest Liver Physiol. 2013;305(6):398-407. doi:10.1152/ajpgi.00450.2012

164. Geidl-Flueck B, Gerber PA. Insights into the hexose liver metabolism—glucose versus fructose. Nutrients. 2017;9(9). doi:10.3390/nu9091026

165. Molinaro A, Wahlström A, Marschall HU. Role of Bile Acids in Metabolic Control. Trends Endocrinol Metab. 2018;29(1):31-41. doi:10.1016/j.tem.2017.11.002

166. Mehal WZ. The gordian knot of dysbiosis, obesity and nafld. Nat Rev Gastroenterol Hepatol. 2013;10(11):637-644. doi:10.1038/nrgastro.2013.146

167. Poeta M, Pierri L, Vajro P. Gut–Liver Axis Derangement in Non-Alcoholic Fatty Liver Disease. Children. 2017;4(8):66. doi:10.3390/children4080066

168. Miura K, Ohnishi H. Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20(23):7381-7391. doi:10.3748/wjg.v20.i23.7381

169. Schilling JD, Machkovech HM, He L, et al. Palmitate and lipopolysaccharide trigger synergistic ceramide production in primary macrophages. J Biol Chem. 2013;288(5):2923-2932. doi:10.1074/jbc.M112.419978

170. Alisi A, Ceccarelli S, Panera N, Nobili V. Causative role of gut microbiota in non-alcoholic fatty liver disease pathogenesis. Front Cell Infect Microbiol. 2012;2(October):132. doi:10.3389/fcimb.2012.00132

171. Spruss A, Bergheim I. Dietary fructose and intestinal barrier: potential risk factor in the pathogenesis of nonalcoholic fatty liver disease. J Nutr Biochem. 2009;20(9):657-662. doi:10.1016/j.jnutbio.2009.05.006

172. Thuy S, Ladurner R, Volynets V, et al. Nonalcoholic Fatty Liver Disease in Humans Is Associated with Increased Plasma Endotoxin and Plasminogen Activator Inhibitor 1 Concentrations and with Fructose Intake. J Nutr. 2008;138(8):1452-1455. doi:10.1093/jn/138.8.1452

173. Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology. 2009;49(6):1877-1887. doi:10.1002/hep.22848

174. Pradere JP, Troeger JS, Dapito DH, et al. Toll-like receptor 4 and hepatic fibrogenesis. Semin Liver Dis. 2010;30(3):232-244. doi:10.1055/s-0030-1255353

175. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761-1772. doi:10.2337/db06-1491

176. Chunyue Yin, Kimberley J. Evason KA et al. Kupffer cells in non-alcoholic fatty liver disease: The emerging view. J Hepatol. 2010;51(1):212-223. doi:10.1016/j.jhep.2009.03.008.

177. Hartzler B, Dombrowski JC, Crane HM, et al. Prevalence and Predictors of Substance Use Disorders Among HIV Care Enrollees in the United States. AIDS Behav. 2017;21(4):1138-1148. doi:10.1007/s10461-016-1584-6

178. Arnsten JH, Demas PA, Farzadegan H, et al. Impact of Active Drug Use on Antiretroviral Therapy Adherence and Viral Suppression in HIV-infected Drug Users. Clin Infect Dis. 2002;33(8):1417-1423. doi:10.1086/323201

179. DeLorenze GN, Weisner C, Tsai AL, et al. Excess Mortality Among HIV-Infected Patients Diagnosed With Substance Use Dependence or Abuse Receiving Care in a Fully Integrated Medical Care Program. Alcohol Clin Exp Res. 2011;35(2):203-210. doi:10.1111/j.1530-0277.2010.01335.x

180. Reback CJ, Larkins S, Shoptaw S. Methamphetamine abuse as a barrier to HIV medication adherence among gay and bisexual men. AIDS Care. 2003;15(6):775-785. doi:10.1080/09540120310001618621

181. Dash S, Balasubramaniam M, Villalta F, et al. Impact of cocaine abuse on HIV pathogenesis. Front Microbiol. 2015;6:1-12. doi:10.3389/fmicb.2015.01111

182. Baum MK, Rafie C, Lai S, et al. Crack-cocaine use accelerates HIV disease progression in a cohort of HIV-positive drug users. J Acquir Immune Defic Syndr. 2009;50(1):93-99. doi:10.1097/QAI.0b013e3181900129

183. Crawford TN, Thornton AC. Alcohol Use and Multimorbidity Among Individuals Living with HIV. AIDS Behav. 2019;23(1):152-160. doi:10.1007/s10461-018-2242-y

184. Shoreibah M, Raff E, Bloomer J, et al. Alcoholic liver disease presents at advanced stage and progresses faster compared to non-alcoholic fatty liver diseas. Ann Hepatol. 2016;15(2):183-189. doi:10.5604/16652681.1193707

185. Deleuran T, Grønbæk H, Vilstrup H, Jepsen P. Cirrhosis and mortality risks of biopsy-verified alcoholic pure steatosis and steatohepatitis: A nationwide registry-based study. Aliment Pharmacol Ther. 2012;35(11):1336-1342. doi:10.1111/j.1365-2036.2012.05091.

186. Tyagi M, Weber J, Bukrinsky M, Simon GL. The effects of cocaine on HIV transcription. J Neurovirol. 2016;22(3):261-274. doi:10.1007/s13365-015-0398-

187. Campa A, Martinez SS, Sherman KE. Cocaine Use and Liver Disease are Associated with All-Cause Mortality in the Miami Adult Studies in HIV (MASH) Cohort. J Drug Abus. 2016;2(4):pii:27. doi:10.21767/2471-853x.100036

188. Lai S, Gerstenblith G, Moore RD, et al. Cocaine use may modify HIV / ART-associated myocardial steatosis and hepatic steatosis. Drug Alcohol Depend. 2017;177:84-92. doi:10.1016/j.drugalcdep.2017.03.029

CHAPTER III: THE TRIGLYCERIDE-GLUCOSE (TYG) INDEX AS A PREDICTOR OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN THE MIAMI ADULT STUDIES ON HIV (MASH) COHORT

Introduction

The prevalence of Non-alcoholic fatty liver disease (NAFLD) in the general population has been estimated to be around 20-24%,[1,2] but some studies have estimated it to be as high as 50% of the population.[3,4] People living with HIV (PLWH) have been previously shown to have higher rates NAFLD compared to the general population.[5,6] The contribution of NAFLD to overall liver disease has increased over the past 20 years from 47-75%.[7] Among obese individuals, the contribution of NAFLD to overall liver disease is even higher, representing between 57-98% total liver disease cases.[2] Individuals of Hispanic ethnicity represent the highest risk of developing NAFLD, followed by whites of European ancestry and it appears Black individuals have the lowest risk of NAFLD development.[8]

The development of NAFLD is thought to be a result of increased insulin resistance (IR),[9] which results most commonly from obesity-related inflammation. A pro-inflammatory state can inhibit the function of hormone sensitive lipase (HSL), thus increasing the mobilization of free fatty acids (FFAs) in the blood steam resulting in their transport to the liver.[10] Furthermore, insulin increases the synthesis of FFAs in the liver by increasing the transcription of sterol regulatory element-binding protein-1c (SREBP1-c).[11] Secondary to an increase in IR, is a subsequent increase in blood glucose concentrations. Increased blood glucose

upregulates carbohydrate responsive element-binding protein (ChREBP) which also increases FFA synthesis in the liver. Dietary factors also appear to play a role in the regulation of SREBP1-c. Cholesterol and saturated fat have both been associated with upregulation of SREBP-1c.[12]

Among PLWH, antiretroviral therapy (ART) use has been a major factor in the development of NAFLD, possibly related to increased IR. Nucleoside reverse transcriptase inhibitors (NRTIs) have traditionally been considered the most harmful classification of ART drugs related to the development of NAFLD. Older NRTIs were associated with increased IR and dyslipidemia.[13-15] NRTIs have been previously shown to decrease lipid oxidative phosphorylation, increase reactive oxygen species (ROS) and lead to an accumulation of lipids in the liver.[16] Newer NRTIs have been shown to have more favorable impacts on metabolic markers.[17-18] Protease inhibitors (PIs) also appear to be detrimental to metabolic health through the development of IR. PIs have been associated with hyperglycemia, hyperinsulinemia and impaired secretion of insulin from beta cells.[19-20] Additionally, PIs may increase adipocyte size due to having an inhibitory effect on glucose transport-4 (GLUT-4).[21] Enlarged adipocytes have been shown to release less adiponectin, worsening liver fat accumulation.[22]

Further complicating the relationship between HIV and metabolic health is cocaine use. The prevalence of cocaine use disorder among PLWH has been estimated between 11-50%.[23,24] Cocaine has been shown to reduce ART adherence and decrease the number of PLWH maintaining a suppressed viral load.[25] Uncontrolled viral load leads to accelerated HIV disease progression and

increased mortality.[23,26] Additionally, HIV VL has been shown to be inversely correlated with BMI in men, explaining one possible mechanism behind reduced bodyweight among PLWH who abuse cocaine.[27]

Combined, multiple stressors including: HIV infection, ART toxicity, insulin resistance, and cocaine use, foster an environment that increases the risk of developing liver disease in PLWH. Due to the difficulty in measuring liver fat in large populations using Magnetic Resonance Imaging – Protein Density Fat Fraction (MRI-PDFF) or liver biopsy, identifying an easier to obtain measure that correlates well with liver steatosis can be a valuable asset in studying liver disease risk in the PLWH population going forward. The triglyceride-glucose index (TyG Index) is a measure of metabolic health that has recently been used as an indicator of IR.[28] The TyG index has been shown to be a superior predictor of NAFLD than the homeostatic model for insulin resistance (HOMA-IR).[29] The TyG Index is composed of fasting triglycerides and blood glucose, both of which are easily obtain using a fasted blood draw and make up two of the five components of metabolic syndrome. Insulin resistance has previously been shown to be a predictor of NAFLD in the general population,[30] but IR was not measured via the TyG index and the study population did not include PLWH. Cross-sectional studies have previously found associations between the TyG Index and NAFLD,[31] but not in a population of PLWH. The purpose of this paper is to determine if the TyG Index is a valid marker of liver steatosis or NAFLD, in PLWH and to determine the effect of HIV infection and cocaine use on liver steatosis risk.

Methods:

Study Participants: All participants for this cross-sectional study were enrolled in the Miami-Adult Studies in HIV (MASH) cohort. The MASH cohort is a longitudinal study cohort with the primary focus of determining the effect of HIV, Hepatitis-C Virus (HCV), HIV/HCV and common co-morbidities associated with these infections on liver disease progression. Inclusion criteria for the current study include HIV monoinfection, referred to as PLWH, or the absence of HIV and HCV infection (uninfected controls). Individuals with HCV were not included in these analyses. To be considered a cocaine user for this study participants had to have either stated they were cocaine users in a drug screening questionnaire, blood metabolite testing or urine toxicology screen (American Bio Medical, Kinderhook, NY). Alcohol use was assessed by the Alcohol Use Disorders Identification Test (AUDIT).[32] For analyses of the PLWH only group, only individuals who reported using ART within the last 6 months were included. All participants were required to complete an MRI scan to assess liver steatosis to be included in final analysis. After exclusion criteria were applied there were a total of 480 participants included in analysis for this study, 211 PLWH, and 269 uninfected controls. All methodologies were approved by the Institutional Review Board (IRB) # IRB-20-0273 at Florida International University.

Metabolic syndrome criteria. The criteria used to determine metabolic syndrome are the same criteria established by the National Cholesterol Education Program-

Adult Treatment Panel III (NCEP-ATP III) cohort.[33] The NCEP-ATP criteria were used to categorize participants dichotomous metabolic syndrome status. The NCEP ATP III defines metabolic syndrome as three or more of the following risk factors: waist circumference (WC) > 40 inches for men or 35 inches for women, blood pressure over 130/85 mmHg, fasting triglycerides (TG) > 150 mg/dl, fasting HDL cholesterol <40 mg/dl in men or <50 mg/dl in women, and fasting blood glucose >100 mg/dl.

HIV Variables: In participants with HIV monoinfection, HIV Viral Load (VL) and $CD4^+$ T Cell counts were obtained from the participants' charts with written permission. ART use was self-reported over the past six months.

Insulin resistance was measured via the TyG Index using the equation below.[28] Both fasting triglycerides and fasting glucose are obtained from the blood draw from the research nurse and sent to Laboratory Corporation of America as described in the section above.

$$Ln\left[\frac{fasting\ TG\ \left(\frac{mg}{dL}\right) * fasting\ glucose\ \left(\frac{mg}{dL}\right)}{2}\right]$$

Dietary Assessment: Each participant completed a 24-hour food recall administered to them by a trained research assistant. Food items were entered into the NutriBase nutrient analysis software. Dietary recalls were analyzed for total calories, total fat, saturated fat, and total cholesterol intake.

Liver steatosis: Liver fat % is calculated using the proton density fat fraction (PDFF) score from the magnetic resonance elastography (MRE) scan. Each MRE scan is performed by a trained technician on participants who are deemed eligible after a standardized MRE pre-screening questionnaire. PDFF is converted to liver fat % with the following calculation.[34] Participants were categorized into the liver steatosis group if they had >5% liver fat.[34]

$$\frac{[100 * (Fat\ to\ Water\ Ratio]}{[1 + (Fat\ to\ Water\ Ratio)]}$$

Statistical Analyses

Descriptive statistics including mean and standard deviation were used to describe participant characteristics. Differences between the PLWH and uninfected control groups were detected using independent t-tests and chi-square tests. HIV VL was not normally distributed and was Log10 transformed to bring distribution closer to normalization. Chi-square analysis was used to compare the odds of meeting metabolic syndrome criteria between study groups as well as to compare odds of meeting metabolic syndrome criteria when participants were separated by liver steatosis status. Unadjusted logistic regression analysis was used to compare the relationship between all relevant demographic variables and covariates with liver steatosis risk. Multivariate logistic regression was performed to combine relevant demographic variables and covariates in a single model to assess whether the relationships between variables of interest, TyG Index and HIV status, were independent of one

another. All covariates included in multivariate regression analysis were included based on previous relevant literature. An ANCOVA was performed to determine the estimated TyG Index mean when participants were divided by liver steatosis status while HIV status and cocaine use were controlled for as covariates. A receiver-operator curve (ROC) analysis was performed to determine the predictive ability of the TyG Index on the outcome of liver steatosis in different study groups. Comparison of ROC Curves between PLWH only and the Uninfected Control group were performed using the DeLong test in XL Stat.[35]

Results

Participant characteristics: There were 480 total participants included in analysis for this study, 211 PLWH and 269 uninfected controls. The control group was older than the PLWH group (54.85 years ± 7.13 vs. 53.18 ± 7.57, P=0.014). The uninfected control group also had a lower BMI than the PLWH group (30.05 kg/m^2 ± 6.37 vs. 28.74 ± 6.05, P=0.022). There were no differences between study groups in terms of sex or race. Only one ATP-III metabolic syndrome criteria were different between study groups. Waist circumference was higher in the control group 38.54 in. ± 6.14 compared to the PLWH group 37.42 ± 5.53, P=0.044. The prevalence of liver steatosis and cocaine use was also not different between study groups.

HIV and metabolic syndrome: Table 2 shows the prevalence of each of the five ATP-III metabolic syndrome criteria by study group. There were no differences between study groups for any of the five metabolic syndrome criteria or for the prevalence of metabolic syndrome overall.

Liver Steatosis and Metabolic Syndrome: Table 3 describes the close relationship between liver steatosis and metabolic syndrome. Individuals with liver steatosis are more likely to meet the ATP-III criteria for metabolic syndrome in four of the five criteria. The only metabolic syndrome criteria not associated with liver steatosis was high blood pressure.

Dietary Intake Pearson Correlations: Table 4. Pearson correlation analysis found no association between the TyG Index or Liver Fat% and any of the four dietary variables studied (Total Calories, Total Fat, Saturated Fat, and Total Cholesterol).

Unadjusted Associations with liver steatosis: Table 5. Unadjusted regression analysis found age, sex, and race/ethnicity were all demographic factors not significantly associated with liver steatosis. Greater BMI [OR= 1.118 (1.075-1.164, P<0.001)] and increased TyG Index [OR= 3.065 (2.130-4.409, P<0.001)] were both strongly associated with greater odds of liver steatosis. Higher Log10 HIV VL was marginally significant associated with lower odds of liver steatosis [OR= 0.718 (0.500-1.030), P=0.072], but this relationship did not reach statistical significance. There was no association between CD4 $^{+}$count and liver steatosis. Cocaine use was associated with a decreased likelihood of having

metabolic syndrome, the prevalence of metabolic syndrome was 12.8% among cocaine users and 20.7% among non-cocaine users respectively (P=0.027).

Multivariate Regression Analyses: Table 5. A one unit increase in the TyG index was independently associated with greater likelihood of liver steatosis [OR= 2.869 (1.960-4.200), P<0.001]. Higher BMI was also associated with greater odds of liver steatosis [1.104 (1.056-1.155), P<0.001]. There was no association between HIV status and liver steatosis.

ANCOVA: Table 6. The estimated mean TyG Index value of the liver steatosis group was 0.517 units higher (8.949 ± 0.068 vs. 8.432 ± 0.031, P<0.001) compared to the non-steatosis group when controlling for cocaine and HIV status.

ROC Analysis: Table 7. When the PLWH group and uninfected control groups were combined in a single analysis, the AUC=0.712 ± 0.031. The AUC of the PLWH group was a marginally better predictor of liver steatosis than the AUC of the uninfected control group (0.738 ± 0.049 vs. 0.702 ± 0.401, P=0.068) (Table 9). The optimal predictive cut-off value for TyG Index to predict liver steatosis in the PLWH group was 8.5938. In PLWH, chi-square analysis found individuals with TyG Index values >8.5938 (High Risk) were more likely to have liver steatosis [4.638 (2.075, 10.368), P<0.001] than participants with TyG Index values <8.5938 (Low Risk) (Table 10).

Discussion

The key finding in these analyses was the TyG Index was a moderately good predictor of liver steatosis in the MASH Cohort. This study was able to

establish an optimum TyG Index cutoff value as 8.5938 in PLWH with a sensitivity of 0.697 and a specificity of 0.669 to predict liver steatosis. In PLWH, individuals with a TyG score > 8.5938 were more than 4x more likely to have liver steatosis compared to individuals with a TyG score <8.5938. The optimal TyG Index cut-off value in the current study was higher than found by Sterling et al. 2021, who found the optimum cut-off value to predict liver steatosis in a cohort of HBV and HBV-HIV coinfected participants was 8.38. In the current study, the total AUC of uninfected controls (0.702) and among PLWH (0.738) were similar to values found by Sterling et al. among HBV only participants (0.70) and HBV-HIV coinfected participants (0.76).[36]

When controlling for cocaine use and HIV infection, the estimated mean TyG Index of participants with liver steatosis was 0.517 higher than participants without liver steatosis. This difference is not as large as was found by Fedchuk et al. 2014, which found a TyG Index value of 8.8 among participants with mild steatosis compared to 8.0 among participants without steatosis present. However, this study had participants with a much greater prevalence of liver steatosis compared to the current study.[37]

The development of NAFLD is closely linked to IR and it has been previously shown that the TyG Index may be a better predictor of NAFLD than the traditional biomarker HOMA-IR.[29] However, to the best of our knowledge, the TyG Index has not been studied in an HIV monoinfected population, or compared an uninfected control group to an HIV monoinfected population. A previous review of NAFLD and HIV identified insulin resistance as a major risk factor for

NAFLD development in PLWH.[38] Interestingly in the current study, HIV infection was not associated with an increased likelihood of liver steatosis in univariate or multivariate models. This may be due to a number of factors including: age, waist circumference, BMI, and improvements in ART technology. The PLWH group was younger than the uninfected control group, had a lower BMI, and had smaller mean waist circumference, each of these factors is likely to decrease the number of participants with liver steatosis.[39,40] Additionally, the overall low rate of liver steatosis in this study population likely made it more difficult to find statistically significant differences between study groups. The current study population was found to have a steatosis prevalence of 17.5%, lower than the majority of studies reviewed by Soti et al.[38] This low steatosis rate is despite all participants in the PLWH group self-reporting ART use in the past 6 months. The effect of ART on NAFLD remains controversial, Crum-Cianflone et al. 2009 found no association between ART and NAFLD,[40] while more recently Vuille-Lessard et al. 2016 found PI use was associated with liver fibrosis, but not NAFLD.[41] In the current study, only participants with self-reported ART adherence were included in the PLWH group analysis, because this group did not have a greater prevalence of NAFLD than uninfected controls, it does not appear ART increased steatosis risk, even though the effect of ART was not directly assessed.

A major difference between this study population compared to most other HIV cohorts studying NAFLD is the racial makeup of the MASH Cohort. The MASH cohort enrolls a predominately Black population (62.1%), and previous studies have found lower rates of liver steatosis among Black participants

compared to Hispanic populations.[8] In the current study, it appeared as though Hispanic ethnicity may be associated with increased risk of liver steatosis compared to Black participants, (21.8% vs. 16.4%), however the sample size in the current study was not large enough to detect a statistically significant difference.

In univariate analysis, cocaine use was associated with lower odds of having liver steatosis among the entire study population [0.563 (0.339-0.936), P=0.027]; however, in multivariate analysis cocaine use was no longer associated with a decreased odds of liver steatosis. Previous research on cocaine and BMI is conflicting. Soni et al. found no association between cocaine use and obesity prevalence and Escobar et al. found no association between severity of crack-cocaine use and BMI.[42,43] However, past reviews have described increased rates of anorexia, malnutrition and weight loss among cocaine users.[44] Further investigation into the effect of cocaine on insulin resistance in PLWH is needed.

There were no associations between any TyG Index or liver fat% and total calories, saturated fat, total fat, or total cholesterol intake. There was no association between total calorie intake and BMI (data not shown). The lack of any dietary associations is likely more related to each participants ability to recall their dietary intake and less associated with actual dietary intake. Further studies looking more directly at the relationship between dietary intake, liver fat and insulin resistance in PLWH should be pursued.

Conclusion

The TyG Index appears to be a moderately good predictor of liver steatosis among PLWH and in the uninfected control group. It also appears the TyG index may be a slightly netter predictor of steatosis among PLWH. This may be due to PLWH being exposed to multiple stressors to their liver that increase the likelihood of metabolic dysfunction and may lead to increased likelihood of steatosis. Furthermore, it does not appear as though HIV infection or cocaine use are independent risk factors for NAFLD. Future studies should look to investigate the relationship between high metabolic risk and TyG Index on the likelihood of developing liver steatosis, fibrosis, and other comorbidities over time.

Table1. Participant Characteristics

Participants	Total N= 480	PLWH (N=211)	Uninfected Controls (N=269)	P-Value
Age(years)	54.12 ± 7.367	53.18 ± 7.574	54.85 ± 7.129	**0.014**
Sex	46.3%F 53.8%M	43.6%F 56.4%M	48.3% 51.7%	0.303
Race				
Black (non-Hispanic)	62.1%	66.4%	58.7%	0.088
White (Non-Hispanic)	7.9%	7.1%	8.6%	0.562
Hispanic	29.6%	25.6%	32.7%	0.090
Other	0.4%	-----	----	----
BMI (kg/m^2)	29.47 ± 6.26	28.74 ± 6.05	30.05 ± 6.37	**0.022**
TyG Index	8.52 ± 0.654	8.585 ± 0.684	8.475 ± 0.628	0.069
Liver Steatosis Prevalence	84/480 17.5%	32/211 15.2%	52/269 19.3%	0.233
Cocaine Use Prevalence	195/580 40.6%	78/211 37.0%	117/269 43.5%	0.148
AUDIT Score	5.05 ± 6.59	4.60 ± 6.21	5.40 ± 6.87	0.185
AUDIT >8	109/480 22.7%	44/211 20.9%	65/269 24.2%	0.390
Log10 HIV VL		0. 759 ± 1.315		
Controlled HIV VL (<200 copies/ml)		88.8%		
CD4$^+$ (cells/μL)		603.79 ± 382.93		
Obesity (BMI >30)	42.1%	39.3%	44.2%	0.280
Waist Circumference (In)	38.06 ± 5.906	37.42 ± 5.528	38.54 ± 6.139	**0.044**
Glucose (mg/dL)	98.11 ± 46.96	99.53 ± 55.31	97.00 ± 39.23	0.559
Triglycerides (mg/dL)	127.2 ± 89.00	133.67 ± 90.71	122.1 ± 87.47	0.159
SBP (mmHg)	130.2 ± 20.58	128.5 ± 19.91	131.5 ± 21.05	0.122
DBP (mmHg)	81.91 ± 12.80	81.86 ± 12.86	81.94 ± 12.77	0.942
HDL (mg/dL)	58.21 ± 18.76	57.30 ± 18.58	58.93 ± 18.90	0.345

Bold P-Values indicate statistical significance (P<0.05). Data for continuous variables is presented as mean ± STD. Statistical differences between study groups was detected using independent t-tests for continuous variables and Chi-Square tests for categorical variables. BMI: Body Mass Index, TyG Index: Triglyceride-Glucose Index, AUDIT: Alcohol Use Disorders Identification Tests, HIV VL: HIV Viral Load, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL: high-density lipoprotein.

Table 2. Chi-Square analysis comparing the likelihood of meeting metabolic syndrome criteria between PLWH and Uninfected Controls

	Control Group	PLWH	Chi-Square	P-Value	OR (95% CI)
High Glucose ≥100 mg/dL	56/268 20.9%	50/211 23.7%	0.538	0.464	1.176 (0.763-1.183)
Low HDL <40 mg/dL men, <50 mg/dL women	57/269 21.2%	47/211 22.3%	0.082	0.775	1.066 (0.689-1.650)
High BP ≥130 mmHg SBP or ≥ 85 mmHg DBP	151/269 56.1%	116/211 55.0%	0.064	0.853	0.954 (0.664-1.371)
High TRG ≥ 150 mg/dL	57/269 21.1%	56/210 26.7%	1.963	0.161	1.352 (0.886-2.065)
High WC ≥40 inches men ≥35 inches women	209/266 78.6%	151/198 76.3%	0.348	0.555	0.876 (0.565-1.359)
ATP-III Met S ≥ 3 Met S Criteria	79/265 29.8%	62/197 31.5%	0.147	0.701	1.081 (0.725-1.612)

Bold P-Values indicate statistical significance (P<0.05).

Table 3. Chi-square analysis describing the relationship between liver steatosis and each of the five ATP-III metabolic syndrome criteria

	No Liver Steatosis	Liver Steatosis Present	Chi-Square	P-Value	OR (95% CI)
High Glucose ≥100 mg/dL	70/395 17.7%	36/84 42.9%	25.397	**<0.001**	3.482 (2.105-5.761)
Low HDL <40 mg/dL men, <50 mg/dL women	76/396 19.2%	28/84 3.3%	8.165	**0.004**	2.105 (1.254-3.534)
High BP ≥130 mmHg SBP or ≥ 85 mmHg DBP	217/396 54.8%	50/84 59.5%	0.627	0.428	1.213 (0.752-1.957)
High TRG ≥ 150 mg/dL	77/395 19.5%	36/84 42.9%	20.976	**<0.001**	3.097 (1.881-5.100)
High WC ≥40 inches men ≥35 inches women	280/380 73.7%	80/84 95.2%	18.378	**<0.001**	7.143 (2.551-20.004)
ATP-III Met S ≥ 3 Met S Criteria	91/378 24.1%	50/84 59.5%	40.730	**<0.001**	4.638 (2.826-7.612)

Bold P-Values indicate statistical significance (P<0.05).

Table 4. Pearson Correlation between TyG Index and Dietary Intake Variables

	Total Calories	Saturated Fat (g)	Total Fat (g)	Total Cholesterol (mg)
TyG Index	-0.051 P=0.483	-0.032 P=0.657	-0.012 P=0.868	0.041 P=0.575
Liver Fat%	-0.067 P=0.359	-0.068 P=0.352	-0.053 P=0.471	0.044 P=0.547

Bold P-Values indicate statistical significance (P<0.05).

Table 5. Unadjusted univariate and multivariate logistic regression analysis displaying the relationship between covariates and liver steatosis (All Participants)

	Prevalence of Liver Steatosis	Unadjusted OR (95% CI)	P-Value	Adjusted OR (95% CI)	P-Value
Age		0.996 (0.964-1.028)	0.782	0.995 (0.959-1.032)	0.786
Sex		0.833 (0.520-1.355)	0.448	0.940 (0.551-1.602)	0.820
Female (ref)	33.3%				
Male	28.1%				
Race/Ethnicity					
White non-Hispanic	4/34 (4.8%)	0.598 (0.203-1.761)	0.351		
Hispanic	31/ 142 (21.8%)	1.419 (0.859-2.345)	0.172		
Black non-Hispanic (reference)	49/298 (16.4%)	-	-		
BMI		1.118 (1.075-1.163)	**<0.001**	1.104 (1.056-1.155)	**<0.001**
TyG Index		3.065 (2.130-4.409)	**<0.001**	2.869 (1.960-4.200)	**<0.001**
Log10 HIV VL		0.718 (0.500-1.030)	0.072		
$CD4^+$ Count (Per 100 Cells)		1.039 (0.934-1.155)	0.482		
Cocaine Use		0.563 (0.339-0.936)	**0.027**	0.639 (0.367-1.114)	0.639
Cocaine Users	12.8%				
Non-Cocaine Users	20.7%				
Heavy Alcohol Users (AUDIT >8)		1.260 (0.733-2.166)	0.402		
AUDIT >8	20.2%				
AUDIT <8	16.7%				

Bold P-Values indicate statistical significance (P<0.05). Adjusted analysis also included HIV status.

Table 6. ANCOVA analysis comparing estimated mean TyG Index of participants with steatosis to those without steatosis.

	Estimated Means	F	P-Value
No Steatosis	8.432 ± 0.031	47.094	**<0.001**
Liver Steatosis	8.949 ± 0.068		

Bold P-Values indicate statistical significance (P<0.05). Model controlled for HIV infection and cocaine use.

Table 7. ROC Curve Analysis describing the relationship between TyG Index and liver steatosis

	AUC	Std. Error	AUC ≠ 0.5 P-Value	95% CI
Uninfected Controls N=268	0.702	0.041	**<0.001**	0.622-0.781
PLWH N=210	0.738	0.049	**<0.001**	0.642-0.834
Groups Combined	0.712	0.031	**<0.001**	0.651-0.774

Bold P-Values indicate statistical significance.

Table 8. Sensitivity and Specificity Analysis of TyG Risk and Liver Steatosis (PLWH Only)

	No Liver Steatosis	Liver Steatosis
Low TyG Risk <8.5938	121	10
High TyG Risk >8.5938	60	23

Sensitivity = 0.697, Specificity = 0.669

Table 9. Comparison of the AUCs between PLWH

Test	PLWH Only	All Participants
P-Value		
PLWH Only	1	0.068
Uninfected Controls	0.068	1

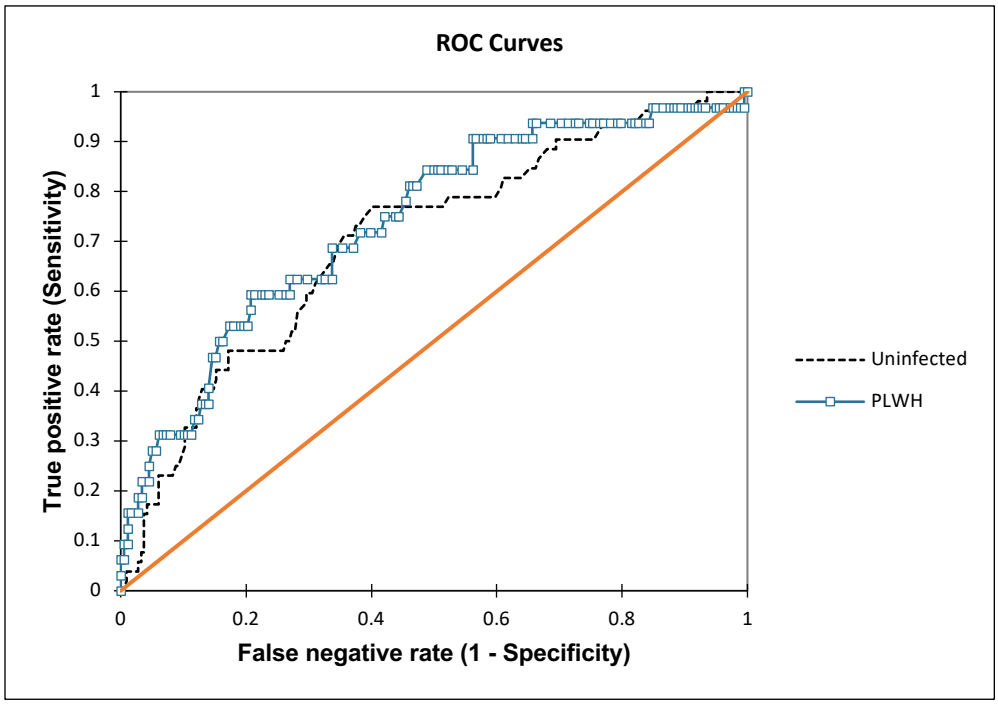
Bold P-Values indicate statistical significance (P<0.05).

Table 10. Chi-Square analysis showing the risk of liver steatosis in low vs. high TyG risk categories (PLWH Only)

	Prevalence of Liver Steatosis	Chi Square	Estimate	P-Value
Low TyG Risk (<8.5938)	10/131 (7.6%)	15.703	4.638 (2.075-10.368)	**<0.001**
High TyG Risk (>8.5938)	23/83 (27.7%)			

Bold P-Values indicate statistical significance.

Figure 1. Comparison of ROC Curves for PLWH and Uninfected Groups.



References

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. doi:10.1002/hep.28431

2. Vernon G, Baranova A, Younossi ZM. Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34(3):274-285. doi:10.1111/j.1365-2036.2011.04724.x

3. Bedogni G, Miglioli L, Masutti F, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: The dionysos nutrition and liver study. Hepatology. 2005;42(1):44-52. doi:10.1002/hep.20734

4. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. Gastroenterology. 2011;140(1):124-131. doi:10.1053/j.gastro.2010.09.038

5. Maurice JB, Patel A, Scott AJ, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. AIDS. 2017;31(11):1621-1632. doi:10.1097/QAD.0000000000001504

6. Seth A, Sherman KE. Fatty liver disease in persons with HIV infection. Top Antivir Med. 2019 May;27(2):75-82. PMID: 31136997; PMCID: PMC6550355.

7. Younossi ZM, Stepanova M, Afendy M, et al. Changes in the Prevalence of the Most Common Causes of Chronic Liver Diseases in the United States From 1988 to 2008. Clin Gastroenterol Hepatol. 2011;9(6):524-530.e1. doi:10.1016/j.cgh.2011.03.020

8. Wagenknecht LE, Scherzinger A, Stamm E, et al. Correlates and heritability of nonalcoholic fatty liver disease in a minority cohort. Obesity (Silver Spring). 2009;17(6):1240-1246. doi:10.1038/oby.2009.4.

9. Sakurai M, Takamura T, Ota T, et al. Liver steatosis, but not fibrosis, is associated with insulin resistance in nonalcoholic fatty liver disease. J Gastroenterology. 2007. 42(4):312-7. doi:10.1007/s00535-006-1948-

10. Hales CN, Luzio JP, Siddle K. Hormonal control of adipose-tissue lipolysis. Biochem Soc Symp. 1978;43:97-135. doi:10.1079/pns19750044

11. Xu X, So JS, Park JG, Lee AH. Transcriptional control of hepatic lipid metabolism by SREBP and ChREBP. Semin Liver Dis. 2013;33(4):301-311. doi:10.1055/s-0033-1358523

12. Jump DB. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr Opin Lipidol. 2002;13(2):155-164. doi:10.1097/00041433-200204000-00007

13. Brown TT, Li X, Cole SR, et al. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. AIDS. 2005;19(13):1375-1383. doi:10.1097/01.aids.0000181011.62385.91

14. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: A 3-year randomized trial. JAMA. 2004;292(2):191- 201. doi:10.1001/jama.292.2.191

15. Crane HM, Grunfeld C, Willig JH, et al. Impact of NRTIs on lipid levels among a large HIV-infected cohort initiating antiretroviral therapy in clinical care. AIDS. 2011;25(2):185-195. doi:10.1097/QAD.0b013e328341f925

16. Gardner K, Hall PA, Chinnery PF, Payne BAI. HIV Treatment and Associated Mitochondrial Pathology:Review of 25 Years of in Vitro, Animal, and Human Studies. Toxicol Pathol. 2014;42(5):811-822. doi:10.1177/0192623313503519

17. Johnson AA, Ray AS, Hanes J, et al. Toxicity of Antiviral Nucleoside Analogs and the Human Mitochondrial DNA Polymerase. J Biol Chem. 2001;276(44):40847-40857. doi:10.1074/jbc.M106743200

18. Birkus G, Hitchcock MJM, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: Comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother. 2002;46(3):716-723. doi:10.1128/AAC.46.3.716-723.2002

19. Schütt M, Zhou J, Meier M, Klein HH. Long-term effects of HIV-1 protease inhibitors on insulin secretion and insulin signaling in INS-1 beta cells. J Endocrinol. 2004;183(3):445-454. doi:10.1677/joe.1.05620

20. Behrens G, Dejam A, Schmidt H, et al. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. AIDS. 1999;13(10):E63-70. doi:10.1097/00002030-199907090-00001

21. Carper MJ, Cade WT, Cam M, et al. HIV-protease inhibitors induce expression of suppressor of cytokine signaling-1 in insulin-sensitive tissues and promote insulin resistance and type 2 diabetes mellitus. Am J Physiol - Endocrinol Metab. 2008;294(3):E558-567. doi:10.1152/ajpendo.00167.2007

22. Maximos M, Bril F, Portillo Sanchez P, et al. The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. Hepatology. 2015;61(1):153-160. doi:10.1002/hep.27395

23. Baum MK, Rafie C, Lai S, et al. Crack-cocaine use accelerates HIV disease progression in a cohort of HIV-positive drug users. J Acquir Immune Defic Syndr. 2009;50(1):93-99. doi:10.1097/QAI.0b013e3181900129

24. Hartzler B, Dombrowski JC, Crane HM, et al. Prevalence and Predictors of Substance Use Disorders Among HIV Care Enrollees in the United States. AIDS Behav. 2017;21(4):1138-1148. doi:10.1007/s10461-016-1584-6

25. Arnsten JH, Demas PA, Farzadegan H, et al. Impact of Active Drug Use on Antiretroviral Therapy Adherence and Viral Suppression in HIV-infected Drug Users. Clin Infect Dis. 2002;33(8):1417-1423. doi:10.1086/323201

26. Campa A, Martinez SS, Sherman KE. Cocaine Use and Liver Disease are Associated with All-Cause Mortality in the Miami Adult Studies in HIV (MASH) Cohort. J Drug Abus. 2016;2(4):pii:27. doi:10.21767/2471-853x.100036

27. Blashill AJ, Mayer KH, Crane HM, et al. Body mass index, immune status, and virological control in HIV-infected men who have sex with men. J Int Assoc Provid AIDS Care. 2013 Sep-Oct;12(5):319-24. doi:10.1177/2325957413488182.

28. Simental-Mendía, LE, Rodriguez-Moran M, Guerro-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. Metab Syndr Relat Disord. 2008;6(4):299-304. doi:10.1089/met.2008.0034

29. Lee SB, Kim MK, Kang S, et al. Triglyceride glucose index is superior to the homeostasis model assessment of insulin resistance for predicting nonalcoholic fatty liver disease in Korean adults. Endocrinol Metab. 2019;34(2):179-186. doi:10.3803/EnM.2019.34.2.179

30. Lallukka S, Sädevirta S, Kallio MT, et al. Predictors of Liver Fat and Stiffness in Non-Alcoholic Fatty Liver Disease (NAFLD) - An 11-Year Prospective Study. Sci Rep. 2017;7(1):1-10. doi:10.1038/s41598-017-14706-0.

31. Zhang S, Du T, Zhang J, et al. The triglyceride and glucose index (TyG) is an effective biomarker to identify nonalcoholic fatty liver disease. Lipids Health Dis. 2017;16(1):15. doi:10.1186/s12944-017-0409-6

32. Saunders, JB, Aasland OG, Babor TF, et al. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption-II. *Addiction*. 1993;88(6):791-804. doi:10.1111/j.1360-0443.1993.tb02093.x

33. Lipsy RJ. The National Cholesterol Education Program Adult Treatment Panel III guidelines. J Manag care Pharm. 2003;9(1 Suppl):2-5. doi:10.18553/jmcp.2003.9.s1.2

34. Tang A, Tan J, Sun M, et al. Nonalcoholic Fatty Liver Disease: MR Imaging of Liver Proton Density Fat Fraction to Assess Hepatic Steatosis. Radiology. 2013;267(2):422-431.doi:10.1148/radiol.12120896

35. Venkatraman ES. A permutation test to compare receiver operating characteristic curves. Biometrics. 2000;56(4):1134-8. doi: 10.1111/j.0006-341x.2000.01134.x.

36. Sterling RK, King WC, Khalili M, et al. Performance of Serum-Based Scores for Identification of Mild Hepatic Steatosis in HBV Mono-infected and HBV-HIV Co-infected Adults. Dig Dis Sci. 2021. Feb 8. doi:10.1007/s10620-021-06860-3

37. Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2014. 40(10):1209-22. doi:10.1111/apt.12963

38. Soti S, Corey KE, Lake JE, et al. NAFLD and HIV: Do Sex, Race, and Ethnicity Explain HIV-Related Risk?. Current HIV/AIDS Rep. 2018. 15(3):212-222. doi:10.1007/s11904-018-0392-1

39. Akl L, Valadares ALR, Moraes MJ, et al. Metabolic syndrome in HIV-infected middle-aged women on antiretroviral therapy: prevalence and associated factors. Braz J Infect Dis. 2017. 21(3):263-269. doi:10.1016/j.bjid.2017.02.003

40. Crum-Cianflone N Dilay A, Collins G, et al. Nonalcoholic fatty liver disease among HIV-infected persons. J Acquir Immune Defic Syndr. 2009. 50(5):464-73.doi:10.1097/QAI.0b013e318198a88a

41. Vuille-Lessard E, Lebouche B, Lennox L, et al. Nonalcoholic fatty liver disease diagnosed by transient elastography with controlled attenuation parameter in unselected HIV monoinfected patients. AIDS. 2016. 30(17):2635-2643.doi:10.1097/QAD.0000000000001241

42. Soni M, Rodriguez VJ, Babayigit S, et al. Blood Pressure, HIV, and Cocaine Use Among Ethnically and Racially Diverse Individuals. South Med J. 2018. 111(11):643-648. doi:10.14423/SMJ.0000000000000893

43. Escobar M, Scherer JN, Ornell F, et al. Leptin levels and its correlation with crack-cocaine use severity: A preliminary study. Neurosci Lett. 671:56-59. doi:10.1016/j.neulet.2018.02.009

44. Cregler LL. Adverse health consequences of cocaine abuse. J Natl Med Assoc.1989;81(1):27-38. doi:10.1037/025942

CHAPTER IV: THE TYG INDEX AND LIVER STEATOSIS ARE ASSOCIATED WITH GREATER LEVELS OF IMMUNE ACTIVATION, AND LIVER FIBROSIS BIOMARKERS IN THE MIAMI ADULT STUDIES ON HIV (MASH) COHORT

Introduction

Human Immunodeficiency Virus (HIV) Infection has been shown to disrupt inflammatory pathways early in the course of infection.[1] Individuals who start early anti-retroviral therapy (ART) within three weeks of HIV infection maintained greater levels of microbial translocation even though their HIV viral load (VL) remained controlled.[2] Microbial translocation promotes liver fibrosis through activation of Kupffer cells and Hepatic Stellate Cells (HSCs).[3] Activated HSCs are associated with higher levels of tissue inhibitor of metalloproteinases (TIMPs) which increase during fibrosis by inhibiting the breakdown of the extracellular matrix (ECM) and not allowing fibrosis to resolve.[4,5]

Microbial translocation also leads directly to the secretion of pro-fibrosis cytokines such as transforming growth factor-β (TGF-β), which promotes collagen production[6] and monocyte activation markers like soluble CD14 (sCD14) and soluble CD163 (sCD163).[7,8] The relationship between microbial translocation and immune activation is likely driven by HIV VL because individuals on ART therapy have reduced levels of immune activation compared to those not on ART, even though a higher level of immune activation persists compared to uninfected individuals.[9,10] Higher VL is evidence of weaker immune function and reduced gut integrity which leads to higher levels of circulating lipopolysaccharide (LPS) which activates the toll-like receptor-4 (TLR-4)

pathway.[11,12] In PLWH, TGF-β has become a well-established marker of liver fibrosis;[13] however, the direct effect of HIV on TGF-β is still not fully understood.[14,15] Kupffer cells produce TGF-β in the liver in response to oxidative stress (OS), activating HSCs and increasing collagen production.[16,17]

In the PLWH, HIV may deplete Kupffer cells and reduce HSC activation, but an overall inflammatory effect remains due to a decreased ability to clear microbial translocation products from the liver.[18,19] Additionally, PLWH have reduced gut microbial diversity compared to uninfected controls.[20] Damage to the mucosa layer of the intestine from HIV infection provides another avenue for microbial products such as LPS to leak from the intestine and travel to the liver via the portal vein.[22] The disruption of the mucosal layer is likely due to a depletion of $CD4^+$ cells in the gut, but also depletion of CD22.[23, 24] A depleted mucosal layer leads to increased production of inflammatory cytokines by gut enterocytes and disruption of the tight junctions between enterocytes which promotes a loss of gut integrity.[25]

Increased microbial translocation products as a result of dysbiosis or gut endothelial damage may also contribute to NAFLD development. Visceral adipose tissue and Kupffer cells respond to TLR-4 with increased production of TNF-α and IL-6, both cytokines may contribute to insulin resistance (IR)[26,27] which is a primary driver of NAFLD development.[28] Individuals with HIV associated NAFLD have been shown have a higher prevalence of NASH than non-HIV associated NAFLD.[29] In PLWH sCD163 has been associated with metabolic syndrome NAFLD and liver fibrosis, likely due to the increased

macrophage infiltration signaled from inflammatory cytokines secreted from adipose tissue.[30,31]

It has been previously shown 32% of participants earlier in the MASH Cohort were using cocaine.[32] Cocaine use has been previously shown to alter the microbial composition of PLWH.[33] Altered microbial translocation, and increased immune activation[34] may explain a portion of the accelerated HIV disease progression among crack cocaine users.[35] However, complicating the relationship between insulin resistance and immune activation is the relationship between cocaine use and lower BMI[33] which typically reflects lower insulin resistance.

The TyG Index is a marker of insulin resistance,[36] previously associated with NAFLD.[37] In PLWH, poor metabolic health has been associated with markers of macrophage activation and fibrosis.[31,32] Because PLWH are at increased risk of immune activation[38] and metabolic abnormalities,[39,40] the relationship between TyG Index, immune activation, and liver fibrosis pathways warrants further investigation. The purpose of this study was to determine the relationship between the insulin resistance, measured via the TyG index, and biomarkers of immune activation and liver fibrosis in PLWH.

Methods

All participants recruited for this study were enrolled as part of the MASH Cohort. For inclusion in the current study, participants were either free of HIV or HCV infections (uninfected control group), or were HIV monoinfected (PLWH group).

Participants in the MASH Cohort with HCV infection were excluded from analysis. Participants were considered cocaine users if they stated they were cocaine users on a drug screening questionnaire, tested positive for cocaine metabolites through a blood draw, or positive urine toxicology screen (American Bio Medical, Kinderhook NY). In the PLWH group, only participants who self-reported ART use in the past 6 months were included in analyses. All participants had to be deemed eligible to complete a Magnetic Resonance Elastography (MRE) scan to measure liver steatosis. After all inclusion and exclusion criteria were applied, there were a total of 480 participants selected that were deemed eligible for inclusion in the study, 211 participants in the PLWH group and 269 in the uninfected control group. When participants were separated into four study groups based on HIV and steatosis, Study Group 1: HIV^+, $Steatosis^+$ n=32, Group 2: HIV^+, $Steatosis^-$ n=179, Group 3: HIV^-, $Steatosis^+$ = 52, Group 4: HIV^-, $Steatosis^-$ = 217. The number of eligible participants with complete biomarker data was more limited, for each statistical analysis, the number of participants eligible for inclusion in that analyses are described within each result. The methodology and analysis for this study has been approved by the IRB (Approval # IRB-20-0273) at Florida International University.

Biomarkers of Immune Activation and Inflammation: The primary outcome variables were analyzed by plasma collected via blood draw at FIU Borinquen research clinic. Plasma samples were separated and stored at -80 degrees Celsius prior to shipment to the University of Cincinnati for analyses. All

biomarker data were quantified by analyte-specific bead-based Lumine Multiplex immunoassays (EMD Millipore Corporation).

HIV: To be included in the PLWH, participants must have had documented HIV infection in their medical chart. HIV VL and $CD4^{+}$ counts were taken from the most recent value documented in each participant's medical record. ART usage was self-reported from participants through an ART adherence questionnaire.

The TyG Index was used to approximate insulin resistance using the equation below.[36] Triglycerides and glucose were obtained via a fasted blood draw from a trained research nurse and sent to Laboratory Corporation of America for final analysis.

$$Ln\left[\frac{fasting\ TG\ \left(\frac{mg}{dL}\right) * fasting\ glucose\ \left(\frac{mg}{dL}\right)}{2}\right]$$

Liver steatosis: Liver steatosis was defined as >5% liver fat calculated from the proton density fat fraction (PDFF) score from an MRE scan.[41]

Dietary Assessment: All participant completed a 24-hour food recall administered at their baseline appointment. All food intake was recorded and analyzed using NutriBase nutrient analysis software. Nutrients included in statistical analysis total calories, total fat, saturated fat, and total cholesterol.

Statistical Analyses

Descriptive statistics including mean and standard deviation were used to describe participant characteristics. Differences between the participant characteristics of the PLWH and uninfected control groups were detected using independent t-tests for continuous variables and chi-square tests for categorical variables. HIV VL was not normally distributed and was Log10 transformed to bring distribution of data closer to normalization. All biomarker data were Ln transformed to make data more normally distributed. All outcome variables were analyzed cross-sectionally. Multiple linear regression models were performed to detect the associations between cocaine use, TyG Index, and HIV infection with biomarkers of immune activation, inflammation, and fibrosis. All linear regression models included age and sex as potential confounding variables. Cocaine use, TyG Index, and HIV status/ HIV VL were all included in linear regression models together to detect independent associations between each independent variable and dependent variable of interest. HIV status was used as a predictor variable on models that included all study participants and Log 10 HIV VL was used in analyses of PLWH only. ANCOVA analysis was used to detect differences between biomarkers when participants were separated by HIV infection and steatosis. All pairwise comparisons between study groups included a Bonferroni correction to adjust for multiple comparisons. All statistical analysis were completed using IBM SPSS version 26.

Results

Participant characteristics and unadjusted biomarker outcomes are shown in table 1. The PLWH group was younger than the uninfected control group (53.18 years ± 7.57 vs. 54.85 ± 7.13, P=0.014) and also had a lower BMI (28.74 kg/m^2 ± 6.05 vs. 30.05 ± 6.37, P=0.022). Unadjusted Analysis of Biomarker Data: The only marker of monocyte or immune activation that was increased in the PLWH group was Ln sCD27 (9.45 ± 5.01 vs. 7.58 ± 3.492, P=0.001), Ln sCD14 and Ln sCD163 were not different between PLWH and Uninfected Controls. Compared to the uninfected group, PLWH had higher levels of both Ln TGF-Beta (2955 ± 3958 vs. 1265 ± 1448, P=0.001) and Ln TIMP-1 (53.07 ± 21.17 vs. 45.71 ± 15.44, P=0.010).

Dietary Intake: Table 2 shows there are no significant correlations between total calories, saturated fat, total fat, or total cholesterol intake an any biomarker analyzed in this study.

Predictors of Primary Biomarker outcomes: Table 3 and 4. Cocaine use was association with increased levels of Ln sCD14 (β =0.216, P<0.001) and Ln sCD27 (β = 0.176, P=0.003). Among PLWH only, cocaine use was only associated with Ln sCD14 (β=0.184, P=0.017). Higher TyG Index values were associated with higher levels of both Ln sCD14 (β =0.080, P<0.050) and Ln sCD163 (β =0.164, P<0.008). Similar to analysis of all study participants, among PLWH only, higher TyG Index values were associated with Ln sCD14 (β =0.116, P<0.024) and Ln sCD163 (β =0.219 P<0.003). Additionally, cocaine use was associated with higher levels of Ln sCD27 (β =0.123, P=0.011) in PLWH only.

Among all study participants, HIV infection was associated with higher levels of Ln sCD27 (β =0.181, P=0.005), Ln TGF- β (β = 0.915, P<0.001), and Ln TIMP-1 (β=0.118, P=0.034). Among PLWH, HIV VL was associated with higher Ln sCD27 (β=0.181, P<0.001).

Pairwise Comparisons of Immune Activation Markers: Table 5. Mean Ln sCD27 was greater in the HIV^+, $Steatosis^+$ group compared to group the HIV^-, Steatosis $^+$ group (2.202 ± 0.531 vs. 1.709 ± 0.604, P=0.020) and was also greater in the HIV^+, $Steatosis^-$, group compared to group HIV^-, Steatosis $^+$ group (2.118 ± 0.468 vs. 1.709 ± 0.604). Plasma levels of Ln sCD163 were higher in the HIV^+, $Steatosis^+$ group (6.645 ± 0.673) compared to both the HIV^+, $Steatosis^-$ group (6.178 ± 0.689, P=0.013) and the HIV^-, $Steatosis^-$ group (6.084 ± 0.626, P=0.005). For the Ln TGF- β pairwise comparisons, the HIV^+, $Steatosis^+$ group (7.681 ± 0.729) had higher mean plasma Ln TGF- β than both the HIV^-, $Steatosis^+$ group (6.849 ± 0.772, P<0.001) and the HIV^-, $Steatosis^-$ group (6.698 ± 0.899, P<0.001). Additionally, the HIV+, $Steatosis^-$ group (7.719 ± 0.692) had higher levels of Ln TGF- β than both the HIV^-, $Steatosis^+$ group, and the HIV^-, $Steatosis^-$ groups. The mean plasma level of Ln TIMP-1 was greater in both the HIV^+, $Steatosis^+$ (3.940 ± 0.429) and HIV^+, $Steatosis^-$ groups (3.897 ± 0.362) than the HIV^-, $Steatosis^+$ group (3.587 ± 0.473), P = 0.034 and P=0.019, respectively.

ANCOVA Analysis Comparing High Risk vs. Low Risk TyG Index Groups: Table 6. Among PLWH, the estimated mean of Ln sCD14 among high TyG Risk individuals was greater than the low TyG risk group (6.942 ± 0.048 vs. 6.786 ± 0.052, P=0.031). There was no difference between mean Ln sCD27 levels

between high and low risk TyG groups. For plasma level of Ln sCD163, the high risk TyG Index group had a higher estimated mean value than the low TyG Risk Group (6.413 ± 0.072 vs. 6.035 ± 0.079, P=0.001).

Discussion

This study demonstrated a direct association between higher levels of insulin resistance and immune activation in the MASH cohort. Participants with TyG Index levels that placed them at increased risk for NAFLD, also had higher mean levels of immune activation than participants at low risk for NAFLD, even after controlling for HIV infection, cocaine use, age, and sex. The relationship between higher TyG Index and increased immune activation appeared to be even stronger in the PLWH only group, despite a smaller sample size. Consistent with previous findings in the MASH Cohort, cocaine use was also independently associated with immune activation among the entire study population and PLWH group. This is likely due to alterations in gut microbial composition[33] and increased microbial translocation.[34]

Also consistent with previous findings of the MASH cohort and others, HIV infection was also associated with higher levels of TGF- β[42] and TIMP-1[43]. Among the three markers of immune activation analyzed for the current study, HIV infection was only associated with higher levels of sCD27, not sCD14 nor sCD163. These findings are in partial, but not full agreement with a previous study performed by Williams et al. that found increased levels of immune activation among all three makers in PLWH on ART.[8] However, this study

excluded participants with substance abuse than may interfere with their ability to complete the study, it is unclear whether or not cocaine use was included in these criteria.[8] Unadjusted analysis comparing immune activation between PLWH and Uninfected controls suggest a likely relationship between HIV infection and immune activation in the current study as well, all three markers of immune activation had P-Values <0.07 even though only sCD27 reached the statistically significant threshold of P<0.05.

The finding that increased sCD27 was associated with higher HIV is suggests HIV disease severity is also related to greater immune activation and not simply a history of controlled HIV infection. This supports findings from previous studies that demonstrate higher levels of immune activation even among PLWH with highly controlled infection. Previous studies have shown an inverse relationship between sCD27 and $CD4^+$ count [44] and higher sCD27 may even predict $CD4^+$ decline.[45] However, even when $CD4^+$ count is at a healthy level, sCD27 remains elevated in PLWH.[46] Combined, these previous findings are in agreement with our current findings that showed elevated sCD27 among PLWH compared to uninfected controls even though our study composed mostly of PLWH with controlled HIV VL (88.8% >200 copies/mL) and healthy $CD4^+$ cell counts (90.4% $CD4^+$ >200 cells/μL). However, among the few participants with uncontrolled VL and low $CD4^+$ count, Log10 HIV VL was inversely associated with sCD27.

The finding of no association between HIV infection and sCD14 contrasts recent literature on HIV infection and microbial translocation. Increased sCD14

levels are an immune response to circulating LPS that increases as gut integrity is reduced.[47] The high level of ART adherence, controlled HIV VL, and high $CD4^+$ cell counts seen in the current study may explain why there was no association between HIV infection and sCD14. However, greater TyG Index was associated with higher levels of sCD14. This may indicate increased microbial translocation related to poor metabolic health is a greater driver of immune activation among PLWH with healthy immune systems. These findings are supportive of previous work by Lemoine et al. 2017, who found increased sCD14 and sCD163 among HIV monoinfected participants with metabolic syndrome compared to participants without metabolic syndrome. However, this study did not have an uninfected control group and was unable to assess the effect of the HIV infection on sCD14.[48] Increased levels of sCD14 have been shown to independently predict mortality in PLWH,[49] our findings suggest increased mortality could be related comorbidities from poor metabolic health among people who have higher TyG Index and controlled HIV infection.

Higher levels of TGF- β and TIMP-1 among PLWH are consistent with previous literature. [42,50]All significant pairwise findings for TGF- β indicate the increase was likely due to HIV infection. What is potentially novel about our current findings, is there appears to be increased levels of TIMP-1 among PLWH with liver steatosis compared to PLWH without steatosis, possibly indicating a role for liver steatosis in direct fibrotic pathways and not just increased immune activation. Among individuals with NAFLD, increased TIMP-1 levels are highly

predictive of NASH,[51] suggesting higher TIMP-1 levels among participants with steatosis may be at greater risk of progressing to NASH.

There were no associations found between any biomarker of immune activation or fibrosis with total caloric intake, saturated fat, total fat, or total cholesterol intake. Dietary information was collected via 24-hr dietary recall and it appears the ability of participants to accurately recall their dietary intake to provide accurate diet was lacking. There is established research on the effect of diet on the gut microbiome [52] and future studies looking more directly at this relationship in PLWH could be beneficial.

Conclusion

The TyG Index was associated with increased levels of immune activation markers independently of cocaine use and HIV infection. Among, PLWH adherent to ART with healthy immune function this finding may point to metabolic health as a primary driver of immune activation in this study population. HIV infection was also associated with greater pro-fibrotic cytokines. Combined, these findings suggest PLWH who have an elevated TyG Index maybe be at increased risk of developing liver fibrosis over time.

Table 1. Participant Characteristics

	Total N= 480	HIV Monoinfected N=211	Uninfected Controls N=269	P-Value
Age (years)	54.12 ± 7.367	53.18 ± 7.57	54.85 ± 7.13	**0.014**
Sex	46.3%F 53.8%M	43.6%F 56.4%M	48.3% 51.7%	0.303
Race/Ethnicity				
Black (non-Hispanic)	62.1%	66.4%	58.7%	0.088
White (Non-Hispanic)	7.9%	7.1%	8.6%	0.562
Hispanic	29.6%	25.6%	32.7%	0.090
BMI (kg/m^2)	29. 47 ± 6.255	28.74 ± 6.05	30.05 ± 6.37	**0.022**
TyG Index	8.52 ± 0.654	8.59 ± 0.684	8.48 ± 0.63	0.069
Liver Steatosis Prevalence	17.5%	15.2%	19.3%	0.233
Cocaine Use Prevalence	40.6%	37.0%	43.5%	0.148
AUDIT Score	5.05 ± 6.59	4.60 ± 6.21	5.40 ± 6.87	0.185
AUDIT >8	109/480 22.7%	44/211 20.9%	65/269 24.2%	0.390
Log10 HIV VL		0.759 ± 1.315		
Controlled HIV VL (<200 copies/ml)		88.8%		
CD4$^+$ (cells/μL)		603.79 ± 382.93		
CD4$^+$ >200 cells/μL		90.4%		
sCD14 (ng/ml)	1100 ± 455.4	1064 ± 454.7	1183 ± 449.2	0.068
sCD27 (ng/ml)	8.891± 4.681	9.45 ± 5.01	7.58 ± 3.492	**0.001**
sCD163 (ng/ml)	617.4 ± 412.2	646.4 ± 443.8	548.8 ± 317.7	0.059
TGF- β (pg/ml)	2441 ± 3482	2955 ± 3958	1265 ± 1448	**0.001**
TIMP-1 (ng/ml)	50.84 ± 19.87	53.07 ± 21.17	45.71 ± 15.44	**0.010**

P-Values in bold indicate statistical significance. Data for continuous variables is presented as mean ± STD. Statistical differences between study groups was detected using independent t-tests for continuous variables and Chi-Square tests for categorical variables. BMI: Body Mass Index, TyG Index: Triglyceride-Glucose Index, AUDIT: Alcohol Use Disorders Identification Tests, HIV VL: HIV Viral Load, sCD14: soluble CD14, sCD27: soluble CD27, sCD163: soluble CD163, transforming growth factor β: TGF- β, tissue inhibitor of metalloproteinases: TIMP-1.

Table 2. Pearson Correlation between Biomarkers and Dietary Intake Variables

	Total Calories (kcal)	Saturated Fat (g)	Total Fat (g)	Total Cholesterol (mg)
sCD14	-0.094 P=0.266	-0.098 P=0.247	-0.125 P=0.138	0.072 P=0.393
sCD27	-0.113 P=0.180	-0.119 P=0.157	-0.115 P=0.174	-0.104 P=0.218
sCD163	0.013 P=0.882	0.068 P=0.424	0.009 P=0.918	0.084 P=0.323
TGF- β	0.065 P=0.452	0.020 P=0.818	0.053 P=0.541	0.084 P=0.327
TIMP-1	0.049 P=0.567	0.032 P=0.708	-0.005 P=0.951	0.004 P=0.968

Bold P-Values Indicate statistical significance (P < 0.05).

Table 3. Multiple Linear Regressions showing associations between TyG Index, Cocaine use, and HIV Infection with biomarker outcomes. (All Participants)

		β	SE	*t*	*p*
TyG Index					
	sCD14	0.080	0.124	1.968	**0.050**
	sCD27	0.069	0.045	1.532	0.090
	sCD163	0.164	0.061	2.697	**0.008**
	TGF- β	0.001	0.071	-0.255	0.799
	TIMP-1	-0.001	0.035	-0.030	0.976
Cocaine					
	sCD14	0.216	0.059	3.658	**<0.001**
	sCD27	0.176	0.065	2.052	**0.003**
	sCD163	-0.071	0.088	-0.801	0.424
	TGF- β	-0.051	0.104	0.081	0.935
	TIMP-1	-0.048	0.051	-0.932	0.352
HIV Infection					
	sCD14	-0.060	0.064	-0.926	0.356
	sCD27	0.181	0.064	2.811	**0.005**
	sCD163	0.123	0.096	1.280	0.202
	TGF- β	0.915	0.103	8.877	**<0.001**
	TIMP-1	0.118	0.055	2.135	**0.034**

All models above included age, sex, TyG Index, and HIV status. Sample size for each biomarker: sCD14: n=234, sCD27: n=234, sCD163: n=134, TGF- β: n=225, TIMP-1: n=226. Bold P-Values Indicate statistical significance (P < 0.05).

Table 4. Multiple Linear Regressions showing associations between TyG Index, Cocaine use, and HIV Infection with biomarker outcomes (PLWH Only)

		β	SE	*t*	*p*
TyG Index					
	sCD14	0.184	0.076	2.422	**0.017**
	sCD27	0.076	0.071	1.064	0.289
	sCD163	-0.071	0.088	-0.801	0.424
	TGF- β	-0.058	0.110	-0.525	0.601
	TIMP-1	-0.013	0.063	-0.016	0.841
Cocaine					
	sCD14	0.116	0.051	2.277	**0.024**
	sCD27	0.123	0.048	2.580	**0.011**
	sCD163	0.219	0.074	2.970	**0.003**
	TGF- β	-0.087	0.072	-1.209	0.229
	TIMP-1	0.028	0.042	0.055	0.497
HIV VL					
	sCD14	-0.001	0.027	-0.041	0.968
	sCD27	0.124	0.026	4.722	**<0.001**
	sCD163	0.072	0.040	1.807	0.073
	TGF- β	-0.031	0.040	-0.89	0.431
	TIMP-1	0.031	0.023	0.114	0.177

Sample size for each biomarker: sCD14: n=165, sCD27: n=165, sCD163: n=165, TGF- β: n=157, TIMP-1: n=157. Bold P-Values Indicate statistical significance (P < 0.05).

Table 5. One-Way ANOVA to analyze difference between mean biomarker values. Groups separated by HIV Status and Steatosis.

	Mean Value	F	P-Value	Differences between four groups	P-Value
Ln sCD14 (ng/ml)		1.437	0.233	Groups 1 and 2 = 0.070	1.000
				Groups 1 and 3 = -0.103	1.000
Group 1: HIV^+, $Steatosis^+$	6.932 ± 0.415			Groups 1 and 4 = -0.056	1.000
Group 2: HIV^+, $Steatosis^-$	6.863 ± 0.480			Groups 2 and 3 = -0.172	1.000
Group 3: HIV^-, $Steatosis^+$	7.034 ± 0.443			Groups 2 and 4 = -0.126	0.486
Group 4: HIV^-, $Steatosis^-$	6.988 ± 0.435			Groups 3 and 4 = 0.046	1.000
Ln sCD27 (ng/ml)		4.465	**0.005**	Groups 1 and 2 = 0.084	1.000
				Groups 1 and 3 = 0.492	**0.020**
Group 1: HIV^+, $Steatosis^+$	2.202 ± 0.531			Groups 1 and 4 = 0.241	0.289
Group 2: HIV^+, $Steatosis^-$	2.118 ± 0.468			Groups 2 and 3 = 0.408	**0.019**
Group 3: HIV^-, $Steatosis^+$	1.709 ± 0.604			Groups 2 and 4 = 0.157	0.260
Group 4: HIV^-, $Steatosis^-$	1.961 ± 0.493			Groups 3 and 4 = -0.252	0.517
Ln sCD163 (ng/ml)		4.105	**0.007**	Groups 1 and 2 = 0.466	**0.013**
				Groups 1 and 3 = 0.335	0.847
Group 1: HIV^+, $Steatosis^+$	6.645 ± 0.673			Groups 1 and 4 = 0.561	**0.005**
Group 2: HIV^+, $Steatosis^-$	6.178 ± 0.689			Groups 2 and 3 = -0.131	1.000
Group 3: HIV^-, $Steatosis^+$	6.310 ± 0.632			Groups 2 and 4 = 0.094	1.000
Group 4: HIV^-, $Steatosis^-$	6.084 ± 0.626			Groups 3 and 4 = 0.226	1.000
Ln TGF- β (pg/ml)		27.377	**<0.001**	Groups 1 and 2 = -0.038	1.000
				Groups 1 and 3 = 0.832	**0.008**
Group 1: HIV^+, $Steatosis^+$	7.681 ± 0.729			Groups 1 and 4 = 0.983	**<0.001**
Group 2: HIV^+, $Steatosis^-$	7.719 ± 0.692			Groups 2 and 3 = 0.870	**<0.001**
Group 3: HIV^-, $Steatosis^+$	6.849 ± 0.772			Groups 2 and 4 = 1.021	**<0.001**
Group 4: HIV^-, $Steatosis^-$	6.698 ± 0.899			Groups 3 and 4 = 0.151	1.000
LnTIMP-1 (ng/ml)		3.760	**0.012**	Groups 1 and 2 = 0.043	1.000
				Groups 1 and 3 = 0.353	**0.034**
Group 1: HIV^+, $Steatosis^+$	3.940 ± 0.429			Groups 1 and 4 = 0.135	0.892
Group 2: HIV^+, $Steatosis^-$	3.897 ± 0.362			Groups 2 and 3 = 0.310	0.019
Group 3: HIV^-, $Steatosis^+$	3.587 ± 0.473			Groups 2 and 4 = 0.092	0.713
Group 4: HIV^-, $Steatosis^-$	3.805 ± 0.333			Groups 3 and 4 = -0.218	0.301

Bold P-Values Indicate statistical significance ($P < 0.05$).

Table 6. ANCOVA Comparing immune activation markers between High and Low TyG Risk.

	Estimated Means	P-Value
sCD14 (ng/ml)		**0.031**
Low TyG Risk	6.786 ± 0.052	
High TyG Risk	6.942 ± 0.048	
sCD27 (ng/ml)		0.386
Low TyG Risk	2.093 ± 0.051	
High TyG Risk	2.154 ± 0.047	
sCD163 (ng/ml)		**0.001**
Low TyG Risk	6.035 ± 0.079	
High TyG Risk	6.413 ± 0.072	

Analysis controlled for HIV status, cocaine use, age, and sex. Bold P-Values Indicate statistical significance ($P < 0.05$).

References

1. Hunt, Peter W. HIV and inflammation: mechanisms and consequences. Current HIV/AIDS Rep. 2012. 9(2):139-147. doi:10.1007/s11904-012-0118-8

2. Utay N, Ananworanich J, Slike B, et al. Inflammation persists despite early initiation of ART in acute HIV infection [abstract 47]. In: Program and abstracts of the 2015 Conference on Retroviruses and Opportunistic Infections, Seattle, Washington, 23–26 February 2015.

3. Joshi D, O'Grady J, Dieterich D, et al. Increasing burden of liver disease in patients with HIV infection. Lancet. 2011; 377:1198-209. doi: 10.1016/S0140-6735(10)62001-6.

4. Robert S, Gicquel T, Bodin A, et al. Characterization of the MMP/TIMP imbalance and collagen production induced by IL-1 β or TNF-α release from human hepatic stellate cells. PLoS One. 2016;11(4):e0153118. doi:10.1371/journal.pone.0153118

5. Roderfeld M. Matrix metalloproteinase functions in hepatic injury and fibrosis. Matrix Biol. 2018;68-69:452-462. doi:10.1016/j.matbio.2017.11.011

6. Page EE, Nelson M, Kelleher P. HIV and hepatitis C co-infection: pathogenesis and microbial translocation. Curr Opin in HIV AIDS 2011;6:472-7. doi: 10.1097/COH.0b013e32834bbc71

7. Operskalski EA, Kovacs J. HIV/HCV co-infection: pathogenesis clinical complications, treatments and new therapeutic technologies. Current HIV/AIDS. 2011;8:12-22. doi: 10.1007/s11904-010-0071-3

8. Williams JC, Zhang X, Karki M, et al. Soluble CD14, CD163, and CD27 biomarkers distinguish ART-suppressed youth living with HIV from healthy controls. J Leukoc Biol. 2018;103(4):671-680. doi:10.1002/JLB.3A0717-294RR

9. Wallet MA, Rodriguez CA, Yin L, et al. Microbial translocation induces persistent macrophage activation unrelated to HIV-1 levels or T-cell activation following therapy. AIDS. 2010;24:1281-1290. doi:10.1097/QAD.0b013e328339e228

10. Burdo TH, Lentz MR, Autissier P, et al. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after antiretroviral therapy. J Infect Dis. 2011;204(1):154-163. doi:10.1093/infdis/jir214

11. Corbitt N, Kimura S, Isse K, et al. Gut bacteria drive kupffer cell expansion via MAMP-mediated ICAM-1 induction on sinusoidal endothelium and influence preservation-reperfusion injury after orthotopic liver transplantation. Am J Pathol. 2013;182(1):180-191. doi:10.1016/j.ajpath.2012.09.010

12. Schnabl B. Interactions Between the Intestinal Microbiome and Liver Diseases. Gastroenterology. 2015;146(6):1513-1524. doi:10.1053/j.gastro.2014.01.020.Interactions

13. Bissell DM, Roulot D, George J. Transforming growth factor β and the liver. Hepatology. 2001. doi:10.1053/jhep.2001.28457

14. Sacchi P, Cima S, Corbella M, et al. Liver fibrosis, microbial translocation and immune activation markers in HIV and HCV infections and in HIV/HCV co- infection. Dig Liver Dis. 2015;47(3):218-225. doi:10.1016/j.dld.2014.11.012

15. Rallón NI, Barreiro P, Soriano V, et al. Elevated TGF-β1 levels might protect HCV/HIV-coinfected patients from liver fibrosis. Eur J Clin Invest. 2011;41(1):70-76. doi:10.1111/j.1365-2362.2010.02381.x

16. Koek GH, Liedorp PR, Bast A. The role of oxidative stress in non-alcoholic steatohepatitis. Clin Chim Acta. 2011;412(15-16):1297-1305. doi:10.1016/j.cca.2011.04.013

17. Fabregat I, Moreno-Càceres J, Sánchez A, et al. TGF-β signalling and liver disease. FEBS J. 2016;283(12):2219-2232. doi:10.1111/febs.13665

18. Housset C, Boucher O, Girard PM, et al. Immunohistochemical evidence for human immunodeficiency virus-1 infection of liver kupffer cells. Hum Pathol. 1990;21(4):404-408. doi:10.1016/0046-8177(90)90202-G

19. Hufert FT, Schmitz J, Schreiber M et al. Human kupffer cells infected with HIV-1 in vivo. J Acquir Immune Defic Syndr. 1993;6(7):772-777.

20. Lozupone CA, Li M, Campbell TB, et al. Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe. 2013;14(3):329-339. doi:10.1016/j.chom.2013.08.006

21. Guadalupe M, Reay E, Sankaran S, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol. 2003;77(21):11708-17 doi:10.1128/jvi.77.21.11708-11717.2003

22. Dandekar S, George MD, Bäumler AJ. Th17 cells, HIV and the gut mucosal barrier. Curr Opin HIV AIDS. 2010;5(2):173-178. doi:10.1097/COH.0b013e328335eda3

23. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med. 2004;200(6):761-770. doi:10.1084/jem.20041196

24. Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. Clin Microbiol Rev. 2013;26(1):2-18. doi:10.1128/CMR.00050-12

25. Nazli A, Chan O, Dobson-Belaire WN, et al. Exposure to HIV-1 Directly Impairs Mucosal Epithelial Barrier Integrity Allowing Microbial Translocation. PLoS Pathog. 2010;6(4):e1000852. doi:10.1371/journal.ppat.1000852

26. Larter CZ, Farrell GC. Insulin resistance, adiponectin, cytokines in NASH: Which is the best target to treat? J Hepatol. 2006;44(2):253-261. doi:10.1016/j.jhep.2005.11.030

27. Johnston AM, Pirola L, Van Obberghen E. Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling. FEBS Lett. 2003;546(1):32-36. doi:10.1016/S0014-5793(03)00438-1

28. Sakurai M, Takamura T, Ota T, et al. Liver steatosis, but not fibrosis, is associated with insulin resistance in nonalcoholic fatty liver disease. J Gastroenterology. 2007. 42(4):312-7. doi:10.1007/s00535-006-1948-

29. Vodkin I, Valasek MA, Bettencourt R, et al. Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: A case-control study. Aliment Pharmacol Ther. 2015;41(4):368-378. doi:10.1111/apt.13052

30. Lidofsky A, Holmes JA, Feeney ER, et al. Macrophage activation marker soluble CD163 is a dynamic marker of liver fibrogenesis in human immunodeficiency virus/Hepatitis C virus coinfection. J Infect Dis. 2018;218(9):1394-1403. doi:10.1093/infdis/jiy331

31. Mueller JL, Feeney ER, Zheng H, et al. Circulating soluble CD163 is associated with steatohepatitis and advanced fibrosis in nonalcoholic fatty liver disease. Clin Transl Gastroenterol. 2015;6(10):e114-8. doi:10.1038/ctg.2015.36

32. Campa A, Martinez SS, Sherman KE. Cocaine Use and Liver Disease are Associated with All-Cause Mortality in the Miami Adult Studies in HIV (MASH) Cohort. J Drug Abus. 2016;2(4):pii:27. doi:10.21767/2471-853x.100036

33. Volpe GE, Ward H, Mwamburi M, et al. Associations of cocaine use and HIV infection with the intestinal microbiota, microbial translocation, and Inflammation. J Stud Alcohol Drugs. 2014;75(2):347-357. doi:10.15288/jsad.2014.75.347

34. Hernandez J. Gut Integrity, Microbial Translocation, Immune Activation and Vitamin D in Drug Users Living with HIV. 2020. Florida International University.

35. Baum M, Rafie C, Lai S, et al. Crack-Cocaine Use Accelerates HIV Disease Progression in a Cohort of HIV-Positive Drug Users. J Acquir Immune Defic Syndr. 2009;50(1):93-99. doi:10.1097/QAI.0b013e3181900129

36. Simental-Mendía, LE, Rodriguez-Moran M, Guerro-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. Metab Syndr Relat Disord. 2008;6(4):299-304. doi:10.1089/met.2008.0034

37. Zhang S, Du T, Zhang J, et al. The triglyceride and glucose index (TyG) is an effective biomarker to identify nonalcoholic fatty liver disease. Lipids Health Dis. 2017;16(1):15. doi:10.1186/s12944-017-0409-6

39. Le MH, Devaki P, Ha NB, et al. Prevalence of non-Alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PLoS One. 2017;12(3). doi:10.1371/journal.pone.0173499

40. Maurice JB, Patel A, Scott AJ, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. AIDS. 2017;31(11):1621-1632. doi:10.1097/QAD.0000000000001504

41. Tang A, Tan J, Sun M, et al. Nonalcoholic Fatty Liver Disease: MR Imaging of Liver Proton Density Fat Fraction to Assess Hepatic Steatosis. Radiology. 2013;267(2):422-431. doi:10.1148/radiol.12120896

42. Stewart T. Lifestyle and Biological Risk Factors for Liver Fibrosis in the Miami Adult Studies on HIV (MASH Cohort: An HIV Infected and HIV/HCV Co-Infected Population. 2016. Florida International University.

43. Bruno R, Galastri, S, Sacchi, P. et al. gp 120 modulates the biology of human hepatic stellate cells: A link between HIV infection and liver fibrogenesis. Gut. 2010;59(4): 513–520. Doi: 10.1136/gut.2008.163287

44. De Milito A, Aleman S,Marenzi R, et al. Plasma levels of soluble CD27: a simple marker to monitor immune activation during potent antiretroviral therapy in HIV-1-infected subjects. Clin Exp Immunol. 2002;127(3):486-94. doi:10.1046/j.1365-2249.2002.01786.x

45. Messele T, Brouwer M, Girma M, et al. Plasma levels of viro-immunological markers in HIV-infected and noninfected Ethio1pians: correlation with cell surface activation markers. Clin Immunol. 2001;98:212–9. doi:10.1006/clim.2000.4958

46. Ghaffari G, Passalacqua D, Caicedo J, et al. Two-year clinical and immune outcomes in human immunodeficiency virus-infected children who reconstitute CD4 T cells without control of viral replication after combination antiretroviral therapy. Pediatrics. 2004;114:e604–611. doi: 10.1542/peds.2004-0274.

47. Brenchley JM, Douek DC. Microbial Translocation Across the GI Tract. Annu Rev Immunol. 2012;30(1):149-173. doi:10.1146/annurev-immunol-020711-075001

48. Lemoine M, Lacombe K, Bastard JP, et al. Metabolic syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected patients. AIDS. 2017;31(14):1955-1964. doi:10.1097/QAD.0000000000001587

49. Sandler NG, Wand H, Roque A, et al. Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection. J Infect Dis. 2011;203(6):780-790. doi:10.1093/infdis/jiq118

50. Abdel-Hameed E, Rouster SD, Kottilil S, Sherman KE. The enhanced liver fibrosis (ELF)-Index predicts hepatic fibrosis superior to FIB4 and APRI in HIV/HCV infected patients. Clin Infect Disease. 2020; May 27;ciaa646 doi:10.1093/cid/ciaa646

51. Abdelaziz R, Elbasel M, Esmat S, et al. Tissue Inhibitors of Metalloproteinase-1 and 2 and Obesity Related Non-Alcoholic Fatty Liver Disease: Is There a Relationship. Digestion. 2015;93(3):130-7. doi:10.1159/000439083

52. Zinocker MK, Lindseth IA. The western diet-microbiome-host interaction and its role in metabolic disease. Nutrients. 2018;10(3):365. doi: 10.3390/nu10030365

CHAPTER V: THE TYG INDEX IS ASSOCIATED WITH GREATER LIVER STIFFNESS AND LIVER FIBROSIS IN PEOPLE LIVING WITH HIV (PLWH)

Introduction

People Living with HIV (PLWH) are 3.7x more likely to die of liver disease than the general population.[1] Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world.[2] Between 5-10% of individuals with NAFLD will develop non-alcoholic steatohepatitis (NASH) and 38% of these individuals with develop liver fibrosis.[3] A previous meta-analysis found the prevalence of NAFLD in PLWH was 35%,[4] compared to 25% in the general population.[5] Another study found a higher NAFLD rate among men living with HIV than uninfected men, but a lower NAFLD rate among women living with HIV than uninfected women.[6] Other studies have found there was no increased risk for NAFLD in PLWH.[7]

Insulin resistance (IR) is one mechanism shown to increase the risk of NAFLD and liver fibrosis.[8,9] Insulin resistance increases hepatic de novo lipogenesis[10] and adipose tissue dysfunction, increasing levels of pro-inflammatory adipokines and cytokines,[11] leading to chronic hepatic inflammation. Insulin resistance is likely related to immune activation, which has been shown to remain increased in PLWH even after successful antiretroviral therapy (ART).[12] The relationship between insulin resistance and NAFLD in PLWH is noteworthy because PLWH with NAFLD have nearly twice the likelihood of developing NASH compared to uninfected individuals.[13] The progression from NAFLD to NASH is likely facilitated by hepatic stellate cell

(HSC) activation and proliferation. Higher levels of insulin and glucose, hallmarks of NAFLD, increase HSC activation and connective tissue growth factor (CTGF), both promoters of liver fibrosis.[14] The activation of HSCs is also increased in response to liver apoptosis and increased reactive oxygen species (ROS).[15]

The HIV virus binds to CXCR4 and CCR5 receptors which are expressed on hepatocytes,[16] activation of these receptors increases production of fibrotic components of the extracellular matrix (ECM) proteins.[17] Proteins in the HIV viral envelop also active tissue-inhibitor metalloproteinase (TIMP) which is associated with increased fibrosis development.[18] Another component of NASH that is distinguishable from NAFLD is the level of oxidative stress present in the liver. Increased ROS damage mitochondrial membranes and ultimately leads diminished mitochondrial function[19,20] and increased hepatic apoptosis.[21,22] Furthermore, individuals with NASH also have reduced antioxidant capacity, which has been shown to be correlated with severity of NASH.[23,24] Increased apoptosis in NASH can be initiated by increased FFA accumulation,[25,26] and exposure to pro-inflammatory cytokines.[27,28] Viral HIV envelope proteins can also increase hepatocyte apoptosis directly or through inflammatory pathways.[29,30]

Confounding the relationship between HIV infection, ROS, and apoptosis is substance abuse. It has been previously shown 32% of selected participants earlier in the MASH Cohort were using cocaine.[31] Cocaine use has been previously shown to induce liver injury through ROS pathways.[32] Also, previous studies from the MASH Cohort have found accelerated HIV disease progression

among cocaine users.[31,33] Substance abuse has been described as a leading reason to delay ART therapy[34], which may further exacerbate gut mucosal integrity issues in PLWH. Recently in the MASH Cohort cocaine use was found to be associated with increased immune activation in the MASH Cohort.[35]

Due to the high level of liver disease mortality among PLWH, it is important to understand the interaction between multiple physiological and lifestyle factors that may lead to liver disease in this population. Poor metabolic health has been previously associated with greater liver fibrosis development in PLWH[9] however, to the best of our knowledge there are no studies that have looked directly at the effect of insulin resistance and liver steatosis on the likelihood of liver fibrosis in PLWH in the context of cocaine use. Therefore, the purpose of this study was to determine the association between two markers of metabolic health, insulin resistance via the TyG Index, and liver steatosis, on oxidative stress, hepatic apoptosis, and liver fibrosis in the MASH Cohort.

Methods

The data in this cross-sectional study was derived from a subset of participants in the Miami Adult Studies on HIV (MASH) cohort and included PLWH and an uninfected control group. Individuals with Hepatitis C Virus (HCV) were excluded from analysis. Eligibility for inclusion included the ability to participate in a liver scan using Magnetic Resonance Elastography (MRE) Imaging to determine liver stiffness and Proton Density Fat Fraction (PDFF) for liver fat. Exclusion criteria for MRE imaging included any presence of metal in the body, claustrophobia,

and excessively high obesity that mechanically obstructed the technician's ability to perform the scan. Cocaine use was confirmed through self-report questionnaire, blood metabolites or urine toxicology (American Bio Medical, Kinderhook NY). After application of all exclusion criteria, 480 participants were eligible for final analysis, 211 in the PLWH and 269 in the uninfected group. When participants were separated by HIV infection and steatosis status, Study Group 1: HIV+, with steatosis = 32, Group 2: HIV +, no steatosis = 179, Group 3: HIV- with steatosis = 52, Group 4: HIV-, no steatosis = 217. Not all participants were able to be analyzed for each outcome biomarker, participants with missing data were not included in analysis. All participants provided their written consent to participate in the study and the study was approved by the IRB (approval # IRB-20-0273) at Florida International University.

Anthropometrics: Height, weight, waist circumference, and BMI were obtained by trained research staff. Patients most recent medical records were used to confirm HIV status and abstract values for $CD4^{+}$ and HIV VL. Blood draws were completed to collect fasting triglycerides and fasting glucose,

Insulin resistance: The Triglyceride-glucose index (TyG Index) equation was calculated using the following equation:[36]

$$Ln\left[\frac{fasting\ TG\ \left(\frac{mg}{dL}\right) * fasting\ glucose\ \left(\frac{mg}{dL}\right)}{2}\right]$$

MRE Variables: Liver fat % was calculated from MR scans using the fat to water

ratio that is produced by the MRI-PDFF value using the following equation.[37]

$$\frac{[100 * (Fat\ to\ Water\ Ratio]}{[1 + (Fat\ to\ Water\ Ratio)]}$$

Liver fat % >5% is considered evidence for liver steatosis.[36] Liver fibrosis, was assessed from magnetic resonance elastography (MRE) scans. MRE values <2.5 kilopascals (kPa) are indicative of a healthy liver, 2.5-2.9kPa indicates normal liver with possible inflammation, > 2.9kPa indicates liver fibrosis.[38]

Dietary Assessment: During their baseline interview each participant completed a 24-hour dietary recall. All reported food intake was recorded using NutriBase nutrient analysis software. Statistical analyses were completed on the following nutrients: total calories, total fat, saturated fat, and total cholesterol.

Biomarker Data: All glutathione variables were measured using Enzyme Linked ImmunoELISA kits (Arbor Assays, 1514 Eisenhower Place, Ann Arbor MI, 48108). Samples are prepared by mix 200ml of whole blood with 200ml of sulfosalicylic acid. Samples were stored at -80 degrees C and thawed immedaitely before analysis performed to manufactorers instructions. 8-oxo-2'-deoxyguanosine (8-oxo-dG) DNA must be extracted from peripheral blood mononuclear cells (PBMCs) using ReliaPrep Blood gDNA Miniprep System (Promega). PBMCs are thawed from the -80 degrees Celsius freezer and washed with phosphate buffered saline and RPMI solutions. After DNA is extracted the concentration of DNA is measured using a spectrophotometer. The

DNA concentration of each sample is used to calculate the volume needed for formamidopyrimidine DNA glycosylase (FPG) enzyme treatment for samples. The FPG enzyme repairs oxidized DNA bases, especially purines such as 8-oxo-dG. For each participant included in analysis there were duplicate DNA samples that remained untreated and duplicate samples treated with the FPG Enzyme. The samples were mixed with SYBR Green mix and amplified using qPCR (Bio-Rad) and threshold cycle values (Ct) were obtained for both treated and untreated samples. The mean difference in Ct values (Ct^{unt}-Ct^{trt}) represents the amount of 8-oxo-dG in the sample. Apoptosis is being measured via a Human CK 18 (Cytokeratin 18)-M30 sandwich enzyme-linked immune-sorbent assay (ELISA) kit (XpressBio). Each well is pre-coated with Anti-CK 18-M30 antibody. Biotin conjugated ant-CK 18-M30 antibody is used as a detection reagent. Standard reagents, samples, and Biotin reagent are also added to each well and then washed away with wash buffer. HRP-Streptavidin reagent is then added and all unbound conjugates are washed away from the plate. TMB substrate is used to produce a bright color in each well that can be detected by a microplate reader to calculate CK 18-M30 concentration.

Statistical Analysis

Statistical analysis was completed using IBM SPSS 26 software. All demographic variables and participant characteristics were reported as mean ± STD for continuous variables and categorical variables were reported as a percentage of cases. For unadjusted analysis of continuous outcome variables independent t-

tests were performed, unadjusted categorical group differences were detected with Chi-Square tests. One-Way ANOVA was performed to detect differences across the four study groups separated by HIV and steatosis status, and HIV and TyG Risk category. All post-hoc pairwise analysis included Bonferroni corrections to adjust for multiple comparisons. Multiple linear regression was performed to determine independent associations between primary independent and dependent variables of interest. Simple and multivariate logistic regression was performed to assess the relationship between TyG Index and liver fibrosis. All multiple linear and logistic regressions were adjusted for Age, Sex, BMI, HIV Status, and Cocaine Use.

Results

Participant characteristics are described in table 1. The mean age of this selected MASH cohort sample was 54.12 ± 7.367 years, the PLWH group was significantly younger than the uninfected control group (53.18 ± 7.574 vs. 54.85 ± 7.129, P=0.014). The mean BMI of all participants was 29.47 ± 6.255, the PLWH group had a significantly lower BMI compared to the uninfected control group (28.74 ± 6.05 vs. 30.05 ± 6.365, P = 0.022). This study enrolled a slightly higher number of males (53.8%) than females. The racial/ethnic makeup of the study was predominantly Black non-Hispanic (62.1%), and 29.6% of the cohort identified as Hispanic. The prevalence of cocaine use among all participants was 40.6%. The prevalence of liver steatosis and fibrosis was 17.5% and 9.2%

respectively. Among PLWH, the number of participants with controlled HIV VL (<200 copies/mL) was 88.8%).

Dietary Intake Variables: Tables 2 and 3. Pearson correlation analysis found Total Calories, Total Fat, and Saturated Fat were all positively associated with increased liver apoptosis (P<0.05). When multiple regression analyses were performed that controlled for Age, Sex, BMI, Cocaine Use, and HIV Status, only saturated fat remained associated with increased apoptosis (β=3.26, P=0.050). Unadjusted analysis found no difference between hazardous alcohol drinkers and non-hazardous drinkers for mean 8-oxo-dG, Total GSH, Free GSH, Apoptosis, or Liver Stiffness (data not shown).

Unadjusted Oxidative Stress, Apoptosis, and Liver Stiffness Analysis: Table 4. Free glutathione (GSH) and Total Glutathione (Total GSH) were both higher among cocaine users than non-users [1045.3 ± 343.5 vs. 945.9 ± 339.9, P=0.002] and (1079.1 ± 344.9 vs. 978.9 ± 339.5, P=0.002), respectively. There was also decreased levels of hepatic apoptosis among cocaine users than non-users (1022.6 ± 324.6 vs. 1115.0 ± 434.2, P=0.050). There were no differences between liver fibrosis rates or 8-oxo-dG levels between cocaine users vs. non-users. Participants with liver steatosis had greater mean liver stiffness than participants without liver steatosis (2.459 ± 0.698 vs. 2.284 ± 0.471, P=0.005). There were no differences in liver fibrosis rates or 8-oxo-dG levels between steatosis groups. Participants with TyG Index values that placed them at high risk for liver steatosis had lower GSH (919.6 ± 286.5 vs. 1003.4 ± 355.6, P=0.015) and Total GSH (952.2 ± 289.4 vs. 1036.8 ± 355.5, P=0.014) than participants with low steatosis risk TyG Index values. Additionally, the high steatosis risk TyG

Index group had increased levels of liver stiffness compared to the low steatosis risk group (2.416 ± 0.67 vs. 2.290 ± 0.71, P=0.033). Levels of 8-oxo-dG were no different when compared between TyG Risk groups (P=0.868).

One-Way ANOVA Across HIV and Steatosis Groups: Table 5. Total GSH, F=4.081, P=0.007; Free GSH, F=4.216, P-Value=0.006, and Liver Stiffness, F=3.647, P=0.013 were all different across groups. For Total GSH, pairwise analysis found the HIV^+, $Steatosis^-$ group had lower Total GSH compared to the HIV^-, $Steatosis^-$ group (969.10 vs. 1073.09, P=0.016). For Free GSH, pairwise analysis found the HIV^+, $Steatosis^-$ Group had lower Free GSH compared to the HIV^-, $Steatosis^-$ Group (935.54 vs. 1040.14, P=0.015). For Liver Stiffness, the HIV^+, $Steatosis^+$ Group (2.588 kPa) had higher liver stiffness measurements than both the HIV^+, $Steatosis^-$ Group (2.588 vs. 2.283 kPa, P=0.015) and the HIV^-, $Steatosis^-$ Group (2.588 kPa vs. 2.285, P=0.014).

One-Way ANOVA Across HIV and TyG Risk Groups: Table 6. When participants were separated by HIV and TyG Risk, Total GSH, F=5.985, P=0.001; Free GSH, F=6.088, P<0.001, and Liver Stiffness, F=3.682, P=0.012 were all different across groups. Pairwise analysis found the HIV^+, Low TyG Risk group had lower Total GSH compared to the HIV^-, Low TyG Risk group (965.38 vs. 1092.40, P=0.016) and the HIV^-, High TyG Risk Group had a lower Total GSH compared to the HIV^-, Low TyG Risk Group (953.00 vs. 1092.40, P=0.041). The HIV^+, Low TyG Risk Group had lower Free GSH compared to the HIV^-, Low TyG Risk Groups (918.85 vs. 1059.70, P=0.002). The HIV^-, High TyG Risk Group had lower Free GSH compared to the HIV^-, Low TyG Risk Group (920.18 vs.

1059.70, P=0.040). The HIV^+, High TyG Risk Group had greater mean liver stiffness (2.557 kPa) than either than HIV^-, Low TyG Risk Group (2.557 kPa vs. 2.270, P=0.007) or the HIV^-, Low TyG Risk Group (2.557 kPa vs. 2.306, P=0.021).

Regression Analysis of Primary Outcomes: Tables 7 and 8. Higher TyG Index was associated with increased liver stiffness in (β = 0.118, P=0.002). Higher TyG Index appeared to be associated with both lower Free GSH and greater hepatic apoptosis (each association had a P-Value <0.010), but neither of these values reached the threshold of statistical significance for this study. Cocaine use was associated with greater levels of Free GSH (β=88.00, P=0.006) and increased hepatic apoptosis (β= 94.62, P=0.050). HIV infection was associated with reduced levels of Free GSH (β= -95.24, P=0.003). Among PLWH, the TyG Index was associated with increased likelihood of liver fibrosis in unadjusted [OR=2.593 (1.442, 4.664), P=0.001], partially adjusted [OR=2.557 (1.403, 4.661), P=0.002] and fully adjusted [OR=2.718 (1.469, 5.028), P=0.001) logistic regression models. Similarly, among all participants the TyG Index was associated with increased likelihood of liver fibrosis in unadjusted [OR=1.610 (1.042, 2.490), P=0.032], partially adjusted [OR=1.628 (1.042, 2.542), P=0.032] and fully adjusted [OR=1.619 (1.027, 2.553), P=0.046) models.

Discussion

The primary finding of this study is the TyG Index was a significant predictor of liver fibrosis among PLWH only and in the combined MASH Cohort.

Additionally, HIV infection was associated with reduced levels of both Free GSH (P=0.003) and Total GSH (P=0.003). Interestingly cocaine use was associated with increased hepatic apoptosis (P=0.050), but also increased Free GSH and Total GSH (P=0.006) and (P=0.005) respectively. The TyG index also trended towards being associated with reduced Free GSH, Total GSH, and increased hepatic apoptosis, but these findings did not reach the statistically significant threshold of P<0.05.

The TyG Index appeared to be a stronger predictor of fibrosis in PLWH only and this finding may highlight the "multiple hit hypothesis" of NASH that has been previously discussed a mechanism of liver fibrosis development.[39] Increased insulin resistance represents an additional stressor to the liver on top of HIV infection, ART use, and possible substance use and food insecurity, all factors that have been previously associated with liver fibrosis.[40-42] Previous research has found an association between HIV monoinfection and increased FIB-4 score;[43] however, our study did not find an independent association between HIV and liver fibrosis. The effect of the HIV virus may have been difficult to assess because the PLWH were younger and had lower BMI compared to the control group. The overall prevalence of fibrosis in this study was also lower than typically reports in cohorts of HIV monoinfection.[44-46]

Pairwise analysis found participants with both HIV and High TyG Risk had increased liver stiffness compared to participants without HIV and low TyG Risk. This finding indicates a potential cumulative effect of HIV infection and IR on liver stiffness. Previous studies have found also found an association between IR and

liver fibrosis,[9] but were unable to compare fibrosis risk in both PLWH and uninfected controls. Another study found increased risk of liver fibrosis among individuals with metabolic syndrome, but neither IR or steatosis severity were associated with increased fibrosis risk.[46]

This study found HIV infection was associated with reduced levels of Free GSH and Total GSH. This supports previous findings of the MASH Cohort that found higher levels of oxidative stress among PLWH.[47] Increased oxidative stress is one pathway of HSC activation that leads to the deposition of fibrotic proteins.[48] Conversely, cocaine use was associated with higher Free GSH and Total GSH. This appears to contradict previous literature in animal studies.[49] However, there were fewer cocaine users among PLWH and cocaine use was associated with decreased odds of liver steatosis (data not shown) which may indicate pro-oxidative stress pathways in cocaine users may be been suppressed due to other factors. Previous studies have found reduced levels of glutathione among participants with NAFLD,[50] in our study, higher TyG Index appeared to be related to lower Free GSH, but this result did not reach statistical significance. Patients with NAFLD may have lower reduced glutathione as a result of increased production of ROS that scavenges glutathione storage.[51]

Dietary fat intake has been previously shown to be associated with increased liver fat content and upregulation of hepatic apoptosis pathways.[52] Our study found a positive association between saturated fat intake and hepatic apoptosis, but no relationship between saturated fat intake and liver fat content (data not shown). The mechanistic link between saturated fat intake and

apoptosis has been previously attributed to increased hepatic fat content and ROS,[53] but this finding is not support by our own data that found no relationship between saturated fat intake and oxidative stress (data not shown). Most previous studies analyzing hepatic apoptosis outcomes have been in animal models. Further investigation into the relationship between saturated fat intake and hepatic apoptosis is necessary to understand this relationship with more clarity.

Conclusion

Higher TyG Index is associated with greater likelihood of liver fibrosis among PLWH and among MASH cohort participants overall. In this cohort of participants with a high rate of ART adherence and well-controlled HIV VL, insulin resistance and metabolic health overall may act as a primary driver of liver disease. Future studies should continue to monitor the relationship between high TyG Index values and liver fibrosis development and progression over time.

Table 1. Participant Characteristics

	Total N= 480	PLWH N-211	Uninfected Controls N=269	P-Value
Age	54.12 ± 7.367	53.18 ± 7.574	54.85 ± 7.129	**0.014**
Sex	46.2% F 53.8% M	43.6% F 56.4% M	48.3% 51.7%	0.303
Race/Ethnicity				
Black (non-Hispanic	62.1%	66.4%	58.7%	0.088
White (Non-Hispanic)	7.9%	7.1%	8.6%	0.562
Hispanic	29.6%	25.6%	32.7%	0.090
BMI (kg/m^2)	29.47 ± 6.255	28.74 ± 6.05	30.05 ± 6.365	**0.022**
TyG Index	8.52 ± 0.654	8.585 ± 0.684	8.475 ± 0.628	0.069
Liver Steatosis Prevalence	84/480 (17.5%)	32/211 (15.2%)	52/269 (19.3%)	0.233
Liver Fat %	4.25 ± 4.43	3.932 ± 3.694	4.499 ± 4.930	0.150
Cocaine Use Prevalence	195/580 (40.6%)	78/211 (37.0%)	117/269 (43.5%)	0.148
AUDIT Score	5.05 ± 6.59	4.60 ± 6.21	5.40 ± 6.87	0.185
AUDIT >8	109/480 22.7%	44/211 20.9%	65/269 24.2%	0.390
Log10 HIV VL	-----	0.759 ± 1.315	-----	-----
Controlled HIV VL (<200 copies/ml)	-----	88.8%	-----	-----
CD4+ (cells/μL)	-----	604.59 ± 381.14	-----	-----
CD4+ >200 cells/μL	-----	90.4%	-----	-----
Free GSH (μM)	986.35 ± 344.50	927.0 ± 330.4	1033.1 ± 341.9	**0.001**
GSSG(μM)	33.34 ± 23.10	34.13 ± 23.32	32.71 ± 22.94	0.505
% GSSG	3.76 ± 3.33	4.101 ± 3.527	3.495 ± 3.138	0.048
Total GSH (μM)	1019.69 ± 344.90	961.1 ± 340.2	1065.8 ± 342.2	**0.001**
Apoptosis (U/L)	1059.14 ± 373.75	1083.1 ± 370.5	1021.9 ± 377.5	0.195
8-oxo-dG (Ct^2-Ct^1)	0.24 ± 0.52	0.219 ± 0.462	0.261 ± 0.552	0.669
Liver Stiffness (kPA)	2.31 ± 0.52	2.328 ± 0.546	2.304 ± 0.501	0.614
Liver Fibrosis Prevalence	44/478 (9.2%)	22/210 (10.5%)	22/268 (8.2%)	0.395

P-Values in bold indicate statistical significance (P <0.05). Data for continuous variables is presented as mean ± STD. Statistical differences between study groups was detected using independent t-tests for continuous variables and Chi-Square tests for categorical variables. BMI: Body Mass Index, TyG Index: Triglyceride-Glucose Index, HIV VL: HIV Viral Load, Free GSH: reduced glutathione, GSSG: oxidized glutathione, Total GSH: total glutathione.

Table 2. Pearson Correlation between Dietary Intake Variables, Oxidative Stress, Apoptosis, and Liver Stiffness

	Total Calories (kcal)	Saturated Fat (g)	Total Fat (g)	Total Cholesterol (mg)
8-oxo-dG (Ct^2-Ct^1)	0.118 P=0.430	0.074 P=0.623	0.100 P=0.503	0.065 P=0.663
Total GSH	-0.107 P=0.141	-0.074 P=0.307	-0.099 P=0.191	-0.026 P=0.722
Free GSH	-0.100 P=0.168	-0.701 P=0.332	-0.096 P=0.186	-0.030 P=0.683
Apoptosis	**0.162** **P=0.045**	**0.176** **P=0.030**	**0.168** **P=0.038**	0.030 P=0.712
Mean Liver Stiffness	0.010 P=0.895	0.008 P=0.909	0.001 P0.990	0.083 0.257

Bold P-Values Indicate statistical significance (P < 0.05).

Table 3. Multiple Linear Regression models showing associations between Dietary Variables and Hepatic Apoptosis.

	β	SE	*t*	*p*
Total Calories (kcal)	0.045	0.026	1.699	0.092
Total Fat (g)	1.158	0.600	1.930	0.056
Saturated Fat (g)	3.26	1.634	1.980	**0.050**

Bold P-Values Indicate statistical significance (P < 0.05). All models adjusted for Age, Sex, BMI, Cocaine, and HIV status.

Table 4. Unadjusted Comparisons of Oxidative Stress, Apoptosis, Liver Stiffness and Fibrosis

	Coc+	Coc-	P	Steat+	Steat-	P	Low TyG	High TyG	P
Free GSH (μM)	1045.3 ± 343.5	945.9 ± 339.9	**0.002**	955.3 ± 306.0	992.9 ± 352.0	0.367	1003.4 ± 355.6	919.6 ± 286.5	**0.015**
GSSG (μM)	33.80 ± 23.24	33.03 ± 23.04	0.720	33.87 ± 17.80	33.23 ± 24.08	0.819	33.39 ± 24.60	32.65 ± 15.82	0.776
% GSSG	3.487 ± 2.825	3.951 ± 3.621	0.134	3.951 ± 3.428	3.722 ± 3.306	0.569	3.742 ± 3.487	3.773 ± 2.542	0.934
Total GSH (μM)	1079.1 ± 344.9	978.9 ± 339.5	**0.002**	989.2 ± 306.7	1026.1 ± 352.4	0.376	1036.8 ± 355.5	952.2 ± 289.4	**0.014**
Apoptosis (U/L)	1115.0 ± 434.2	1022.6 ± 324.6	**0.050**	1140.2 ± 368.2	1044.2 ± 373.7	0.131	1042.2 ± 367.1	1125.0 ± 398.9	0.146
8-oxo-dG (Ct^2-Ct^1)	0.300 ± 0.471	0.223 ± 0.533	0.474	0.177 ± 0.527	0.261 ± 0.515	0.491	0.234 ± 0.532	0.250 ± 0.505	0.868
Liver Stiffness (kPa)	2.300 ± 0.403	2.325 ± 0.588	0.589	2.459 ± 0.698	2.284 ± 0.471	**0.005**	2.290 ± 0.71	2.416 ± 0.67	**0.033**
Liver Fibrosis	62/194 (32.0%)	82/284 (28.9%)	0.470	31/83 (37.3%)	113/395 (28.6%)	0.115	69/238 (29.0%)	75/238 (31.5%)	0.549

Bold P-Values indicate statistical significance (P <0.05).

Table 5. One-Way ANOVA. Groups separated by HIV Status and Steatosis (All Post-Hoc Analysis included Bonferroni Correction)

	Mean Value	F	P-Value	Differences between four groups	P-Value
Total GSH (μM)		4.081	**0.007**	Groups 1 and 2: -52.51	1.000
				Groups 1 and 3: -118.13	0.755
Group 1: HIV$^+$, Steatosis$^+$	916.59			Groups 1 and 4: -156.50	0.096
Group 2: HIV$^+$, Steatosis$^-$	969.10			Groups 2 and 3: -65.62	1.000
Group 3: HIV$^-$, Steatosis$^+$	1034.72			Groups 2 and 4: -104.00	**0.016**
Group 4: HIV$^-$, Steatosis$^-$	1073.09			Groups 3 and 4: -38.38	1.000
Free GSH (μM)		4.216	**0.006**	Groups 1 and 2: -56.28	1.000
				Groups 1 and 3: -123.77	0.650
Group 1: HIV$^+$, Steatosis$^+$	879.26			Groups 1 and 4: -160.88	0.078
Group 2: HIV$^+$, Steatosis$^-$	935.54			Groups 2 and 3: -67.49	1.000
Group 3: HIV$^-$, Steatosis$^+$	1003.03			Groups 2 and 4: -104.60	**0.015**
Group 4: HIV$^-$, Steatosis$^-$	1040.14			Groups 3 and 4: -37.11	1.000
Apoptosis (U/L)		1.547	0.203	Groups 1 and 2: 66.16	1.000
				Groups 1 and 3: 0.08	1.000
Group 1: HIV$^+$, Steatosis$^+$	1140.21			Groups 1 and 4: 145.10	0.631
Group 2: HIV$^+$, Steatosis$^-$	1074.05			Groups 2 and 3: -66.08	1.000
Group 3: HIV$^-$, Steatosis$^+$	1140.13			Groups 2 and 4: 78.93	0.762
Group 4: HIV$^-$, Steatosis$^-$	995.11			Groups 3 and 4: 145.02	0.760
Liver Stiffness (kPa)		3.647	**0.013**	Groups 1 and 2: 0.305	**0.015**
				Groups 1 and 3: 0.207	0.471
Group 1: HIV$^+$, Steatosis$^+$	2.588			Groups 1 and 4: 0.303	**0.014**
Group 2: HIV$^+$, Steatosis$^-$	2.283			Groups 2 and 3: -0.098	1.000
Group 3: HIV$^-$, Steatosis$^+$	2.382			Groups 2 and 4: -0.002	1.000
Group 4: HIV$^-$, Steatosis$^-$	2.285			Groups 3 and 4: 0.096	1.000

Bold P-Values indicate statistical significance (P <0.05). Models shown above did not control for any covariates due to smaller sample sizes when separated into four study groups. Group 1: 32, Group 2: 179, Group 3: 52, Group 4: 217.

Table 6. One-Way ANOVA. Groups separated by HIV Status and TyG Risk (All Post-Hoc Analysis included Bonferroni Correction)

	Mean Value	F	P-Value	Differences between four groups	P-Value
Total GSH (μM)		5.985	**0.001**	Groups 1 and 2: -14.09	1.000
				Groups 1 and 3: -1.706	1.000
Group 1: HIV$^+$, High TyG	951.29			Groups 1 and 4: -141.10	0.069
Group 2: HIV$^+$, Low TyG	965.38			Groups 2 and 3: 12.39	1.000
Group 3: HIV$^-$, High TyG	953.00			Groups 2 and 4: -127.01	**0.002**
Group 4: HIV$^-$, Low TyG	1092.40			Groups 3 and 4: -139.40	**0.041**
Free GSH (μM)		6.088	**<0.001**	Groups 1 and 2: -12.25	1.000
				Groups 1 and 3: -1.34	1.000
Group 1: HIV$^+$, High TyG	918.85			Groups 1 and 4: -140.86	0.069
Group 2: HIV$^+$, Low TyG	931.10			Groups 2 and 3: 10.92	1.000
Group 3: HIV$^-$, High TyG	920.18			Groups 2 and 4: -128.60	**0.002**
Group 4: HIV$^-$, Low TyG	1059.70			Groups 3 and 4: -139.52	**0.040**
Apoptosis (U/L)		1.477	0.221	Groups 1 and 2: 48.17	1.000
				Groups 1 and 3: -10.65	1.000
Group 1: HIV$^+$, High TyG	1121.80			Groups 1 and 4: 128.26	0.604
Group 2: HIV$^+$, Low TyG	1073.62			Groups 2 and 3: -58.83	1.000
Group 3: HIV$^-$, High TyG	1132.45			Groups 2 and 4: 80.08	0.788
Group 4: HIV$^-$, Low TyG	993.54			Groups 3 and 4: 138.91	0.777
Liver Stiffness (kPa)		3.682	**0.012**	Groups 1 and 2: 0.288	**0.007**
				Groups 1 and 3: 0.256	0.090
Group 1: HIV$^+$, High TyG	2.557			Groups 1 and 4: 0.252	**0.021**
Group 2: HIV$^+$, Low TyG	2.270			Groups 2 and 3: -0.032	1.000
Group 3: HIV$^-$, High TyG	2.302			Groups 2 and 4: -0.036	1.000
Group 4: HIV$^-$, Low TyG	2.306			Groups 3 and 4: -0.003	1.000

Bold P-Values indicate statistical significance (P <0.05). Models shown above did not control for any covariates due to smaller sample sizes when separated into four study groups. Group 1: 45, Group 2: 165, Group 3: 55, Group 4: 213.

Table 7. Multiple Linear Regression showing associations between TyG Index, Cocaine Use, and HIV Infection with primary outcomes.

Free GSH	β	SE	*t*	*p*
TyG Index	-40.60	24.52	-1.656	0.098
Cocaine Use	88.00	31.88	2.761	**0.006**
HIV Status	-95.24	31.75	-3.000	**0.003**
Total GSH				
TyG Index	-39.73	24.57	-1.617	0.107
Cocaine Use	89.20	31.95	2.792	**0.005**
HIV Status	-93.60	31.82	-2.942	**0.003**
Apoptosis				
TyG Index	64.46	36.51	1.766	0.079
Cocaine Use	94.62	48.11	1.967	**0.050**
HIV Status	58.39	38.52	1.203	0.230
Liver Stiffness				
TyG Index	0.118	0.038	3.100	**0.002**
Cocaine Use	-0.001	0.049	-0.017	0.986
HIV Status	0.023	0.049	0.472	0.637

Bold P-Values indicate statistical significance (P <0.05). All Models Adjusted for Age, Sex, and BMI. Free GSH (n=276), Total GSH (n=476), Apoptosis (n=261), Liver Stiffness (n=475).

Table 8. TyG Index predicts liver fibrosis in both PLWH and combined study groups.

TyG Index	PLWH Only (n=211)	P Value	All Participants (n=476)	P Value
Unadjusted	2.593 (1.442, 4.664)	**0.001**	1.610 (1.042, 2.490)	**0.032**
Adjusted for age, sex, and BMI	2.557 (1.403, 4.661)	**0.002**	1.628 (1.042, 2.542)	**0.032**
Adjusted for age, sex, BMI, Cocaine and HIV VL	2.718 (1.469, 5.028)	**0.001**	1.593 (1.009, 2.515)	**0.046**

Bold P-Values indicate statistical significance (P <0.05).

References

1. Croxford S, Kitching A, Desai S, et al. Mortality and causes of death in people diagnosed with HIV in the era of highly active antiretroviral therapy compared with the general population: an analysis of a national observational cohort. Lancet Public Health. 2017;2(1):e35-e46. doi:10.1016/S2468-2667(16)30020-2

2. Blachier M, Leleu H, Peck-Radosavljevic M, et al. The burden of liver disease in Europe: A review of available epidemiological data. J Hepatol. 2013;58(3):593-608. doi:10.1016/j.jhep.2012.12.005

3. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038-1048. doi:10.1016/j.metabol.2015.12.012

4. Maurice JB, Patel A, Scott AJ, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. AIDS. 2017;31(11):1621-1632. doi:10.1097/QAD.0000000000001504

5. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. doi:10.1002/hep.28431

6. Kardashian A, Ma Y, Scherzer R, et al. Sex differences in the association of HIV infection with hepatic steatosis. AIDS. 2017;31(3):365-373. doi:10.1097/QAD.0000000000001334

7. Price JC, Seaberg EC, Latanich R, et al. Risk factors for fatty liver in the multicenter AIDS Cohort. 2014;2014(5):695-704. doi:10.1038/ajg.2014.32.Risk

8. Blanco F, Barreiro P, Ryan P, et al. Risk factors for advanced liver fibrosis in HIV-infected individuals: Role of antiretroviral drugs and insulin resistance. J Viral Hepat. 2011;18(1):11-16. doi:10.1111/j.1365-2893.2009.01261.x

9. Lemoine M, Lacombe K, Bastard JP, et al. Metabolic syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected patients. AIDS. 2017;31(14):1955-1964. doi:10.1097/QAD.0000000000001587

10. Bugianesi E, Moscatiello S, Ciaravella MF, Marchesini G. Insulin Resistance in Nonalcoholic Fatty Liver Disease. Curr Pharm Des. 2010;16(17):1941-1951. doi:10.2174/138161210791208875

11. Guilherme A, Virbasius J V., Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(5):367-377. doi:10.1038/nrm2391

12. Williams JC, Zhang X, Karki M, et al. Soluble CD14, CD163, and CD27 biomarkers distinguish ART-suppressed youth living with HIV from healthy controls. J Leukoc Biol. 2018;103(4):671-680. doi:10.1002/JLB.3A0717-294RR

13. Vodkin I, Valasek MA, Bettencourt R, et al. Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: A case-control study. Aliment Pharmacol Ther. 2015;41(4):368-378. doi:10.1111/apt.13052

14. Paradis V, Perlemuter G, Bonvoust F, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: A potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001;34(4 I):738-744. doi:10.1053/jhep.2001.28055

15. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2011;6:425-456. doi:10.1146/annurev-pathol-011110-130246

16. Nomiyama H, Hieshima K, Nakayama T, et al. Human CC chemokine liver-expressed chemokine/CCL16 is a functional ligand for CCR1, CCR2 and CCR5, and constitutively expressed by hepatocytes. Int Immunol. 2001;13(8):1021-1029. doi:10.1093/intimm/13.8.1021

17. Tuyama AC, Hong F, Saiman Y, et al. Human Immunodeficiency Virus (HIV)-1 infects human hepatic stellate cells and promotes collagen I and monocyte chemoattractant protein-1 expression: Implications for the pathogenesis of HIV/hepatitis C virus-induced liver fibrosis. Hepatology. 2010;52(2):612-622. doi:10.1002/hep.23679

18. Gupta D, Rani M, Khan N, Jameel S. HIV-1 infected peripheral blood mononuclear cells modulate the fibrogenic activity of hepatic stellate cells through secreted TGF-b and JNK signaling. PLoS One. 2014;9(3):1-11. doi:10.1371/journal.pone.0091569

19. Pessayre D, Fromenty B, Berson A, et al. Central role of mitochondria in drug-induced liver injury. Drug Metab Rev. 2012;44(1):34-87. doi:10.3109/03602532.2011.604086

20. Fromenty B, Robin MA, Igoudjil A, et al. The ins and outs of mitochondrial dysfunction in NASH. Diabetes Metab. 2004;30(2):121-138. doi:10.1016/S1262-3636(07)70098-8

21. Begriche K, Massart J, Robin MA, et al. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. Hepatology. 2013;58(4):1497-1507. doi:10.1002/hep.26226

22. Luedde T, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: Mechanisms and clinical relevance. Gastroenterology. 2014;147(4):765-783. doi:10.1053/j.gastro.2014.07.018

23. Liu W, Baker S, Baker R, Zhu L. Antioxidant Mechanisms in Nonalcoholic Fatty Liver Disease. Curr Drug Targets. 2015;16(12):1301-1314. doi:10.2174/1389450116666150427155342

24. Videla LA, Rodrigo R, Orellana M, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci. 2004;106(3):261-268. doi:10.1042/CS20030285

25. Mota M, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism. 2016;65(8):1049-1061. doi:10.1016/j.metabol.2016.02.014

26. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. Semin Liver Dis. 2008;28(4):360-369. doi:10.1055/s-0028-1091980

27. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998;94(4):491-501. doi:10.1016/S0092-8674(00)81590-1

28. Milhas D, Cuvillier O, Therville N, et al. Caspase-10 triggers bid cleavage and caspase cascade activation in fasL-induced apoptosis. J Biol Chem. 2005;280(20):19836-19842. doi:10.1074/jbc.M414358200

29. Babu CK, Suwansrinon K, Bren GD, et al. HIV induces TRAIL sensitivity in hepatocytes. PLoS One. 2009;4(2):1-9. doi:10.1371/journal.pone.0004623

30. Forrester JE, Rhee MS, McGovern BH, et al. The association of HIV viral load with indirect markers of liver injury. J Viral Hepat. 2012;19(2):e202-e211. doi:10.1111/j.1365-2893.2011.01529.x

31. Campa A, Martinez SS, Sherman KE. Cocaine Use and Liver Disease are Associated with All-Cause Mortality in the Miami Adult Studies in HIV (MASH) Cohort. J Drug Abus. 2016;2(4):pii:27. doi:10.21767/2471-853x.100036

32. Vitcheva V. Cocaine toxicity and hepatic oxidative stress. Curr Med Chem. 2012;19(33):5677-82. doi: 10.2174/092986712803988929. PMID: 22856658.

33. Baum M, Rafie C, Lai S, et al. Crack-Cocaine Use Accelerates HIV Disease Progression in a Cohort of HIV-Positive Drug Users. J Acquir Immune Defic Syndr. 2009;50(1):93-99. doi:10.1097/QAI.0b013e3181900129

34. Beer L, Valverde EE, Raiford JL, et al. Clinician perspectives on delaying initiation of antiretroviral therapy for clinically eligible HIV-infected patients. J Int Assoc Provid AIDS Care. 2015;14(3):245-254. doi:10.1177/2325957414557267

35. Hernandez J. Gut Integrity, Microbial Translocation, Immune Activation and Vitamin D in Drug Users Living with HIV. 2020. Florida International University.

36. Simental-Mendía, LE, Rodriguez-Moran M, Guerro-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. Metab Syndr Relat Disord. 2008;6(4):299-304. doi:10.1089/met.2008.0034

37. Tang A, Tan J, Sun M, et al. Nonalcoholic Fatty Liver Disease: MR Imaging of Liver Proton Density Fat Fraction to Assess Hepatic Steatosis. Radiology. 2013;267(2):422-431. doi:10.1148/radiol.12120896

38. Venkatesh SK, Ehman RL. Magnetic resonance elastography of liver. Magn Reson Imaging Clin N Am. 2014. doi:10.1016/j.mric.2014.05.001

39. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038-1048. doi:10.1016/j.metabol.2015.12.012

40. Soti S, Corey KE, Lake JE, et al. NAFLD and HIV: Do Sex, Race, and Ethnicity Explain HIV-Related Risk?. Current HIV/AIDS Rep. 2018. 15(3):212-222. doi:10.1007/s11904-018-0392-1

41. Tamargo JA, Sherman KE, Campa A, et al. Food insecurity is associated with magnetic resonance-determined nonalcoholic fatty liver and liver fibrosis in low-income, middle-aged adults with and without HIV. Am J Clin Nutr. 2021 Jan 29:nqaa362. doi: 10.1093/ajcn/nqaa362.

42. Baum MK, Tamargo JA, Ehman RL, et al. Heroin use is associated with liver fibrosis in the Miami Adult Studies on HIV (MASH) cohort. Drug Alcohol Depend. 2021 Mar 1;220:108531. doi:10.1016/j.drugalcdep.2021.108531.

43. Blackard JT, Welge JA, Taylor LE, et al. HIV mono-infection is associated with FIB-4 - A noninvasive index of liver fibrosis - In women. Clin Infect Dis. 2011;52(5):674-680. doi:10.1093/cid/ciq199

44. Morse C, McLaughlin M, Matthews L, et al. Nonalcoholic Steatohepatitis and Hepatic Fibrosis in HIV-1-Monoinfected Adults With Elevated Aminotransferase Levels on Antiretroviral Therapy. Clin Infect Dis. 2015;60(10):1569-78. Doi: 10.1093/cid/civ101

45. Lui G, Wong VWS, Wong GLH, et al. Liver fibrosis and fatty liver in Asian HIV-infected patients. Aliment Pharmcol Ther. 2016.;44(4):411-21. doi:10.1111/apt.13702

46. Lombardi R, Sambatakou H, Mariolis I, et al. Prevalence and predictors of liver steatosis and fibrosis in unselected patients with HIV mono-infection. Dig Liver Dis. 2016;48(12):1471-1477. doi:10.1016/j.dld.2016.08.117

47. Shin DH, Martinez SS, Parsons M, et al. Relationship of oxidative stress with HIV disease progression in HIV/HCV co-infected and HIV mono-infected adults in Miami. Int J Biosci Biochem Bioinforma. 2012;2(3):217-223. doi: 10.7763/iijbbbb.2012.v2.104

48. Sanchez-Valle V, Chavez-Tapia NC, Uribe M, Mendez-Sanchez N. Role of oxidative stress and molecular changes in liver fibrosis: A review. Curr Med Chem. 2012;19(28):4850-4860. doi:10.2174/092986712803341520.

49. Aksenov M, Aksenova MV, Nath A, et al. Cocaine-mediated enhancement of Tat toxicity in rat hippocampal cell cultures: the role of oxidative stress and D1 dopamine receptor. Neurotoxicology. 2006;27(2):217-28. doi:10.1016/j.neuro.2005.10.003

50. Videla LA, Rodrigo R, Orellana M, et al. Oxidative stress-related parameters in the liver of nonalcoholic fatty liver disease patients. Clin Sci (Lond). 2004;106(3):261-268. doi:10.1042/CS20030285

51. Malaguarnera L, Madeddu R, Palio E, et al. Heme oxygenase-1 levels and oxidative stress-related parameters in non-alcoholic fatty liver disease patients. J Hepatol. 2005;42(4):585-591. doi:10.1016/j.hep.2004.11.040

52. Hernández EA, Kahl S, Seelig A, et al. Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. J Clin Invest. 2017;127(2): 695-708. doi:10.1172/JCI89444

53. Dandona P, Aljada A,Chaudhuri A, et al. et al. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation. 2005;111(11):1448-54. doi:10.1161/01.CIR.0000158483.13093.9D

Chapter VI: SUMMARY OF CONCLUSIONS AND IMPACT ON PRACTICE

This study investigated the ability of the triglyceride-glucose (TyG) Index to predict liver steatosis in people living with HIV (PLWH) and the association between the TyG Index and biomarkers of immune activation, inflammation, oxidative stress, and liver fibrosis in the Miami Adult Studies on HIV (MASH) cohort.

Over the past two decades, the disease burden of PLWH has shifted from advanced immunodeficiency syndrome (AIDS), to a disease that has been correlated with similar chronic disease states as seen in the general population.[1] The MASH Cohort has been closing studying the relationship been HIV infection and liver disease for over a decade, and we know that PLWH are more than 3.7x more likely to die of liver disease than individuals in the general population.[2] The most common form of liver disease in the world is non-alcoholic fatty liver disease (NAFLD), also known as liver steatosis. Previous estimates have reported around 35% of PLWH have steatosis compared to 25% of the general population.[3] The presence of steatosis increases the likelihood of individuals developing liver fibrosis.[4] In PLWH in particular, these individuals present a unique set of risk factors that may explain the increased risk of liver steatosis and fibrosis in this population; direct effects of from HIV infection, anti-retroviral therapy (ART) related hepatotoxicity, food insecurity, and substance abuse.[5-10]

The gold standards of measuring liver steatosis are liver biopsy and magnetic resonance imaging-protein density fat fraction (MRI-PDFF) score. Each of these measures can be invasive, expensive, and difficult to apply to a large

population. This study found the TyG Index was a good predictor of liver steatosis in both PLWH and uninfected controls. Similar findings have been previously shown in HBV-HIV coinfected adults, but not among HIV monoinfected individuals.[11] Our study found identified a TyG Index score of 8.5938 as the optimal cut-off value to distinguish between individuals with and without liver steatosis in PLWH, the sensitivity of this value = 0.697 with a specificity of 0.669. The total area under the curve (AUC) of the Receiver-Operator Curve (ROC) was marginally higher in the PLWH compared to the uninfected control group (P=0.068). In PLWH, participants with a TyG Index score >8.5835 were 4.638x more likely to have liver steatosis than participants with TyG Index scores < 8.5835.

It has been previously shown that PLWH have increased levels of microbial translocation by through activation of Kupffer cells and Hepatic Stellate Cells (HSCs) in the liver.[12,13] Greater microbial translocation activation is associated with both increased metabolic syndrome and lipid accumulation in the liver[14] while simultaneously activating pro-fibrotic pathways.[15,16] Markers of immune activation related to increased microbial translocation include sCD14 and sCD163,[17] both of which were increased in the current study. Liver fibrosis pathway markers TGF-β and TIMP-1 were also increased among PLWH in the current study, suggesting the HIV virus actives collagen deposition pathways[18,19] and inhibits the breakdown of extracellular matrix (ECM) proteins.[20,21]

In multiple regression analysis, the TyG Index was associated with both sCD14 (β =0.080, P=0.050) and sCD163 (β = 0.164, P=0.008). We found HIV

infection was associated with both increased immune activation and fibrosis pathway markers. Increased plasma levels of sCD27 (β = 0.181, P=0.005), TGF-β, (β =0.915, P<0.001) and TIMP-1 (β = 0.118, P=0.034) were all associated with HIV infection. Furthermore, among PLWH only, higher HIV VL was associated with increased sCD27 (β=0.124, P<0.001). Together, these findings indicate even well controlled HIV infection is associated with immune activation but more severe HIV disease also contributes to increased immune activation as well. Cocaine was associated with increased levels of sCD14 (β = 0.116, P=0.024), sCD27 (β=0.123, P=0.011), and sCD163 (β =0.219, P=0.003), consistent with previous MASH cohort findings.[22]

Previous studies have found IR[14] and metabolic syndrome[23] to be associated with liver fibrosis in PLWH. However, to the best of our knowledge, there have not been any studies that have measured IR using the TyG Index. Because the TyG Index has been previously shown to be a better predictor of NAFLD than HOMA-IR, and it is calculated using only fasting triglycerides and glucose, both laboratory values typically obtain at a routine health screening, we chose to examine the relationship between TyG Index and liver fibrosis. Chapter 3 of this work found the TyG Index value of 8.5938 was an optimal cut-off value to determine steatosis risk in PLWH. We found High TyG risk was associated with greater liver stiffness (P=0.012) in PLWH. We also found HIV infection was associated with lower levels of Free GSH (β= -95.24, P=0.003) and Total GSH (β= -93.60, P=0.003), consistent with previous MASH Cohort findings of increased oxidative stress among PLWH.[24]

In conclusion, we found the TyG Index can be a good proxy measurement for estimating steatosis risk in PLWH. When used either as a marker of insulin resistance or liver steatosis, higher TyG Index levels are indicative of poor metabolic health. This study found the TyG Index was associated with both immune activation and liver fibrosis in the MASH Cohort. It also appears as though there may be an association between increased TyG Index and lower Total Glutathione as well. The current MASH cohort sample consistent of PLWH with high rates of ART adherence and well controlled HIV disease. These individuals have a higher risk of developing cardiometabolic co-morbidities than HIV-AIDS related health issues, therefore the focus on the TyG Index as a determinant of liver disease is representative of a concern that many PLWH are living with. These findings suggest the TyG Index as a marker of metabolic health is a primary determinant of liver disease outcomes in PLWH. This index is an easy to obtain measurement that can be primary treatment target among healthcare practitioners in the prevention of liver disease.

References

1. Weber R, Sabin CA, Friis-Moller N, et al. Liver-Related Deaths in Persons Infected With the Human Immunodeficiency Virus: The D:A:D Study. Arch Intern Med. 2006;166:1632-1641. doi:10.1001/archinte.166.15.1632

2. Croxford S, Kitching A, Desai S, et al. Mortality and causes of death in people diagnosed with HIV in the era of highly active antiretroviral therapy compared with the general population: an analysis of a national observational cohort. Lancet Public Health. 2017;2(1):e35-e46. doi:10.1016/S2468-2667(16)30020-2

3. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. doi:10.1002/hep.28431

4. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038-1048. doi:10.1016/j.metabol.2015.12.012

5. Forrester JE, Rhee MS, McGovern BH, et al. The association of HIV viral load with indirect markers of liver injury. J Viral Hepat. 2012;19(2):e202-e211. doi:10.1111/j.1365-2893.2011.01529.x

6. Brown TT, Li X, Cole SR, et al. Cumulative exposure to nucleoside analogue reverse transcriptase inhibitors is associated with insulin resistance markers in the Multicenter AIDS Cohort Study. AIDS. 2005;19(13):1375-1383. doi:10.1097/01.aids.0000181011.62385.91

7. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: A 3-year randomized trial. J Am Med Assoc. 2004;292(2):191-201. doi:10.1001/jama.292.2.191

8. Baum MK, Raflie C, Lai S, et al. Alcohol use accelerates HIV disease progression. AIDS Re Hum Retroviruses. 2010;26(5):511 doi:10.1089/aid.2009.0211

9. Campa A, Martinez SS, Sherman KE. Cocaine Use and Liver Disease are Associated with All- Cause Mortality in the Miami Adult Studies in HIV (MASH) Cohort. J Drug Abus. 2016;2(4):pii:27. doi:10.21767/2471-853x.100036

10. Tamargo JA, Sherman KE, Campa A, et al. Food insecurity is associated with magnetic resonance-determined nonalcoholic fatty liver and liver fibrosis in low-income, middle-aged adults with and without HIV. Am J Clin Nutr. 2021 Jan 29:nqaa362. doi: 10.1093/ajcn/nqaa362.

11. Sterling RK, King WC, Khalili M, et al. Performance of Serum-Based Scores for Identification of Mild Hepatic Steatosis in HBV Mono-infected and HBV-HIV Co-infected Adults. Dig Dis Sci. 2021. Feb 8. doi:10.1007/s10620-021-06860-3

12. Utay N, Ananworanich J, Slike B, et al. Inflammation persists despite early initiation of ART in acute HIV infection [abstract 47]. In: Program and abstracts of the 2015 Conference on Retroviruses and Opportunistic Infections, Seattle, Washington, 23–26 February 2015.

13. Joshi D, O'Grady J, Dieterich D, et al. Increasing burden of liver disease in patients with HIV infection. Lancet. 2011; 377:1198-209. doi: 10.1016/S0140-6735(10)62001-6.

14. Lemoine M, Lacombe K, Bastard JP, et al. Metabolic syndrome and obesity are the cornerstones of liver fibrosis in HIV-monoinfected patients. Aids. 2017;31(14):1955-1964. doi:10.1097/QAD.0000000000001587

15. Corbitt N, Kimura S, Isse K, et al. Gut bacteria drive kupffer cell expansion via MAMP-mediated ICAM-1 induction on sinusoidal endothelium and influence preservation-reperfusion injury after orthotopic liver transplantation. Am J Pathol. 2013;182(1):180-191. doi:10.1016/j.ajpath.2012.09.010

16. Schnabl B. Interactions Between the Intestinal Microbiome and Liver Diseases. Gastroenterology. 2015;146(6):1513-1524. doi:10.1053/j.gastro.2014.01.020.Interactions

17. Williams JC, Zhang X, Karki M, et al. Soluble CD14, CD163, and CD27 biomarkers distinguish ART-suppressed youth living with HIV from healthy controls. J Leukoc Biol. 2018;103(4):671-680. doi:10.1002/JLB.3A0717-294RR

18. Koek GH, Liedorp PR, Bast A. The role of oxidative stress in non-alcoholic steatohepatitis. Clin Chim Acta. 2011;412(15-16):1297-1305. doi:10.1016/j.cca.2011.04.013

19. Fabregat I, Moreno-Càceres J, Sánchez A, et al. TGF-β signalling and liver disease. FEBS J. 2016;283(12):2219-2232. doi:10.1111/febs.13665

20. Robert S, Gicquel T, Bodin A, et al. Characterization of the MMP/TIMP imbalance and collagen production induced by IL-1 β or TNF-α release from human hepatic stellate cells. PLoS One. 2016;11(4):e0153118. doi:10.1371/journal.pone.015311

21. Roderfeld M. Matrix metalloproteinase functions in hepatic injury and fibrosis. Matrix Biol. 2018;68-69:452-462. doi:10.1016/j.matbio.2017.11.011

22. Hernandez J. Gut Integrity, Microbial Translocation, Immune Activation and Vitamin D in Drug Users Living with HIV. 2020. Florida International University.

23. Lombardi R, Sambatakou H, Mariolis I, et al. Prevalence and predictors of liver steatosis and fibrosis in unselected patients with HIV mono-infection. Dig Liver Dis. 2016;48(12):1471-1477. doi:10.1016/j.dld.2016.08.117

24. Shin DH, Martinez SS, Parsons M, et al. Relationship of oxidative stress with HIV disease progression in HIV/HCV co-infected and HIV mono-infected adults in Miami. Int J Biosci Biochem Bioinforma. 2012;2(3):217-223.

CHAPTER VII: FUTURE RESEARCH

Our study demonstrated the TyG Index was a good predictor of liver steatosis, and was associated with immune activation and liver fibrosis in PLWH. However, many biomarkers analyzed in this study including sCD14, sCD27, sCD163, TGF-β, TIMP-1 and hepatic apoptosis had a more limited sample size than liver steatosis and fibrosis measurements and appeared to be statistically underpowered. Continuing to analyze more participants in the MASH Cohort and improving the statistical power to detect differences between study groups would provide extensive value towards understanding the relationship between metabolic health and liver disease in PLWH. Furthermore, this work used only cross-sectional analysis, the COVID-19 pandemic ceased the ability of the MASH Cohort research team to continuously collect data on the development of liver fibrosis. Future MASH Cohort studies should compare participants with high TyG Risk at baseline to participants with low TyG Risk and compare the likelihood of developing liver fibrosis over time. Additionally, the MASH Cohort and other similar cohort shave identified many factors independent factors associated with liver fibrosis; including: HIV viral infection, ART use, substance abuse, micronutrient status, food insecurity, and metabolic health. The development of a liver fibrosis risk score that combines fibrosis risk factors into a single index could be a convenient tool for health practitioners to use in determine fibrosis risk in PLWH. Lastly, the impact of TyG and TyG Risk on mortality risk, would provide an excellent insight into the overall impact of the TyG Index and metabolic health overall on health outcomes in PLWH.

VITA

COLBY S. TEEMAN

Born, North Kansas City, Missouri

2009-2013	B.S., Exercise Science University of Central Missouri Warrensburg, MO
2013-2016	Graduate Research Assistant Kansas State University Manhattan, KS
2013-2016	M.S., Human Nutrition Kansas State University Manhattan, KS
2016-2020	Graduate Research Assistant Florida International University Miami, FL
2016-2021	Doctoral Candidate Florida International University Miami, FL
2021	Clinical Dietitian Palmetto General Hospital Miami, FL

PUBLICATIONS

Teeman CS, Kurti SP, Cull BJ, Emerson SR, Haub MD, Rosenkranz SK. Postprandial lipemic and inflammatory responses to high-fat meals: a review of the roles of acute and chronic exercise. Nutrition and Metabolism. 2016. 13:80.

Emerson SR, Haub MD, Teeman CS, Kurti SP, Rosenkranz SK. Summation of blood glucose and TAG to characterise the ‘metabolic load index’. British Journal of Nutrition. 2016. 13:26.

Kurti SP, Rosenkranz SK, Chapes SK, Teeman CS, Cull BJ, Emerson SR, Levitt MH, Smith JR, Harms CA. Does chronic physical activity level modify the airway inflammatory response to an acute bout of exercise in the postprandial period? Applied Physiology, Nutrition and Metabolism. 2016. 18:1-8.

Kurti SP, Emerson SR, Rosenkranz SK, Teeman CS, Emerson EM, Cull BJ, Smith JR, Harms CA. Post-prandial systemic 8-isoprostane increases after consumption of moderate and high-fat meals in insufficiently active males. Nutrition Research. 2017. 39:61-68

Emerson SR, Kurti SP, Teeman CS, Emerson EM, Cull BJ, Haub MD, Rosenkranz SK. Realistic Test-Meal Protocols Lead to Blunted Postprandial Lipemia but Similar Inflammatory Responses Compared with a Standard High-Fat Meal. Curr Dev Nutr. 2017. 22;1(4):e000232.

Sherman KE, Abdel-Hameed EA, Ehman RL, Rouster SD, Campa A, Martinez SS, Huang Y, Zarini GG, Hernandez J, Teeman C, Tamargo J, Liu Q, Mandler R, Baum MK. Validation and Refinement of Noninvasive Methods to Assess Hepatic Fibrosis: Magnetic Resonance Elastography Versus Enhanced Liver Fibrosis Index. Dig Dis Sci. 2020;65(4):1252-1257.

Zarini G, Sales Martinez S, Campa A, Sherman K, Tamargo J, Hernandez Boyer J, Teeman C, Johnson A, Degarege A, Greer P, Liu Q, Huang Y, Mandler R, Choi D, Baum MK. Sex Differences, Cocaine Use, and Liver Fibrosis Among African Americans in the Miami Adult Studies on HIV Cohort. J Womens Health (Larchmt). 2020 Sep;29(9):1176-1183.

Tamargo JA, Sherman KE, Campa A, Martinez SS, Li T, Hernandez J, Teeman C, Mandler RN, Chen J, Ehman RL, Baum MK. Food insecurity is associated with magnetic resonance-determined nonalcoholic fatty liver and liver fibrosis in low-income, middle-aged adults with and without HIV. Am J Clin Nutr. 2021 Jan 29:nqaa362. doi: 10.1093/ajcn/nqaa362.

Baum MK, Tamargo JA, Ehman RL, Sherman KE, Chen J, Liu Q, Mandler RN, Teeman C, Martinez SS, Campa A. Heroin use is associated with liver fibrosis in the Miami Adult Studies on HIV (MASH) cohort. Drug Alcohol Depend. 2021 Mar 1;220:108531. doi: 10.1016/j.drugalcdep.2021.108531. Epub 2021 Jan 19. PMID: 33508691; PMCID: PMC7889727.