ASSOCIATION OF LIPOPOLYSACCHARIDE-BINDING PROTEIN WITH INCREASES IN LIVER FAT IN AFRICAN ANCESTRY MEN

by

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a disease with increasing prevalence worldwide, especially in developed countries. While the etiology of NAFLD is complex, the intestinal microbiome may play a role in the development of liver steatosis through bacterial-induced inflammation. Lipopolysaccharide-binding protein (LBP) is an acute-phase protein produced by the liver and which facilitates bacterial-induced inflammation. LBP levels in the serum tend to be low but are raised during infection or in the presence of bacterial components; thus, levels of LBP are considered to be reflective of bacterial-induced inflammation. While the association of LBP with NAFLD has been investigated in cross-sectional studies, no longitudinal studies have been done to determine if LBP levels are associated with liver fat accumulation. Furthermore, no studies have been performed which show the heritability of LBP levels, which may affect susceptibility to the inflammatory response. Elucidation of the role of LBP in NAFLD can provide insights into the etiology of NAFLD and provide a mechanistic link between the intestinal microbiome and liver fat accumulation, yielding public health importance for the understanding and treatment of NAFLD. This study is the first longitudinal study to look at the associations of LBP with the development of steatosis and it is the first study to determine the

heritability of LBP in any population, thus supplying novel information on the associations between LBP and liver fat accumulation.

A large family-based study of African ancestry men and women (N = 470) and a large prospective cohort study of African ancestry men (N = 2853) from Tobago were used for this study. LBP was measured in all individuals in the family study. Heritability analyses were performed using SOLAR. In the prospective cohort study, LBP levels were measured at baseline visit (2004-2007), and liver fat was assessed at the follow-up visit (2013-2016, ~10 years later) by computerized tomography (CT) scan, with an overlap of 204 men having both LBP and liver CT completed. Associations were assessed using Spearman correlations and regression analyses. LBP levels in the families were found to have no residual heritability, suggesting that LBP might be entirely environmentally determined in this population. In the prospective cohort, LBP was associated with liver fat infiltration in multivariable analyses which included BMI (p = 0.0469), but not in models which instead included waist circumference. In conclusion, we determined that among African ancestry individuals, LBP levels are completely environmentally determined and that levels may be associated with increases in liver fat accumulation.

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PREFACE

I would like to firstly thank the staff and participants of the Tobago Health Study and the Tobago Family Study, whose hard work and selfless giving are critical for the pursuit of knowledge and betterment of health. I would also like to thank the members of the Heinz Nutrition and Zmuda Laboratories, especially Cara Nestlerode, who helped me with learning to work with serum samples, running assays, and troubleshooting freezer issues.

Additionally, I would like to thank my thesis committee members, Dr. Zmuda and Dr. Minster, for your guidance, patience, and encouragement throughout both the masters and thesis process. Finally, I want to extend a special thank you to my thesis advisor Dr. Miljkovic, who always made the time to help me through my questions and results (even when she was extremely busy), who encouraged me to pursue projects of my interest, and who was a never-ending font of positivity that kept me sane throughout my MS. I look forward to barging into your office as a PhD student!

1.0 INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as the buildup of hepatic fat in the absence of heavy alcohol drinking[1]. NAFLD is extremely common, with a worldwide prevalence rate of 6%–35% and a median of 20%[2]. NAFLD serves as an umbrella term for a spectrum of liver disease, ranging from the relatively benign non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis and liver cancer[1]. NAFLD is associated with many of the components of metabolic syndrome and so is thought to be the manifestation of metabolic syndrome in the liver[3,4]. In addition, individuals with NAFLD are at increased risk of developing type 2 diabetes and cardiovascular disease[4].

Pathogenesis and progression of NAFL and NASH are complex and not currently not clear, involving a complex interplay between diet, genetics, insulin resistance, adipose tissue and inflammation[3-5]. One hypothesis of progression to NASH is the “two-hit hypothesis”, where the first hit is the accumulation of fat in the liver and the second hit is either lipid peroxidation or cytokine-mediated injury leading to liver inflammation and fibrosis[6]. Of recent interest to the development of NAFLD is the role of the gut microbiota. In mouse models, the colonization of germ-free mouse intestines with bacteria was associated with a 2.3-fold increase in hepatic triglycerides with no changes in total liver free fatty acids or cholesterol[7]. Small intestinal bacterial overgrowth was more prevalent among NASH patients than in matched healthy controls[8].

Additionally, individuals with NAFLD have been found to have greater intestinal permeability, and this is associated with NAFLD severity[9].

One method by which intestinal bacteria could impact NAFLD pathogenesis is through effects on systemic inflammation. Lipopolysaccharide (LPS) is a lipid constituent of the Gram-negative bacterial membrane[10]. Bacteria shed LPS throughout their lifecycle, and this LPS can be absorbed in the intestinal tract and circulate through the body via the hepatic portal vein[11]. Once in the circulation, it has the potential to bind with the cell receptor toll-like receptor 4 (TLR4) and to initiate an inflammatory cytokine response[12]. Lipopolysaccharide-binding protein (LBP) is an acute-phase protein produced primarily by the liver and plays a role in LPS-induced inflammation[11,13]. LBP and LBP-like proteins are found widely across the animal kingdom and are thought to originate from a family of ancient lipid-binding proteins, and while a few residues are conserved across some species, most LBP proteins share common motifs[14]. LBP binds to LPS, complexes with protein CD14, and then transfers LPS to the TLR4[12]. It has been demonstrated in mice that chronic LPS infusion promotes weight gain similar to eating a high fat diet[15]; additionally, obesity is commonly found to be associated with chronic low-grade inflammation[16]. Thus, it is believed that gut bacteria-derived LPS and subsequent transference by LBP is thought to be linked to systemic low-grade inflammation and inflammatory diseases.

LBP has been associated with obesity, insulin resistance, and metabolic syndrome[17-21]. Due to the associations with metabolic syndrome, it is not surprising that LBP is also associated with NAFLD. Ruiz et al. demonstrated that, compared to healthy controls, obese patients with NASH had elevated levels of LBP, and individuals with NASH had higher levels of LBP than those with just steatosis. Additionally, the study showed that levels of LBP correlated with liver TNF-α

mRNA production[22]. A study by Wong et al. found in a prospective Chinese cohort that individuals with incident NAFLD had higher LBP levels at baseline[23].

Despite associations with NAFLD itself, it is controversial whether LBP is associated with liver fat progression or just with the other metabolic phenotypes often accompanying NAFLD. In the same study by Wong et al., it was seen in a larger cross-sectional group that LBP was positively correlated with intrahepatic triglycerides in a univariate analysis; however, the association with intrahepatic triglycerides became insignificant in a multivariable analysis[23]. Similarly, in a study of bariatric surgery patients with different severities of NAFLD, LBP was found to be higher in all NAFLD groups compared to lean healthy controls, but could neither discriminate between severities of NAFLD nor between those with normal liver and those with NASH/NASH fibrosis, indicating that it may only be tracking components of the metabolic syndrome in those individuals[24].

It is possible that LBP may only be associated with earlier increases in liver fat but may not play a role in later stages of NASH. It appears that no prospective studies have looked at LBP in relation to hepatic fat accumulation; rather, all studies have been cross-sectional or have only reported on incident cases of NAFLD and not on unit change in liver fat. Furthermore, studies looking at LBP and liver fat accumulation have not been performed in African ancestry populations. Thus, this study set out to determine if baseline levels of LBP could predict liver fat accumulation in an African ancestry population.

2.0 METHODS

2.1 THE TOBAGO HEALTH STUDIES

Between 1997 and 2003, 3,170 previously unscreened men were recruited for a population-based prostate cancer screening study on the Caribbean island of Tobago, Trinidad and Tobago[25]. To be eligible, men had to be ambulatory, noninstitutionalized, and not terminally ill. Recruitment for the survey was accomplished by flyers, public service announcements, and posters, informing health care workers at local hospitals and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated, and participation was similar across the island parishes. This group of age-eligible men served as the pool of potential probands for the Tobago Family Health Study and as the first visit of the Tobago Health Study (the men recruited and followed for the population cohort).

Men in the Tobago health studies cohort are primarily of West African Ancestry, similar to African Americans. Genetic ancestry markers have determined ~94% West African ancestry in the Tobago population[26]. In addition to sharing West African ancestry, Tobagonian Afro-Caribbean men and African American men also share similarities in skeletal muscle quality and body fat distribution[27-31]. However, low gene flow due to limited in-migration and out-migration from the island, as well as cultural differences as compared to the U.S. and Africa, make the Tobagonian men a unique and informative African Ancestry study population.

2.1.1 *Study Population: Tobago Family Health Study*

Probands for the Tobago Family Health Study were identified from the Tobago Health Study. To be eligible, a proband had to be Afro-Caribbean (determined by self-report of having four Afro-Caribbean grandparents), have had a spouse who was willing to participate in the study, and have at least six living offspring and/or siblings aged 18+ years who were residing in Tobago. Probands and families were recruited without regard to health status, resulting in 7 multigenerational families (mean family size $N = 51$) with 401 individuals spanning 18–103 years old (mean age = 43). Among the families, we have the following relationships: 361 parent–offspring, 495 full siblings, 101 grandparent–grandchildren, 1,137 avuncular, 61 half-siblings, and 1,380 cousins (3,535 relative pairs). All participants provided written informed consent.

2.1.2 *Study Population: Tobago Prostate Cohort Study*

Between 2004 and 2007, all men from the Tobago Health Study were invited to participate in a follow-up clinic examination. 2,031 men (70% of survivors) and 451 new participants completed the visit. This visit represented the baseline for the current study.

Between 2014 and 2017, we invited these men to return for repeat clinical examinations and CT scans. The baseline and follow-up visits followed the same procedures for questionnaire interviews and biospecimen collection. The Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services approved this study. All participants provided written informed consent before data collection.

2.2 STUDY-SPECIFIC VARIABLES: FAMILY STUDY

2.2.1 *Dual-Energy X-ray Absorptiometry Measures*

Dual-energy X-ray absorptiometry (DXA) measurement of total body adipose tissue was made using a Hologic QDR 4500W densitometer (Hologic Inc., Bedford MA). Scans were analyzed with QDR software version 8.26a.

2.2.2 *Anthropometric and Lifestyle Measurements*

Body weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. BMI was calculated from body weight and standing height (kg/m^2). Information on lifestyle habits (smoking status [current/not current], minutes walked per week, and alcohol drinking [yes/no]), medication use, and post-menopausal status for women (yes/no), were assessed using standardized interviewer-administered questionnaires. Hypertension was defined as a systolic blood pressure (SBP) of ≥ 140 mmHg and/or diastolic blood pressure (DBP) of ≥ 90 mmHg. Type 2 diabetes was defined as fasting serum glucose ≥ 126 mg/dL and/or currently taking antidiabetic medication.

2.3 STUDY-SPECIFIC VARIABLES: COHORT STUDY

2.3.1 *Liver CT Scans*

CT scanning of the liver was performed using dual slice, high-speed NX/I scanner, with gantry speed 0.7 seconds (GE Medical Systems, Waukesha, WI) to assess liver fat. CT images at

T12-L1 were obtained and used to measure the liver located below the right diaphragm corresponding to superior aspects of the right and medial lobes or hepatic segments 4a, 7 and 8 using the Couinaud system. The analysts deposit 3 regions of interest (ROIs) within homogenous portions of the liver at two levels. Liver attenuation measured in Houndsfield units (HU) was calculated as the average density of three regions. CT scans were read at the Wake Forest University (WFU) Imaging Center and analyzed using Medical Image Processing, Analysis, and Visualization (MIPAV) software with custom programmed subroutines (a.k.a."plug-ins") coded at WFU Health Sciences (WFUHS). MIPAV is a software application produced by the National Institute of Health's Center for Information Technology; Biomedical Imaging Research Services Section lead by Dr. Matthew McAuliffe[32].

2.3.2 *Anthropometric, Lifestyle, and Medicinal Measurements*

Body weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. BMI was calculated from body weight and standing height (kg/m^2). Waist circumference was measured at the narrowest point of the waist using an inelastic fiberglass tape. If there was no narrowest point, waist circumference was measured at the umbilicus. Information on lifestyle habits (smoking status [never/ever/current], hours walked per week, hours of television watching, and current intake of alcohol of more than 8 drinks per week [yes/no]), and medication use (non-steroidal anti-inflammatory drugs [NSAIDs], anti-hypertensives, and antidiabetics) were assessed using standardized interviewer-administered questionnaires. Men were asked to bring all prescription medications taken in the past 30 days to their clinic visit. Participants also rated their overall health status compared with men their own age. Prediabetes was defined as having a fasting serum glucose level of 100–125 mg/dL without being on any antidiabetic medication. Type 2

diabetes was defined as currently taking an antidiabetic medication, regardless of fasting serum glucose level, or having a fasting serum glucose level of ≥ 126 mg/dL. Hypertension was defined as a SBP of ≥ 140 mmHg and/or DBP of ≥ 90 mmHg and/or currently taking antihypertensive medication. Fatty liver disease was defined as HU < 40.

2.4 SHARED STUDY VARIABLES: INFLAMMATION AND METABOLIC MARKERS

All biochemical assays in fasting serum samples were performed in the Heinz Nutrition Laboratory at the University of Pittsburgh. Fasting serum glucose was measured using an enzymatic procedure; the coefficient of variation percentage (CV%) between runs was 1.8%. Insulin was measured using a radioimmunoassay procedure developed by Linco Research, Inc.; the CV% between runs was 2.1%. The degree of insulin resistance (IR) was estimated by homeostatic model assessment (HOMA) according to the method described by Matthews et al.[33]. In previous studies, HOMA-IR has correlated reasonably well with insulin clamp techniques[34]. High-density lipoprotein cholesterol (HDL-c) was determined using the selective heparin/manganese chloride precipitation method and had an inter-assay coefficient of variation (CV) of 2.1%. Low density lipoprotein cholesterol (LDL-c) was calculated by means of the Friedewald equation[35]. Triglycerides were determined enzymatically using the procedure of Bucolo and David[36] and had an interassay CV of 1.7%. Baseline fasting serum LBP was measured using a Human LBP Enzyme-Linked Immunosorbent Assay (ELISA) kit (Cell Sciences, Canton, MA) according to the manufacturer's protocol. Manufacturer-reported inter- and intra-assay CV% were 9.8%–17.8% and 6.1%, respectively.

2.5 STATISTICAL ANALYSES

Heritability analyses were performed using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program. Two models were generated: one sex- and age-adjusted, and one where all covariates were screened and where only significant covariates were retained. The residual heritability (h^2_r) for LBP was estimated as well as the variance attributable to the fixed-covariance effects (r^2) for each of the additional variables.

To be included in the cohort liver analysis, men must have fasted at least 8 hours prior to baseline interview blood draw, must have had LBP and lipids measured at the baseline visit, and must have had liver CT scans analyzed at the follow-up. 1385 men had lipids measured, 582 men had LBP measured at baseline, and 550 men had liver CT scans analyzed at follow-up; overlap between these two populations was 199 individuals who were eligible for analysis. One individual was found to have fasted < 8 hours and so was dropped from analysis. One individual was also found to have an outlier >3 standard deviations from the mean in LBP and was dropped from analysis; two individuals had missing LBP values. This resulted in a final sample size of 195 for analysis.

Associations between liver attenuation and other variables were determined using Spearman correlations. For multiple linear regression analyses, liver attenuation was cube-transformed to normalize the residuals. Multiple logistic regression models were constructed to examine one of two outcome variables: (1) dichotomizing liver attenuation values based on the median liver attenuation value and (2) membership in the highest and lowest quartiles of liver attenuation value. Statistical significance was based on an $\alpha = 0.05$, and analyses were performed using SAS 9.3 software (SAS Institute, Inc., Cary, NC).

3.0 RESULTS

3.1 HERITABILITY OF LBP

3.1.1 *Family Study Population Characteristics*

Population characteristics of the families can be found in Table 1. Participants on average were 42 years of age and were slightly overweight with a median BMI of 27.55 kg/m^2. They were relatively healthy with low rates of drinking (13.22%) and smoking (4.91%) and with low rates of hypertension (28.63%) and type 2 diabetes (15.84%).

3.1.2 *Results of Heritability Analysis*

In the age- and sex-adjusted models, the heritability of LBP was extremely low and non-significant ($h^2_r = 0.067$, $p = 0.237$), and the proportion of variance due to age and sex was 0.0483. A second model was generated in which all covariates (all variables listed in Table 1; excluded BMI from analysis since trunk fat was used) were tested but only significant covariates were retained (age, trunk fat, alcohol drinking category, menopause status, HDL-c, and walking); in this model, LBP was not heritable ($h^2_r = 0.000$, $p = 0.500$), and the proportion of variance due to the covariates was 0.142.

Table 1. Family Study Population Characteristics

Variable	*N*	Median (IQR) or *N* (%)
General Characteristics		
Age (years)	471	42 (28, 54)
Sex (Female)	471	284 (60.30)
Post-Menopausal	463	88 (19.01)
BMI (kg/m^2)	466	27.55 (23.48, 31.95)
Trunk Fat (kg)	444	10.03 (6.296, 13.92)
DBP (mmHg)	461	74 (67, 82.67)
SBP (mmHg)	461	116.67 (105.33, 138.67)
Lifestyle Factors		
Walking (min/week)	466	20 (0, 60)
Drink Alcohol	469	62 (13.22)
Current Smoker	468	23 (4.91)
Hypertensive	461	132 (28.63)
Type 2 Diabetes	461	73 (15.84)
Metabolic Measures		
HDL-c (mg/dL)	397	39.20 (31.10, 48.50)
LDL-c (mg/dL)	398	127.55 (104, 157.90)
Triglycerides (mg/dL)	401	77 (59, 104)
HOMA-IR	394	2.77 (1.95, 4.17)
LBP (µg/mL)	462	25.04 (17.68, 34.61)

IQR = Interquartile Range

3.2 LBP AND LIVER FAT

3.2.1 *Cohort Population Characteristics*

General baseline characteristics of men in the cohort are reported in Table 2. Men in the longitudinal cohort were relatively young with a median age of 53. They were slightly overweight (median BMI = 27.03 kg/m^2), and about half were hypertensive (54.36%). Incidence of NAFLD at follow-up was low (4.10%). 23.59% of the men were prediabetic and about 15% diabetic at baseline.

As can be seen in the population comparison table (Appendix Table 8), compared to the overall baseline population, the analyzed men were slightly healthier (lower levels of drinking, lower levels of current smoking, fewer type 2 diabetics, less medication usage, and greater perception of good health status). LBP values were similar between the analyzed cohort and the 582 men in which LBP was measured; similarly, mean liver attenuation was similar between the analyzed cohort and the men in which liver CTs had been analyzed.

Table 2. LBP/Liver Cohort Population Characteristics

Variable	Median (IQR) or *N* (%)
LBP (µg/mL)	20.89 (16.30, 27.11)
Mean Liver Attenuation (HU)	59.36 (54.69, 61.87)
Age (years)	53 (48, 64)
Waist Circumference (cm) [a]	91 (84.3, 98)
BMI (kg/m^2)	27.03 (24.59, 29.56)
Lifestyle	
Hours Walked per Week [b]	1.5 (0, 5)
TV Watching (≥ 14 hrs/week) [c]	67 (34.54)
Previous Smoker	39 (20)
Current Smoker	10 (5.13)
Any Alcohol Consumption	113 (57.95)
4+ Alcoholic Drinks / Week	14 (7.18)
8+ Alcoholic Drinks / Week	7 (3.59)
Comorbidities	
SBP (mmHg)	139 (126, 154)
DBP (mmHg)	80 (73, 89)
Hypertension	106 (54.36)
Prediabetic	46 (23.59)
Diabetic	29 (14.87)
HOMA-IR	2.75 (1.85, 4.11)
HDL-c (mg/dL)	48 (41.10, 57.10)
LDL-c (mg/dL)	134.50 (103.10, 162.20)
Triglycerides (mg/dL)	103 (77, 131)
Fatty Liver Disease	8 (4.10)
Perceived Good Health	183 (94.33)
Medication Use	
Antihypertensive Medication	42 (21.54)
Antidiabetic Medication	19 (9.74)
NSAIDs Medication	13 (6.67)

[a,b,c]: Variables that had less than *N* = 195. Waist circumference, *N* = 193. Walking, *N* = 191. TV watching, *N* = 194. Abbreviation: TV = Television.

3.2.2 *Correlation Analyses*

Spearman correlations were calculated between covariates and mean liver attenuation values. The lower the attenuation value, the higher the liver fat content; thus, inverse correlations are interpreted as an increase in liver fat for every unit increase in the variable.

LBP, BMI, waist circumference, insulin resistance, triglycerides, diastolic blood pressure, and anti-hypertensive medications were all significantly and negatively correlated with mean liver attenuation, while HDL was significantly and positively correlated (Table 3). The association between LBP and mean liver attenuation was then determined after adjustment for the other covariates. For this analysis, hypertensive status was not included as a covariate (as SBP and DBP were in the model), and the model was run twice using either BMI or waist circumference. After adjustment for the other covariates, the association between LBP and increases in liver fat were attenuated but remained significant in both models (BMI adjusted model: $N = 190$, $\rho = -0.182$, $p = 0.0158$; waist-circumference adjusted model: $N = 188$, $\rho = -0.174$, $p = 0.0223$).

Table 3. Spearman Correlations with Mean Liver Attenuation

Variables	ρ	p
LBP (µg/mL)	−0.20	0.0043
Age (years)	0.04	0.5479
BMI (kg/m^2)	−0.37	< 0.0001
Waist Circumference (cm) [a]	−0.37	< 0.0001
HOMA-IR	−0.29	< 0.0001
HDL-c (mg/dL)	0.24	0.0006
LDL-c (mg/dL)	−0.09	0.2121
Triglycerides (mg/dL)	−0.21	0.0026
Walking (hr/week) [b]	0.001	0.9924
TV (≥ 14 hrs/week) [c]	0.07	0.3376
Alcohol 8+	0.10	0.1689
Smoking Status	0.07	0.3102
Hypertension	−0.06	0.3822
DBP (mmHg)	−0.15	0.0362
SBP (mmHg)	−0.07	0.3694
Anti-diabetic Use	−0.13	0.0736
NSAID Use	−0.09	0.2309
Anti-hypertensive Use	−0.15	0.0328

[a,b,c]: Variables that had less than $N = 195$. Waist circumference, $N = 193$. Walking, $N = 191$. TV watching, $N = 194$

3.2.3 *Linear Regression*

A simple linear regression analysis was performed between LBP and mean liver attenuation. Mean liver attenuation was very heavily left-skewed, resulting in non-normally distributed residuals (Figure 1). Therefore, a cube-transformed liver value was used for all linear regression analyses, which corrected this distribution. In the univariable model, LBP was significantly associated with having higher liver fat values at followup ($\beta = -11.5$, $p = 0.0071$).

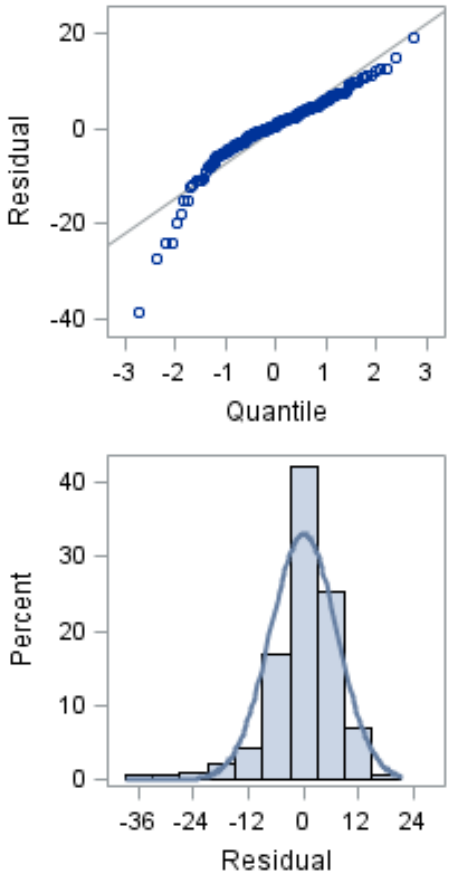

Figure 1. Quantile-Quantile Plot and Histogram of Residuals

Multivariable linear regressions were then performed (Tables 4). Importantly, waist circumference is thought to be more important for liver fat accumulation than general obesity[37]; however, with our small sample size and with waist circumference having been measured in two fewer individuals than BMI, any reduction in sample size could reduce power; thus, two analyses were performed, with models differing only in the use of BMI or waist circumference.

In both models, no variance inflation or collinearity was detected according to diagnostics run in SAS. In the BMI model, the adjusted R^2 was 0.184. In this model, mean liver attenuation was negatively associated with LBP ($p = 0.0469$) and BMI ($p = 0.0006$), indicating that increasing

LBP and BMI are associated with increases in liver fat. Interestingly, mean liver attenuation was almost statistically significantly and positively associated with heavy drinking (p = 0.0501); however, the standard error and confidence intervals are large, likely due to the very low number of heavy drinking in this population.

In the waist circumference model, the adjusted R^2 was 0.171. Mean liver attenuation was only significantly and negatively associated with waist circumference (p = 0.0023) but not significantly with LBP (p = 0.0547). Additionally, heavy drinking was significantly and positively associated with mean liver attenuation (p = 0.0226), indicating that those who drank the most in this population were protected from liver fat accumulation. Again, the standard error and confidence intervals were quite large for this estimate due to the low number of heavy drinkers.

Table 4. Linear Regression Models

Baseline Variable	Model with BMI (N = 190)			Model with Waist Circumference (N = 188)		
	β (SE)	95% CI	p	β (SE)	95% CI	p
LBP (µg/mL)	−10.36 (8.22)	(−13.03, −2.49)	0.0469	−10.30 (8.27)	(−13.02, 2.81)	0.0547
Age (years)	7.55 (7.94)	(−8.24, 11.24)	0.3927	8.42 (7.98)	(−7.41, 11.70)	0.2424
BMI (kg/m^2)	−16.38 (10.78)	(−19.00, −12.43)	0.0006	—	—	—
WC (cm)	—	—	—	−11.44 (7.85)	(−13.49, −8.16)	0.0023
HOMA-IR	−12.33 (13.58)	(−18.96, 14.54)	0.4556	−13.43 (13.62)	(−19.50, 13.69)	0.3392
HDL-c (mg/dL)	5.66 (7.45)	(−8.59, 9.99)	0.6610	5.48 (7.50)	(−8.73, 9.99)	0.6961
LDL-c (mg/dL)	1.06 (4.70)	(−5.89, 5.91)	0.9908	−2.44 (4.72)	(−6.05, 5.78)	0.8895
Triglycerides (mg/dL)	−4.78 (4.59)	(−6.69, 4.34)	0.2605	−4.58 (4.62)	(−6.63, 4.62)	0.3316
DBP (mmHg)	−7.24 (8.65)	(−11.83, 9.64)	0.5576	−7.30 (8.71)	(−11.92, 9.71)	0.5571
SBP (mmHg)	6.51 (7.33)	(−7.94, 10.17)	0.4841	5.89 (7.40)	(−8.41, 10.01)	0.6147
Drink 8+	35.99 (28.70)	(−3.13, 45.36)	0.0501	38.23 (28.95)	(19.95, 46.99)	0.0226
Walking (hr/week)	5.38 (9.85)	(−12.01, 12.69)	0.8712	−3.98 (9.88)	(−12.53, 12.26)	0.9479
Smoking Status	17.94 (19.97)	(−21.51, 27.81)	0.4693	19.50 (20.13)	(−20.55, 28.65)	0.3645
TV (≥ 14 hrs/week)	13.23 (21.17)	(−25.41, 27.61)	0.8077	10.87 (21.32)	(−26.13, 27.33)	0.8948
Anti-diabetics	−22.45 (25.27)	(−35.08, 27.38)	0.4839	−24.47 (25.43)	(−36.12, 26.12)	0.3744
NSAIDs	7.02 (26.58)	(−33.24, 33.45)	0.9853	10.67 (27.09)	(−33.63, 34.33)	0.9514
Anti-hypertensives	−26.06 (22.84)	(−34.54, 17.99)	0.1393	−24.09 (23.01)	(−33.63, 21.59)	0.2526

Abbreviations: SE = Standard Error, WC = Waist Circumference.

In observing the regression diagnostics, it was noticed that there were several influential points as determined by Cook's $D > 0.02$ (Figure 2). To investigate these further, a plot of studentized r^2 by

leverage for each observation was created (Figure 3). An $r^2 > 4$ and a leverage $h_i > 0.17$ were used as criteria for determining points which had potentially problematic residual values and whose leverage may affect the regression model. Following these criteria, two observations were found which could be problematic.

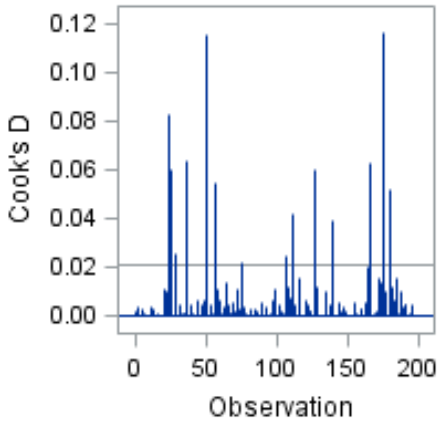

Figure 2. Cook's D Plot of Influential Points

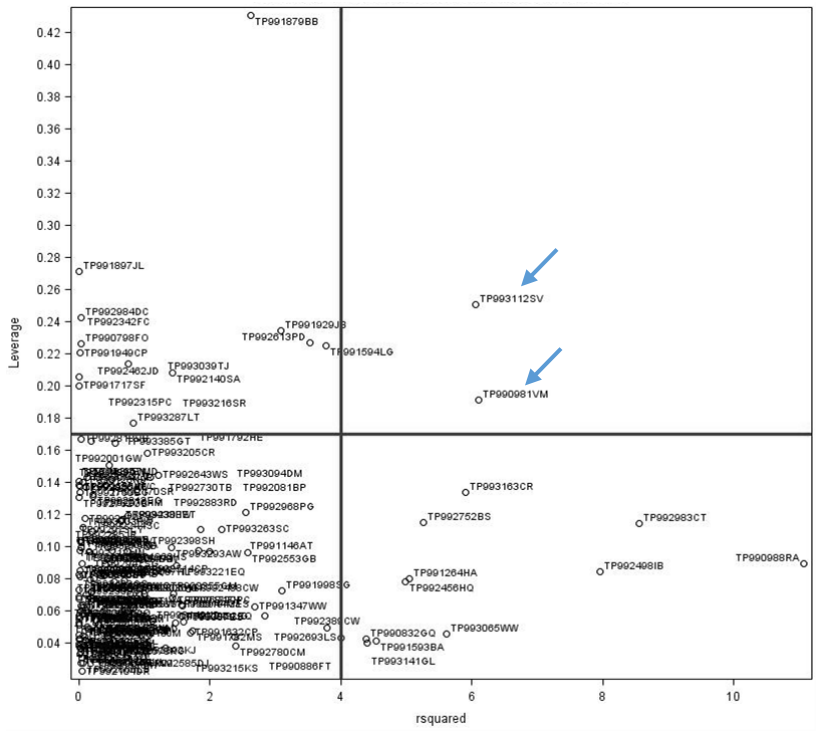

Figure 3. Residual Squared by Leverage Plot

The two observations found in the above analysis were inspected further to determine what was driving their influence and to determine if they should be dropped from the analysis (Table 5). For each individual, covariate values and DFBETA (the amount that observation contributes to the standard error of the estimate) was listed. In the first individual, ID = TP990981VM, the BMI was higher than most men and most influence on an estimate (DFBETA = −0.7008); additionally, mean liver attenuation was very low compared to most men. However, these values were not likely to be errors, and therefore the participant should not be dropped from analysis. The second participant had no outstanding values but was one of the individuals who was a heavy drinker, which may be the reason for his being flagged. It was therefore determined that this participant should also not be dropped from the analysis.

Table 5. Influential Participant Characteristics and DFBetas

TP990981VM		
Variable	**Value**	**DFBETA**
LBP (µg/mL)	14.72	0.15
Age (years)	50	−0.02
BMI (kg/m^2)	40.21	−0.70
HOMA-IR	6.49	−0.24
HDL-c (mg/dL)	65.50	−0.49
LDL-c (mg/dL)	92.70	0.25
Triglycerides (mg/dL)	79	0.12
DBP (mmHg)	79	0.07
SBP (mmHg)	124	0.24
Drink 8+	No	0.12
Walking (hr/week)	10	−0.13
Smoking Status	Ever	−0.39
TV (≥14 hrs/week)	No	0.07
Anti-diabetics	No	0.32
NSAIDs	No	0.26
Anti-hypertensives	Yes	−0.53
Mean Liver Attenuation (HU)	18.54	—

TP993112SV		
Variable	**Value**	**DFBETA**
LBP (µg/mL)	22.20	0.21
Age (years)	49	−0.08
BMI (kg/m^2)	26.30	0.36
HOMA-IR	8.81	−0.68
HDL-c (mg/dL)	45.40	0.38
LDL-c (mg/dL)	173.60	−0.05
Triglycerides (mg/dL)	85	0.38
DBP (mmHg)	93	0.25
SBP (mmHg)	168	−0.37
Drink 8+	Yes	−1.09
Walking (hr/week)	7	−0.08
Smoking Status	Never	0.24
TV (≥14 hrs/week)	Yes	−0.07
Anti-diabetics	No	0.11
NSAIDs	No	0.12
Anti-hypertensives	No	0.17
Mean Liver Attenuation (HU)	49.65	—

3.2.4 *Logistic Regression*

Two sets of logistic regression analyses were performed to determine if LBP could predict categorization by liver fat values at follow-up. The first set divided the population into high/low based off the median liver attenuation value, 59.3553 HU. Two logistic regression models were again done, using BMI or waist circumference (Table 6). In the univariable analyses, 97 people had liver attenuations below the median and 98 had liver attenuations above the median. In the multivariable analyses, the population was split 95 above the median and 95 below. Only baseline LBP (OR = 1.063, 95% CI: 1.017–1.112) and BMI (OR = 1.228, 95% CI: 1.091–1.382) could significantly predict being in the top 50% of fatty livers. In the waist circumference model, the population was split with 95 above the median and 93 below. Again, only baseline LBP (OR = 1.059, 95% CI: 1.014–1.106) and waist circumference (OR = 1.059, 95% CI: 1.014–1.106) could significantly predict being in the top 50% of fatty livers.

Mean liver attenuation was then divided into quartiles. Logistic regression analysis was run to determine if LBP could predict being in the highest liver fat quartile (mean liver attenuation ≤ 54.6946 HU) versus the lowest liver fat quartile (mean liver attenuation ≥ 61.8709 HU) (Table 7). In univariable analyses, 48 people were in the high-fat quartile and 49 in the low-fat quartile. In multivariable analyses, 46 people were in the high-fat quartile and 49 in the low-fat quartile. Similar to the median-based analysis, in the BMI model only LBP (OR = 1.106, 95% CI: 1.019–1.200) and BMI (OR = 1.336, 95% CI: 1.087–1.642) could significantly predict high-fat quartile. In the WC model, only LBP could significantly predict high/low fatty liver quartile (OR = 1.089, 95% CI: 1.008–1.176).

Table 6. Logistic Regression (Median)

Baseline Variable	Univariable Analysis (*N* = 195)		Multivariable Analysis (BMI, *N*=190)		Multivariable Analysis (WC, *N* = 188)	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
LBP (µg/mL)	1.06 (1.02, 1.10)	0.0025	1.06 (1.02, 1.11)	0.0072	1.06 (1.01, 1.11)	0.0101
Age (years)	1.00 (0.98, 1.03)	0.9043	1.01 (0.97, 1.05)	0.5793	1.00 (0.96, 1.04)	0.9538
BMI (kg/m^2)	1.23 (1.13, 1.34)	<.0001	1.23 (1.09, 1.38)	0.0007	—	—
WC (cm) [a]	1.07 (1.04, 1.11)	<.0001	—	—	1.06 (1.01, 1.11)	0.0094
HOMA-IR	1.25 (1.06, 1.49)	0.0100	0.89 (0.73, 1.09)	0.2602	0.94 (0.77, 1.15)	0.5326
HDL-c (mg/dL)	0.96 (0.93, 0.98)	0.0009	0.97 (0.94, 1.01)	0.1371	0.97 (0.94, 1.01)	0.1133
LDL-c (mg/dL)	1.01 (0.998, 1.01)	0.1573	1.00 (0.99, 1.01)	0.5891	1.00 (0.995, 1.01)	0.4589
Triglycerides (mg/dL)	1.01 (1.00, 1.02)	0.0071	1.00 (0.995, 1.01)	0.5595	1.00 (0.99, 1.01)	0.6878
DBP (mmHg)	1.02 (0.999, 1.05)	0.0630	1.00 (0.95, 1.05)	0.9498	1.00 (0.95, 1.05)	0.9243
SBP (mmHg)	1.01 (0.997, 1.03)	0.1187	1.00 (0.97, 1.03)	0.9233	1.01 (0.97, 1.04)	0.7491
Drink 8+	0.39 (0.07, 2.07)	0.2699	0.27 (0.04, 2.01)	0.2015	0.21 (0.03, 1.67)	0.1383
Walking (hr/week) [b]	1.03 (0.96, 1.09)	0.4502	1.03 (0.95, 1.11)	0.5267	1.04 (0.97, 1.12)	0.3117
Past Smoker	0.81 (0.40, 1.65)	0.5631	0.52 (0.23, 1.19)	0.1230	0.52 (0.22, 1.20)	0.1225
Current Smoker	0.63 (0.17, 2.33)	0.4898	0.92 (0.20, 4.37)	0.9198	0.77 (0.17, 3.55)	0.7345
TV (≥14 hrs/week) [c]	0.73 (0.40, 1.32)	0.2912	0.73 (0.35, 1.52)	0.3999	0.78 (0.38, 1.61)	0.5018
Anti-diabetics	1.84 (0.69, 4.88)	0.2236	1.63 (0.45, 5.91)	0.4576	1.77 (0.50, 6.26)	0.3768
NSAID Use	2.40 (0.71, 8.08)	0.1567	1.82 (0.40, 8.31)	0.4382	1.60 (0.34, 7.54)	0.5512
Anti-hypertensive Use	2.13 (1.05, 4.33)	0.0355	1.39 (0.55, 3.51)	0.4877	1.19 (0.47, 3.01)	0.7130

[a,b,c]: Variables that had less than *N* = 195 for univariable analyses.
Waist circumference, *N* = 193. Walking, *N* = 191. TV watching, *N* = 194

Table 7. Logistic Regression (Quartiles)

Baseline Variable	Univariable Analysis (*N* = 98)		Multivariable Analysis (BMI, *N* = 95)		Multivariable Analysis (WC, *N* = 93)	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
LBP (µg/mL)	1.08 (1.02, 1.14)	0.0124	1.11 (1.02, 1.20)	0.0157	1.09 (1.01, 1.18)	0.0301
Age (years)	0.97 (0.93, 1.01)	0.1791	1.00 (0.94, 1.07)	0.9461	0.99 (0.93, 1.06)	0.8325
BMI (kg/m^2)	1.32 (1.15, 1.51)	<.0001	1.34 (1.09, 1.64)	0.0059	—	—
WC (cm)	1.09 (1.04, 1.15)	0.0004	—	—	1.07 (0.999, 1.14)	0.0532
HOMA-IR	1.44 (1.11, 1.88)	0.0069	0.96 (0.66, 1.40)	0.8240	1.08 (0.74, 1.59)	0.6823
HDL-c (mg/dL)	0.94 (0.90, 0.98)	0.0039	0.97 (0.91, 1.03)	0.2880	0.98 (0.92, 1.04)	0.4249
LDL-c (mg/dL)	1.00 (0.995, 1.01)	0.4269	1.00 (0.99, 1.01)	0.9853	1.00 (0.99, 1.01)	0.9510
Triglycerides (mg/dL)	1.01 (1.00, 1.02)	0.0239	1.00 (0.99, 1.01)	0.7990	1.00 (0.99, 1.01)	0.7947
DBP (mmHg)	1.03 (0.999, 1.06)	0.0579	1.01 (0.94, 1.09)	0.7764	1.03 (0.96, 1.11)	0.3820
SBP (mmHg)	1.01 (0.99, 1.03)	0.1948	1.01 (0.95, 1.06)	0.8323	1.00 (0.95, 1.05)	0.8794
Drink 8+	0.32 (0.03, 3.18)	0.3306	0.26 (0.01, 4.78)	0.3614	0.13 (0.004, 3.69)	0.2309
Walking (hr/week) [a]	0.96 (0.88, 1.05)	0.4079	1.00 (0.88, 1.14)	0.9919	1.01 (0.90, 1.14)	0.8267
Past Smoker	0.62 (0.24, 1.58)	0.3124	0.48 (0.14, 1.63)	0.2397	0.51 (0.16, 1.64)	0.2593
Current Smoker	0.65 (0.13, 3.11)	0.5857	0.78 (0.08, 7.46)	0.8299	0.58 (0.07, 4.99)	0.6187
TV (≥14 hrs/week)	0.71 (0.31, 1.60)	0.4076	0.85 (0.28, 2.60)	0.7817	0.82 (0.28, 2.40)	0.7127
Anti-diabetics	60.25 (1.25, 29.14)	0.0256	6.83 (0.80, 58.32)	0.0790	8.14 (0.97, 68.06)	0.0529
NSAIDs	2.09 (0.36, 11.97)	0.4083	1.09 (0.06, 19.94)	0.9545	1.08 (0.07, 16.59)	0.9548
Anti-hypertensives	2.49 (0.95, 6.52)	0.0644	1.38 (0.30, 6.34)	0.6794	1.07 (0.23, 5.10)	0.9308

[a]: Walking had *N* = 97 for univariable analysis

4.0 DISCUSSION

To the best of our knowledge, our study is the first study to investigate the heritability of LBP in any population and the first to investigate longitudinal associations between LBP and liver fat in an African Ancestry population. We found that LBP was entirely environmentally determined in a group of large Afro-Caribbean families. We also found that higher baseline levels of LBP were independently associated with higher liver fat content at follow-up visits in linear regression models using BMI as an obesity measure, but that this association was attenuated when instead adjusting for waist circumference. Additionally, we found that levels of LBP differed between the highest and lowest quartile of liver attenuation. These data help to support the role of intestinal bacteria-induced inflammation in the progression of NAFLD.

Lipopolysaccharide (LPS) is a cell-wall lipid constituent of Gram-negative bacteria[10]. LPS binds to cell-surface receptor toll-like receptor 4 (TLR4) and induces inflammation[12]. Intestinal bacterial-derived LPS can enter the circulation by moving between intestinal epithelial cells or by incorporation into chylomicrons[38], and its first point of contact is the liver via the hepatic portal vein[11]. Levels of circulating LPS have been shown to increase after eating energy-rich meals[38], and chronic LPS infusion mimics the weight gain of eating a high fat diet[15], linking LPS to systemic low-grade inflammation and increased adiposity.

LBP is an acute phase protein which is responsible for binding to LPS, complexing with protein CD14, and ultimately transferring LPS to the TLR4[12]. In the presence of LBP, macrophages produced inflammatory cytokines at a 1000-fold lower level of LPS, demonstrating the importance of LBP to this pathway[39]. Importantly, inflammatory cytokines can upregulate production of LBP[11], suggesting that LBP may serve as a marker of LPS-induced inflammation.

There are several limitations to measuring LPS in the blood owing to interference by many factors[40]. LBP can be measured using a simple ELISA, and has been shown to have a good correlation with coefficients ≥ 0.6[17]. Therefore, it has widely been used as a surrogate marker for LPS-induced inflammation[18-22,41]. However, given that LBP is produced in response to inflammatory cytokines, it can be produced during non–Gram-negative bacterial infections or other acute-phase reactions[42].

LBP is constitutively expressed at low levels by liver cells[11]; however, various genetic polymorphisms in LBP may affect protein levels and have functional outcomes. For example, SNP 1683 C allele (rs2232571; *LBP*:c.−836T>C) is a CAAT box promoter variant which has been associated with higher basal levels of LBP and a 5-fold increase in death by Gram-negative bacteremia following a hematopoietic cell transplant[43]. Another study also found that *LBP* variants were associated with being susceptible to severe sepsis[44]. Given this knowledge, it is possible that our measured LBP levels could also be influenced by genetic factors, thus reducing our claims of LBP serving as a biomarker for LPS-induced inflammation.

Our heritability analysis results suggest that this is not the case in our population. The Tobago population is primarily of African descent, with little in- or out-migration and with ~6% European admixture (unpublished data). Thus, this makes them a unique population to study using large family study genetic analyses. Our analysis shows that LBP has virtually no residual heritability, indicating that levels of LBP are due entirely to environmental factors. This would suggest that no variants affecting LBP production are to be found in several of the large families comprising this Caribbean island population. It has been found that toll-like receptor (TLR) SNPs vary regionally and that the high prevalence of a pro-inflammatory variant in *TLR4* in sub-Saharan African populations is due to a protective advantage against malaria[45]; additionally, African

ancestry individuals from the Cardiovascular Health Study have been found to have a *CD14* SNP which was associated with lower levels of CD14[46]. It is possible, then, that familial differences in levels of other proteins involved in LPS recognition and subsequent inflammation may be present in our population; this will need to be investigated in future work.

Our findings that LBP was associated with BMI and waist circumference agree with the current literature[17-21]. Obesity is a strong risk factor for NAFLD and has been reported to be present in as high as 80% of obese adults[47]. We found, however, that there were differences in significance of covariates in linear regression models when using either BMI or waist circumference (Table 4). Waist circumference could be more important predictor than general obesity with regards to liver fat accumulation[37]. Still, it is possible that differences in these models may be due to the difference in sample sizes for each model. The waist circumference model had two fewer individuals in it, and given that the sample size was small to begin with, this reduction could have an impact. Indeed, we found that reducing the BMI model by the same two individuals missing waist circumference values changed the significance of LBP to become non-significant (Appendix Table 9). A larger sample size after future data release can help to resolve this discrepancy.

We found that in our cohort that insulin resistance was correlated with liver fat (Table 3) and that it was associated with liver fat in univariable linear and logistic regression analyses (Table 4, Table 6, and Table 7). This is to be expected, as insulin resistance is a common pathological feature of NAFLD[3,4]. However, after adjustment for other covariates, this association became insignificant. This may in part be due to the role of LBP in promoting insulin resistance. We have previously reported that LBP was associated with insulin resistance in this population[21], and this finding is also supported by the literature[17,41,48,49]. TLR4 activation can interrupt insulin signaling through insulin receptor substrate (IRS) deactivation and degradation, as reviewed by Lontchi-

Yimagou et al.[50]. Mice with hematopoietic cell-specific deletion of *Tlr4* fed a high fat diet became obese but became more insulin sensitive in hepatic and adipose tissues, demonstrating that hematopoietic TLR4 may be necessary for hepatic insulin resistance[51]. It is possible, then, that LBP serves as the promoter of liver insulin resistance which can then lead to liver fat accumulation.

We report for the first time that levels of LBP are positively and significantly associated with longitudinal liver fat infiltration. We also found that levels of LBP can discriminate between the highest and lowest quartiles of liver attenuation value. In a mouse model, $Lbp^{-/-}$ mice had less diet-induced liver damage than wild-type mice when fed a western-style diet[52]. LBP has also been found to correlate with liver portal and interface inflammation, with myofibroblasts and fibrosis[53]. In humans, LBP has been found to show associations with the incidence or presence of NAFLD[22-24]. However, to the best of our knowledge, this is the first study which has looked at a longitudinal association between LBP and liver fat. A study by Wong et. al.[23] found that LBP was not cross-sectionally associated with liver fat after adjustment for other factors, and while liver fat was measured in a subset of the population at a later time (~1.5–2.5 years later), the authors only reported associations with incident NAFLD but not with measures of liver fat. The time between our visits was much longer (~10 years), and thus participants may have had greater liver fat accumulation.

Our study has several limitations. First, we are using LBP as a surrogate biomarker for LPS-induced inflammation; however, as discussed previously, while it is not an infallible marker it is a better alternative than trying to measure LPS directly. Still, our results are limited by a lack of acute inflammatory markers. Second, we did not collect extensive dietary data in either the family or the cohort populations, and dietary components could independently affect liver fat or affect the microbiome. Third, we only investigated heritability of one component of the LPS-

induced inflammatory pathway. Fourth, the sample size of our cohort population is quite small. We are waiting for additional data releases to see if the associations we found can hold in a larger population. Fifth, we only were able to adjust for baseline measures of variables and did not have baseline levels of liver fat. It is possible that change in levels of liver fat or in levels of LBP may be more informative predictors and outcomes. Finally, both of our populations are of Afro-Caribbean background, and our cohort population is comprised completely of middle-aged and older men. African ancestry men have been found to have lower rates of NAFLD than whites or hispanics[54], and it has been suggested that this could be due to differences in lipid homeostasis[55]; additionally, NAFLD shows a slightly higher prevalence among the elderly compared to younger adults, with 15%–30% among the younger and 35.1% in the older[56]. Thus, our findings may not apply to other ethnic groups, ages, or to women.

In conclusion, we report for the first time an independent association between levels of LBP and future liver fat content, and that these LBP levels are likely due to environmental effects and not genetics. This association provides one plausible mechanistic role for the effects of the intestinal microbiome on liver fat accumulation. The public health significance of this work is in the supplying of greater insights into the etiology of NAFLD, an understanding which is necessary for effective treatments of NAFLD. Future research will need to be done to confirm these findings in our larger population size when data has been released, and these findings will also need to be confirmed in other populations of different age, sex, and ethnic background.

APPENDIX: TABLES

Table 8. Comparisons of Cohorts

Variable	Tobago Health Study Full Cohort		LBP/Liver Cohort		Men with Liver Scans		Men with LBP Measures	
	N	**Median (IQR) or *N* (%)**	***N***	**Median (IQR) or *N* (%)**	***N***	**Median (IQR) or *N* (%)**	***N***	**Median (IQR) or *N* (%)**
General and Lifestyle Risk Factors								
Age (years)	2161	57 (50, 67)	195	53 (48, 64)	539	53 (48, 60)	581	58 (49, 67)
BMI (kg/m^2)	2163	27.02 (24.39, 29.94)	195	27.03 (24.59, 29.56)	540	27.04 (24.65, 30.02)	582	26.90 (24.54, 29.46)
Perceived Good Health	2149	1967 (91.53)	194	183 (94.33)	537	515 (95.90)	579	541 (93.44)
Walking (hrs/week)	2134	1.5 (0, 3.5)	191	1.5 (0, 5)	536	1.25 (0, 4)	570	1.5 (0, 4)
TV Watching ≥ 14 hrs/week	2156	824 (38.22)	194	67 (34.54)	536	205 (38.25)	579	219 (37.82)
Any Alcohol Consumption	2164	1315 (60.77)	195	113 (57.95)	540	346 (64.07)	582	348 (59.79)
8+ Alcoholic Drinks / Week	2168	140 (6.46)	195	7 (3.59)	541	37 (6.84)	582	31 (5.33)
Previous Smoker	2165	483 (22.31)	195	39 (20)	541	116 (21.44)	582	126 (21.65)
Current Smoker	2165	229 (10.58)	195	10 (5.13)	541	51 (9.43)	582	44 (7.56)

Table 8 (continued)

Prediabetic	2086	376 (18.02)	195	46 (23.59)	526	108 (20.53)	582	119 (20.45)
Diabetic	2086	438 (21)	195	29 (14.87)	526	65 (12.36)	582	121 (20.79)
Hypertension	2167	1125 (51.92)	195	106 (54.36)	541	259 (47.87)	582	313 (53.78)
Cardiometabolic Markers								
LBP (µg/mL)	—	—	195	20.89 (16.30, 27.11)	—	—	580	21.35 (16.84, 27.21)
HDL-c (mg/dL)	1813	47.8 (40.8, 57)	195	48 (41.1, 57.1)	470	48.6 (41.1, 56.8)	561	46.9 (41, 56.2)
LDL-c (mg/dL)	1811	130 (105.2, 156.1)	195	134.5 (103.1, 162.2)	468	131.25 (107.65, 158.05)	561	132.4 (106.9, 161)
Triglycerides (mg/dL)	1813	97 (75, 131)	195	103 (77, 131)	470	100 (75, 131)	561	99 (75, 135)
Glucose (mg/dL)	2086	94 (86, 108)	202	93 (85, 105)	526	92 (84, 102)	582	94 (86, 108)
Insulin (mg/dL)	2083	11.1 (8.3, 15)	202	11.1 (8.3, 15.9)	526	10.8 (8.3, 15.4)	582	10.6 (8.2, 14.8)
HOMA-IR	2083	2.72 (1.91, 4.02)	195	2.75 (1.85, 4.11)	526	2.63 (1.82, 3.97)	582	2.68 (1.88, 4.00)
Liver Parameters								
Mean Liver Attenuation (HU)	—	—	195	59.36 (54.69, 61.87)	545	58.64 (53.77, 61.68)	—	—
Fatty Liver Disease at Follow-up	—	—	195	8 (4.10)	545	22 (4.04)	—	—
Medications								

Table 8 (continued)

Antidiabetic Medication	2168	313 (14.44)	195	19 (9.74)	541	45 (8.32)	582	78 (13.40)
NSAIDs Medication	2168	187 (8.63)	195	13 (6.67)	541	31 (5.73)	582	50 (8.59)
Antihypertensive Medication	2167	486 (22.43)	195	42 (21.54)	541	96 (17.74)	582	132 (22.68)

Table 9. Linear Regression with BMI or WC, *N* = 188

Baseline Variable	Model with BMI		Model with Waist Circumference	
	***β* (SE)**	***p***	***β* (SE)**	***p***
LBP (µg/mL)	−10.34 (8.25)	0.0505	−10.30 (8.27)	0.0547
Age (years)	7.62 (7.97)	0.3828	8.42 (7.98)	0.2424
WC (cm)	—	—	−11.44 (7.85)	0.0023
BMI (kg/m^2)	−16.4 (10.8)	0.0006	—	—
HOMA-IR	−12.42 (13.62)	0.4498	−13.43 (13.62)	0.3392
HDL-c (mg/dL)	5.69 (7.46)	0.6580	5.48 (7.50)	0.6961
LDL-c (mg/dL)	0.96 (4.71)	0.9933	−2.44 (4.72)	0.8895
Triglycerides (mg/dL)	−4.75 (4.6)	0.2727	−4.58 (4.62)	0.3316
DBP (mmHg)	−7.12 (8.68)	0.5814	−7.30 (8.71)	0.5571
SBP (mmHg)	6.36 (7.36)	0.5213	5.89 (7.40)	0.6147
Drink 8+	35.98 (28.76)	0.0518	38.23 (28.95)	0.0226
Walking (hr/week)	5.34 (9.88)	0.8747	−3.98 (9.88)	0.9479
Smoking Status	18.15 (20.08)	0.4611	19.50 (20.13)	0.3645
TV (≥14 hrs/week)	12.89 (21.23)	0.8230	10.87 (21.32)	0.8948
Anti-diabetics	−22.79 (25.35)	0.4683	−24.47 (25.43)	0.3744
NSAIDs	11.72 (27.02)	0.9351	10.67 (27.09)	0.9514
Anti-hypertensives	−25.9 (22.93)	0.1513	−24.09 (23.01)	0.2526

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