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RESEARCH ARTICLE

Lipoprotein Profiles in Class III Obese Caucasian and African American Women with Nonalcoholic Fatty Liver Disease

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Abstract

Triglyceride content in the liver is regulated by the uptake, production and elimination of lipoproteins, and derangements in these processes contribute to nonalcoholic fatty liver disease (NAFLD). Previous studies show a direct relationship between intrahepatic fat and production of apolipoprotein B100 (apoB100) containing particles, VLDL and LDL, but little consensus exists regarding changes in lipoprotein production in the development of simple steatosis (SS) versus nonalcoholic steatohepatitis (NASH). Further, ethnic variations in lipoproteins among SS and NASH are unknown as is how such variations might contribute to the differential prevalence of disease among Caucasians versus African Americans. In this study, we assessed plasma lipoprotein profiles by nuclear magnetic resonance (NMR) spectroscopy in 70 non-diabetic class III obese females recruited from the surgical weight loss clinic. Of these, 51 females were stratified by biopsy-staged NAFLD severity (histolog-ically normal, SS, or NASH). NASH females displayed increased circulating triglycerides and increased VLDL particle number and size relative to those with histologically normal livers, while total and large LDL concentration decreased in SS versus NASH and corre-lated with increased insulin resistance (via HOMA2-IR). When Caucasian women were examined alone (n = 41), VLDL and triglycerides increased between normal and SS, while total LDL and apoB100 decreased between SS and NASH along with increased insulin resistance. Compared to Caucasians with SS, African American women with SS displayed reduced triglycerides, VLDL, and small LDL and a more favorable small to large HDL ratio despite having increased BMI and HOMA2-IR. These findings suggest that ApoB100 and lipoprotein subclass particle number and size can delineate steatosis from NASH in obese Caucasian females, but should be interpreted with caution in other ethnicities as African Americans with SS display relatively improved lipoprotein profiles. This may reflect

variation in the relationship between dyslipidemia and NAFLD progression across gender and ethnicity.

Introduction

Hepatic steatosis stems from an imbalance between lipid supply and demand where excess fatty acids are stored in the liver as fat [1]. Obese individuals in particular have surplus fatty acids and increased hepatic steatosis due to dietary intake and the tendency to become insulin resistant. With decreased insulin sensitivity, insulin can no longer inhibit lipolysis in peripheral tissues, resulting in more circulating free fatty acids for potential storage in the liver [2]. Once accumulated, hepatic triglyceride (TG) is eliminated from the liver via lipid oxidation or the secretion of TG-rich very-low density lipoproteins (VLDL) depending on local or peripheral energy needs [3, 4]. However, this lipid and lipoprotein balance can become perturbed in the setting of obesity and type 2 diabetes (T2D), and may contribute to the development of nonalcoholic fatty liver disease (NAFLD) [5].

NAFLD encompasses a spectrum of disease ranging from excess fat in more than 5% of hepatocytes (termed simple steatosis; SS) to nonalcoholic steatohepatitis (NASH), an advanced inflammatory phenotype hallmarked by hepatocyte ballooning and necrosis [6]. NAFLD, especially NASH, is associated with hepatic and global metabolic dysfunction, including increased insulin resistance [5]. While over 25% of the general population has NAFLD, this number jumps to 90% in class III obese subjects (BMI $\geq$ 40) and to 100% in those with T2D [7, 8]. The prevalence of the disease differs among ethnicities, with African Americans exhibiting a lower prevalence of steatosis and NASH relative to Caucasians and Hispanics [8, 9]. Elevated levels of TG, VLDL and small dense low-density lipoprotein (LDL) and decreased levels of high-density lipoprotein (HDL) are associated with the development of cardiovascular disease and diabetes. [3, 10]. Interestingly, African Americans exhibit lower levels of TG and higher levels of HDL for a given BMI and degree of insulin resistance [11]. However, it is unclear how lipoproteins vary in SS versus NASH of any ethnicity, with some studies reporting increased production of VLDL and LDL in NASH relative to SS while others report a decline [12–17].

In this study, we examined lipoprotein profile differences among class III obese females with histologically-determined SS or NASH and compare them to lipoprotein profiles from histologically normal-weight-matched female controls. We found distinct differences in lipoprotein and lipid profiles that may aid in noninvasively determining NAFLD diagnoses and severity progression [12, 17–19].

Materials and Methods

Subjects

The Internal Review Board of Vanderbilt University approved this study which is registered at ClinicalTrials.gov (NCT00983463). All studies met NIH and institutional guidelines for human subject research and the Declaration of Helsinki. All subjects gave informed written consent prior to participation. Caucasian and African American Class III obese females (BMI > 40 kg/m^2) scheduled for bariatric surgery were recruited from the Vanderbilt Center for Surgical Weight Loss (Nashville, TN). Inclusion criteria included non-sedentary, non-diabetic women between the ages of 23 and 60 years. Screening included anthropomorphic measures, clinical history, physical exam, and laboratory tests including complete blood count with differential, liver function tests, and fasting blood glucose. Ethnicity was self-reported.

Exclusion criteria included presence of viral hepatitis, autoimmune hepatitis, hemochromatosis, significant alcohol use (>14 drinks per week over a 2-year period), intercurrent infections or cancer, previous gastric, duodenal, proximal jejunum or pancreatic resection surgery, and positive serum pregnancy test. Lean females (BMI $\leq$ 27 kg/m^2) were recruited from the Vanderbilt community and had no significant past medical history. Liver biopsies were not available for these women as it is discouraged to obtain non-clinically indicated liver biopsies due to the risks associated with the procedure. Further, we did not utilize AST and ALT to screen for liver disease in control patients given the values are not sensitive enough to screen for NAFLD nor are they a reliable indicative of liver disease severity [20–22].

Blood and tissue sampling

Women were studied after an overnight fast ($\geq$10 hours). Blood was collected from lean patients by the laboratory nurse practitioner in the morning following an overnight fast. Blood was collected from patients receiving bariatric surgery preoperatively prior to induction of anesthesia. The attending surgeon performed intra-operative wedge liver biopsies collected from the left lobe of the liver for these patients as well.

Plasma analyses

Plasma glucose concentration was determined using an automated glucose analyzer (YSI 2300 STAT plus, Yellow Spring Instrument Co., Yellow Springs, OH, USA). Plasma insulin concentrations were measured by radioimmunoassay (Linco Research, St. Louis, MO, USA). Plasma insulin and glucose were used to calculate insulin resistance using the Homeostatic Model Assessment of Insulin Resistance with physiologic adjustments (HOMA2-IR) [23]. Hospital lab services assessed aspartate transaminase (AST) and alanine transaminase (ALT) in surgical patients via enzymatic methods, albumin via a bromo-cresol green albumin assay, and platelets via Sysmex Automated Hematology System (Sysmex America, Lincolnshire, IL. USA).

Lipoprotein subclass concentration and particle size were assessed via NMR (nuclear magnetic resonance) spectroscopy (Liposcience/LabCorp, Raleigh, NC, USA) using the Lipoprofile-3 algorithm. Concentrations (nanomoles/L) of total VLDL, LDL, HDL and the following subclasses were determined according to particle size (nanometers, nm): large VLDL (>60.0 nm), medium VLDL (42.0–60.0 nm), small VLDL (29.0–42.0 nm), IDL (23.0–29.0 nm), large LDL (20.5–23.0 nm), small LDL (18.0–21.2 nm), large HDL (9.4–14.0 nm), medium HDL (8.2–9.4 nm) and small HDL (7.3–8.2 nm) [24]. Weighted average particle size for VLDL, LDL, and HDL was calculated based on subclass concentration and associated diameters. NMR calculated lipid levels highly correlate with respective chemically measured levels of plasma triglyceride (r>0.9), HDL cholesterol (r>0.9), and LDL cholesterol (r = ~0.8–0.9) while providing the added benefit of quantifying particle concentrations and size [25]. Because each VLDL and LDL possesses one apolipoprotein B100 (apoB100) molecule, previous methods used a combination of centrifugation and apoB100 quantification assays to separate and quantify the lipid classes. NMR-derived levels strongly correlate with these methods (r = 0.928) as well as LDL and HDL levels determined by gradient gel electrophoresis (r = 0.946 and r = 0.953)[24].

In addition, we employed a novel means of describing insulin sensitivity. Classically, insulin resistance is defined by the degree of insulin's effect on glucose metabolism, but several investigators have emphasized the importance of lipid abnormalities in the pathogenesis of insulin resistance [26, 27]. NMR studies suggest a more "lipocentric" view of insulin resistance after finding significant associations between specific lipoprotein profile constituents and insulin resistance [28, 29]. The 6 parameters most associated with insulin resistance include: increased

number of individual particles of large VLDL and small LDL, decreased number of large HDL particles, bigger VLDL sizes and smaller LDL and HDL overall sizes. Shalaurova and Otvos et al. used this information to create an equation that can use lipoprotein profiles to describe insulin sensitivity based on each parameter's individual association with insulin resistance. This Lipoprotein Insulin Resistance (LP-IR) Index or Score assigns each lipid parameter an individual weight as follows: VLDL size, 32; large VLDL, 22; HDL size, 20; large HDL, 12; small LDL, 8; and LDL size, 6. The sum of these weights creates the LP-IR score, which ranges from 1–100, with 100 being the most insulin resistant [30]. The scores were calculated by the Liposcience group during NMR analysis, and the algorithm used is described in detail in the manuscript by Shalaurova and Otvos et al. [30].

Histopathologic Determination of NAFLD Severity

Diagnosis of normal, simple steatosis, or steatohepatitis pathology was made by retrospective chart review of pathology reports for the liver biopsies collected at the time of surgery by the attending. Women were diagnosed as having simple steatosis when fat was present in greater than 5% hepatocytes or NASH if the pathologist described the sample as "steatohepatitis" based on the degree of lobular inflammation and ballooning [31]. Of note, the current investigators were able to secure slides for 31 of the 51 women in the study in order to evaluate the biopsies via the NAFLD Activity SCORE (NAS) (Table A in S1 File) and confirm hepatopathologist findings with the help of an independent hepatopathologist. The NAS Score is a sum of independent scores for ballooning, lobular inflammation and steatosis [32]. Women with a NAS score of 0–1 were categorized as "normal," 2–3 as "simple steatosis" (SS) 4 or above as "NASH". Fibrosis was scored as an independent variable. Unfortunately, due to the retrospective nature of this study, slides were not available for all samples. Because of this, we ultimately elected to classify livers based on the original histopathological classification at the time of surgery that was recorded in the electronic medical record, as this classification was uniformly determined and available for all women.

Statistics

Results for two groups were compared by unpaired, two-tailed Mann-Whitney test (t-test) using Prism 5 (Graphpad Software). Differences among three groups were analyzed by analysis of variance (ANOVA) when variables were normally distributed by the Kolmogorov-Smirnov test or upon log transformation. All data are presented as mean ± standard error (SEM). Statistical significance was established at P-value ≤ 0.05. Regression analyses (Table B in S1 File) were used to estimate linear relationships among variables after log transformation of skewed data.

Results

Anthropometric Measurements in Class III Obese

Lipoprotein profiles consisting of VLDL, IDL, LDL and HDL concentration and particle size (Fig 1) from 70 class III obese females undergoing bariatric surgery and 9 lean female subjects were measured by H-NMR spectroscopy. Of our 70 class III obese females, 54 were Caucasian and 16 were African American. While the cohorts did not differ in demographics such as body weight, BMI, or insulin sensitivity, African American women exhibited a significant reduction in liver enzymes (Table 1). When lipid profiles were compared, African Americans exhibited a less atherogenic lipoprotein profiles including decreased levels of triglycerides, VLDL, and small LDL as well as improved LDL and HDL size ratios (Table 2).
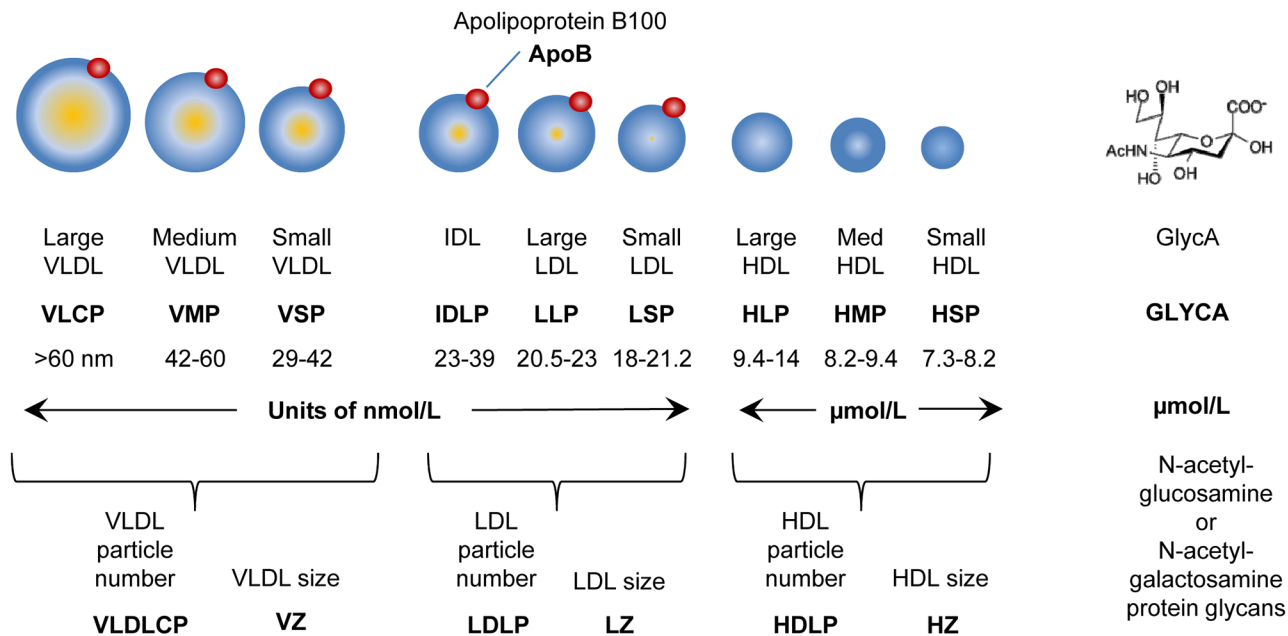
Fig 1. Schematic illustrating lipoproteins particles under investigation in this study.

Liver histology reports were available for 51 women (41 Caucasians and 10 African Americans). Class III obese females were stratified into subcohorts based on liver biopsy-determined NAFLD diagnoses of histologically normal (non-steatotic; n = 18), simple steatosis (SS; n = 16), or NASH (n = 17) via NAFLD Activity Score (NAS) and histological determination by pathologist as simple steatosis or steatohepatitis. Weight, BMI, and fasting glucose levels were similar across the three obese groups. NASH females were slightly older and exhibited increased levels of AST, ALT and fasting insulin, and insulin resistance per HOMA2-IR, but these results were adjusted for age (Table 3). Histology was not available for lean women, but

Table 1. Baseline characteristics of class III obese females stratified by ethnicity.

	Caucasian	African Americans	*P*-value
n	54	16	
Age	43.4 ± 1.2	39.5 ± 2.2	0.116
Weight (kg)	117.3 ± 2.4	122.0 ± 6.0	0.711
BMI (kg/m^2)	43.8 ± 0.8	43.9 ± 2.0	0.566
Insulin (µU/mL)	22.9 ± 1.7	19.9 ± 2.7	0.426
Glucose (mg/dL)	102 ± 2.7	98.2 ± 7.1	0.121
HOMA2-IR (AU)	2.9 ± 0.2	2.5 ± 0.3	0.365
AST (normal range: 0–65 U/mL)	29.3 ± 3.1	18.7 ± 1.4	0.074
ALT (normal range: 0–65 U/mL)	33.2 ± 3.0	15.8 ± 0.9	0.003*
Alkaline Phosphatase (U/L)	80.5 ± 3.0	82.4 ± 6.3	0.857
Albumin (g/dL)	4.1 ± 0.04	4.1 ± 0.1	0.624
Platelets (1000/mm^3)	276.1 ± 7.9	282.2 ± 9.8	0.632
Bilirubin (mg/dL)	0.5 ± 0.03	0.4 ± 0.03	0.874

*Statistically significant changes; Data presented as mean ± SEM. AU, arbitrary units. BMI, body mass index; HOMA2-IR, updated homeostasis model assessment of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NAS, NAFLD Activity Score.

Table 2. Lipid and lipoprotein measures in class III obese females stratified by ethnicity.

	Caucasian	African American	*P*-value
n	54	16	
NMR calculated lipids			
Triglycerides (mg/dL)	157.7 ± 7.5	78.3 ± 4.7	< 0.001*
HDL cholesterol (mg/dL)	44.3 ± 1.2	51.9 ± 4.0	0.049*
VLDL & chylomicron TG (mg/dL)	123.5 ± 7.4	44.0 ± 3.9	< 0.001*
Triglycerides/HDL-C ratio	4.2 ± 0.2	1.7 ± 0.5	< 0.001*
Lipoprotein particles			
TOTAL VLDL (nmol/L)	71.4 ± 4.0	30.6 ± 3.3	< 0.001*
Large VLDL & chylomicrons	8.3 ± 0.7	2.0 ± 0.3	< 0.001*
Medium VLDL	26.7 ± 2.4	8.1 ± 1.3	< 0.001*
Small VLDL	36.3 ± 2.1	20.5 ± 2.9	< 0.001*
TOTAL LDL (nmol/L)	1,256 ± 57.7	1,083 ± 69.2	0.134
IDL	118.8 ± 12.3	85.1 ± 10.7	0.235
Large LDL	348 ± 35	566 ± 77	0.012*
Small LDL	789 ± 47	431 ± 77	0.001*
TOTAL HDL (µmol/L)	33.9 ± 0.8	33.3 ± 1.4	0.897
Large HDL	3.7 ± 0.2	6.6 ± 1.0	0.007*
Medium HDL	7.7 ± 0.7	9.1 ± 1.4	0.407
Small HDL	20.4 ± 0.8	16.4 ± 1.1	0.015
ApoB100 (mg/dL)	113.7 ± 3.7	96.1 ± 7.8	0.063
Lipoprotein particle size (nm)			
VLDL	53.9 ± 0.9	47.0 ± 1.8	0.001*
LDL	20.4 ± 0.1	20.8 ± 0.1	0.011*
HDL	8.9 ± 0.04	9.3 ± 0.1	0.005*
Particle ratios			
LDL small/large ratio	4.8 ± 0.8	2.6 ± 1.4	0.004*
HDL small/large ratio	6.5 ± 0.5	3.7 ± 0.7	0.001*
Lipoprotein Insulin Resistance Score	67.1 ± 2.6	37.6 ± 3.1	< 0.001*

*Statistically significant changes; Data presented as mean ± SEM. AU, arbitrary units.

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demographics and lipoprotein profiles were compared to obese individuals with normal histology.

Lipoprotein Profiles in Class III Obese Women by Histology

Compared to lean females, obese females with normal histology had an elevated HOMA2-IR, driven by increased fasting glucose and insulin levels (Table 3). Obese women with normal liver histology were more insulin resistant and had a more atherogenic lipid profile than lean females manifest by increased large and medium VLDL, increased total and small LDL, and increased small to large HDL ratio (Table 4). The Lipoprotein Insulin Resistance Score (LP-IR) was also elevated in the pathologically normal women compared to leans.

Among obese women in the cohort with NAFLD, NASH patients exhibited higher HOMA2-IR driven primarily by a significant increase in fasting insulin compared to SS and normal females. Further, NMR determined lipoprotein profiles differed across histology. NASH females had significantly elevated levels of triglycerides, VLDL cholesterol, large VLDL particle number, and VLDL size compared to normal and SS females (Table 4). Women with

Table 3. Baseline characteristics of class III obese females stratified by histology.

	Leans	Obese Normal	Obese SS	Obese NASH	P-Value
n	9	18	16	17	-
Gender (F/M)	9/0	18/0	16/0	17/0	-
Caucasian/African American	7/2	13/5	5-Nov	17/0	-
Age	40.2 ± 2.0	38.8 ± 1.4	43.7 ± 2.9	47.2 ± 6.3[b]	0.009
Weight (kg)	59.5 ± 2.2****	122.7 ± 5.2	116.0 ± 4.7	116.8 ± 2.9	0.553
BMI (kg/m^2)	22.5 ± 0.8****	45.4 ± 1.3	43.1 ± 1.3	43.9 ± 1.6	0.400
Fasting insulin (μU/mL)	7.6 ± 1.0****	18.7 ± 1.8	20.3 ± 3.2	28.8 ± 3.5[a]	0.026
Fasting glucose (mg/dL)	81.6 ± 1.9***	96.4 ± 2.6	102.9 ± 6.5	109.8 ± 6.2	0.241
HOMA2-IR (AU)	0.9 ± 0.2***	2.4 ± 0.2	2.5 ± 0.4	3.7 ± 0.4[c]	0.018
AST (normal range: 0–65 U/mL)	-	20.2 ± 1.5	26.5 ± 3.5	46.6 ± 9.0[a]	0.022
ALT (normal range: 0–65 U/mL)	-	22.3 ± 2.7	29.3 ± 4.9	49.2 ± 7.1[b]	0.009
Alkaline Phosphatase (U/L)	-	72.6 ± 5.0	91.1 ± 7.0	82.3 ± 4.3	0.271
Albumin (g/dL)	-	4.1 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	0.936
Bilirubin (mg/dL)	-	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.532
NAS	-	0.8 ± 0.2	2.7 ± 0.3[a]	5.0 ± 0.2[d]	<0.001

Data presented as mean ± SEM. AU, arbitrary units. Asterisks represent significant difference between lean and obese:

***P<0.001

****P<0.0001

One-way ANOVA P values among obese groups (far-right). Dunn's Multiple Comparison test results for obese group:

[a]P <0.05 vs. normal

[b]P <0.01 vs. normal

[c]P <0.05 vs. SS

[d]P <0.001 vs. normal

Abbreviations: BMI, body mass index; HOMA2-IR, updated homeostasis model assessment of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NAS, NAFLD Activity Score.

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SS had increased large LDL levels relative to normal females, while NASH patients interestingly had decreased large LDL levels. LP-IR was elevated in obese NASH relative to the obese with normal histology. There were no differences in HDL particle number, LDL size, HDL size, or ApoB100 concentration in the obese cohort as a whole. We also examined the potential association between lipoprotein measures and NAS using linear regression with NAS score as outcome and lipoprotein measure as predictor. Other adjusted clinical variables include age, race, weight and BMI. VLDL size, large VLD concentration as well as serum AST, LP-IR, ALT, TG were found to be positively associated with NAS score. LDL size was found to be inversely associated with NAS score. Furthermore, we also examined the association between each individual component of NAS and lipoprotein measures. The full results of this analysis can be viewed in data in Table B in S1 File.

Impact of Cohort Selection on Lipoprotein Profiles in NAFLD

When the 41 female Caucasians with NAFLD were examined without the African Americans in the cohort, additional differences in lipoprotein profiles across pathology emerged compared to those seen in the cohort as a whole. In the Caucasian cohort, 17 had NASH, 11 had SS, and 13 had normal liver histology (Table 5). Caucasian women with SS showed significant increases in TG concentration, VLDL concentration, and VLDL size versus normal histology (Table 4). However, women with NASH demonstrated a decline in TG and VLDL concentrations relative

Table 4. Lipid and lipoprotein measures in class III obese females stratified by histology.

Variable	Lean	Obese Normal	Obese SS	Obese NASH	*P*-value
n	9	18	16	17	
NMR calculated lipids					
Triglycerides (mg/dL)	76.9 ± 2.9***	119.0 ± 12.9	157.0 ± 18.8	168.2 ± 9.9[a]	0.017
HDL cholesterol (mg/dL)	60.6 ± 4.2*	45.3 ± 2.6	51.0 ± 4.2	44.8 ± 1.8	0.58
VLDL & chylomicron TG (mg/dL)	42.9 ± 4.1***	85.1 ± 13.1	115.7 ± 17.4	136.8 ± 10.2[a]	0.016
Triglycerides/HDL-C ratio	1.3 ± 0.1****	2.8 ± 0.4	3.5 ± 0.6	3.9 ± 0.3	0.081
Lipoprotein particles					
TOTAL VLDL (nmol/L)	34.9 ± 4.0**	52.7 ± 8.5	63.5 ± 8.7	72.5 ± 5.6	0.165
Large VLDL & chylomicrons	1.0 ± 0.2***	4.7 ± 0.9	8.2 ± 1.4	10.4 ± 1.0[b]	0.002
Medium VLDL	7.4 ± 1.8**	11.2 ± 2.5	23.2 ± 4.8	25.3 ± 2.8	0.254
Small VLDL	26.5 ± 3.2	22.4 ± 2.6	32.1 ± 4.6	36.7 ± 3.2	0.109
TOTAL LDL (nmol/L)	853 ± 117**	1,177 ± 76	1,487 ± 101[a]	1,157 ± 141	0.05
IDL	104 ± 18	111 ± 17	140. ± 29	102 ± 15	0.839
Large LDL	675 ± 114*	457 ± 69	527 ± 73	264 ± 62[c]	0.018
Small LDL	72 ± 12****	608 ± 90	820 ± 115	789 ± 75	0.379
TOTAL HDL (µmol/L)	35.3 ± 1.7	31.7 ± 1.1	35.7 ± 1.9	35.2 ± 1.4	0.168
Large HDL	9.2 ± 0.9****	5.0 ± 0.7	5.2 ± 1.0	3.6 ± 0.4	0.452
Medium HDL	17.1 ± 1.6***	7.2 ± 1.1	6.9 ± 1.0	7.2 ± 1.1	0.979
Small HDL	9.0 ± 1.6****	17.5 ± 1.3	20.9 ± 1.6	21.6 ± 1.3	0.066
ApoB100 (mg/dL)	-	106.2 ± 5.1	120.8 ± 7.5	103.6 ± 6.1	0.127
Lipoprotein particle size (nm)					
VLDL	43.5 ± 0.7***	50.4 ± 1.1	54.0 ± 2.2	57.3 ± 1.1[b]	0.003
LDL	21.3 ± 0.1***	20.6 ± 0.1	20.6 ± 0.2	20.2 ± 0.1	0.053
HDL	9.8 ± 0.1****	9.1 ± 0.1	8.9 ± 0.1	8.9 ± 0.1	0.668
Particle ratios					
LDL small/large ratio	0.1 ± 0.0****	3.9 ± 1.5	3.9 ± 1.9	5.6 ± 1.1	0.047
HDL small/large ratio	1.1 ± 0.3****	4.9 ± 0.8	6.0 ± 1.0	6.9 ± 0.9	0.286
Lipoprotein Insulin Resistance Score	13.4 ± 1.7****	51.8 ± 5.3	63.2 ± 6.1	73.4 ± 2.8[a]	0.021

Data presented as mean ± SEM. AU, arbitrary units. Asterisks represent significant difference between lean and obese.

* P<0.05

**P<0.01

***P<0.001

****P<0.0001

One-way ANOVA P values among obese groups (far-right). Dunn's Multiple Comparison test results for obese group:

[a]P < 0.05 vs. normal

[b]P < 0.01 vs. normal

[c]P < 0.05 vs. SS

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to SS females, and this was accompanied by significant reductions in total LDL and apoB100 particle numbers (Table 4). Simultaneously, there were increases in HOMA2-IR and insulin in NASH versus SS, and elevated LP-IR in NASH versus normal.

Interestingly, despite enrolling every African American woman who came through clinic during our proposed study period, none of the females had a diagnosis of NASH. In this small African American cohort, 5 had SS and 5 had non-steatotic normal liver histology. There were no differences in demographics or lipoprotein profiles (Table 6) between the groups.

Table 5. Lipid and lipoprotein measures in class III obese Caucasian females stratified by histology.

Variable	Obese Normal	Obese SS	Obese NASH	*P*-value
n	13	11	17	
HOMA2-IR	2.5 ± 0.3	2.0 ± 0.5	3.6 ± 0.5[d]	0.004
Insulin	18.6 ± 1.9	17.2 ± 4.1	28.8 ± 3.8[d]	0.006
Glucose	95.1 ± 3.0	98.0 ± 4.5	109.8 ± 6.2	0.099
NMR calculated lipids				
Triglycerides (mg/dL)	136.5 ± 15.8	195.5 ± 19.2[a]	168.2 ± 9.9	0.034
HDL cholesterol (mg/dL)	42.8 ± 2.6	46.4 ± 3.7	44.8 ± 1.8	0.427
VLDL & chylomicron TG (mg/dL)	102.7 ± 16.5	152.7 ± 17.2	136.8 ± 10.2	0.0651
Lipoprotein particles				
TOTAL VLDL (nmol/L)	65.3 ± 10.3	81.6 ± 8.7	72.48 ± 5.6	0.895
Large VLDL & chylomicrons	5.8 ± 1.2	11.2 ± 1.4[b]	10.4 ± 1.0[b]	0.006
Medium VLDL	28.0 ± 6.8	31.1 ± 6.0	25.3 ± 2.8	0.736
Small VLDL	31.5 ± 4.4	39.2 ± 5.7	36.7 ± 3.2	0.472
TOTAL LDL (nmol/L)	1241 ± 84	1636 ± 117	1157 ± 101[c]	0.014
IDL	114 ± 22	173 ± 41	102 ± 15	0.377
Large LDL	417 ± 68	443 ± 85	264 ± 62	0.076
Small LDL	708 ± 98	1019 ± 119	789 ± 75	0.228
TOTAL HDL (µmol/L)	31.6 ± 1.5	35.5 ± 2.6	35.3 ± 1.4	0.264
Large HDL	4.0 ± 0.6	3.6 ± 0.6	3.6 ± 0.4	0.815
Medium HDL	6.4 ± 1.1	6.1 ± 1.1	7.2 ± 1.1	0.899
Small HDL	19.3 ± 1.2	22.7 ± 2.2	21.6 ± 1.4	0.311
ApoB100 (mg/dL)	111.5 ± 4.7	131.0 ± 7.6	103.6 ± 6.1[c]	0.020
Lipoprotein particle size (nm)				
VLDL	50.6 ± 1.4	57.0 ± 1.9[b]	57.3 ± 1.0[b]	0.002
LDL	20.5 ± 0.2	20.4 ± 0.2	20.2 ± 0.1	0.182
HDL	8.9 ± 0.1	8.7 ± 0.08	8.9 ± 0.09	0.497
Particle ratios				
LDL small/large ratio	3.7 ± 1.2	5.9 ± 3.0	5.6 ± 1.1	0.511
HDL small/large ratio	5.7 ± 0.9	6.6 ± 0.6	7.2 ± 0.9	0.459
Lipoprotein Insulin Resistance Score	45.9 ± 6.4	56.6 ± 7.2	73.4 ± 4.7[a]	0.027

Data presented as mean ± SEM. AU, arbitrary units. P values represent difference between groups.

[a]P < 0.05 vs. normal

[b]P < 0.01 vs. normal

[c]P < 0.05 vs. SS

[d]P < 0.01 vs. SS

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Comparing African Americans and Caucasians with SS

Despite having higher BMI's and insulin resistance measured by HOMA2-IR compared to Caucasians with SS, the African American women with SS had decreased triglycerides and liver enzymes (Fig 2A–2C). African Americans with SS had lower total VLDL and all sub-fractions (Fig 2D) and decreased total and small LDL compared to Caucasians (Fig 2E). African Americans also had improved HDL profiles as evidenced by increased large HDL and less small HDL particle numbers compared to Caucasians with SS (Fig 2F). Further, Caucasians with SS had a much higher LP-IR score than African Americans with SS (Table 6).

Table 6. Lipid and lipoprotein measures in class III obese African American females stratified by histology.

Variable	Obese Normal	Obese SS	P-value
n	5	5	
HOMA2-IR	2.2 ± 0.5	3.4 ± 0.6	0.222
Insulin	18.7 ± 4.8	26.6 ± 5.2	0.530
Glucose	99.8 ± 5.2	113.6 ± 18.9	0.917
NMR calculated lipids			
Triglycerides (mg/dL)	77.0 ± 11.5	80.0 ± 7.7	0.401
HDL cholesterol (mg/dL)	51.2 ± 6.5	60.2 ± 10.2	0.802
VLDL & chylomicron TG (mg/dL)	42.96 ± 8.6	41.72 ± 5.1	0.667
Lipoprotein particles			
TOTAL VLDL (nmol/L)	22.66 ± 5.4	27.5 ± 3.5	0.421
Large VLDL & chylomicrons	2.3 ± 0.8	2.3 ± 0.7	0.917
Medium VLDL	8.5 ± 2.0	7.2 ± 2.0	0.841
Small VLDL	11.8 ± 4.1	18.0 ± 4.4	0.421
TOTAL LDL (nmol/L)	1026 ± 168	1191 ± 139	0.691
IDL	103 ± 27	74 ± 16	0.548
Large LDL	555 ± 184	694 ± 131	0.691
Small LDL	367 ± 180	422 ± 155	0.841
TOTAL HDL (μmol/L)	31.8 ± 1.6	36.1 ± 3.0	0.295
Large HDL	7.4 ± 1.7	8.5 ± 2.3	0.737
Medium HDL	9.3 ± 2.6	8.5 ± 2.3	0.691
Small HDL	13.04 ± 2.5	17.4 ± 1.4	0.310
ApoB100 (mg/dL)	92.6 ± 12.5	98.4 ± 13.4	0.472
Lipoprotein particle size (nm)			
VLDL	49.5 ± 2.7	48.0 ± 4.8	0.714
LDL	20.6 ± 0.3	21.1 ± 0.2	0.139
HDL	9.5 ± 0.2	9.4 ± 0.2	0.325
Particle ratios			
LDL small/large ratio	5.3 ± 4.6	0.9 ± 0.4	0.366
HDL small/large ratio	2.9 ± 1.4	2.7 ± 0.6	0.916
Lipoprotein Insulin Resistance Score	33.8	36.2	0.397

Data presented as mean ± SEM. AU, arbitrary units.

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Discussion

This study in a controlled population of histologically proven NAFLD identified novel changes in fasting lipoprotein subclass particle number and size that delineated simple steatosis from NASH. Namely, we found that circulating VLDL particle number, VLDL size, and TG content were increased in SS relative to weight-matched obese normal females, whereas NASH females trended toward decreased VLDL with significant declines in LDL particle number and apoB100 concentrations compared to SS. We also identified disparities in the relationship between lipoprotein profiles and NAFLD in African American women relative to Caucasians. The relationship parallels the differential prevalence of dyslipidemia across ethnicities and may explain the variation in studies of NAFLD epidemiology and dyslipidemia in the literature to date [9]. These findings suggest a role for monitoring lipoprotein profiles in NAFLD progression, but that ethnicity should be considered in the process [33].
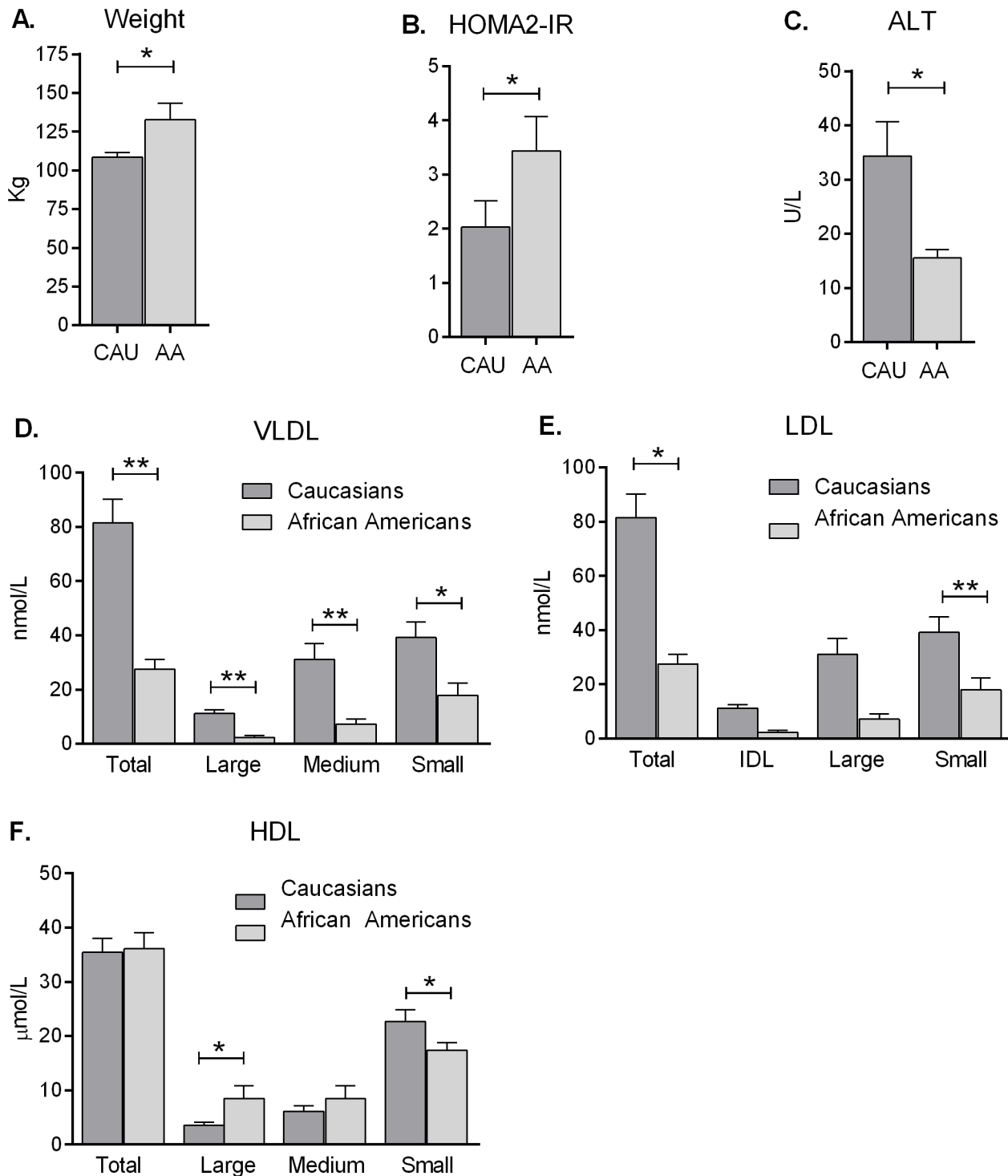
Fig 2. Ethnic Lipoparticle Comparisons. African American women with simple steatosis had less atherogenic lipoprotein profiles relative than Caucasian women with simple steatosis. A) African Americans with simple steatosis (SS) were heavier (*P<0.05) and more insulin resistant B) as measured by homeostatic model assessment index 2 (HOMA2-IR, *P<0.05), yet displayed lower triglycerides (***P<0.001, not shown) and C) lower alanine aminotransferase (ALT, *P<0.05) than Caucasian SS. D) African Americans with SS displayed lower total very-low-density lipoprotein (VLDL) and VLDL sub-fractions (**P<0.01 for all, except small VLDL particle concentration, *P<0.05), E) decreased total and small low-density lipoprotein (LDL; *P<0.05 and **P<0.01, respectively), and F) improved high-density lipoprotein (HDL) profiles (increased large HDL, p = 0.002, and lower small LDL, P = 0.04) relative to Caucasian SS (E-F). Values are mean ± SEM. Data were analyzed by one-way ANOVA, followed by Mann-Whitney t Tests.

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Individual lipoprotein parameters such as increased small LDL, small HDL, and ApoB100 have been associated with increased atherogenic risk and diabetes independent of obesity, but less is known about lipoprotein characteristics relative to NAFLD progression [28, 29, 34–36]. Given the liver is the site of a majority lipoprotein secretion and uptake, namely VLDL and LDL, alterations in lipoprotein profiles may reflect changes in NAFLD progression [1, 37–40]. Studies using imaging such as CT or MRI [10] to diagnose NAFLD found that the lipoprotein profile changes accompanying NAFLD are similar to the dyslipidemia that precedes the onset of T2D including increased triglycerides, large VLDL, VLDL size, and small LDL, and decreased large HDL [5, 12–14, 18]. Further, they found that triglycerides and total cholesterol increase and HDL decreases with hepatic fat. However, these studies were insufficient to describe NASH, as only liver biopsy can detect the inflammation, fibrosis, and ballooning that distinguish NASH from steatosis [9, 32, 41, 42]. Other studies have evaluated lipoprotein using AST and ALT as surrogate markers of hepatic dysfunction, but these enzymes are normal in up to 78% of patients with NAFLD and thus cannot be used to accurately diagnose the disease or distinguish steatosis from NASH [20–22].

Of the studies in biopsy confirmed NASH, plasma lipoparticle analyses by HPLC or ultra-centrifugation have shown an increase in total triglycerides, VLDL, LDL, and ApoB100 and a decrease in HDL in NASH relative to normal livers [4]. Studies of steatosis versus NASH however have been less congruent, as some show decreases in VLDL (and HDL), and others an increase. Recently, the use of NMR-derived lipoprotein profiles revealed previously undetected changes in particle number and size that are associated with metabolic disease [34]. We are only the second study to employ this technology to compare biopsy driven NAFLD diagnoses to lipoprotein profiles [43]., but this other work did not use the standard subclass classifications employed by the existing literature and available commercial methods [25], thus limiting its applicability [24]. In addition, the group did not report differences between SS and NASH, only differences relative to normal histology, nor did they stratify their cohort by ethnicity or gender. Similar to ethnicity, the relationship between dyslipidemia and metabolic diseases such as diabetes, cardiovascular disease, and NAFLD differs by gender, with females displaying improved lipid profiles relative to men [11, 44]. Hence, the study may have missed significant differences in lipoprotein subclass profiles across pathology due to cohort heterogeneity.

In this study we found that changes in steatosis versus NASH were most pronounced when Caucasian women were examined alone. Among Caucasians, increased VLDL size and TG content were the first changes associated with the development of NAFLD, similar to other in ethnically heterogeneous reports [15, 16, 45]. Here progression from SS to NASH was accompanied by more global dysfunction, with decreased total LDL and apoB100 production and increased insulin resistance. These findings suggest a role for deranged lipid production in disease progression, in particular ApoB100 [15, 16, 46]. ApoB100 is a lipid-binding protein produced in the liver that is necessary for the assembly and secretion of VLDL [39]. Because one apoB100 is required for each VLDL particle, its availability determines hepatic lipoprotein production, thus deficiency in the protein synthesis or release could contribute to hepatic TG retention [5]. Fujita et al. came to a similar conclusion when they found that VLDL and ApoB100 mRNA was decreased in NASH relative to simple steatosis patients, but they found that LDL was increased. However, they did not evaluate lipoprotein parameters via H-NMR nor did they stratify their results by gender, both of which might explain the different results [16].

In addition to the decline in lipoprotein parameters in NASH, we found differences in demographics and lipid profiles between African Americans and Caucasian women with SS. Here none of the African American women had NASH. This is similar to the epidemiological literature which reports decreased rates of NAFLD and NASH among African Americans, an

observation that has been attributed to variation in genetics and body fat distribution [47]. We found that African Americans with SS weighed more and were more insulin resistant than Caucasians with SS, yet had a less atherogenic lipid profile. This reflects the "triglyceride paradox" of improved dyslipidemia in African Americans relative to other ethnicities despite higher rates of obesity and insulin resistance [48]. Attempts to explain this discrepancy have implicated increased lipoprotein lipase (LPL) activity and lower levels of the LPL inhibitory protein, apolipoprotein CII, in African Americans as a cause [47, 49] as well as an apoB100 gene variant that is associated with VLDL diameter in Caucasians and Hispanics, but not African Americans [50]. While the mechanisms are yet to be elucidated, these findings point to potential ethnic differences in hepatic lipid metabolism [44, 47, 50].

A final novel facet of this study was the use of the lipoprotein-insulin resistance (LP-IR) score to quantify the relationship between dyslipidemia and insulin sensitivity. The importance of lipids in the pathogenesis of insulin resistance has been highlighted by Randle et al. who described a glucose-fatty cycle where increased byproducts of lipid oxidation lead to inhibition of glucose uptake in type I diabetics [26]. Other studies utilizing H-NMR techniques found significant associations between lipoprotein profile constituents and insulin sensitivity [28, 29]. Six parameters in particular have been used to create an overall picture of insulin resistance known as the LP-IR Score [26, 30]. Here we found a positive relationship between LP-IR and HOMA2-IR including a significant increase in NASH females. Further, increases in LP-IR in Caucasians accompanied or preceded increases in HOMA2-IR and were associated with progression to NASH. Given dyslipidemia's tendency to precede the onset of frank diabetes, the LP-IR score and the lipocentric view of insulin resistance might be of use in the early identification of T2D or NASH [5], but additional studies are needed to further validate this new technique.

This study has some limitations. While liver biopsy remains the "gold standard" for assessing NAFLD severity, the procedure carries risk and therefore was not available for all females [19]. This also rendered our study cohort smaller than those used in larger population-driven studies. Our African American population is especially small, but is reflective of the larger bariatric surgery population, which is currently predominately Caucasian [51, 52]. This work should be repeated in a larger, ethnically diverse cohort of both sexes. Similar to ethnicity, differences in NAFLD severity and dyslipidemia are described in males versus females, thus our decision to recruit only females [53]. Other considerations include more detailed analysis of insulin resistance via hyperinsulinemic euglycemic clamp or lipoprotein production via tracer studies. It would also be useful to assess liver histology longitudinally, but unfortunately, non-clinically indicated liver biopsies are rarely warranted based on ethical concerns.

In conclusion, this study identified differences in total lipoprotein and subclass levels across NAFLD severity that differed in Caucasians versus African American women. The combination of NMR lipoprotein analysis and biopsy driven NASH diagnoses provided the opportunity to appreciate previously undetected changes in lipoprotein profiles, namely changes in VLDL, LDL, and apoB100 that can be used to delineate steatosis from NASH in Caucasian women. These findings suggest a potential role for lipoprotein subclass concentration and size as markers of NAFLD progression, particularly apoB100. Monitoring for relative reductions in these parameters, as well as changes in the LP-IR index, may enable clinicians to better identify and monitor those at increased risk for developing NASH. However, if this strategy is to be employed clinically, our observations in African Americans with SS highlight the need for separate parameters for each ethnicity, as the relationship between dyslipidemia and NAFLD does not appear to be racially homogenous.

Supporting Information

S1 File.
(DOCX)

Author Contributions

Conceived and designed the experiments: AEG NNA CRF. Performed the experiments: AEG RG JA EAE PM-S CRF JD. Analyzed the data: AEG CRF NNA TW. Contributed reagents/materials/analysis tools: NK RAT JW CRF NNA. Wrote the paper: AEG NNA CRF.

References

1. Fon Tacer K, Rozman D. Nonalcoholic Fatty liver disease: focus on lipoprotein and lipid deregulation. J Lipids. 2011; 2011:783976. Epub 2011/07/21. doi: 10.1155/2011/783976 PMID: 21773052; PubMed Central PMCID: PMC3136146.

2. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005; 115(5):1343–51. Epub 2005/05/03. doi: 10.1172/JCI23621 PMID: 15864352; PubMed Central PMCID: PMC1087172.

3. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. Nat Rev Gastroenterol Hepatol. 2013; 10(6):330–44. Epub 2013/03/20. doi: 10.1038/nrgastro.2013.41 PMID: 23507799.

4. Ginsberg HN, Zhang YL, Hernandez-Ono A. Metabolic syndrome: focus on dyslipidemia. Obesity (Silver Spring). 2006; 14 Suppl 1:41S–9S. Epub 2006/04/29. doi: 10.1038/oby.2006.281 PMID: 16642962.

5. Cohen DE, Fisher EA. Lipoprotein metabolism, dyslipidemia, and nonalcoholic fatty liver disease. Semin Liver Dis. 2013; 33(4):380–8. Epub 2013/11/14. doi: 10.1055/s-0033-1358519 PMID: 24222095; PubMed Central PMCID: PMC3988578.

6. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis. 2001; 21(1):3–16. Epub 2001/04/12. PMID: 11296695.

7. Ruhl CE, Everhart JE. Epidemiology of nonalcoholic fatty liver. Clin Liver Dis. 2004; 8(3):501–19, vii. Epub 2004/08/28. doi: 10.1016/j.cld.2004.04.008 PMID: 15331060.

8. Bellentani S, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). Ann Hepatol. 2009; 8 Suppl 1:S4–8. Epub 2009/06/25. PMID: 19381118.

9. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004; 40(6):1387–95. Epub 2004/11/27. doi: 10.1002/hep.20466 PMID: 15565570.

10. Adiels M, Olofsson SO, Taskinen MR, Boren J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler Thromb Vasc Biol. 2008; 28(7):1225–36. Epub 2008/06/21. doi: 10.1161/atvbaha.107.160192 PMID: 18565848.

11. Osei K. Metabolic syndrome in blacks: are the criteria right? Curr Diab Rep. 2010; 10(3):199–208. doi: 10.1007/s11892-010-0116-4 PMID: 20425583.

12. Chan DC, Watts GF, Gan S, Wong AT, Ooi EM, Barrett PH. Nonalcoholic fatty liver disease as the transducer of hepatic oversecretion of very-low-density lipoprotein-apolipoprotein B-100 in obesity. Arteriosclerosis, thrombosis, and vascular biology. 2010; 30(5):1043–50. Epub 2010/02/13. doi: 10.1161/ATVBAHA.109.202275 PMID: 20150556.

13. Magkos F, Fabbrini E, Mohammed BS, Patterson BW, Klein S. Increased whole-body adiposity without a concomitant increase in liver fat is not associated with augmented metabolic dysfunction. Obesity (Silver Spring). 2010; 18(8):1510–5. doi: 10.1038/oby.2010.90 PMID: 20395947; PubMed Central PMCID: PMC3319734.

14. Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology. 2008; 134(2):424–31. Epub 2008/02/05. doi: 10.1053/j.gastro.2007.11.038 PMID: 18242210; PubMed Central PMCID: PMC2705923.

15. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS. Apolipoprotein synthesis in nonalcoholic steatohepatitis. Hepatology. 2002; 35(4):898–904. Epub 2002/03/27. doi: 10.1053/jhep.2002.32527 PMID: 11915037.

16. Fujita K, Nozaki Y, Wada K, Yoneda M, Fujimoto Y, Fujitake M, et al. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. Hepatology. 2009; 50(3):772–80. Epub 2009/08/04. doi: 10.1002/hep.23094 PMID: 19650159.

17. Toledo FG, Sniderman AD, Kelley DE. Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. Diabetes Care. 2006; 29(8):1845–50. Epub 2006/07/29. doi: 10.2337/dc06-0455 PMID: 16873790.

18. DeFilippis AP, Blaha MJ, Martin SS, Reed RM, Jones SR, Nasir K, et al. Nonalcoholic fatty liver disease and serum lipoproteins: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 2013; 227(2):429–36. Epub 2013/02/20. doi: 10.1016/j.atherosclerosis.2013.01.022 PMID: 23419204.

19. El-Badry AM, Breitenstein S, Jochum W, Washington K, Paradis V, Rubbia-Brandt L, et al. Assessment of hepatic steatosis by expert pathologists: the end of a gold standard. Ann Surg. 2009; 250(5):691–7. Epub 2009/10/07. doi: 10.1097/SLA.0b013e3181bcd6dd PMID: 19806055.

20. Lorenzo C, Hanley AJ, Rewers MJ, Haffner SM. The association of alanine aminotransferase within the normal and mildly elevated range with lipoproteins and apolipoproteins: the Insulin Resistance Atherosclerosis Study. Diabetologia. 2013; 56(4):746–57. Epub 2013/01/25. doi: 10.1007/s00125-012-2826-4 PMID: 23344727; PubMed Central PMCID: PMC3615715.

21. Ghamar-Chehreh ME, Amini M, Khedmat H, Moayed Alavian S, Daraei F, Mohtashami R, et al. Elevated alanine aminotransferase activity is not associated with dyslipidemias, but related to insulin resistance and higher disease grades in non-diabetic non-alcoholic fatty liver disease. Asian Pac J Trop Biomed. 2012; 2(9):702–6. Epub 2013/04/10. doi: 10.1016/S2221-1691(12)60213-5 PMID: 23569998; PubMed Central PMCID: PMC3609376.

22. Oh SY, Cho YK, Kang MS, Yoo TW, Park JH, Kim HJ, et al. The association between increased alanine aminotransferase activity and metabolic factors in nonalcoholic fatty liver disease. Metabolism. 2006; 55(12):1604–9. Epub 2006/12/05. doi: 10.1016/j.metabol.2006.07.021 PMID: 17142131.

23. Geloneze B, Vasques AC, Stabe CF, Pareja JC, Rosado LE, Queiroz EC, et al. HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). Arq Bras Endocrinol Metabol. 2009; 53(2):281–7. Epub 2009/05/26. PMID: 19466221.

24. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. Clin Lab. 2002; 48(3–4):171–80. Epub 2002/04/06. PMID: 11934219.

25. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clin Lab Med. 2006; 26(4):847–70. Epub 2006/11/18. doi: 10.1016/j.cll.2006.07.006 PMID: 17110242.

26. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963; 1(7285):785–9. Epub 1963/04/13. PMID: 13990765.

27. McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. Science. 1992; 258(5083):766–70. Epub 1992/10/30. PMID: 1439783.

28. Goff DC Jr., D'Agostino RB Jr., Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. Metabolism. 2005; 54(2):264–70. Epub 2005/02/04. doi: 10.1016/j.metabol.2004.09.002 PMID: 15690322.

29. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes. 2003; 52(2):453–62. Epub 2003/01/24. PMID: 12540621.

30. Shalaurova I, Connelly MA, Garvey WT, Otvos JD. Lipoprotein Insulin Resistance Index: A Lipoprotein Particle-Derived Measure of Insulin Resistance. Metab Syndr Relat Disord. 2014. Epub 2014/06/25. doi: 10.1089/met.2014.0050 PMID: 24959989.

31. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology. 2012; 142(7):1592–609. Epub 2012/06/05. doi: 10.1053/j.gastro.2012.04.001 PMID: 22656328.

32. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005; 41(6):1313–21. Epub 2005/05/26. doi: 10.1002/hep.20701 PMID: 15915461.

33. Maximos M, Bril F, Portillo Sanchez P, Lomonaco R, Orsak B, Biernacki D, 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):153–60. Epub 2014/08/26. doi: 10.1002/hep.27395 PMID: 25145475.

34. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. J Clin Lipidol. 2011; 5(2):105–13. Epub 2011/03/12. doi: 10.1016/j.jacl.2011.02.001 PMID: 21392724; PubMed Central PMCID: PMC3070150.

35. Mackey RH, Greenland P, Goff DC Jr., Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). J Am Coll Cardiol. 2012; 60(6):508–16. Epub 2012/07/17. doi: 10.1016/j.jacc.2012.03.060 PMID: 22796256; PubMed Central PMCID: PMC3411890.

36. Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE, Ridker PM. Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. Diabetes. 2010; 59 (5):1153–60. Epub 2010/02/27. doi: 10.2337/db09-1114 PMID: 20185808; PubMed Central PMCID: PMC2857895.

37. Targher G, Byrne CD. Diagnosis and management of nonalcoholic fatty liver disease and its hemostatic/thrombotic and vascular complications. Semin Thromb Hemost. 2013; 39(2):214–28. Epub 2013/02/12. doi: 10.1055/s-0033-1334866 PMID: 23397556.

38. Ellington AA, Kullo IJ. Atherogenic lipoprotein subprofiling. Advances in clinical chemistry. 2008; 46:295–317. Epub 2008/11/14. PMID: 19004193.

39. Shelness GS, Ledford AS. Evolution and mechanism of apolipoprotein B-containing lipoprotein assembly. Curr Opin Lipidol. 2005; 16(3):325–32. Epub 2005/05/14. PMID: 15891394.

40. Verges B. Abnormal hepatic apolipoprotein B metabolism in type 2 diabetes. Atherosclerosis. 2010; 211(2):353–60. Epub 2010/03/02. doi: 10.1016/j.atherosclerosis.2010.01.028 PMID: 20189175.

41. Obika M, Noguchi H. Diagnosis and evaluation of nonalcoholic fatty liver disease. Exp Diabetes Res. 2012; 2012:145754. Epub 2011/11/24. doi: 10.1155/2012/145754 PMID: 22110476; PubMed Central PMCID: PMC3205741.

42. Morling JR, Fallowfield JA, Guha IN, Nee LD, Glancy S, Williamson RM, et al. Using non-invasive biomarkers to identify hepatic fibrosis in people with type 2 diabetes mellitus: the Edinburgh type 2 diabetes study. J Hepatol. 2014; 60(2):384–91. Epub 2014/02/12. doi: 10.1016/j.jhep.2013.10.017 PMID: 24512822.

43. Mannisto VT, Simonen M, Soininen P, Tiainen M, Kangas AJ, Kaminska D, et al. Lipoprotein subclass metabolism in nonalcoholic steatohepatitis. J Lipid Res. 2014; 55(12):2676–84. doi: 10.1194/jlr.P054387 PMID: 25344588; PubMed Central PMCID: PMC4242459.

44. Sumner AE. The relationship of body fat to metabolic disease: influence of sex and ethnicity. Gender medicine. 2008; 5(4):361–71. doi: 10.1016/j.genm.2008.11.003 PMID: 19108808; PubMed Central PMCID: PMC3485399.

45. Musso G, Cassader M, De Michieli F, Rosina F, Orlandi F, Gambino R. Nonalcoholic steatohepatitis versus steatosis: adipose tissue insulin resistance and dysfunctional response to fat ingestion predict liver injury and altered glucose and lipoprotein metabolism. Hepatology. 2012; 56(3):933–42. Epub 2012/06/12. doi: 10.1002/hep.25739 PMID: 22684858.

46. Siddiqui MS, Fuchs M, Idowu M, Luketic VA, Boyett S, Sargeant C, et al. Severity of Nonalcoholic Fatty Liver Disease and Progression to Cirrhosis Associate With Atherogenic Lipoprotein Profile. Clin Gastroenterol Hepatol. 2014. doi: 10.1016/j.cgh.2014.10.008 PMID: 25311381.

47. Pan JJ, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. World J Hepatol. 2014; 6(5):274–83. Epub 2014/05/29. doi: 10.4254/wjh.v6.i5.274 PMID: 24868321; PubMed Central PMCID: PMC4033285.

48. Yu SS, Castillo DC, Courville AB, Sumner AE. The triglyceride paradox in people of African descent. Metab Syndr Relat Disord. 2012; 10(2):77–82. Epub 2012/01/10. doi: 10.1089/met.2011.0108 PMID: 22224930; PubMed Central PMCID: PMC3311911.

49. Kallwitz ER, Guzman G, TenCate V, Vitello J, Layden-Almer J, Berkes J, et al. The histologic spectrum of liver disease in African-American, non-Hispanic white, and Hispanic obesity surgery patients. Am J Gastroenterol. 2009; 104(1):64–9. Epub 2008/12/23. doi: 10.1038/ajg.2008.12 PMID: 19098851.

50. Frazier-Wood AC, Manichaikul A, Aslibekyan S, Borecki IB, Goff DC, Hopkins PN, et al. Genetic variants associated with VLDL, LDL and HDL particle size differ with race/ethnicity. Hum Genet. 2013; 132 (4):405–13. Epub 2012/12/25. doi: 10.1007/s00439-012-1256-1 PMID: 23263444; PubMed Central PMCID: PMC3600091.

51. Wee CC, Huskey KW, Bolcic-Jankovic D, Colten ME, Davis RB, Hamel M. Sex, race, and consideration of bariatric surgery among primary care patients with moderate to severe obesity. J Gen Intern Med. 2014; 29(1):68–75. Epub 2013/09/21. doi: 10.1007/s11606-013-2603-1 PMID: 24048655; PubMed Central PMCID: PMC3889969.

52. Lynch CS, Chang JC, Ford AF, Ibrahim SA. Obese African-American women's perspectives on weight loss and bariatric surgery. J Gen Intern Med. 2007; 22(7):908–14. Epub 2007/04/21. doi: 10.1007/s11606-007-0218-0 PMID: 17447097; PubMed Central PMCID: PMC2583799.

53. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988–1994. Am J Epidemiol. 2013; 178(1):38–45. Epub 2013/05/25. doi: 10.1093/aje/kws448 PMID: 23703888; PubMed Central PMCID: PMC3698993.