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Nonalcoholic Fatty Liver Disease and serum lipoproteins: The Multi-Ethnic Study of Atherosclerosis

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Abstract

Objective—While non-alcoholic fatty liver disease (NAFLD) is associated with the metabolic syndrome, it is not known if NAFLD plays an independent role in the atherogenic dyslipidemia phenotype.

Methods and Results—The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based prospective cohort study of adults free of clinical cardiovascular disease at enrollment. We tested for a relationship between NAFLD, defined as a liver/spleen (L/S) attenuation ratio of <1 on

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a non-contrast cardiac CT scan, and multiple measures of fasting serum lipoprotein size, cholesterol and particle concentrations.

NAFLD was present in 569 (17%) of 3,362 participants. After adjustment for multiple metabolic risk factors, adiposity and measures of insulin resistance (HOMA-IR), NAFLD was independently associated with higher fasting serum triglycerides and lower serum HDL-C. Despite a lack of association with LDL-C, NAFLD was associated with higher LDL particle concentration and lower LDL particle size. Modeling the L/S ratio as a continuous variable, a severity dependent association was observed between atherogenic lipoprotein abnormalities and NAFLD.

Conclusion—In a large, multi-ethnic, gender balanced cohort, CT-diagnosed NAFLD was associated with the atherogenic dyslipidemia phenotype in a dose dependent fashion. These relationships persisted after adjustment for several metabolic risk factors and HOMA-IR, suggesting a possible independent pathophysiologic role between NAFLD and dyslipidemia.

Keywords

Nonalcoholic Fatty Liver Disease; Lipoproteins; MESA; NMR; cardiac CT scan

INTRODUCTION

The prevalence of obesity has increased and it now affects more than 1 billion individuals worldwide.[1] Non-alcoholic fatty liver disease (NAFLD) is a condition associated with obesity in which there is ectopic accumulation of triglycerides in the liver parenchyma.[2] NAFLD affects about 30% of adults in the United States[3, 4] and has become the leading cause of liver disease[5]. NAFLD is associated with both subclinical atherosclerosis[5] as well as overt cardiovascular events.[6–9] This association is independent of obesity-related comorbidities including diabetes mellitus, hypertension, and hyperlipidemia.[5, 6, 8, 9] Indeed, cardiovascular disease (CVD) contributes more than liver disease to the burden of morbidity and mortality of patients with NAFLD.[5]

Lipoprotein and cholesterol metabolism occurs primarily in the liver.[10] NAFLD is associated with hypertriglyceridemia and reductions in high density lipoprotein (HDL) cholesterol,[11, 12] which may be secondary to an increase in the size of very low density lipoproteins (VLDL).[13] However, the independent impact of NAFLD on low density lipoproteins, apolipoprotein B, size and particle concentration of major lipoproteins and the commonly used measures of athrogenic dyslipidemia remains poorly defined.[11, 12, 14, 15]

Although liver biopsy is the gold standard for the assessment of liver fat, the procedure is invasive and carries risk of substantial morbidity. Non-contrast computed tomography (CT) has been adapted as a noninvasive alternative to assess for the presence of fatty infiltration of the liver and quantify its severity.[16] Quantitative radiographic attenuation of the liver is normally greater than that of the spleen. Reversal of this ratio suggests the presence of a fatty liver.[17, 18] This application of CT has shown good correlation (r=0.77) with histological samples.[19, 20]

Prior studies examining NAFLD's association with lipoproteins have been limited by study size and an inability to adequately control for factors known to impact apolipoprotein

metabolism, including lipid and diabetes medications, obesity, and diabetes/insulin resistance. The Multi-Ethnic Study of Atherosclerosis (MESA) is a NIH/NHLBI-funded population-based prospective cohort study in which a baseline measure of NAFLD was made using non-contrast cardiac-gated CTs originally aimed at measuring coronary calcification. We hypothesized that NAFLD, as assessed on non-contrast CT scans obtained in this context, would be independently associated with atherogenic patterns in serum lipoproteins even after adjustment for gender, race/ethnicity, and other metabolic factors including insulin resistance. Defining the relationship between liver fat and serum lipoproteins is a critical step in understanding the link between NAFLD pathophysiology, lipoprotein metabolism and cardiometabolic risk.

METHODS

Study Population

The design and methods of the MESA study have been previously published.[21] Briefly, 6,814 participants aged 45 to 84 years representing four different ethnic backgrounds (Caucasian, Chinese, African American, Hispanic) were recruited from six communities in the United States (Forsyth County, North Carolina; Northern Manhattan and the Bronx, New York; Baltimore City and Baltimore County, Maryland; St. Paul, Minnesota; Chicago, Illinois; and Los Angeles County, California) between 2000 to 2002. All participants were free of clinical cardiovascular disease at study enrollment. An approximately equal number of men and women were recruited according to pre-specified age and race/ethnicity/ethnicity strata. All participants gave informed consent, and the study protocol was approved by the institutional review board at each site.

Medical history, anthropometric measurements, laboratory testing, and coronary calcium CT scans were taken during the first examination (July 2000 to August 2002). Lipoprotein cholesterol concentrations and lipoprotein particle concentration and size were measured at the first examination (July 2000 to August 2002).

A total of 4,384 participants had scans with adequate field of view to assess attenuation of the liver and spleen. To limit confounding, of the 4,384 participants with adequate scans, we excluded subjects on cholesterol lowering medication (N=781), and those who reported cirrhosis, heavy drinking (>7 drinks per week in women, >14 drinks per week in men), or use of oral steroids and/or class III antiarrhythmics, including amiodarone (N=241). Thus, our final study population consisted of 3,362 MESA participants.

Laboratory and Risk Factor Measurement

Total cholesterol, HDL-C and triglyceride measurements were made at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). Total cholesterol was measured from plasma using a cholesterol oxidase method (Roche Diagnostics, Indianapolis, IN) on a Roche COBAS FARA centrifugal analyzer. The laboratory coefficient of variance (CV) for this test was 1.6%. HDL cholesterol was measured in EDTA plasma using the cholesterol oxidase method (Roche Diagnostics) after precipitation of non-HDL-C with magnesium/dextran. The laboratory CV for this test was

2.9%. Triglycerides were measured in EDTA plasma using Triglyceride GB reagent (Roche Diagnostics) on the Roche COBAS FARA centrifugal analyzer. The laboratory CV for this test was 4.0%. LDL-C was calculated in plasma specimens having a triglyceride value <400 mg/dL using the formula of Friedewald et al.[22]

Individual lipoprotein subclasses were measured by Nuclear Magnetic Resonance (NMR) spectroscopy using the commercially available LipoProfile-II spectral analysis process (LipoScience, Inc.; Raleigh, NC). The instrument employs proton NMR spectroscopy to measure the particle concentrations of 11 subclasses of VLDL, LDL, and HDL. In addition, calculated values for mean VLDL, LDL, and HDL particle size and estimates of total and VLDL triglycerides and HDL cholesterol are provided. The CVs for the particle concentrations of VLDL, LDL, and HDL were 4% or less. CVs for mean VLDL, LDL, and HDL mean particle size were 2.0% or less. For calculated total triglycerides, VLDL triglycerides, and HDL cholesterol, CVs ranged from 1.1–1.4%.

Hyper-chylomicronemia was diagnosed if an individual had triglycerides greater than 500 mg/dl and a triglycerides/total cholesterol ratio >10:1.[23] Diabetes mellitus was defined as a fasting blood glucose ≥126 mg/dl or the use of insulin or oral hypoglycemic medications. Impaired fasting glucose was defined as a fasting glucose between 100 and 125 mg/dl. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting glucose (mg/dl) × fasting insulin (μU/mL)/405. Hypertension was defined in accordance with JNC VI guidelines: a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg, or the use of medications for hypertension.[24] Waist circumference at the umbilicus was measured to the nearest 0.1 cm using a steel measuring tape. Height and weight were measured with participants wearing light clothing and no shoes, and body mass index was calculated (in kg/m^2). Metabolic syndrome was defined by the joint American Heart Association/National Heart, Blood and Lung Institute guidelines: >40 inches in men or 35 inches in women, triglycerides >150 mg/dl, HDL-C <40 mg/dl in men or 50 mg/dl in women, blood pressure ≥135/85 mmHg and a fasting glucose >100 mg/dl.[25] Atherogenic dyslipidemia was defined in two ways, 1) an HDL-C <40 mg/dl in men, <50 mg/dl in women and triglycerides ≥150 mg/dl[26] and 2) a ratio of triglycerides to HDL-C ≥3.[27, 28]

CT Imaging

Each participant underwent two consecutive non-enhanced cardiac-gated computed tomography scans during a single session for the primary purpose of coronary artery calcium scoring. Each scan was obtained during a single breath hold at end inspiration to reduce motion artifacts and improve image quality of the coronary arteries. The participants were scanned using either electron-beam tomography (EBT) (3 sites) or multi-detector CT (3 sites). Scans were performed from the carina to below the apex of the heart, including images of the liver and spleen in most patients. The protocol of scanner parameters and scanning details were reported previously.[29] The images were read at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

Liver Fat Measurement and Definition of NAFLD

CT scans were evaluated by two independent, experienced readers blinded to the demographic data. Duplicate scans of each participant were examined; the scan with the greatest coverage of the liver and spleen was selected for liver fat measurement. Hepatic and splenic Hounsfield attenuation were measured using regions of interest (ROI) greater than 100 mm^2 in area. Two ROI were placed in the right liver lobe along the anterior-posterior dimension and one ROI was placed in the spleen. ROI with larger areas were used, when possible, to include a greater area of the liver and spleen while taking care to exclude regions of non-uniform parenchymal attenuation, including hepatic vessels. During initial planning of this MESA ancillary study, the liver/spleen attenuation ratio (L/S ratio) was selected as the most stable measure of hepatic fat content. This was calculated by taking mean HU measurement of both right liver lobe ROIs and dividing it by the spleen HU measurement.[30] A L/S ratio <1.0 was defined a priori as the cut-point for diagnosing NAFLD.[30] Liver fat severity was graded as mild (<1.0 to ≥0.7), moderate (<0.7 to ≥0.5) and severe (<0.5) based on prior research, and as the absolute L/S ratio.[31] Based on data from Park et al.[20], the CT L/S based designations of mild, moderate and severe NAFLD would correspond to < 30.1%, 30.1% to 41% and > 41% macrovesicular steatosis. Imaging of the liver and spleen was adequate to evaluate the L/S ratio in 64% of subjects (4384 of 6814) with non-contrast cardiac CT scans.

Statistical Analysis

Baseline characteristics were assessed among those with and without NAFLD, with Pearson Chi-square and ANOVA testing used for the evaluation of statistically significant differences among categorical and normally distributed continuous variables respectively. The Kruskal-Wallis test was used to determine statistical significance for variables with a non-normal distribution.

We assessed the relationship between liver fat presence/severity and three distinct sets of lipid parameters: 1) Routine lipid tests found on a standard lipid profile; 2) Advanced testing quantifying lipoprotein particle and size by NMR; and 3) atherogenic dyslipidemia. After calculating means of the raw lipid parameters for the two sets of data, we present age, gender, and race/ethnicity adjusted p-values for significant differences by presence of L/S<1. To model the effect of increasing liver fat severity, we used robust linear regression to calculate absolute beta-coefficients of change in raw lipid values per one standard deviation of change in the L/S ratio, after hierarchical adjustment - model 1: unadjusted; model 2: age, gender, race/ethnicity; model 3: age, gender, race/ethnicity, body mass index (BMI), waist circumference, hypertension, smoking, CRP (log transformed), diabetes, HOMA-IR (log transformed), oral hypoglycemics, and insulin therapy. An interaction term for diabetes therapies (oral hypoglycemics or insulin) x log-HOMA-IR was tested, but it did not have a significant impact on results and therefore it was discarded.

We modeled the relationship of liver fat severity assessed using the ordinal L/S ratio (≥1 normal, 1.0-0.7 mild, 0.7-0.5 moderate, <0.5 severe) and a clinical “atherogenic dyslipidemia” definition defined as above. Multivariable prevalence odds ratios were calculated in the three hierarchical models using multivariable logistic regression. Finally,

we conducted hypothesis-generating, stratified analyses examining for effect modification by race/ethnicity.

This data analysis plan was peer reviewed and approved by the MESA Publications and Presentations committee. All statistical analyses were completed using Stata software version 11 (College Station, TX).

RESULTS

The prevalence of NAFLD in the final study population was 17% [569 with NAFLD (L/S<1) and 2793 without NAFLD (L/S≥1)]. Subjects with NAFLD were younger and more frequently Hispanic (Table 1). They had a higher average BMI, greater waist circumference, higher blood pressure, higher median CRP level, and higher prevalence of metabolic syndrome. Subjects with NAFLD had a higher median HOMA-IR, were more likely to have impaired fasting glucose, diabetes (treated or untreated) or to be taking oral hypoglycemics but were less likely to be on insulin therapy or to be a current or past smoker. No subjects met criteria for hyper-chylomicronemia (triglycerides greater than 500 mg/dl and a triglycerides to total cholesterol ratio >10:1).

Standard Lipid Measures (Cholesterol Concentration)

When stratified by the presence of NAFLD (L/S <1) or not and adjusted for age, gender, and race/ethnicity, there was no association between total cholesterol or LDL-C with the diagnosis of NAFLD (Table 2). However, the presence of NAFLD was associated with lower HDL-C, higher triglycerides, and higher non-HLD-C (Table 2). Similarly, ratios of total cholesterol/HDL-C and triglycerides/HDL-C were higher in subjects with NAFLD.

The relationship between the degree of NAFLD (L/S ratio as a continuous variable) and standard lipid measures is shown in Table 3. Consistent with findings of NAFLD as a binary variable, no association between total cholesterol or LDL-C with NAFLD was observed, but the degree of NAFLD was inversely associated with HDL-C, and positively associated with triglycerides, and non-HLD-C. These associations were present in the unadjusted model and persisted after adjustment for age, gender, and race/ethnicity, as well as multiple direct and indirect correlates of insulin resistance (Table 3).

Lipoprotein size and particle concentration

Particle concentration of large VLDL and chylomicrons, as well as VLDL, and IDL were progressively higher across higher levels of NAFLD severity, after adjustment for age, gender and race/ethnicity (Table 4). Despite similar LDL-C levels, LDL particle concentrations were higher and LDL particle size was smaller across progressive levels of NAFLD severity. In contrast, HDL particle concentrations were similar across NAFLD severity categories. VLDL particle size was progressively higher, while LDL and HDL particle size was progressively smaller across categories of higher NAFLD severity, after adjustment for age, gender and race/ethnicity (Table 4).

The relationship between NAFLD (L/S ratio as a continuous variable) and NMR lipid measures was further evaluated (Table 5). Consistent with findings of NAFLD as a

categorical variable, large VLDL & Chylomicrons, VLDL, IDL and LDL particle concentrations were positively associated with NAFLD severity while HDL particle concentration was not associated with NAFLD severity. These associations were present in the unadjusted model, when adjusted for age, gender, and race/ethnicity, and persisted after multivariable adjustment, including multiple direct and indirect measures of insulin resistance (Table 5).

Arthrogenic Dyslipidemia

Two commonly used definitions of arthrogenic dyslipidemia derived from a combination of standard lipid measures were evaluated in relation to NAFLD severity (Table 6). The odds ratio for having an atherogenic dyslipidemia (HDL-C <40 mg/dl in men, <50 mg/dl in women and triglycerides ≥150 mg/dl) or a Triglyceride/HDL-C ratio ≥3, increased substantially with increasing L/S ratio when compared to a normal L/S ratio (Table 6). This relationship was not significantly attenuated by age, gender, and race/ethnicity, however there was moderate attenuation when measures of insulin resistance and inflammation were incorporated (model 3: age, gender, race/ethnicity, BMI, waist circumference, hypertension, anti-hypertensive medication, smoking, log CRP, diabetes status, log HOMA-IR, oral hypoglycemic use, insulin use adjusted).

Race

Overall, there was no statistical evidence of heterogeneity for the effect of liver fat on atherogenic dyslipidemia (p-value for interaction term 0.48 and 0.80, for the measures of atherogenic dyslipidemia). However, testing interaction terms in individual race/ethnicity, there was evidence of a statistically significant weaker association of African Americans with both measures of atherogenic dyslipidemia compared to the other race/ethnicity (data not shown). These analyses are limited by small sample sizes.

DISCUSSION

In this large multi-ethnic cohort of adults with no known CVD at the time of enrollment, NAFLD, as diagnosed by the L/S ratio, was associated with higher fasting serum triglycerides, lower serum HDL-C but no difference in total cholesterol or LDL-C. Although no association was observed between NAFLD and LDL-C, NAFLD was positively associated with LDL particle concentration and negatively associated with LDL particle size assessed by NMR. The cardiovascular risk association with NAFLD that may be attributed to high LDL particle concentration was appreciated by NMR and indirectly by the association of NAFLD with atherogenic dyslipidemia defined with standard lipid measurements which correlate with direct measures of LDL particle concentration. A severity or “dose” dependent association was apparent between lipoproteins and NAFLD by both absolute and ordinal CT L/S ratios available from non-contrast cardiac-gated CTs originally aimed at measuring coronary calcification. These relationships persisted after adjustment for age, gender, race/ethnicity, BMI, waist circumference, hypertension, anti-hypertensive medications, smoking, log CRP, diabetes status, log HOMA-IR, oral hypoglycemic use, and insulin use. These findings suggest that NAFLD, as diagnosed via an abnormal L/S ratio and frequently available from a non-contrast cardiac-gated CTs aimed at

measuring coronary calcification, is associated with lipoprotein abnormalities appreciated via standard and NMR measures that are independent of insulin resistance.

Strengths of this study include the use of standard coronary calcium CT (a commonly done, non-invasive procedure) for an assessment of NAFLD via L/S attenuation ratios. CT offers a noninvasive modality to assess for the presence of fatty infiltration of the liver and quantify its severity.[16] This methodology has shown good correlation (r=0.77) with histological samples.[19, 20] In our population based study, imaging on a standard coronary calcium CT was adequate to evaluate the L/S ratio in 64% of subjects (4384 of 6814 subjects with coronary calcium CT). Furthermore, this cohort was characterized with both standard and NMR evaluations for a more complete assessment of the association of NAFLD with the cholesterol content and particle concentration of lipoproteins.

While causality cannot be established from a cross sectional analysis as performed in this study, our findings suggest that the association between NAFLD and dyslipidemia is in part independent of insulin resistance. Independence from insulin resistance is an important question because it is widely believed that insulin resistance resulting in enhanced peripheral lipolysis (from increased intra-cellular lipoprotein lipase activity), increased triglyceride synthesis and increased hepatic uptake of fatty acids leading to the accumulation of hepatocellular triglyceride is responsible for NAFLD.[32–35] Alternatively, primary lipoprotein abnormalities resulting in hepatic triglyceride over production or impaired secretion, independent of insulin resistance may be responsible for NAFLD. Liver fat content is directly associated with VLDL-apoB100 concentrations[36] and a defect in postprandial apolipoprotein B secretion, leading to triglyceride accumulation has been demonstrated in steatohepatitis.[37] Impaired VLDL synthesis and secretion were also more apparent in patients with nonalcoholic steatohepatitis.[38]

The lipoprotein derangements associated with NAFLD observed in this study have been associated with increased cardiovascular events. Based upon strong epidemiologic data, each 1 mg/dL higher HDL-C level is associated with a 2–3% lower risk of coronary heart disease.[39] An association between fasting and postprandial triglycerides and coronary heart disease risk has been established.[40–42] An examination of participants in the Framingham Offspring Study, free of known cardiovascular disease at enrollment and free of diabetes at the time of lipoprotein assessment, found a relative risk of 2.1 (CI 1.2–3.6) for coronary heart disease events in individuals who fulfilled a definition of atherogenic dyslipidemia used in this study (HDL-C <40 mg/dl in men, <50 mg/dl in women and triglycerides ≥150 mg/dl).[26] Triglyceride to HDL-C ratio is also associated with cardiovascular risk.[27, 28] Finally, multiple studies have established LDL particle concentration as a superior predictor of both subclinical atherosclerosis and major adverse cardiovascular events to that of LDL-C.[43–49]

Our findings that NAFLD is associated with increased triglycerides and reduced HDL-C is consistent with prior studies.[11, 12] Furthermore, our study is consistent with a prior study which demonstrated that the increase in triglycerides is secondary to both an increase in VLDL concentration and particle size.[13] Our study is unique in that it demonstrates these associations after accounting for multiple factors known to influence both liver fat

accumulation and lipoprotein metabolism –including multiple measures of insulin resistance.

The multiple differences in lipoprotein levels observed among individuals with NAFLD in our cohort are consistent with current understanding of lipid metabolism. An overproduction of apolipoprotein B100 containing particles observed in our cohort (large VLDL, VLDL, IDL) are released by the liver and converted to cholesterol rich LDL particles via lipoprotein lipase. The predilection for small, dense LDL is not completely understood but may be secondary to enhanced cholesterol ester transfer protein (CETP) activity leading to intermediate lipoproteins with greater triglyceride content - the preferred substrate for hepatic lipase.[13] Resultant increased activity of hepatic lipase also favors the production of small, dense LDL.[50–53] The lower HDL-C observed in NAFLD subjects is expected secondary to the marked hypertriglyceridemia leading to increased CETP mediated incorporation of triglycerides into the HDL particle. These triglyceride enriched HDL particles tend to be cleared more rapidly from the circulation.[54] Our findings of preserved HDL concentration and reduction in HDL particle size with increasing NAFLD severity underscore the complexity of HDL metabolism. Carefully designed in vivo isotope labeled lipoprotein kinetic studies will be needed to further delineate the altered metabolism of lipoproteins in NAFLD.

NAFLD is closely associated with insulin resistance and present in up to 90% of individuals with diabetes and/or morbid obesity.[5] However, an increased risk of cardiovascular events has been associated with NAFLD in those with and without type 2 diabetes/insulin resistance as assessed by glycosylated hemoglobin and measures of obesity.[7–9] Therefore, factors independent of insulin resistance/obesity, accounting for the increased risk of cardiovascular disease among patients with NAFLD has remained an area of need in the study of NAFLD and cardiovascular disease.[5] This large multi-ethnic cohort of adults has allowed us to show the importance of dyslipidemia, including elevated triglycerides, reduced HDL-C, elevated non-HDL-C, and increased LDL particle concentration in the cardiovascular risk profile associated with NAFLD. The associations between NAFLD and dyslipidemic factors, that have been shown to impart increased cardiovascular risk in multiple mechanistic, observational and experimental trials, remain significant after accounting for multiple other cardiovascular risk factors, including: age, gender, race/ethnicity, BMI, waist circumference, hypertension, anti-hypertensive medication, smoking, log CRP, diabetes status, log HOMA-IR, oral hypoglycemic use, and insulin use. These data help establish dyslipidemia as a significant factor in explaining the observed increased cardiovascular risk among those with NAFLD. Surveillance and treatment of dyslipidemia, beyond LDL-C, is therefore paramount among those with NAFLD for the prevention and treatment of cardiovascular disease. Lifestyle modification (diet, exercise, weight loss) and statin therapy have been shown to safely improve many of the lipid abnormalities associated with NAFLD and cardiovascular outcomes in multiple populations.[55–57]

Limitations

This study utilizes L/S attenuation of non-contrast CT scan to estimate liver fat content. Although liver biopsy with histological examination is the gold standard for the diagnosis of

NAFLD and NAFLD severity, the CT-scan methodology used in this study has shown good correlation with histological samples.[19, 20] Furthermore, the assessment of liver fat by non-contrast CT scan has been previously examined in this study cohort.[30] This evaluation found excellent inter-reader and intra-reader variability for both liver attenuation (r = 0.96 and 0.99, respectively) and spleen attenuation (r = 0.99 and 0.99, respectively).[30]

We cannot exclude the possibility that the available measures of insulin resistance, including HOMA-IR, did not fully capture insulin resistance information. However, while no single measure address insulin resistance adequately, our model included multiple direct and indirect measures of insulin resistance, including; BMI, waist circumference, log CRP, diabetes status, log HOMA-IR, oral hypoglycemic use and insulin use.

Although we excluded participants on cholesterol lowering medication, oral steroids and/or class III antiarrhythmics; unless omega-3 fatty acids were reported as a lipid lowering medication, no record of omega-3 fatty acid use was available. Given that omega-3 fatty acid supplement use may reduce liver fat this is a limitation of the study.

We did not have sufficient power to fully explore differences in the relationship between NAFLD and lipoproteins in an analysis stratified by race/ethnicity. However, race/ethnicity was included in both multivariable models.

CONCLUSION

In a large, multi-ethnic, gender balanced cohort, NAFLD, as assessed by L/S attenuation from a standard non-contrast coronary calcium CT scan was associated with standard and NMR measures of atherogenic dyslipidemia in a severity dependent fashion. While these relationships were moderately attenuated after adjustment for metabolic risk factors and a measure of insulin resistance, there remained an up to 3-fold increase in the prevalence of standard clinical definitions of atherogenic dyslipidemia when more severe NAFLD was present.

While causality cannot be established from a cross sectional analysis as performed in this study, these findings do raise the possibility that NAFLD may influence lipoprotein derangements associated with atherosclerosis and cardiovascular risk independent of obesity and insulin resistance.

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Non-alcoholic fatty liver disease (NAFLD)

Multi-Ethnic Study of Atherosclerosis (MESA) – a large, multi-ethnic, gender balanced cohort.

NAFLD assessment by non contrast CT scan

NAFLD was independently associated with higher fasting serum triglycerides and lower serum HDL-C after adjustment for multiple metabolic risk factors and measures of insulin resistance (HOMA-IR).

Possible independent pathophysiologic role for NAFLD in dyslipidemia.

Table 1

Baseline characteristics of those with and without NAFLD by L/S ratio.

Variable	NAFLD Liver/Spleen Ratio <1 (n=569)	No NAFLD Liver/Spleen Ratio ≥1 (n=2793)	p-value
Age, years	60.5 ± 9.6	62.5 ± 10.7	<0.001
Gender, men	47%	45%	0.38
Race			<0.001
• White	30%	37%	
• Chinese	11%	10%	
• African-American	19%	33%	
• Hispanic	40%	21%	
BMI, kg/m^2	31.2 ± 5.6	27.9 ± 5.2	<0.001
Waist Circumference, cm	105.7 ± 14	96.7 ± 14	<0.001
Hypertension	49%	42%	0.001
Systolic Blood Pressure, mmHg	129.6 ± 20.8	125.8 ± 21.6	<0.001
Diastolic Blood Pressure, mmHg	73.6 ± 10.5	71.4 ± 10.2	<0.001
Anti-hypertensives	40%	33%	0.002
Smoking			0.12
• Former	33%	36%	
• Current	11%	12%	
Fasting Glucose, mg/dL	96 (88 – 112)	88 (82 – 96)	<0.001
HOMA-IR	2.3 (1.5 – 3.7)	1.1 (0.71 – 1.7)	<0.001
Diabetes Status			<0.001
• Impaired fasting glucose	24%	11%	
• Untreated Diabetes	7%	2%	
• Treated Diabetes	12%	7%	
Oral Hypoglycemics	11%	6%	<0.001
Insulin Use	1.1%	1.4%	0.48
CRP, mg/L	3.0 (1.5 – 6.6)	1.8 (0.8 – 4.2)	<0.001
Metabolic Syndrome	59%	28%	<0.001

BMI = body mass index; HOMA-IR = homeostasis model assessment of insulin resistance; CRP = C-reactive protein

Table 2

Standard lipid measures and ratios in those with and without NAFLD by L/S ratio

Variable	NAFLD Liver/Spleen Ratio <1 (n=569)	No NAFLD Liver/Spleen Ratio ≥1 (n=2793)	Adjusted p-value*
Standard Lipid Measures			
Total Cholesterol, mg/dL	195.9 ± 40	195.9 ± 34	0.84
LDL-C, mg/dL	117.7 ± 31.3	120.3 ± 31.0	0.23
HDL-C, mg/dL	44.4 ± 12.3)	51.6 ± 14.8	<0.0001
Triglycerides, mg/dL	150 (104 – 208)	102 (73 – 148)	<0.0001
Non-HDL-C	151.5 ± 39.4	144.3 ± 34.8)	<0.0001
Standard Lipid Ratios			
Total Cholesterol/HDL-C	4.7 ± 1.5	4.1 ± 1.2	<0.001
Triglycerides/HDL-C	4.7 ± 6.0	2.7 ± 2.1	<0.011

* p-values adjusted for age, gender, and race/ethnicity. Derived from multivariable robust linear regression model (values log transformed where appropriate).

Data are presented a mean +/− SD and as median (interquartile range)

Table 3

Association of standard lipid measures and ratios with L/S ratio as a continuous variable (representative of NAFLD severity).

Beta coefficient of change per 1 standard deviation decrease in L/S ratio (95% confidence interval)*			
Variable	**Model 1**	**Model 2**	**Model 3**
Standard Lipid Measures			
Total Cholesterol, mg/dL	0.57 (−0.60 – 1.73)	0.95 (−0.20 – 2.10)	0.84 (−0.40 – 2.08)
LDL-C, mg/dL	−0.31 (−1.36 – 0.74)	−0.30 (−1.35 – 0.76)	−0.54 (−1.68 – 0.59)
HDL-C, mg/dL	**−2.73 (−3.21 – −2.26)**	**−2.41 (−2.82 – −2.01)**	**−0.78 (−1.20 – −0.37)**
† Triglycerides, mg/dL	**0.15 (0.13 – 0.17)**	**0.15 (0.13 – 0.17)**	**0.07 (0.05 – 0.09)**
Non-HDL-C	**3.05 (1.89 – 4.21)**	**3.00 (1.83 – 4.17)**	**1.37 (0.13 – 2.62)**
Standard Lipid Ratios			
Total Cholesterol/HDL-C	**0.24 (0.20 – 0.28)**	**0.21 (0.17 – 0.25)**	**0.08 (0.04 – 0.12)**
† Triglycerides/HDL-C	**0.21 (0.18 – 0.23)**	**0.20 (0.17 – 0.22)**	**0.09 (0.06 – 0.11)**

Model 1: Unadjusted

Model 2: Age, gender, race/ethnicity adjusted

Model 3: Age, gender, race/ethnicity, BMI, waist circumference, hypertension, HTN meds, smoking, log CRP, diabetes status, log HOMA-IR, oral hypoglycemic use, insulin use adjusted

BOLD indicates a statistically significant result, ($p<0.05$)

* Note: cannot directly compare coefficient across different lipid measures, only between models

† log transformed

Table 4

Lipoprotein particle concentration, size, and ratios across categories of NAFLD severity.

Variable	No NAFLD L/S Ratio ≥1 (n=2793)	Mild NAFLD L/S Ratio 1.0 – 0.7 (n=432)	Moderate NAFLD L/S Ratio 0.7 – 0.5 (n=291)	Severe NAFLD L/S Ratio <0.5 (n=64)	Adjusted p-value*
Particle Concentration (nmol/L)					
Large VLDL &	3.1 ± 4.5	6.9 ± 7.8	7.2 ± 7.9	9.6 ± 6.6	<0.001
Chylomicrons					
VLDL	72.5 ± 39	82.7 ± 45	80.5 ± 35	90.1 ± 37	<0.001
IDL	18.4 ± 23	28.0 ± 30	34.6 ± 34	40.1 ± 25	<0.001
LDL	1307 ± 368	1418 ± 393	1476 ± 390	1505 ± 390	<0.001
HDL	30.8 ± 5.7	30.5 ± 6.1	30.0 ± 5.2	30.2 ± 5.6	0.31
Particle Size (nm)					
VLDL	49.8 ± 8.4	55.3 ± 9.2	56.1 ± 10	59.2 ± 10	<0.001
LDL	20.9 ± 0.77	20.5 ± 0.74	20.4 ± 0.69	20.4 ± 0.79	<0.001
HDL	9.19 ± 0.42	8.98 ± 0.36	8.95 ± 0.29	8.97 ± 0.39	<0.001
Particle Ratios					
LDL small/large ratio	4.3 ± 15	9.8 ± 47	8.5 ± 15	11.2 ± 17	<0.001
HDL small/large ratio	5.6 ± 9.9	9.2 ± 17	8.4 ± 9.1	11.0 ± 14	<0.001

* p-values adjusted for age, gender, and race/ethnicity. Derived from multivariable robust linear regression model (values log transformed where appropriate).

Table 5

Association of particle concentration, size, and ratios with L/S ratio as a continuous variable (representative of NAFLD severity).

Beta coefficient of change per 1 standard deviation decrease in L/S ratio (95% confidence interval)*			
Variable	**Model 1**	**Model 2**	**Model 3**
Particle Concentration (nmol/L)			
Large VLDL & Chylomicrons	**0.83 (0.75 – 0.92)**	**0.83 (0.75 – 0.92)**	**0.54 (0.45 – 0.63)**
VLDL	**4.49 (3.22 – 5.77)**	**4.15 (2.88 – 5.42)**	1.22 (−0.12 – 2.55)
IDL	**4.19 (3.53 – 4.85)**	**3.91 (3.25 – 4.58)**	**1.86 (1.17 – 2.54)**
LDL	**55.0 (42.6 – 67.4)**	**49.9 (37.5 – 62.3)**	**22.4 (9.4 – 35.4)**
HDL	−0.15 (−0.34 – 0.04)	0.01 (−0.16 – 0.19)	0.12 (−0.06 – 0.31)
Particle Size (nm)			
VLDL	**2.20 (1.95 – 2.45)**	**2.19 (1.96 – 2.43)**	**1.46 (1.21 – 1.70)**
LDL	**−0.19 (−0.21 – −0.16)**	**−0.16 (−0.19 – −0.14)**	**−0.05 (−0.08 – −0.03)**
HDL	**−0.09 (−0.11 – −0.08)**	**−0.08 (−0.09 – −0.07)**	**−0.02 (−0.03 – −0.00)**
Particle Ratios			
LDL small/large ratio	**0.30 (0.24 – 0.37)**	**0.25 (0.19 – 0.31)**	*0.06 (0.00 – 0.13)*
HDL small/large ratio	**0.47 (0.39 – 0.56)**	**0.43 (0.35 – 0.52)**	**0.12 (0.04 – 0.20)**

Model 1: Unadjusted

Model 2: Age, gender, race/ethnicity adjusted

Model 3: Age, gender, race/ethnicity, BMI, waist circumference, hypertension, HTN meds, smoking, log CRP, diabetes status, log HOMA-IR, oral hypoglycemic use, insulin use adjusted

BOLD indicates a statistically significant result, ($p<0.05$)

Italics indicates $p=0.06$

* Note: cannot directly compare coefficient across different lipid measures, only between models

Table 6

Prevalence of arthrogenic dyslipidemia across categories of NAFLD severity.

Prevalence Odds Ratio (CI) for association with atherogenic dyslipidemia*	**Mild NAFLD L/S ratio 1.0 – 0.7**	**Moderate NAFLD L/S ratio 0.7-0.5**	**Severe NAFLD L/S ratio <0.5**
Low HDL & High Triglycerides (HDL<40 mg/dL in men, <50 mg/dL in women, triglycerides ≥150 mg/dL)			
• Model 1	2.91 (2.33 – 3.65)	3.68 (2.39 – 5.68)	6.70 (3.71 – 12.1)
• Model 2	2.86 (2.28 – 5.62)	3.64 (2.35 – 5.62)	6.74 (3.73 – 12.2)
• Model 3	1.62 (1.25 – 2.10)	1.87 (1.15 – 3.03)	3.17 (1.63 – 6.15)
Triglyceride/HDL-C ratio ≥3			
• Model 1	3.17 (2.57 – 3.90)	4.07 (2.63 – 6.29)	6.56 (3.38 – 12.7)
• Model 2	3.17 (2.56 – 3.92)	4.23 (2.70 – 6.65)	7.44 (3.80 – 14.5)
• Model 3	1.87 (1.48 – 2.37)	2.28 (1.39 – 3.73)	3.08 (1.56 – 6.10)

*Reference group is patients with no NAFLD.

Model 1: Unadjusted

Model 2: Age, gender, race/ethnicity adjusted

Model 3: Age, gender, race/ethnicity, BMI, waist circumference, hypertension, HTN meds, smoking, log CRP, diabetes status, log HOMA-IR, oral hypoglycemic use, insulin use adjusted