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Relationship between resolution of non-alcoholic steatohepatitis and changes in lipoprotein sub-fractions: A post-hoc analysis of the PIVENS trial

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Summary

Background: Dyslipidemia is frequent in nonalcoholic steatohepatitis (NASH); however, it is unclear if improvement in liver histology is associated with favorable changes in cardiovascular disease (CVD) risk.

Aims: We evaluated the relationship of NASH resolution and lipoprotein subfraction levels, markers of endothelial dysfunction, and macrophage activation.

Methods: 117 individuals with NASH who participated in the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with NASH (PIVENS) trial with paired liver biopsies and serum samples available at baseline and after 96 weeks of treatment were

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Author Contributions

Kathleen Corey: Guarantor of article, study concept and design; interpretation of data; drafting of the manuscript; statistical analysis; obtained funding

Laura Wilson: study concept and design; statistical analysis; critical revision of manuscript for important intellectual content

Akif Altinbas: acquisition of data, critical revision of the manuscript for important intellectual content

Katherine Yates: statistical analysis; critical revision of manuscript for important intellectual content

David E. Kleiner: acquisition of data, critical revision of manuscript for important intellectual content

Raymond Chung: acquisition of data, critical revision of manuscript for important intellectual content

Ronald M. Krauss: acquisition of data, critical revision of manuscript for important intellectual content

Naga Chalasani: study concept and design; analysis and interpretation of data; drafting of the manuscript; statistical analysis.

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included. Participants in the PIVENS trials received vitamin E, pioglitazone or placebo for 96 weeks. Lipoprotein subfraction levels, intracellular adhesion molecule 1 (ICAM-1), vascular cellular adhesion molecule 1 (VCAM-1), E-selectin, and sCD163 levels were assessed at baseline and week 96 and their relationship with NASH resolution was examined.

Results: Fifty-seven individuals had NASH resolution and 60 individuals did not have resolution of NASH. NASH resolution was associated with favorable changes in lipoprotein subfraction levels compared to those without NASH resolution. Individuals with resolution of NASH had a significantly increased mean peak LDL diameter (ratio of geometric means [96 weeks vs. baseline] 1.007 vs. 0.996, P=0.004), and higher frequency of LDL phenotype A (58% vs. 33%, P=0.003) at week 96, after adjustment for relevant co-variates including treatment group. No differences in VCAM, ICAM, E-selectin, or sCD163 levels by NASH resolution were found.

Conclusions: NASH resolution is associated with favorable changes in a subset serum lipoprotein levels. More studies are warranted to understand if these favorable changes are associated with decreased risk of CVD. (NCT00063622)

Keywords

Non-alcoholic fatty liver disease (NAFLD); non-alcoholic steatohepatitis (NASH); low-density lipoprotein (LDL); cardiovascular disease (CVD); small dense low-density lipoprotein level; lipoprotein subfraction

Introduction

Nonalcoholic steatohepatitis (NASH) is thought to be a risk factor for cardiovascular disease (CVD), independent of traditional CVD risk factors. [1–4] Additionally, dyslipidemia, another important CVD risk factor, is frequent in both adults and children with NASH [5,6]. Resolution of NASH is associated with increased high-density lipoprotein (HDL-C) levels and decreased triglyceride levels, but is not associated with improvements in several, stronger lipid predictors of CVD, including low-density lipoprotein (LDL-C), total cholesterol, or non-high-density lipoprotein cholesterol (non-HDL-C) levels[5].

While standard lipid profiles provide important information about CVD risk, they do not offer a comprehensive picture of all circulating lipoproteins. Evaluation of lipoprotein sub-fractions allows for the quantification of lipoprotein particle size and concentration, and provides the ability to measure very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), allowing for a more comprehensive assessment of lipid-related CVD risk[7–11].

Cross-sectional studies have identified differences in lipoprotein subfraction levels among patients with steatosis and NASH when compared to controls. Steatosis and NASH are characterized by smaller LDL, IDL, and HDL particle sizes, larger VLDL particle size, increased concentrations of small LDL III and LDL IV subclasses, and decreased levels of larger LDL I concentration, features which are associated with increased CVD risk[12–18]. While these lipoprotein subfraction profiles characterize NASH in cross-sectional studies, the relationship between NASH resolution and changes in lipoprotein subfractions has not been systematically evaluated.

In addition to lipids, endothelial dysfunction and monocyte activation may contribute to CVD risk[19]. Intracellular adhesion molecule 1 (ICAM-1), vascular cellular adhesion molecule 1 (VCAM-1), and E-selectin are cell adhesion molecules present on vascular endothelial cells, lymphocytes, and macrophages whose activation promotes binding of inflammatory cells and tissue migration[20]. These markers are increased in individuals with nonalcoholic fatty liver disease (NAFLD), associated with steatosis grade and NAFLD activity score (NAS), and may contribute to increased prevalence of CVD among individuals with NAFLD[21,22]. Macrophages and macrophage activation also play a role in atherosclerotic disease, particularly in the formation of coronary arterial plaques. Levels of sCD163, a marker of monocyte activation that is associated with coronary atherosclerosis, are increased in NASH[23,24]. However, the relationship between NASH resolution and these CVD markers is unknown. The aim of the present study was to assess the relationship of NASH resolution with changes over 96-weeks in serum lipoprotein subfraction particle size and concentration, markers of endothelial dysfunction (ICAM-1, VCAM-1, and E-selectin), and macrophage activation (sCD163).

Materials and Methods

Study Design and Population

The data for the present study were derived from 117 patients sampled from the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with NASH (PIVENS) Trial (NCT00063622), a randomized, controlled trial that compared the efficacy of 30 mg pioglitazone daily, 800 units vitamin E daily, or placebo for 96 weeks in biopsy-proven NASH in individuals without diabetes.[25] In the PIVENS trial, 222 participants underwent liver biopsy at baseline and 96 weeks. Of these participants, 117 individuals had serum available at both timepoints. Definite or possible NASH were defined using the NASH Clinical Research Network scoring system.[26] Definite or borderline NAS were defined by an NAS ≥ 5 with a finding of possible or definite NASH by pathologist or an NAS = 4 with a finding of definite NASH on review by local pathology and central review. All NASH diagnoses required a score of at least 1 for hepatocyte ballooning. NASH resolution was defined as the absence of steatohepatitis at 96 weeks among patients who had NASH at baseline. Compared to the placebo group (21%), NASH resolution occurred more frequently in patients treated with pioglitazone (47%, P=0.001) and a trend toward increased NASH resolution with vitamin E (36%, P=0.05, P value for significance <0.025). Histologic improvement was assessed by a reduction in NAS. NAS is a composite score for evaluating NASH in clinical trials and includes steatosis (0–2), lobular inflammation (0–3) and hepatocyte ballooning (0–3).[26] Reduction in NAS occurred more frequently in the vitamin E group compared to placebo (43% vs. 19%, P=0.001), while the difference between pioglitazone and placebo groups did not reach the pre-specified P-value (34% vs. 19%, P=0.04. The sampling design of the post hoc analysis ensured equal numbers of patients with and without resolution of NASH.

Laboratory Methods

Blood samples from the PIVENS trial were collected after a 12 hour fast and, after processing, immediately stored at −70C prior to batch shipping on dry ice to the NIDDK

Biosample Repository where they were stored at −80C. Ion mobility analysis (IM) was used to directly quantify the full spectrum of lipoprotein particles in baseline and week 96 serum samples, from the smallest, densest HDL particles to large buoyant VLDL particles, as described previously[27]. LDL phenotype A was defined as an LDL peak particle diameter ≥ 218.8 Å while LDL phenotype B was defined as an LDL peak particle diameter below ≤ 215.5 Å, with intermediate LDL phenotype I defined as an LDL peak particle diameter between these values. Serum sCD163, ICAM-1, VCAM-1, and E-selectin levels were analyzed in duplicate using the Quantikine© enzyme-linked immunosorbent assay system (R&D Systems, Minneapolis).

Statistical Analysis

We determined that a sample size of 116 individuals (58 per group) would be sufficient using the following assumptions: (1) type I error=0.05, (2) power=0.80, (3) equal size groups of resolved and not resolved NASH, (4) minimum clinically important difference (MCID) in concentrations were 12 mmol/L in LDL-IVb (37 ± SD=16 mmol/L in those with NASH resolution vs. 49 ± SD=28 mmol/L in those without NASH resolution), (5) method of sample size calculation: 2-sample t-test, (6) comparison groups: those with NASH resolution vs. those without, and (7) the above assumptions regarding MCID and standard deviations (SDs) were supported by data from prior work[16].

Baseline characteristics between groups were compared using two-sample t-tests for continuous measurements, and Fisher's exact tests for categorical measurements (Table 1). Lipoprotein subfractions were log transformed, due to non-normality, and geometric means (95% confidence intervals) at baseline and after 96 weeks are presented for those with and without NASH resolution. The ratio of lipoprotein subfraction geometric means at 96-weeks vs. baseline (GM_{96}/GM_{BL}) are also presented for those with and without NASH resolution. Adjusted relative geometric mean ratios and P-values were derived from robust multiple linear lognormal regression, controlling for treatment group, age at biopsy, gender, ethnicity, baseline body mass index (BMI), Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), baseline triglyceride level, use of statin and non-statin lipid lowering medications at baseline and/or during follow-up and for ratio measures, the baseline value of the lipoprotein subfraction. Adjusted odds ratios and P-values for LDL phenotype were determined from multiple logistic regression, controlling for the same set of covariates. Due to the large number of multiple comparisons among the lipoprotein subfraction measures, the Benjamini-Hochberg false discovery rate adjustment for P-value thresholds for statistical significance were as follows: $P<0.044$ for analysis of lipoprotein subfractions geometric means, geometric mean ratios (Resolved vs. not resolved) and resolution of NASH at baseline and 96-weeks, and $P<0.007$ for lipoprotein subfraction geometric mean ratios (GM_{96}/GM_{BL}) were considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC) and Stata release 14 (StataCorp, College Station, TX).

Results

Baseline Characteristics

A total of 117 patients with NASH at baseline were included (Table 1). Of these, 57 individuals had resolution of NASH whereas 60 did not have NASH resolution. There were no significant differences in age, gender, race, ethnicity, serum biochemistries, use of lipid-lowering medications (statin or other), or baseline liver histology between patients with and without NASH resolution. However, we found that those with NASH resolution had lower HOMA-IR levels at baseline (4.0 ± 2.8 vs. 6.5 ± 6.4, P=0.006). Individuals with resolution of NASH were more likely to have received pioglitazone (73% vs. 34%, P=0.002) or vitamin E (73% vs. 47%, P=0.02).

Standard Serum Lipid Levels and Resolution of NASH

There was no difference by resolution of NASH in mean serum total cholesterol, LDL-C or non-HDL-C levels at baseline or week 96 (Supplemental Table 1). At week 96, individuals with NASH resolution had significantly lower serum triglyceride levels (136 mg/dL ± 72 vs. 172 mg/dL ± 102, P=0.03) and higher serum HDL-C levels (48 ± 17 mg/dL vs. 39 ± 10, P=<0.001) compared to those without NASH resolution.

Baseline Lipoprotein Levels and Resolution of NASH

Individuals who experienced NASH resolution had a more favorable baseline serum lipid profile than those without NASH resolution (Table 2). Individuals with NASH resolution had significantly lower serum LDL IVa, IIIb, and IIIa, small dense LDL subclasses, compared to individuals without NASH resolution. Comparing those with NASH resolution to those without NASH resolution, the baseline LDL IVa geometric mean (GM) was 59.2 nmol/L vs. 69.7 nmol/L, LDL IIIb was 59.7 nmol/L vs. 75.3 nmol/L, and LDL IIIa was 157.1 nmol/L vs. 201.2 nmol/L. The adjusted ratio of geometric means (resolved vs. not resolved) were 0.80 for LDL IVa (P=0.01), 0.74 for LDL IIIb (P=0.007), and 0.73 for LDL IIIa (P=0.003), indicating that the baseline LDL IVa was 20% lower in those with NASH resolution compared to those without NASH resolution, the baseline LDL IIIb was 26% lower, and the baseline LDL IIIa was 27% lower, controlling for relevant covariates. Similarly, individuals with NASH had significantly lower serum medium VLDL (GM 51.3 vs. 58.1 nmol/L, P=0.03) and large VLDL levels (GM 16.5 vs. 20.1 nmol/L, P=0.003) compared to individuals without NASH resolution, after adjusting for covariates.

Resolution of NASH and Lipoprotein Levels at 96 Weeks

Individuals with resolution of NASH had favorable changes in serum LDL particle characteristics after 96 weeks. LDL diameter increased significantly in the NASH resolution group when compared to those without NASH resolution (ratio of geometric means [96 weeks vs. baseline] 1.007 vs. 0.996, P=0.004) and at 96 weeks those with NASH resolution had significantly higher mean peak LDL particle diameter than those without NASH resolution (GM 220.0 Å vs. 216.2 Å, P<0.001) (Table 2). By 96 weeks, NASH resolution was associated with higher frequency of the favorable serum LDL phenotype A (58% vs. 33%, P=0.003).

In addition, at week 96, serum medium VLDL and large VLDL levels were significantly lower in those with NASH resolution compared to those without NASH resolution: **medium VLDL:** 52.1 nmol/L vs. 64.7 nmol/L, P=0.01; **large VLDL:** 16.3 nmol/L vs. 21.8 nmol/L, P=0.004. Large and medium VLDL levels decreased from baseline in those with NASH resolution while they increased in those without NASH resolution, but this did not meet statistical significance (Large VLDL: adjusted ratio of geometric means [96 weeks vs. baseline] 0.99 vs. 1.08, P=0.23).

At week 96, patients without NASH resolution had more unfavorable serum lipoprotein subfraction levels, including increased LDL IVa, LDL IIIb and LDL IIIa, and compared to those with NASH resolution (Table 2) after adjusting for co-variates: **LDL IVa:** GM 76.7 nmol/L vs. 60.4 nmol/L, P=0.004. **LDL IIIb**: GM 81.0 nmol/L vs. 56.4 nmol/L, P=0.002; **LDL IIIa**: GM 210.3 nmol/L vs. 145.2 nmol/L, P=0.003; However, while these levels differed between NASH resolution status at 96 weeks, the change in serum levels of LDL IVa, LDL IIIb, and LDL IIIa from baseline to 96 weeks was not significantly different by NASH resolution status. (Table 2)

Resolution of NASH and Lipoprotein Levels at 96 Weeks by Treatment Group

The relationship between NASH resolution and changes in serum lipoprotein subfraction levels at 96 weeks were assessed by treatment group (Supplemental Table 2). In the pioglitazone group, the absence of NASH resolution was associated with significantly increased small VLDL levels from baseline compared to those with NASH resolution (ratio of geometric means [96 weeks vs. baseline]: 1.23 vs. 1.04, P=0.04. In the vitamin E group, those without NASH resolution had a larger decrease in LDL peak mean compared to those with NASH resolution (ratio of geometric means [96 weeks vs. baseline]: 0.99 vs. 1.00, P=0.03). Among those receiving placebo, the LDL phenotype A was more common at 96 weeks among those with NASH resolution compared to those without NASH resolution (89% vs. 36%, P=0.02) although the change in LDL peak mean from baseline in this group was not statistically significant by NASH resolution status (P=0.13).

Markers of Endothelial Dysfunction, sCD163 and NASH Histology

At baseline and 96 weeks, individuals with NASH resolution had lower geometric mean baseline serum E-selectin and lower serum sCD163, after adjusting for co-variates, but did not reach statistical significance after adjusting for multiple comparisons. No differences were seen in serum ICAM or VCAM levels by NASH resolution status either at baseline or 96 weeks (Data not shown).

Discussion

Main Findings.

The present study demonstrates that, despite the lack of change in the standard LDL-C level, resolution of NASH is associated with a favorable change in LDL size with an increase in mean peak LDL particle diameter and a predominance of LDL phenotype A. Further, at 96 weeks, patients without NASH resolution had persistently unfavorable lipoprotein subfraction levels, including increased levels of small dense LDL (LDL IVa, LDL IIIb, LDL

IIIa) and large and medium VLDL levels, compared to those with NASH resolution. Results remained significant after adjustment for important co-variates including treatment group and HOMA-IR. These findings suggest that NASH resolution is associated with improvements in lipid-related CVD risk, while persistent NASH may be associated with persistently elevated CVD risk. In addition, these findings suggest that standard LDL-C measurements may be insufficient to determine CVD risk in individuals with NASH, while lipoprotein subfraction level measurements, specifically mean peak LDL diameter and LDL phenotype, may provide more insight into CVD risk. The impact of NASH resolution by treatment group was difficult to assess due to small numbers in each treatment group. But lack of significant interaction between treatment group, the relationship between NASH resolution and changes in lipoprotein characteristics suggests NASH resolution may be associated with favorable alterations in certain lipoproteins independent of vitamin E or pioglitazone.

Context of Published Literature

Dyslipidemia characterized by increased levels of LDL-C, total cholesterol, triglycerides, and non-HDL-C, as well as decreased HDL-C is frequent in NASH patients[5,16,28]. When assessing lipids using a standard lipid profile, resolution of NASH is associated with improvements in only HDL-C and triglyceride levels. Non-HDL-C, LDL-C, and total cholesterol do not differ between patients by NASH resolution, and the persistence of elevated LDL may contribute to enduring CVD risk.

Of note, the standard lipid panel, which measures LDL-C, HDL-C, total cholesterol, and triglyceride levels, does not capture circulating levels of lipid particle subfractions, which provide additional information about CVD risk. Lipoprotein particle levels, particularly LDL particle number and small dense LDL concentration are strong predictors of future CVD risk in a variety of populations[7–11]. Further, among individuals *without* elevated LDL-C levels, LDL subfraction particle levels are predictive of CVD events. A post-hoc analysis of the JUPITER trial found that while baseline standard LDL-C (median 109 mg/dL) was not associated with future CVD events, total LDL particle number, LDL IIb, LDL IIIa, LDL IIIb, LDL IVa, LDL IVb, and LDL IVc were associated with incident CVD events[29]. This study demonstrating the value of lipoprotein sub-fractions as predictors of CVD in those without elevated LDL-C levels are particularly relevant to patients with NAFLD, in whom LDL-C is not commonly elevated.

In our previous work, we evaluated lipid levels in 247 participants in the PIVENS trial and found that the mean LDL-C level was only 121.5 mg/dL, with the majority of participants (57.9%) having LDL-C <130 mg/dL[5]. Thus, lipoprotein subfraction particle levels provide additional information about an individual's burden of CVD risk and may be particularly relevant in a NASH population where the high frequency of normal LDL-C levels may lead to an underestimation of CVD risk. Our findings of improved mean peak LDL particle diameter and increased LDL phenotype A, suggest that NASH resolution is associated with reduced lipoprotein-related CVD risk, despite a lack of improvement in standard LDL-C level. Further, persistently elevated atherogenic LDL IIIb, LDL IIIa, LDL IVa, and large and

medium VLDL levels found in patients without NASH resolution suggests that lipoprotein-related CVD risk may persist over time.

The present study adds to the previous literature related to lipoprotein subfraction profiles in individuals with NAFLD. Several groups have conducted cross-sectional studies to compare lipoprotein subfraction profiles between individuals with NASH and steatosis. Among individuals with steatosis, those with NASH generally have higher levels of small, more atherogenic LDL types (LDL III and IV) and decreased larger, more buoyant LDL types[12,16,17]. Several studies have also found that NASH is characterized by high concentrations of large VLDL particles [18]. Mannisto et al. found that among individuals undergoing obesity surgery, total concentrations of VLDL and LDL subclasses were directly associated with NASH, and these concentrations decreased following surgery [30]. In the present study, NASH was characterized by high concentrations of small LDL and large VLDL subtractions, as well as low mean peak LDL diameter. These works together suggest that the atherogenic dyslipidemia of NASH is characterized by decreased mean peak LDL particle diameter and increased concentrations of small LDL (types III and IV) and large VLDL particles.

Strengths and Limitations

The strengths of the present study include its use of data from a prospective study that included a well-phenotyped population with biopsy-proven NASH. Participants underwent a follow-up biopsy at 96 weeks, allowing for assessment of changes in histology. In addition, lipoprotein subfraction profiles were performed using IM, a well-validated method that determines lipoprotein number across the spectrum of lipoproteins and is not impacted by the lipid composition of a lipoprotein [31]. Previous work has also demonstrated the lipoproteins from stored blood samples were stable for up to 5 freeze/thaw cycles when stored an −20ºC without loss of integrity after more than 10 years of storage.[27,29,31]

This study also has limitations. It is notable that participants who experienced NASH resolution had lower baseline HOMA-IR and more favorable baseline levels in a subset of lipoproteins than those without NASH resolution which may influence 96 week lipoprotein values. However, our findings remained significant after adjustment for these co-variates and at 96 weeks individuals without NASH resolution had a more significant decrease in HOMA-IR than those with NASH resolution, arguing against HOMA-IR playing a key role in our findings (Supplementary Table 3). However, further studies with matched HOMA-IR and baseline lipoprotein levels would be of value. While we evaluated the association of NASH resolution and markers of CVD risk, we did not assess the relationship between resolution of NASH and CVD outcomes. Thus, while several validated markers of CVD risk did improve, we were not able to determine the impact of histological changes on hard CVD outcomes. Additional prospective studies are needed to assess this relationship and to determine what aspects of histological improvement (i.e. ballooning, inflammation, steatosis, or fibrosis) most impact CVD risk. In addition, patients in the PIVENS trial did not have diabetes, and lipoprotein subfraction profiles in individuals with NASH and diabetes may be different from what was observed here and may also vary among patients with diabetes. Finally, our ability to evaluate the impact of individual treatments among

those with NASH resolution on lipoprotein subfraction levels was limited by small group numbers and, thus, no consistent trend found (Supplemental Table 2). Further studies are needed to better delineate the interaction between treatment type, NASH resolution and lipoprotein subfraction levels.

Impact of Clinical Practice and Future Directions

This study demonstrates that despite the lack of change in standard LDL-C levels, NASH resolution is associated with improvements in mean peak LDL diameter and LDL phenotype, which are associated with lower CVD risk. Furthermore, persistent NASH is associated with elevated levels of lipoproteins associated with higher CVD risk, including elevated LDL IVa, IIIb, LDL IIIa, and large and medium VLDL. Thus, we conclude that resolution of NASH is associated with favorable changes in a subset of serum lipoprotein profiles. More studies are warranted to understand if these favorable lipoprotein changes are associated with change in cardiovascular health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Conflict of Interest/Disclosures: Dr. Chalasani has ongoing consulting activities and research grant from several pharmaceutical companies, but none represent a potential conflict of interest for this paper. Dr. Krauss holds a licensed patent for ion mobility analysis. He has received grant support and honoraria from Quest Diagnostics. Dr. Corey has ongoing consulting activities with Novo Nordisk.

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Abbreviations:

NAFLD	Non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
LDL	low-density lipoprotein
HDL	high-density lipoprotein
CVD	cardiovascular disease
VLDL	very low-density lipoprotein
IDL	intermediate density lipoprotein

References

1. Mantovani A, Mingolla L, Rigolon R, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular disease in adult patients with type 1 diabetes. Int J Cardiol 2016; 225: 387–91. [PubMed: 27768965]

2. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. J Hepatol 2016; 65(3): 589–600. [PubMed: 27212244]

3. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005; 129(1): 113–21. [PubMed: 16012941]

4. Puchner SB, Liu T, Mayrhofer T, et al. High-risk plaque detected on coronary CT angiography predicts acute coronary syndromes independent of significant stenosis in acute chest pain: results from the ROMICAT-II trial. J Am Coll Cardiol 2014; 64(7): 684–92. [PubMed: 25125300]

5. Corey KE, Vuppalanchi R, Wilson LA, Cummings OW, Chalasani N, Nash CRN. NASH resolution is associated with improvements in HDL and triglyceride levels but not improvement in LDL or non-HDL-C levels. Aliment Pharmacol Ther 2015; 41(3): 301–9. [PubMed: 25429853]

6. Corey KE, Vuppalanchi R, Vos M, et al. Improvement in liver histology is associated with reduction in dyslipidemia in children with nonalcoholic fatty liver disease. J Pediatr Gastroenterol Nutr 2015; 60(3): 360–7. [PubMed: 25714579]

7. Cromwell WC, Otvos JD, Keyes MJ, et al. LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study - Implications for LDL Management. J Clin Lipidol 2007; 1(6): 583–92. [PubMed: 19657464]

8. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002; 106(15): 1930–7. [PubMed: 12370215]

9. Kuller L, Arnold A, Tracy R, et al. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the cardiovascular health study. Arterioscler Thromb Vasc Biol 2002; 22(7): 1175–80. [PubMed: 12117734]

10. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988; 260(13): 1917–21. [PubMed: 3418853]

11. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 1996; 276(11): 875–81. [PubMed: 8782636]

12. Siddiqui MS, Fuchs M, Idowu MO, et al. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. Clin Gastroenterol Hepatol 2015; 13(5): 1000–8 e3. [PubMed: 25311381]

13. 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. [PubMed: 16873790]

14. Adiels M, Taskinen MR, Packard C, et al. Overproduction of large VLDL particles is driven by increased liver fat content in man. Diabetologia 2006; 49(4): 755–65. [PubMed: 16463046]

15. DeFilippis AP, Blaha MJ, Martin SS, et al. Nonalcoholic fatty liver disease and serum lipoproteins: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2013; 227(2): 429–36. [PubMed: 23419204]

16. Corey KE, Misdraji J, Gelrud L, Zheng H, Chung RT, Krauss RM. Nonalcoholic steatohepatitis is associated with an atherogenic lipoprotein subfraction profile. Lipids Health Dis 2014; 13: 100. [PubMed: 24952382]

17. Sonmez A, Nikolic D, Dogru T, et al. Low- and high-density lipoprotein subclasses in subjects with nonalcoholic fatty liver disease. J Clin Lipidol 2015; 9(4): 576–82. [PubMed: 26228676]

18. Jiang ZG, Tapper EB, Connelly MA, et al. Steatohepatitis and liver fibrosis are predicted by the characteristics of very low density lipoprotein in nonalcoholic fatty liver disease. Liver Int 2016; 36(8): 1213–20. [PubMed: 26815314]

19. Hwang SJ, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997; 96(12): 4219–25. [PubMed: 9416885]

20. Davies MJ, Gordon JL, Gearing AJ, et al. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. J Pathol 1993; 171(3): 223–9. [PubMed: 7506307]
21. Thakur ML, Sharma S, Kumar A, et al. Nonalcoholic fatty liver disease is associated with subclinical atherosclerosis independent of obesity and metabolic syndrome in Asian Indians. Atherosclerosis 2012; 223(2): 507–11. [PubMed: 22748277]
22. Sookoian S, Castano GO, Burgueno AL, et al. Circulating levels and hepatic expression of molecular mediators of atherosclerosis in nonalcoholic fatty liver disease. Atherosclerosis 2010; 209(2): 585–91. [PubMed: 19896127]
23. Aristoteli LP, Moller HJ, Bailey B, Moestrup SK, Kritharides L. The monocytic lineage specific soluble CD163 is a plasma marker of coronary atherosclerosis. Atherosclerosis 2006; 184(2): 342–7. [PubMed: 15979079]
24. Mueller JL, Feeney ER, Zheng H, et al. Circulating Soluble CD163 is Associated with Steatohepatitis and Advanced Fibrosis in Nonalcoholic Fatty Liver Disease. Clin Transl Gastroenterol 2015; 6: e114. [PubMed: 26448455]
25. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010; 362(18): 1675–85. [PubMed: 20427778]
26. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41(6): 1313–21. [PubMed: 15915461]
27. Caulfield MP, Li S, Lee G, et al. Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. Clin Chem 2008; 54(8): 1307–16. [PubMed: 18515257]
28. Corey KE, Lai M, Gelrud LG, et al. Non-high-density lipoprotein cholesterol as a biomarker for nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2012; 10(6): 651–6. [PubMed: 22330232]
29. Mora S, Caulfield MP, Wohlgemuth J, et al. Atherogenic Lipoprotein Subfractions Determined by Ion Mobility and First Cardiovascular Events After Random Allocation to High-Intensity Statin or Placebo: The Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) Trial. Circulation 2015; 132(23): 2220–9. [PubMed: 26408274]
30. Mannisto VT, Simonen M, Soininen P, et al. Lipoprotein subclass metabolism in nonalcoholic steatohepatitis. J Lipid Res 2014; 55(12): 2676–84. [PubMed: 25344588]
31. Musunuru K, Orho-Melander M, Caulfield MP, et al. Ion mobility analysis of lipoprotein subfractions identifies three independent axes of cardiovascular risk. Arterioscler Thromb Vasc Biol 2009; 29(11): 1975–80. [PubMed: 19729614]

Table 1.

Baseline characteristics of the study population

	NASH Resolution (n=57)	No NASH Resolution (n=60)	P*
Demographics			
Age (years)	47 ± 12	46 ± 13	0.57
Female sex	32 (56%)	32 (53%)	0.85
Race			0.39
White	48 (86%)	52 (91%)	
Non-white	8 (14%)	5 (9%)	
Ethnicity			0.79
Hispanic	8 (14%)	7 (12%)	
Non-Hispanic	49 (86%)	53 (88%)	
Using lipid lowering medication (statin or other) at enrollment	19 (33%)	18 (30%)	0. 84
Treatment Group			
Pioglitazone	24 (73%)	13 (34%)	0.002
Placebo	9 (27%)	25 (66%)	
Vitamin E	24 (73%)	22 (47%)	0.02
Placebo	9 (27%)	25 (53%)	
Serum biochemistries			
ALT (U/L)	79 ± 39	91 ± 51	0.16
AST (U/L)	55 ± 30	58 ± 29	0.65
Alkaline phosphatase (U/L)	79 ± 43	80 ± 25	0.93
Total bilirubin (mg/dL)	0.8 ± 0.3	0.8 ± 0.4	0.84
Metabolic factors			
HOMA-IR	4.0 ± 2.8	6.5 ± 6.4	0.006
BMI (kg/m^2)	32 ± 6	33 ± 7	0.18
Lipid Panel			
Total cholesterol (mg/dL)	191 ± 44	196 ± 38	0.51
Triglycerides (mg/dL)	165 ± 108	160 ± 79	0.78
HDL-C (mg/dL)	45 ± 12	41 ± 10	0.12
LDL-C (mg/dL)	114 ± 37	125 ± 34	0.12
Non-HDL-C (mg/dL)	146 ± 44	155 ± 35	0.26
Liver histology			
Fibrosis stage	1.4 ± 0.9	1.6 ± 1.0	0.23
NAFLD Activity Score	4.8 ± 1.3	5.0 ± 1.4	0.46

Values are means ± standard deviations or number (%).

*P-values derived from two-sample t-tests for continuous variables and Fisher's exact test for categorical variables.

Table 2.

Lipoprotein subclasses at baseline and 96 weeks in individuals with and without NASH resolution

	Resolution of NASH at 96 weeks		Adjusted*	
Lipoprotein Subclass and Particle Size	**Resolved (N=57)**	**Not resolved (N=60)**	**Geometric Mean Ratio (Resolved vs. Not resolved)**	**P**
More Atherogenic				
Small and Medium LDL				
IVa (nmol/L)				
Geometric Mean (95% CI)				
Baseline	59.2 (51.1, 68.5)	69.7 (60.4, 80.3)	0.80	0.01
96 weeks	60.4 (52.3, 69.7)	76.7 (66.4, 88.6)	0.79	0.004
Ratio of geometric means (GM_{96}/GM_{BL})	1.021 (0.892, 1.168)	1.101 (0.979, 1.237)	0.87	0.16
IIIb (nmol/L)				
Geometric Mean (95% CI)				
Baseline	59.7 (49.2, 72.5)	75.3 (62.5, 90.7)	0.74	0.007
96 weeks	56.4 (46.9, 68.0)	81.0 (68.1, 96.4)	0.70	0.002
Ratio of geometric means (GM_{96}/GM_{BL})	0.945 (0.814, 1.097)	1.076 (0.940, 1.232)	0.83	0.11
IIIa (nmol/L)				
Geometric Mean (95% CI)				
Baseline	157.1 (131.2, 188.1)	201.2 (172.1, 235.2)	0.73	0.003
96 weeks	145.2 (122.5, 172.0)	210.3 (181.1, 244.1)	0.70	0.003
Ratio of geometric means (GM_{96}/GM_{BL})	0.924 (0.803, 1.063)	1.045 (0.933, 1.171)	0.88	0.28
VLDL Subclass				
Small (nmol/L)				
Geometric Mean (95% CI)				
Baseline	54.6 (49.3, 60.3)	56.7 (51.7, 62.3)	0.98	0.72
96 weeks	55.7 (50.0, 62.0)	61.7 (56.5, 67.3)	0.90	0.11
Ratio of geometric means (GM_{96}/GM_{BL})	1.020 (0.923, 1.128)	1.086 (1.006, 1.174)	0.91	0.17
Medium (nmol/L)				
Geometric Mean (95% CI)				
Baseline	51.3 (44.4, 59.3)	58.1 (51.4, 65.6)	0.86	0.03
96 weeks	52.1 (45.3, 60.0)	64.7 (57.8, 72.5)	0.81	0.01
Ratio of geometric means (GM_{96}/GM_{BL})	1.015 (0.885, 1.165)	1.115 (1.008, 1.232)	0.88	0.16
Large (nmol/L)				
Geometric Mean (95% CI)				
Baseline	16.5 (13.6, 20.0)	20.1 (17.1, 23.7)	0.77	0.003
96 weeks	16.3 (13.8, 19.3)	21.8 (18.5, 25.6)	0.75	0.004
Ratio of geometric means (GM_{96}/GM_{BL})	0.989 (0.834, 1.173)	1.081 (0.961, 1.216)	0.88	0.23
Less Atherogenic (Large Peak and Phenotype A)				

Lipoprotein Subclass and Particle Size	Resolution of NASH at 96 weeks		Adjusted*	
	Resolved (N=57)	Not resolved (N=60)	Geometric Mean Ratio (Resolved vs. Not resolved)	P
LDL Size Measurements				
LDL Peak mean (Å)				
Geometric Mean (95% CI)				
Baseline	218.4 (216.7, 220.2)	217.0 (215.4, 218.6)	1.01	0.009
96 weeks	220.0 (218.2, 221.8)	216.2 (214.5, 217.8)	1.02	<0.001
Ratio of geometric means (GM_{96}/GM_{BL})	1.007 (1.000, 1.014)	0.996 (0.992, 1.001)	1.01	0.004
LDL Phenotype			OR	
Baseline	30 (53%)	26 (43%)	1.00	
A (Favorable)	9 (16%)	6 (10%)	0.67	0.04
I (Intermediate)	18 (32%)	28 (47%)	0.22	
B (Atherogenic)				
96 weeks				
A (Favorable)	33 (58%)	20 (33%)	1.00	
I (Intermediate)	8 (14%)	10 (17%)	0.33	0.003
B (Atherogenic)	16 (28%)	30 (50%)	0.12	

*Adjusted for treatment group, age at biopsy (years), gender, ethnicity, baseline BMI, HOMA-IR, baseline triglyceride level (mg/dL), use of statin or non-statin lipid lowering medication at baseline and/or during follow-up, and for ratio measures, the baseline value of the lipoprotein subfraction. Benjamini-Hochberg false discovery rate adjusted threshold for statistical significance at baseline: P<0.044; 96-weeks: P<0.044, and GM_{96}/GM_{BL} ratios: P<0.007.