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Non-High Density Lipoprotein Cholesterol as a Biomarker for Non-Alcoholic Steatohepatitis

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Abstract

Background & Aims—There are no clinically available biomarkers for non-alcoholic steatohepatitis (NASH); differentiating between steatosis and NASH requires histologic evaluation. Non-invasive methods are needed to replace liver biopsy and its associated risks. Production of very-low density lipoprotein (VLDL) contributes to the development of NASH, and might be used to distinguish steatosis from NASH. However, it is not possible to measure levels of VLDL directly, in the clinic. Non-high density lipoprotein cholesterol (non-HDL-C) encompasses all apolipoprotein-B-containing lipoproteins, including VLDL, and can be calculated from standard lipid panels without additional cost.

Methods—We evaluated the ability of non-HDL-C to differentiate steatosis from NASH in a prospective study of 218 patients with suspected NASH (steatosis, n=100 and NASH, n=118).

Results—Patients with NASH had a trend toward increased levels of non-HDL cholesterol, compared to those with steatosis ($P=.08$). However, among subjects not on lipid-lowering medications, those with NASH had significantly higher levels of non-HDL-C (144.6mg/dL), than those with steatosis (129.3mg/dL; $P=.025$). This difference remained significant when adjusted for levels of cholesterol and triglycerides, indicating that the difference results from increased levels of apolipoprotein B, including VLDL. These findings were validated in a cohort of 40 patients with steatosis or NASH who were not taking lipid-lowering agents. The NASH group had a significantly higher levels of non-HDL-C than the steatosis group (162.8mg/dL vs 145.9 mg/dL; $P=.04$).

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Conclusion—NASH is associated with significantly higher levels of non-HDL-C than steatosis in patients who do not take lipid-lowering agents. This low-cost biomarker could be used in non-invasive differentiation between steatosis and NASH.

Keywords

non-HDL cholesterol; non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; diagnostic; blood test; clinical trial

Background

Nonalcoholic fatty liver disease (NAFLD) affects 40% of the United States population and nearly 90% of obese Americans. Until recently NAFLD was felt to be a benign condition with few health consequences. However, accumulating data from community-based cohorts has demonstrated that persons with NAFLD experience increased all-cause and liver-related mortality when compared to unaffected subjects.² It is also increasingly appreciated that rather than a single condition, NAFLD encompasses a spectrum of disease ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and end-stage liver disease. NASH affects 2–5% of the population and 18–20% of obese persons.^{3,4} While steatosis does not carry the risk of progressive liver disease, patients with NASH are at risk of developing end stage liver disease with cirrhosis developing in 20–30% of NASH patients.⁵ Currently, there are no clinically available biomarkers for NASH and the differentiation between steatosis and NASH requires histologic evaluation through liver biopsy. Liver biopsy is an invasive procedure potentially complicated by organ perforation and is not an appropriate NASH screening tool.⁶ With the progressive nature of NASH and its rising prevalence there is a significant need for non-invasive methods to diagnose NASH.

Alterations in lipid metabolism may account for the differential development of non-alcoholic steatohepatitis (NASH) and steatosis. Recent studies have highlighted the role of triglyceride and very low density lipoprotein (VLDL) production in the development of NASH and associated metabolic syndrome.^{7–9} Successful export of triglycerides from the liver requires packaging as VLDL and intermediate density lipoprotein (IDL).¹⁰ VLDL synthesis increases with increased flux of free fatty acids to the liver. Alterations in the export of triglycerides as VLDL and IDL may impact hepatic lipid storage and the development of NAFLD.^{7,11,12} However, measures of VLDL and IDL are costly and not readily available to clinicians.

Non HDL-cholesterol (non-HDL-C) has recently been recognized as an important measure which encompasses all apolipoprotein-B containing lipoproteins, including LDL, VLDL, IDL, chylomicrons and Lp(a).¹³ Non-HDL-C is a calculated value derived by subtracting HDL cholesterol from the total cholesterol (TC) level, both available on traditional lipid panels and requires no additional cost. In addition, because it is derived from TC and HDL levels, which are not impacted by fasting, non-HDL-C does not require fasting for accuracy. Recently, the value of non-HDL cholesterol has been demonstrated in predicting coronary heart disease.¹⁴ Non-HDL-C has been shown to be a superior predictor of incidence cardiovascular events and cardiac death to the traditional marker, LDL.¹⁵ The Adult Treatment Panel III of the National Cholesterol Education Program has added non-HDL-C to its recommended screening algorithm for assessing cardiovascular disease risk.^{16,17}

With the potential role of VLDL and IDL in the differential development of steatosis and NASH we sought to evaluate the value of non-HDL-C as an inexpensive and easily accessible marker of NASH.

Methods

We conducted a prospective study of patients seen at 2 institutions, Massachusetts General Hospital (MGH) in Boston, MA and Bon Secours Medical Center in Richmond, VA with suspected non-alcoholic fatty liver disease (NAFLD) from January 2010 to August 2011. Patients were seen in both the MGH Hepatology Clinic as well as the Bon Secours Weight Center prior to weight loss surgery. Patients with suspected NAFLD and who gave written informed consent were enrolled in the NAFLD disease registry. Patients with cirrhosis, without steatosis on liver biopsy, with excess alcohol use (defined by >1 drink/day in women or 2 drinks/day in men), other causes of liver disease and those without lipids were excluded. Patients in this primary cohort were included regardless of use of lipid lowering therapy. A validation cohort of patients seen at the Beth Israel Deaconess Medical Center, Boston, MA was also enrolled. Patients with NAFLD diagnosed on liver biopsy and with available lipid data were included. Based on our findings in the primary cohort, the validation cohort was limited to patients who were not receiving lipid lowering therapy. Patients in the validation cohort were stratified by non-alcoholic fatty liver disease activity score (NAS). Patients with an NAS score of 4 or less were classified as having steatosis and with an NAS of 5 or above having NASH.

Subjects were assessed by their treating physician for the presence of co-morbid diseases including diabetes mellitus, hypertension, obstructive sleep apnea and dyslipidemia. Diabetes mellitus was defined by a fasting glucose ≥ 110 mg/dL or known diagnosis of diabetes previously. Hypertension was defined by a blood pressure $\geq 135/85$ or undergoing treatment for hypertension.

Subjects underwent fasting blood samples within 6 months of their liver biopsy. Samples were analyzed for liver function tests, insulin level, glucose level, lipid panel including TC, LDL, HDL and triglycerides. Non-HDL-C was calculated by subtracting HDL from TC levels. Low HDL level was defined by an HDL level <50mg/dL in women, and <40mg/dL in men. Hypertriglyceridemia was defined as fasting triglycerides ≥ 150 mg/dL.

Liver biopsies were performed at the discretion of the treating physician or in the case of weight loss surgery in all patients at the time of surgery. Liver biopsies were reviewed in a blinded fashion by experienced hepatopathologists and judged by grade of steatosis, necroinflammation and stage of fibrosis. NASH was defined as a necroinflammatory grade ≥ 1 with >5% macrovesicular steatosis and hepatocyte ballooning. Steatosis was defined by the reviewing pathologist as the presence of greater than 5% steatosis without evidence of hepatocyte ballooning, lobular inflammation or fibrosis. In the validation cohort a NAS was assessed for each subject.

Statistical analysis was performed using SAS software, version V.9.2 (SAS Institute, Cary, NC). Continuous variables were analyzed using a Student's t-Test while categorical variables were analyzed using a Chi square test or Fisher's exact test as appropriate. Multivariate linear regression model was used to analyze correlations among TC, non-HDL-C, HDL and triglycerides. Multivariate logistic regression model was performed to determine the effect of TC and triglycerides on the presence of NAFLD.

This study was approved by the Partners Human Research Committee.

Results

Baseline Characteristics

Three hundred seventy-one patients were enrolled in the NAFLD Disease Registry. Two hundred eighteen patients underwent liver biopsy and received a diagnosis of either steatosis (n=100) or non-alcoholic steatohepatitis (NASH, n=118). Mean age in the steatosis group was 47.1 years and 47.4 years in the NASH group (p=0.85). Patients were predominantly women in both the steatosis and NASH groups (74.6% and 73.0% respectively, p=0.88). Mean BMI was 45.6 in the steatosis group and 44.5 in the NASH group (p=0.36). Caucasians predominated but there were significantly more African Americans in the steatosis group than the NASH group (29.7% vs. 13.0%, respectively, p=0.008).

There was no significant difference between the steatosis and NASH groups in the prevalence of hypertension, obstructive sleep apnea, coronary artery disease or diabetes mellitus. In addition, mean insulin, glucose and ALT levels were not significantly different between the two groups (Table 1).

Subjects in the NASH group had more steatosis (p=0.053) and fibrosis (p<0.0001) than subjects in the steatosis group.

Of the subjects in the steatosis group, 36.4% were on lipid lowering therapy compared to 54.5% of the NASH subjects (p=0.5). The majority of patients on lipid lowering therapy were on HMG CoA reductase inhibitors (HMG CoARI) (n=86, 86.9%) with 8 patients on fish oil (8.1%), 3 on ezetimibe (3%) and one each on niacin (1%) and fenofibrate (1%).

Lipid Levels in Entire Cohort

Basic lipid panels were performed in all subjects.(Table 2) Mean TC levels were 170.2 mg/dl in the steatosis group and 178.9mg/dL in the NASH group (p=0.115). Mean HDL levels were 43.2 mg/dL in both the steatosis and NASH groups (p=0.97). Mean LDL levels were 99.2 mg/dL in the steatosis group and 103.9mg/dL in the NASH group (p=0.30). Triglyceride levels were 147.7mg/dL in the steatosis group and 159.0mg/dL in the NASH group (p=0.34). There was no difference between the two groups in the prevalence of low HDL levels or the prevalence of elevated triglycerides.

Lipids Levels Stratified by Use of Lipid Lowering Medications

Thirty-seven patients with steatosis and 46 patients in the NASH group were on lipid lowering medications. Among these patients there was no difference in cholesterol, HDL, LDL or triglyceride levels. There was no difference in the prevalence of hypertriglyceridemia and low HDL levels.

Seventy-four subjects with steatosis and 46 patients with NASH were not on lipid lowering medications. Among these patients there was no difference in cholesterol, HDL, LDL or triglyceride levels. Further, there was no significant difference in the prevalence of hypertriglyceridemia or low HDL.

Non-HDL-Cholesterol Levels

Non-HDL-C is not a standard component of the lipid profile but was calculated for each subject from TC and HDL levels (Non-HDL-C = TC-HDL). In the entire cohort there was a trend toward a higher mean non-HDL-C level in the NASH group compared to the steatosis group but did not reach statistical significance (127.0mg/dL vs 135.7mg/dL, p=0.08). Among subjects on lipid lowering medications there was no difference seen in mean non-

HDL-C levels or in the percentage of subjects with a non-HDL-C level >130mg/dL in those with steatosis and those with NASH.

However, among the 74 subjects with steatosis and 46 patients with NASH not on lipid lowering medications, those with NASH had significant higher non-HDL-C levels at 144.6mg/dL than those with steatosis at 129.3mg/dL ($p=0.025$). This remained significant when adjusted for both TC level and triglyceride level, suggesting that a significant proportion of the difference was driven by the presence of elevated levels of VLDL and IDL levels. (Table 5)

Validation Cohort Baseline Characteristics

Forty patients with histologically confirmed NAFLD who were not receiving lipid lowering therapy were evaluated to validate the findings in the primary cohort. Twenty-three patients had NAS ≤ 4 and 17 patients had NAS >4 (Table 6). There was no significant difference between the NAS ≤ 4 and NAS >4 groups in regards to mean age, BMI or gender. The NAS ≤ 4 group had a higher proportion of Caucasian patients compared to the NAS >4 group which had a higher percentage of Asian and Hispanic patients. In addition, the NAS >4 group had more severe steatosis than the NAS ≤ 4 group ($p=0.009$).

Lipid Levels in the Validation Cohort

As in the primary cohort, there was no significant difference in levels of HDL, TC or triglyceride levels between the NAS ≤ 4 and NAS >4 groups (Table 6). Distinct from our primary cohort, in the validation cohort the NAS ≤ 5 group did have a significantly higher LDL level than the NAS ≤ 4 group (107.5mg/dL vs 130.0 mg/dL, $p=0.04$). As with the primary cohort, the NAS ≤ 5 group had a significantly higher non-HDL-C level than the NAS ≤ 4 group (162.8mg/dL vs 145.9mg/dL, $p=0.04$). These data confirm that in patients who are not receiving lipid lowering therapy, elevated non-HDL-C is associated with NAS ≤ 5 .

Use of Non-HDL-Cholesterol To Discriminate Steatosis and NASH

We evaluated the use of various non-HDL-C levels to discriminate NAS ≤ 4 and NASH. The highest sensitivity for non-HDL-C was seen at a level of >125mg/dL but reached only 56.4%. The highest specificity was seen with a non-HDL-C level of ≥ 155 . A level of ≥ 145 provided the highest combined sensitivity (36.6%) and specificity (72.9%).

Discussion

In this prospective study, non-HDL-C levels were higher in subjects with biopsy proven NASH than in patients with steatosis. Lipid lowering medications are known to alter non-HDL-C and may obliterate the difference in non-HDL-C levels between steatosis and NASH.^{15,18} When stratified by the presence of lipid lowering medications, non-HDL-C levels were significantly higher in NASH subjects when compared to steatosis subjects in those not on lipid lowering medications. There was no difference in non-HDL-C levels in patients on lipid lowering medications. To evaluate these findings in an independent cohort we evaluated non-HDL-C levels in NAFLD patients from a third institution. Even in a limited cohort size we found that non-HDL-C levels were significantly higher in NASH patients.

Our study is the first to our knowledge to explore the difference between non-HDL-C in patients with steatosis and NASH. As was seen in our primary cohort, no consistent difference has been reported in the standard lipid panel (TC, LDL, triglycerides, HDL) between patients with steatosis and NASH and these levels cannot be used to stratify patients for risk of NASH and need for liver biopsy. Other biomarkers have been evaluated

to aid in this risk stratification and have proven to be useful in distinguishing steatosis from NASH.^{19–24} However, none are yet clinically available, all require cost outside of the standard laboratory evaluation and none have sufficient performance characteristics alone to replace liver biopsy.^{19,25} Non-HDL-C is an attractive marker since it is easily calculated from the standard lipid panel and can be determined with no additional cost or testing for the patient. Alone, non-HDL-C does not have sufficient specificity and sensitivity to diagnose NASH. However, non-HDL-C may have value in risk-stratifying patients and targeting high risk patients for biopsy. It is also possible that non-HDL-C, coupled with other, emerging biomarkers, could successfully non-invasively diagnose NASH.

In addition to its use in aiding the prediction of NASH, non-HDL-C is also an important marker for the prediction of cardiovascular disease beyond that determined by the standard lipid panel.²⁶ As patients with NAFLD have a high prevalence of cardiovascular disease the use on non-HDL-C provides an important value in cardiovascular risk stratification and as a target for lipid lowering therapy.^{2,27}

The difference in non-HDL-C between steatosis and NASH suggests a potential role for unmeasured lipids in the pathogenesis on NASH. An elevated non-HDL-C level was seen despite the lack of significant differences in LDL, TC and triglycerides between those with steatosis and NASH. This suggests that the difference in non-HDL-C levels is driven by differences in lipids that are unaccounted for by traditional lipid panels, specifically VLDL and IDL. Studies have suggested that alterations in the production and export of VLDL and IDL may be associated with and account for the differential development of steatosis and NASH.^{7,9} Our study lends evidence to these studies and further emphasizes the need for evaluation of VLDL and IDL in the pathogenesis of NAFLD.

As ours was a cross-sectional study, the prospect of non-HDL-C as a target for NAFLD therapy could not be directly addressed. HMG CoARI have been associated with a decrease in steatosis on imaging but studies of the impact of HMG CoARI on histology have been small and conflicting. None have examined the correlation between non-HDL-C levels and histologic changes.^{5,6,28} Our study suggests that steatosis is associated with lower non-HDL-C levels but cannot determine whether lowering of non-HDL-C levels in NASH would be beneficial.

Our study used two distinct cohorts, which varied in their definition of NASH. In our primary cohort NASH was defined, as it is in the community, by the evaluating pathologist determining the presence of any grade of lobular inflammation, any grade of hepatocyte ballooning and zone 3 >5% macrovesicular steatosis, rather than by NAS score¹. This is the recommended method of NASH diagnosis, using an overall assessment of the individual histologic characteristics rather than inferring a diagnosis solely from the NAS score.¹ However, in order to more stringently evaluate the use of non-HDL-C as a biomarker, in the validation cohort we used the NAS score in addition to the standard definition of NASH used in our primary cohort. Several subjects in this cohort with NAS of 4 or less were noted to have advanced fibrosis. The non-HDL-C levels were analyzed both with and without patients with advanced fibrosis (data not shown) and there was no significant difference in the mean non-HDL-C levels between these groups. By both methods, elevated non-HDL-C was a marker of NASH.

In conclusion, non-HDL-C levels are increased in patients with NASH compared to those with steatosis, particularly in those persons who are not receiving lipid lowering medications. In the future, non-HDL-C levels could be added to additional biomarkers to estimate a patient's risk of NASH and need for a liver biopsy. Further study is needed to

determine whether non-HDL-C levels should be targeted in the management of NASH treatment and to further define the role of VLDL in the pathogenesis of NASH.

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List of Abbreviations

Non-HDL-C	non-high density lipoprotein cholesterol
NASH	non-alcoholic steatohepatitis
NAFLD	non-alcoholic fatty liver disease
KEC	study design, data acquisition, analysis and interpretation of data, statistical analysis, study supervision, drafting of manuscript, revision of manuscript, important intellectual content
RTC	study design, data acquisition, analysis and interpretation of data, study supervision, revision of manuscript, important intellectual content
LLB	data acquisition, important intellectual content
HZ	study design, analysis and interpretation of data, statistical analysis, revision of manuscript
KA	data acquisition, revision of manuscript, important intellectual content
DSP	data acquisition, revision of manuscript, important intellectual content
MT	data acquisition, revision of manuscript, important intellectual content
ML	data acquisition, revision of manuscript, important intellectual content
LG	study design, revision of manuscript, important intellectual content
JM	revision of manuscript, important intellectual content

References

1. Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology*. 54:344–353. [PubMed: 21520200]
2. Dunn W, Xu R, Wingard DL, et al. Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. *Am J Gastroenterol*. 2008; 103:2263–2271. [PubMed: 18684196]
3. Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. *Am J Gastroenterol*. 2007; 102:399–408. [PubMed: 17311652]
4. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. *J Hepatol*. 2006; 45:600–606. [PubMed: 16899321]
5. Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006; 44:865–873. [PubMed: 17006923]
6. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. *Hepatology*. 2009; 49:1017–1044. [PubMed: 19243014]
7. Fujita K, Nozaki Y, Wada K, et al. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology*. 2009; 50:772–780. [PubMed: 19650159]

8. 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; 28:1225–1236. [PubMed: 18565848]
9. Choi SS, Diehl AM. Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. *Curr Opin Lipidol.* 2008; 19:295–300. [PubMed: 18460922]
10. Sundaram M, Yao Z. Recent progress in understanding protein and lipid factors affecting hepatic VLDL assembly and secretion. *Nutr Metab (Lond).* 7:35. [PubMed: 20423497]
11. Yamaguchi K, Yang L, McCall S, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology.* 2007; 45:1366–1374. [PubMed: 17476695]
12. Choi CS, Savage DB, Kulkarni A, et al. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. *J Biol Chem.* 2007; 282:22678–22688. [PubMed: 17526931]
13. Arsenault BJ, Rana JS, Stroes ES, et al. Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healthy men and women. *J Am Coll Cardiol.* 2009; 55:35–41. [PubMed: 20117361]
14. Orakzai SH, Nasir K, Blaha M, Blumenthal RS, Raggi P. Non-HDL cholesterol is strongly associated with coronary artery calcification in asymptomatic individuals. *Atherosclerosis.* 2009; 202:289–295. [PubMed: 18452924]
15. Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease risk. *J Am Coll Cardiol.* 2009; 53:316–322. [PubMed: 19161879]
16. Grundy SM, Cleeman JI, Merz CN, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. *J Am Coll Cardiol.* 2004; 44:720–732. [PubMed: 15358046]
17. Brunzell JD, Davidson M, Furberg CD, et al. Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. *J Am Coll Cardiol.* 2008; 51:1512–1524. [PubMed: 18402913]
18. Charlton-Menys V, Betteridge DJ, Colhoun H, et al. Targets of statin therapy: LDL cholesterol, non-HDL cholesterol, and apolipoprotein B in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS). *Clin Chem.* 2009; 55:473–480. [PubMed: 19147732]
19. Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: A multicenter validation study. *Hepatology.* 2009; 50:1072–1078. [PubMed: 19585618]
20. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol.* 2008; 103:1372–1379. [PubMed: 18510618]
21. Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology.* 2006; 44:27–33. [PubMed: 16799979]
22. Hossain N, Afendy A, Stepanova M, et al. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* 2009; 7:1224–1229. 1229 e1221–1222. [PubMed: 19559819]
23. Alkhouri N, Tamimi TA, Yerian L, Lopez R, Zein NN, Feldstein AE. The Inflamed Liver and Atherosclerosis: A Link Between Histologic Severity of Nonalcoholic Fatty Liver Disease and Increased Cardiovascular Risk. *Dig Dis Sci.* 2009; 55:2644–2650. [PubMed: 19960252]
24. Lemoine M, Ratzliff V, Kim M, et al. Serum adipokine levels predictive of liver injury in non-alcoholic fatty liver disease. *Liver Int.* 2009; 29:1431–1438. [PubMed: 19422483]
25. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of Noninvasive Markers of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol.* 2009

26. Robinson JG. Are you targeting non-high-density lipoprotein cholesterol? *J Am Coll Cardiol.* 2009; 55:42–44. [PubMed: 20117362]
27. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia.* 2008; 51:1947–1953. [PubMed: 18762907]
28. Foster T, Budoff MJ, Saab S, Ahmadi N, Gordon C, Guerci AD. Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis Heart Study randomized clinical trial. *Am J Gastroenterol.* 106:71–77. [PubMed: 20842109]

Table 1

Baseline Characteristics of Steatosis and NASH patients

	Steatosis	NASH	P Value
N (%)	118	100	
Age (years)	47.1 (13.0)	47.4 (12.0)	0.85
Gender (%women)	74.6% (88)	73.0% (73)	0.88
BMI (kg/m²)	45.6 (8.4)	44.5 (8.5)	0.36
Ethnicity			
White	68.8% (80)	83.0% (83)	0.008
Black	29.7% (35)	13.0% (13)	
Other	2.5% (3)	3.0% (3%)	
HTN	54% (54)	58.2 % (53)	0.56
Steatosis Score			
1	79.66 (94)	65.7 (65)	0.053
2	12.7% (15)	24.2% (24)	
3	5.1% (6)	9.1% (9)	
Fibrosis Stage			
0	93.2% (110)	44.0% (44)	<0.0001
1	5.1% (6)	40.0% (40)	
2	0% (0)	9.0% (9)	
3	1.7% (2)	6.0% ()	
Mean ALT (U/L)	74.9 (39.2)	90.2 (61.9)	0.48
Mean glucose (mg/dL)	116.7 (38.8)	127.5 (60.4)	0.12
Mean Insulin (μU/mL)	26.6 (15.2)	29.5 (15.8)	0.24
% with OSA	49.6% (58)	48.5% (49)	0.88
% with CAD	12.8% (15)	7.2% (7)	0.18
% with DM	32.5% (38)	45.5% (46)	0.52
% on lipid lowering medications	36.4% (43)	54.5% (55)	0.35

Table 2

Mean Lipid Levels In All Patients with Steatosis and NASH

	Steatosis	NASH	P Value
Mean Total Cholesterol, mg/dL	170.2 (37.6)	178.9 (42.9)	0.115
Mean HDL, mg/dL	43.2 (11.1)	43.2 (12.0)	0.97
Mean LDL, mg/dL	99.2 (29.6)	103.9 (36.9)	0.30
Mean Triglycerides, mg/dL	147.7 (98.5)	159.0 (74.7)	0.34
% with Low HDL *	39.3% (42)	42.4% (39)	0.65
% with triglycerides >150mg/dL	37.3% (44)	49.5% (50)	0.07

* Defined as HDL<50mg/dL in women, HDL<40mg/dL in men

Table 3Mean Lipid Levels in Patients with Steatosis and NASH On Lipid Lowering Therapy

	Steatosis (n=37)	NASH (n=46)	P Value
Mean Total Cholesterol, mg/dL	161.2 (38.0)	168.0 (40.5)	0.44
Mean HDL, mg/dL	41.9(11.2)	43.0 (12.1)	0.68
Mean LDL, mg/dL	90.6 (30.5)	96.3 (34.9)	0.44
Mean Triglycerides, mg/dL	136.9 (85.3)	163.5 (75.4)	0.08
% with Low HDL *	53.1% (17)	65.2%(28)	0.29
% with triglycerides >150mg/dL	37.8% (14)	44.9% (22)	0.92

* Defined as HDL<50mg/dL in women, HDL<40mg/dL in men

Table 4Mean Lipid Levels in Patients with Steatosis and NASH Not on Lipid Lowering Therapy

	Steatosis (n=74)	NASH (n=46)	P Value
Mean Total Cholesterol, mg/dL	173.1 (36.0)	187.3 (43.7)	0.06
Mean HDL, mg/dL	43.8 (10.8)	42.7 (12.0)	0.58
Mean LDL, mg/dL	103.6 (27.7)	111.9 (38.2)	0.17
Mean Triglycerides, mg/dL	136.9 (85.3)	163.5 (75.4)	0.08
% with Low HDL *	67.7% (46)	53.5% (23)	0.13
% with triglycerides >150mg/dL	32.4% (24)	47.38% (22)	0.92

* Defined as HDL<50mg/dL in women, HDL<40mg/dL in men

Table 5

Non-HDL-C Levels in Patients By Lipid Lowering Medication Status

Mean Non-HDL Cholesterol, mg/dL	Steatosis	NASH	P Value
Entire Cohort	127.0 (33.1)	135.7 (40.9)	0.08
Subjects on Lipid Lowering Medications	119.4 (32.7)	125.0 (38.4)	0.48
Subjects NOT on Lipid Lowering Medications	129.3 (31.7)	144.6 (41.5)	0.025

Table 6

Baseline Characteristics and Lipid Levels in Validation Cohort

	NAS≤4	NAS>4	P Value
N (%)	23	17	
Age, years (SD)	48.7 (10.8)	46.0 (11.8)	0.45
Gender (%women)	39.1% (9)	41.1% (7)	0.25
BMI, kg/m ² (SD)	34.4 (7.2)	35.7 (6.7)	0.56
Ethnicity			0.0016
White	70.4% (19)	52.9% (9)	
Black	4.3 % (1)	0% (0)	
Hispanic	8.7% (2)	29.4% (5)	
Asian	4.3 % (1)	17.6% (3)	
HTN	12 (52.2%)	5 (29.4%)	0.15
Steatosis Score			0.009
1	9	0	
2	13	14	
3	1	3	
Fibrosis Stage			0.48
0	9 (39.1%)	5 (29.4%)	
1	5 (21.7%)	4 (23.5%)	
2	7 (30.4%)	8 (47.1%)	
3	2 (8.7%)	0 (0%)	
Mean ALT (U/L)	63.0 (33.1)	72.5 (52.1)	0.50
Mean glucose (mg/dL)	110.4 (28.6)	119.1 (52.0)	0.56
Mean Total Cholesterol, mg/dL	185.3 (33.1)	211.9 (54.9)	0.064
Mean HDL, mg/dL	45.0 (11.7)	49.2 (18.2)	0.38
Mean LDL, mg/dL	107.5 (26.7)	130.0 (39.3)	0.04
Mean Triglycerides, mg/dL	163.1 (79.9)	186.6 (156.0)	0.54
Non-HDL Cholesterol	145.9 (34.8)	162.8 (48.3)	0.04
Mean Total Cholesterol, mg/dL	185.3 (33.1)	211.9 (54.9)	0.064