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Circulating Interleukin-6 is a Biomarker for Coronary Atherosclerosis in Nonalcoholic Fatty Liver Disease: Results from the Multi-Ethnic Study of Atherosclerosis

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Abstract

BACKGROUND—Biomarkers to predict the presence and severity of subclinical cardiovascular disease (CVD) in nonalcoholic fatty liver disease (NAFLD) are lacking.

METHODS—3,876 participants from the Multi-Ethnic Study of Atherosclerosis (MESA), without known chronic liver disease underwent baseline non-contrast cardiac CT, with NAFLD defined by validated liver:spleen ratio (L:S)<1.0, and subclinical CVD defined by coronary artery calcium (CAC) score>0. Randomly-selected subgroups underwent detailed inflammatory marker testing, including LpPLA2 mass (N=2,951), activity (N=3,020), high-sensitivity C-reactive protein (hsCRP; N=3,849), and interleukin-6 (IL-6; N=3,764). Among those with NAFLD, we estimated the prevalence of CAC>0 and CAC>100 for each SD biomarker increase, using multivariable log-binomial regression models adjusted for cardiometabolic risk factors.

RESULTS—Seventeen percent (N=668) of participants met criteria for NAFLD. NAFLD participants were younger (mean age 61±10 vs. 63±10 years, p<0.0001) but more likely to have an

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elevated BMI (mean 31.1 ± 5.5 vs. 28.0 ± 5.2 kg/m², $p < 0.0001$), diabetes (22% vs. 11%, $p < 0.0001$), and increased inflammatory biomarkers, including LpPLA2 activity, hsCRP and IL-6 (all $p < 0.0001$). Among NAFLD participants, IL-6 was the only biomarker independently associated with prevalent CAC >0 (PR=1.06 [1.00–1.11]), or CAC >100 (PR=1.09 [1.02–1.17]). In contrast, circulating LpPLA2 mass/activity and hsCRP were not associated with either the prevalence or severity of subclinical CVD (all $p > 0.05$).

CONCLUSION—In a large, multi-ethnic population with NAFLD, IL-6 is independently associated with the prevalence and severity of subclinical atherosclerosis. Further research into the longitudinal effects of NAFLD on progressive CVD will determine whether IL-6 is a marker or mediator of NAFLD-related atherosclerosis.

Keywords

fatty liver; nonalcoholic fatty liver disease; inflammation; atherosclerosis; cardiovascular disease; biomarker

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents the leading cause of chronic liver disease in the United States, where it affects approximately 90 million adults¹. Among those with NAFLD, cardiovascular disease (CVD) is the most common cause of mortality², and recent data demonstrate that NAFLD is also associated with the development of atherosclerosis and subclinical CVD³. Given that not all patients with NAFLD develop progressive CVD, it has been hypothesized that inflammatory mediators common to the vasculature and the liver may accelerate atherogenesis in certain individuals with NAFLD⁴. However, validated biomarkers for predicting NAFLD-associated atherosclerosis are lacking, and no previous study has examined the relationship between inflammatory markers and subclinical CVD, in a NAFLD population. An improved understanding of CVD risk indicators in NAFLD would allow providers to appropriately identify individuals at highest risk of progressive atherosclerotic disease.

Interleukin-6 (IL-6) is a pleiotropic cytokine that bridges innate and adaptive immunity and serves to regulate the acute-phase response and chronic inflammation⁵. Within the general population, circulating concentrations of IL-6 are associated with subclinical CVD, including endothelial dysfunction, arterial stiffness and coronary atherosclerosis^{6, 7}. Mendelian randomization studies lend further support to the causal role played by IL-6 in CVD pathogenesis^{8, 9}, and in a recent meta-analysis, each standard deviation (SD) increase in IL-6 corresponded to a 25% increased risk of CVD events in the general population¹⁰.

In contrast, clinical biomarkers of CVD risk in NAFLD remain largely uncharacterized. While preliminary clinical studies demonstrate that plasma inflammatory biomarkers correlate with NAFLD severity¹¹, to date no published study has examined whether these biomarkers are also associated with atherosclerosis, in NAFLD. The purpose of this study was to evaluate a set of candidate inflammatory markers known to predict CVD within the general population, and to determine their association with the prevalence and severity of coronary atherosclerosis among individuals with NAFLD.

METHODS

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based, observational cohort of 6,814 Caucasian, African American, Hispanic, and Chinese adults, aged 45–84 years, without known CVD at the time of enrollment. Participants were enrolled at one of six field centers across the United States from July 2000 through September 2002, using a study design that has previously been described¹². MESA was approved by the institutional review board of each participating site, and all participants provided their written informed consent.

A total of 4,389 MESA participants underwent non-contrast cardiac computed tomography (CT) and had adequate imaging for the quantification of liver and spleen fat, using measured attenuation (Hounsfield units). Compared to those excluded for inadequate imaging, the included population was more likely to be older, female, black and Hispanic, with a higher prevalence of obesity, diabetes and hypertension, as has previously been described in MESA¹³. Five hundred thirteen individuals were excluded: 405 had a history of heavy alcohol use (>7 drinks/week in women or >14 drinks/week in men), 59 reported alcohol consumption but did not quantify intake, 39 reported infection with hepatitis B virus or hepatitis C virus, 8 reported cirrhosis and 2 were amiodarone users, leaving a final population of 3,876 participants.

Measurement of IL-6 and other inflammatory biomarkers

Assays for serum inflammatory biomarkers were obtained at baseline from randomly-selected MESA participants in both the main cohort and in selected ancillary studies, as has previously been reported¹⁴. All samples were obtained following a 12 hour fast. IL-6 was measured in 3,764 randomly-selected participants from the full MESA cohort, using ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis MN), with a lower limit of detection of <0.094pg/mL (range 0.156–10.00pg/mL), and a 6.3% coefficient of variability. Gamma glutamyltransferase (GGT) activity was measured from frozen samples¹⁵, and high-sensitivity C-reactive protein (hsCRP) was measured by particle-enhanced immunophotometric assay on the BNII nephelometer (Dade-Behring, Inc., Deerfield IL), also from the full MESA cohort¹⁶. Additional inflammatory biomarkers included soluble intercellular adhesion molecule-1 (sICAM-1), soluble E-selectin, plasminogen activator inhibitor-1 (PAI-1) and lipoprotein associated phospholipase A2 (Lp-PLA2) mass and activity, each of which were measured for an ancillary study, using methods that have previously been described^{17, 18}.

Outcome ascertainment

Details of the MESA scanning protocol have been reported in detail¹⁹. All MESA participants underwent unenhanced cardiac CT scans at the baseline examination, with either a cardiac-gated electron-beam CT (Chicago, Los Angeles, New York), or a multidetector CT (Baltimore, Forsyth County, St. Paul). Individuals were scanned twice, with all images interpreted at the MESA CT reading center (Los Angeles Biomedical Research Institute, Torrance CA), where mean CAC (Agatston) score was calculated²⁰. For the present study,

subclinical atherosclerosis was defined as CAC>0 (vs. CAC=0). For the analysis of CAC severity, comparison groups included CAC>100 vs. CAC=0.

Liver Fat Quantification

Details of the assessment of hepatic steatosis in MESA have previously been reported²¹. Using regions of interest $\geq 100\text{mm}^2$ on baseline cardiac CTs, hepatic and splenic attenuation measurements (in Hounsfield Units) were obtained. The mean of two regions in the right hepatic lobe was divided by the spleen measurement, to calculate the liver:spleen (L:S) attenuation ratio, and hepatic steatosis was defined by $\text{L:S} < 1.0$ ²¹.

Assessment of covariates

MESA collected clinical and socio-demographic variables, including age, sex, race and ethnicity, behavioral risk factors including smoking status (current, former or prior smoking and total pack-years) and alcohol consumption (number of drinks/day)¹². Participants also reported detailed medical histories at baseline, including diabetes, cardiovascular conditions, cancer, and regular physical activity, estimated by metabolic equivalent minutes (MET-MIN) per week¹². Anthropomorphic measurements including waist circumference (centimeters) and body mass index (BMI, kg/m^2) were obtained from trained examiners. Obesity was defined as $\text{BMI} \geq 30\text{kg/m}^2$. Using an automated sphygmomanometer (Critikon, Tampa FL), systolic and diastolic blood pressure measurements were obtained three times, and the mean of the last two measurements was used. Diabetes was defined as a self-reported physician diagnosis of diabetes, diabetic medication use, or a fasting glucose $\geq 126\text{ mg/dL}$. Serum glucose was assayed by a hexokinase/glucose-6-phosphate dehydrogenase method, and triglycerides and cholesterol were assayed by enzymatic methods following a 12-hour fast. HDL cholesterol (HDL-C) was measured after precipitation of non-HDL-C with magnesium/dextran and low-density lipoprotein cholesterol (LDL-C) was calculated via the Friedewald equation. Lipid-lowering medication use was defined as use of prescribed statins, ezetimibe, fibrates, niacin and/or other lipid-lowering medications; in this cohort, as in the MESA cohort as a whole, more than 90% of those taking any lipid-lowering medication were taking statins. Metabolic syndrome was defined by the American Heart Association/National Heart, Lung and Blood Institute criteria²².

Statistical analysis

Baseline demographic and clinical characteristics were compared in individuals with and without NAFLD using descriptive statistics and frequency distributions. Levels of inflammatory biomarkers were calculated by mean ($\pm\text{SD}$) and median [interquartile range, IQR]. Among those with NAFLD ($N=668$), we tested correlations between log-transformed biomarker concentrations using Pearson's correlation. We also tested the relationship between continuous levels of each biomarker and cardiometabolic risk factors chosen *a priori* for their association with subclinical CVD (i.e. age, sex, ethnicity, BMI, waist circumference, triglycerides, HDL-C, dyslipidemia, HOMA-IR, alcohol intake and smoking status), using univariable linear regression, in which regression coefficients and p-values were generated by regressing each clinical factor on the biomarker of interest.

We constructed a series of multivariable log-binomial regression models to estimate the prevalence of (1) CAC>0 vs. CAC=0, and (2) CAC>100 vs. CAC=0, for each one SD increase in biomarker. The following multivariable models were constructed: Model 1, adjusted for age (years), sex, ethnicity and MESA site; Model 2, adjusted for Model 1 + smoking status (current, former, never) and servings/day of alcohol; Model 3, adjusted for Model 2 + BMI (kg/m²) and diabetes; Model 4, adjusted for Model 3 + systolic blood pressure, use of anti-hypertensive medications, total cholesterol, HDL cholesterol, use of lipid-lowering medications, and regular physical activity (MET-MIN/week). Multiplicative interactions between the inflammatory biomarkers, age and ethnicity were assessed in the fully-adjusted adjusted models.

Due to the known relationship between IL-6 and both visceral adiposity and insulin resistance²³, we constructed two additional models that further accounted for (a) waist circumference (Model 5), or (b) HOMA-IR (Model 6). Finally, due to the observed correlation between IL-6 and high-sensitivity C-reactive protein (hsCRP), we constructed a final model that also adjusted for continuous hsCRP, in addition to traditional cardiovascular risk factors (Model 7). All P-values were two-tailed and a P < 0.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Among 3,876 eligible participants, 668 (17.2%) had CT evidence of NAFLD (L:S<1.0). Baseline clinical and demographic characteristics are presented according to NAFLD status (Table 1). Participants with NAFLD were younger (mean age 61±10 vs. 63±10 years, p<0.0001), with a larger mean BMI (31.2±5.5 vs. 28.0±5.2 kg/m², p<0.0001) and more likely to be Hispanic (35.8% vs. 20.5%, p<0.0001), with diabetes (22% vs. 11%, p<0.0001) and hypertension (53% vs. 46%, p<0.0001). However, there was no significant difference in diagnosed dyslipidemia or in use of lipid-lowering medications, when NAFLD vs. non-NAFLD participants were compared (both p=0.338).

Inflammatory biomarkers in NAFLD and non-NAFLD participants

Table 2 outlines the sample size (N) and the mean (SD) and median [IQR] concentration for each biomarker, in individuals with and without NAFLD. Overall, NAFLD participants had increased median [IQR] inflammatory biomarker concentrations, including soluble ICAM-1, PAI-1, hsCRP and IL-6 (all p<0.0001; Table 2).

Inflammatory biomarkers and prevalent CAC, in NAFLD

To evaluate determinants of prevalent CAC in NAFLD, the cohort was restricted to those individuals with steatosis (N=668; Table 3). In this group, after accounting for age, sex, race and MESA site, each one SD increase in IL-6 was associated with a 6.2% increased risk of prevalent CAC (Model 1, prevalence ratio (PR)=1.06 [95% CI 1.01–1.12], p=0.020). Further adjustment for smoking and alcohol consumption did not significantly change the results (Model 2, PR=1.06 [95% CI 1.01–1.12], p=0.026), nor were the effects materially altered when continuous pack-years of smoking was included, in the place of categorical smoking

status (data not shown). In the final fully-adjusted model, accounting for the remaining cardiometabolic risk factors, each SD increase in IL-6 was associated with a statistically significant, 5.6% increase in prevalent CAC>0 (Model 4, PR=1.06 [95% CI 1.00–1.11], $p=0.047$) (Table 3).

Given the known associations between IL-6, central adiposity and insulin resistance, a series of additional multivariable models were constructed to test for potential confounding (Table S3). However, after further adjustment for continuous waist circumference, the positive association between IL-6 and CAC>0 remained statistically significant (Model 5, PR=1.06 [1.00–1.11], $p=0.049$). Similarly, the introduction of HOMA-IR into the multivariable model did not meaningfully alter the results (Model 6, PR=1.06 [1.00–1.11], $p=0.048$) (Table S3).

In models stratified by gender, obesity or by baseline elevation in hsCRP (>5 vs. ≤ 5 mg/dL), the positive association between IL-6 and prevalent CAC>0 did not vary, and formal testing for effect modification was not statistically significant (all p -interaction>0.60). When the relationship between IL-6 and prevalent CAC>0 was compared in persons with and without NAFLD, the association did not vary significantly between groups ($PR_{NAFLD} = 1.049$ [1.004, 1.095] vs. $PR_{non-NAFLD} = 1.034$ [1.006, 1.063]), and formal interaction testing was not statistically significant (p -interaction=0.58) (Table S4).

Inflammatory biomarkers and CAC severity, in NAFLD

To test the relationship between circulating biomarkers and CAC severity among individuals with NAFLD, we constructed log-binomial regression models with CAC>100 entered as the dependent variable (Table 4). In the fully-adjusted multivariable model, each SD increase in IL-6 was associated with an 8.3% increased prevalence of CAC>100 (PR=1.09 [95% CI 1.02–1.16], $p=0.015$) (Table 4). The positive association between IL-6 and CAC>100 remained statistically significant in sequential models that were further adjusted for waist circumference, HOMA-IR or hsCRP (Table S5).

Correlation between inflammatory biomarkers and CVD indicators

Among individuals with NAFLD, traditional cardiovascular risk factors correlated with IL-6 included age ($r=0.10$, $P<0.001$), BMI ($r=0.04$, $P<0.001$), waist circumference ($r=0.02$, $P<0.001$), HOMA-IR ($r=0.07$, $P<0.001$), and hypertension ($r=0.18$, $P<0.001$), whereas male sex was inversely correlated with IL-6 ($r=-0.13$, $P=0.006$) (Table S2). Despite this, as shown in Tables 3 and 4, multivariable adjustment for these factors had minimal impact on the relationship between circulating IL-6 and either prevalent CAC or CAC severity.

Table S1 outlines the relationship between log-transformed concentrations of IL-6 and other inflammatory biomarkers, in individuals with NAFLD (N=668). As expected, the strongest correlate of IL-6 was log-normalized hsCRP ($r=0.52$, $P<0.001$) (Table S1). Notably, further adjustment for continuous hsCRP in the full multivariable model did not materially alter the relationship between IL-6 and CAC>0 (PR=1.07 [1.02–1.14], $p=0.010$, Table S3) or between IL-6 and CAC>100 (PR=1.10 [1.03–1.18], $p=0.005$, Table S5).

DISCUSSION

These data indicate that among asymptomatic, community-dwelling individuals with NAFLD, levels of the inflammatory cytokine IL-6 are significantly elevated in the setting of coronary atherosclerosis. IL-6 was independently associated with both the prevalence and severity of subclinical CVD, and this relationship remained significant after accounting for traditional cardiometabolic risk factors and measures of adiposity and insulin resistance, which are known confounders of circulating IL-6²⁴. In contrast, among individuals with NAFLD, concentrations of sICAM-1, LpPLA2, hsCRP and GGT were notably not associated with either prevalent CAC or CAC severity.

Our findings are supported by studies from the general population, in which circulating IL-6 has been linked to risk of both subclinical and overt CVD. Elevated IL-6 is associated with coronary plaque initiation and destabilization²⁵, aberrations in microvascular function²⁶ and with prevalent CAC²⁷. Increased IL-6 has also been shown to predict future major CVD events in patients with established coronary artery disease²⁸ and in otherwise healthy adults²⁹. More recently, in Mendelian randomization studies, loss of function IL-6 pathway polymorphisms at the rs2228145 and rs7529229 loci were correlated with a reduced lifetime risk of incident vascular events^{8, 9}. In contrast, such causal associations have not been found in population-based Mendelian Randomization studies of other downstream inflammatory markers, including hsCRP^{30, 31}. Together, these data suggest that individual vascular risk varies widely due to heritable differences in IL-6 signaling, and they support a causal role for IL-6 in the pathogenesis of CVD.

A growing body of data also support a role for IL-6 in the pathogenesis and progression of NAFLD²³. Hepatocyte expression of IL-6 is increased in patients with both simple steatosis and NASH, compared to healthy controls³², and peripheral levels of IL-6 correlate with histological NAFLD severity and with systemic insulin resistance^{4, 11, 32}. Although the precise mechanisms have not been fully characterized, it is hypothesized that increased IL-6 may stimulate activation of the interleukin-17 (IL-17) axis and transforming growth factor beta (TGF- β) signaling pathways, resulting in pro-inflammatory activation and immune dysregulation, which in turn promote insulin resistance and NAFLD progression^{33, 34}.

To our knowledge, this represents the first study to evaluate the relationship between circulating IL-6 and subclinical CVD, in individuals with NAFLD. Nevertheless, several lines of evidence support this association. First, epidemiological studies from the general population demonstrate that IL-6 may be a more consistent predictor of early-stage, subclinical atherosclerosis than other inflammatory markers, including hsCRP or LpPLA2^{7, 35, 36}. C-reactive protein is synthesized primarily in the liver, and while it is persistently elevated in individuals with NAFLD, it lacks sufficient variability to serve as a useful biomarker of NAFLD severity or other complications, in this population³⁷. In contrast, IL-6 production occurs in a variety of cell types both within and outside of the liver, including hepatic fibroblasts, macrophages and monocytes²³, as well as cells intrinsic to the vasculature³⁸, the myocardium³⁹, and adipose tissue⁴⁰. Thus, while hepatic IL-6 represents the primary stimulant for CRP synthesis, IL-6 itself is derived from numerous sources, and therefore the two biomarkers do not necessarily operate in parallel. Indeed, it has been

shown that IL-6 accounts for less than 25% of the variability in circulating hsCRP^{29, 36}. In the current study, we similarly observed an R-squared value of 26.8% between IL-6 and hsCRP. Together, these findings support the hypothesis that IL-6 and hsCRP may have different prognostic utility in the setting of NAFLD, and may exert independent effects.

Our study demonstrates that in NAFLD, circulating IL-6 is an independent biomarker for subclinical CVD. Most importantly, IL-6 also accurately stratifies NAFLD patients by their likelihood of having an increased coronary plaque burden. Together, these findings support the hypothesis that inflammatory factors common to both the liver and the vasculature may mediate CVD pathogenesis in NAFLD⁴. On the one hand, it is possible that preclinical atherosclerosis is itself an inflammatory stimulus, given the abundance of monocyte-derived macrophages and IL-6 gene transcripts found in atheromatous plaques⁴¹. On the other hand, it is plausible that increased hepatic IL-6 production related to NAFLD exerts direct effects on plaque deposition and proliferation. This analysis revealed a significant relationship between IL-6 and CAC in both NAFLD and non-NAFLD participants, however the cross-sectional nature of this study prevents us from determining if IL-6 represents a marker or mediator of disease. To fully characterize the variability in stimuli for IL-6 in NAFLD patients with and without atherosclerosis, future prospective studies with well-phenotyped populations and adjudicated cardiovascular outcomes are needed.

A key strength of this study is the size and ethnic diversity of the MESA population. Previous reports assessing the relationship between NAFLD and inflammatory markers have been limited by highly-selected and homogenous populations. Importantly, none have examined inflammatory determinants of subclinical CVD in NAFLD populations. Recently, the CANTOS trial demonstrated that among individuals with established CVD and systemic inflammation, targeted IL-1B inhibition with canakinumab reduces the risk for recurrent CV events⁴⁴. Our findings suggest that such an approach may also be applicable for patients with NAFLD. Future prospective studies are needed to assess the impact of IL-6 and its upstream determinant, IL-1B, on the pathogenesis of atherosclerosis in NAFLD, and to determine whether targeted IL-6 or IL-1B inhibition might represent a strategy for atheroprotection in this high-risk population.

Several important limitations must be considered. First, CT is insensitive for identifying steatohepatitis or assessing fibrosis severity⁴², and liver chemistry data for the non-invasive estimation of NAFLD severity are not available in the MESA cohort. However, CT has been widely used in population-based cohorts including MESA¹³ for the reliable diagnosis of hepatic steatosis, with comparable performance to other imaging modalities for the quantification of hepatic fat⁴². Second, it is possible that multiple testing could have increased the probability of chance findings, however in this analysis a limited set of a priori biomarkers was selected, and the findings were consistent across multiple models. On the other hand, with smaller sample sizes and limited CAC outcomes in some of the biomarker subgroups, it is possible that those analyses were underpowered to detect meaningful differences. Third, this was an observational, cross-sectional analysis, which precludes any conclusions regarding causality. Finally, IL-6 was only measured at one point in time, and that measurement took place at various times during the day¹⁴, which could result in

misclassification due to intra-individual variability or diurnal variation⁴³. Nevertheless, we would expect such random misclassification to have biased our results toward the null⁴³.

In conclusion, among individuals with NAFLD, circulating IL-6 is associated with both the prevalence and severity of subclinical atherosclerosis. Future research into the longitudinal effects of NAFLD upon subclinical and overt CVD will help elucidate whether IL-6 is a marker or a mediator of NAFLD-related atherosclerotic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Author contributions:

- TGS: study design, data interpretation, drafting
- MEPT: data analysis, interpretation, critical revision
- RLM: data analysis, interpretation, critical revision
- RB: interpretation of data, critical revision
- MB: interpretation of data, critical revision
- IZ: study design, critical revision
- KEC: interpretation of data, critical revision
- MJB: study design, critical revision, study oversight
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Table 1

Clinical and demographic characteristics of the included MESA population (N=3,876), according to the presence of NAFLD on baseline computed tomography (CT) scan

Variable *	Full cohort N = 3,876	Non-NAFLD N=3,208	NAFLD N=668	P-value
Age, years	63.0 (10.4)	63.3 (10.5)	61.2 (9.6)	<0.00001
Female sex, %	2192 (56.6%)	1822 (56.8%)	370 (55.4%)	0.445
Race/ethnicity, %				<0.00001
• Caucasian	1438 (37.1%)	1221 (38.1%)	217 (32.5%)	
• African American	1157 (29.9%)	1024 (31.9%)	133 (19.9%)	
• Hispanic	896 (23.1%)	657 (20.5%)	239 (35.8%)	
• Chinese American	385 (9.9%)	306 (9.5%)	79 (11.8%)	
Body mass index, kg/m ²	28.6 (5.4)	28.0 (5.2)	31.2 (5.5)	<0.00001
Waist circumference, cm	98.7 (14.1)	97.3 (13.8)	105.8 (13.5)	<0.00001
Diabetes, %	507 (13.1%)	359 (11.2%)	148 (22.2%)	<0.00001
Hypertension, %	1821 (47.0%)	1466 (45.7%)	355 (53.1%)	<0.00001
Systolic blood pressure	127.4 (21.6)	126.9 (21.7)	129.8 (20.8)	0.0017
Diastolic blood pressure	71.7 (10.1)	71.4 (10.0)	73.3 (10.5)	<0.00001
Metabolic syndrome ³ , %	1490 (38.4%)	1054 (32.9%)	436 (65.3%)	<0.00001
Dyslipidemia, %	646 (16.7%)	526 (16.4%)	120 (18.0%)	0.338
Any lipid-lowering medication use, %	646 (16.7%)	526 (16.4%)	120 (18.0%)	0.338
CKD ⁴ , %	396 (10.2%)	339 (10.6%)	57 (8.5%)	0.114
Daily alcohol intake ¹	0.291 (0.46)	0.295 (0.45)	0.274 (0.448)	0.279
Current Smoking, %	435 (11.2%)	363 (11.3%)	72 (10.8%)	0.158
No. of pack-years ²	21.3 (22.7)	21.2 (22.5)	22.0 (24.1)	0.590
Physical activity, MET-MIN/week	5813 (5848)	5833 (5846)	5715 (5857)	0.635
Laboratory parameters				
HOMA-IR	1.78 (2.2)	1.55 (2.1)	2.88 (2.1)	<0.00001
Fasting glucose, mg/dL	97.9 (31.0)	95.8 (28.8)	108.2 (38.4)	<0.00001
Estimated GFR	80.7 (17.7)	79.9 (17.4)	84.6 (18.8)	<0.00001
HDL cholesterol, mg/dL	50.7 (14.7)	51.9 (14.9)	44.6 (12.0)	<0.00001

Variable *	Full cohort N = 3,876	Non-NAFLD N=3,208	NAFLD N=668	P-value
LDL cholesterol, mg/dL	117.4 (31.4)	117.8 (31.4)	115.7 (31.1)	0.126
Triglycerides, mg/dL	132.6 (94.5)	122.3 (70.2)	182.0 (158.7)	<0.00001
Total cholesterol, mg/dL	194.2 (35.6)	194.1 (34.8)	194.6 (39.2)	0.757

Abbreviations: NAFLD, nonalcoholic fatty liver disease; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; GFR, glomerular filtration rate; CKD, chronic kidney disease; MET-MIN/week, metabolic equivalent minutes per week

* All variables reported as mean (SD), unless listed otherwise

¹ Daily alcohol intake defined as the number of drinks per day among individuals reporting current and/or prior consumption of alcohol

² Number of pack-years among current and former smokers

³ Metabolic syndrome was defined as per the American Heart Association/National Heart, Lung and Blood Institute criteria as ≥ 3 of the following: waist circumference (≥ 102 cm in men and ≥ 88 cm in women); triglycerides ≥ 150 mg/dL or use of medication for hypertriglyceridemia; high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL in men or < 50 mg/dL in women or use of medication for reduced HDL-C; hypertension defined by systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg or anti-hypertensive medication use; and elevated fasting blood glucose ≥ 100 mg/dL or anti-diabetic medication use ²¹.

⁴ CKD defined as glomerular filtration rate (GFR) ≤ 60 (<http://www.renal.org/information-resources/the-uk-ekkd-guide/ckd-stages#sthash.FpCujZ1A.dpbs>)

Table 2

Mean (SD) and Median [IQR] of serum biomarkers, according to the presence of hepatic steatosis on coronary CT scan¹

Biomarker	N.	Total N=3,876	Non-NAFLD N=3,208		NAFLD N=668		P-value*
		Mean (SD)	Mean (SD)	Median [IQR]	Mean (SD)	Median [IQR]	
s ICAM-1, ng/mL	1349	274.8 (78.8)	N = 1090 269.0 (76.6) 265.4 [226, 307.6]		N = 259 299.2 (83.4) 286.9 [252.7, 340]		<0.0001
E-selectin, ng/mL	533	55.1 (26.4)	N = 437 52.1 (23.0) 48.8 [35.9, 65.1]		N = 96 68.7 (35.3) 59.2 [46.3, 86.3]		<0.0001
CD40 ligand, ng/mL	533	4.12 (2.64)	N = 437 4.06 (2.70) 3.43 [2.08, 5.39]		N = 96 4.38 (2.33) 4.18 [2.38, 5.99]		0.076
LpPLA2			N = 2428 (mass), 2489 (activity)		N = 523 (mass), 532 (activity)		
• Mass, ng/mL	2951	178.1 (43.2)	177.6 (41.7)	175.4 [150.2, 201.5]	180.4 (49.7)	176 [150, 202]	0.617
• Activity, nmol/min/mL	3020	149.2 (36.8)	147.8 (36.8)	145.6 [121.7, 171.1]	155 (36.3)	154.7 [128.5, 178.2]	<0.0001
IL-2, pg/mL	1578	984.9 (497.7)	N = 1291 982.6 (490.8) 891 [713, 1131]		N = 287 995.3 (501.8) 911 [719, 1182]		0.602
IL-6, pg/mL	3764	1.61 (1.24)	N = 3108 1.56 (1.21) 1.22 [0.78, 1.86]		N = 656 1.89 (1.32) 1.52 [1.03, 2.37]		<0.0001
PAI-1, ng/mL	521	27.5 (28.4)	N = 426 23.8 (27.5) 17 [8, 29]		N = 95 44.0 (26.6) 43 [21, 59]		<0.0001
MMP-3, ng/mL	533	15.3 (14.6)	N = 437 15.6 (14.6) 11.4 [7.8, 18.5]		N=96 14.3 (15.0) 9.9 [7.4, 15.2]		0.139
MMP-9, ng/mL	533	248.1 (145.9)	N = 437 251.7 (148.6) 207.2 [151.4, 317.9]		N = 96 231.9 (132.1) 196.8 [147.8, 265.9]		0.381
			N = 3186		N = 663		

Biomarker	N.	Total N=3,876			Non-NAFLD N=3,208			NAFLD N=668			P-value*
		Mean (SD)	Median [IQR]	Mean (SD)	Mean (SD)	Median [IQR]	Mean (SD)	Median [IQR]	Mean (SD)	Median [IQR]	
hsCRP, mg/L	3849	3.96 (6.2)		3.72 (6.14)	1.81 [0.83, 4.1]		5.13 (6.2)	2.96 [1.35, 6.48]			<0.0001
GGT, U/L	3838	12.3 (15.4)		11.3 (14.6)	7.50 [4.66, 12.3]		16.8 (17.9)	11.5 [7.74, 19.4]			<0.0001

* P-value obtained from the Mann-Whitney test for the difference between median levels of each candidate biomarker among MESA participants with and without NAFLD

Abbreviations: SD, standard deviation; IQR, interquartile range; NAFLD, nonalcoholic fatty liver disease; N., number; sICAM, soluble intracellular adhesion molecule; LpPLA2, lipoprotein associated phospholipase A2; hsCRP, high-sensitivity C-reactive protein; GGT, gamma glutamyltransferase; IL-6, interleukin-6; IL-2, interleukin-2; PAI-1, plasminogen activator inhibitor-1; MMP-3, matrix metalloproteinase 3; MMP-9, matrix metalloproteinase-9

[†] Hepatic steatosis defined by the liver:spleen ratio (L:S < 1.0) on non-contrast coronary computed tomography (CT) scans

Table 3

Association between levels of inflammatory biomarkers and prevalent CAC>0, among MESA participants with NAFLD (N=668)

Biomarker	N.	N. of cases ¹	SD ²	Model 1 [‡]		Model 2 [§]		Model 3		Model 4 [†]	
				RP (95% CI)	P-value	RP (95% CI)	P-value	RP (95% CI)	P-value	RP (95% CI)	P-value
IL-6, pg/mL	656	348	1.32	1.06 (1.01, 1.12)	0.020	1.06 (1.01, 1.12)	0.026	1.06 (1.00, 1.11)	0.048	1.06 (1.00, 1.11)	0.047
hsCRP mg/L	663	353	6.2	0.99 (0.93, 1.06)	0.88	1.00 (0.93, 1.07)	0.92	0.99 (0.92, 1.06)	0.76	0.99 (0.92, 1.06)	0.74
sICAM-1, ng/mL	259	124	83.4	1.14 (1.02, 1.28)	0.024	1.12 (1.00, 1.26)	0.048	1.10 (0.97, 1.25)	0.13	1.11 (0.96, 1.27)	0.16
LpPLA2 mass, ng/mL	523	290	49.8	1.01 (0.94, 1.08)	0.86	1.01 (0.94, 1.08)	0.79	1.01 (0.94, 1.08)	0.80	1.02 (0.96, 1.10)	0.49
LpPLA2 activity, nmol/min/mL	532	292	36.3	1.02 (0.95, 1.10)	0.52	1.02 (0.95, 1.10)	0.53	1.02 (0.95, 1.10)	0.55	1.06 (0.97, 1.15)	0.19
GGT, U/L	662	351	17.9	1.04 (0.98, 1.10)	0.15	1.03 (0.97, 1.09)	0.33	1.03 (0.97, 1.10)	0.31	1.03 (0.97, 1.10)	0.30
IL-2, pg/mL	287	150	501.8	1.09 (0.99, 1.20)	0.08	1.10 (0.98, 1.20)	0.06	1.10 (1.00, 1.20)	0.053	1.09 (0.98, 1.21)	0.12

Abbreviations: NAFLD, nonalcoholic fatty liver disease; CAC, coronary artery calcium; N., number; SD, standard deviation; sICAM, soluble intracellular adhesion molecule; LpPLA2, lipoprotein associated phospholipase A2; hsCRP, high-sensitivity C-reactive protein; GGT, gamma glutamyltransferase; IL-6, interleukin-6; IL-2, interleukin-2; PR, prevalence ratio; CI, confidence interval

¹Number of study participants with CAC >0

²Standard deviation (SD) for each candidate biomarker, taken from the SD of the NAFLD sub-group (N=668)

[‡]**Model 1:** Multivariable log-binomial regression model adjusted for age, sex, ethnicity and MESA study site.

[§]**Model 2:** Model 1 + smoking history, and alcohol intake (servings/day).

[†]**Model 3:** Model 2 + body mass index (BMI) and diabetes.

[†]**Model 4:** Model 3 + systolic blood pressure, use of anti-hypertensive medications, total cholesterol, high density lipoprotein (HDL) cholesterol, use of lipid-lowering medications and physical activity (continuous metabolic equivalent minutes [MET-MIN] per week)

Table 4

Association between levels of inflammatory markers and prevalent CAC>100 (vs. CAC=0) in MESA participants with NAFLD (N=460)

Biomarker	N. of cases ¹	SD ²	Model 1 [‡]		Model 2 [§]		Model 3		Model 4 [†]	
			PR (95% CI)	P-value	PR (95% CI)	P-value	PR (95% CI)	P-value	PR (95% CI)	P-value
IL-6, pg/mL (N=451)	143	1.27	1.06 (0.99, 1.13)	0.078	1.065 (0.999, 1.14)	0.054	1.061 (0.99, 1.13)	0.081	1.09 (1.02, 1.16)	0.015
hsCRP mg/L (N=456)	146	6.5	1.010 (0.91, 1.12)	0.849	1.018 (0.92, 1.13)	0.736	1.012 (0.90, 1.13)	0.843	1.02 (0.92, 1.13)	0.670
slCAM-1, ng/mL (N=179)	44	76.2	1.300 (1.06, 1.60)	0.012	1.134 (0.95, 1.36)	0.168	1.117 (0.87, 1.44)	0.393	1.10 (0.90, 1.35)	0.353
LpPLA2 mass, ng/mL (N=354)	121	52.0	1.029 (0.93, 1.13)	0.570	1.037 (0.94, 1.15)	0.470	1.037 (0.94, 1.15)	0.483	1.05 (0.94, 1.17)	0.380
LpPLA2 activity, nmol/min/mL (N=358)	118	37.4	1.054 (0.95, 1.16)	0.299	1.049 (0.95, 1.16)	0.359	1.043 (0.94, 1.16)	0.419	1.11 (0.98, 1.27)	0.107
GGT, U/L (N=454)	143	16.8	1.059 (0.98, 1.15)	0.155	1.036 (0.96, 1.12)	0.391	1.041 (0.96, 1.13)	0.339	1.03 (0.95, 1.12)	0.498
IL-2, pg/mL (N=202)	65	466.7	1.074 (0.92, 1.25)	0.360	1.117 (0.96, 1.31)	0.162	1.136 (0.99, 1.31)	0.077	1.09 (0.94, 1.26)	0.259

Abbreviations: NAFLD, nonalcoholic fatty liver disease; CAC, coronary artery calcium; slCAM, soluble intracellular adhesion molecule; LpPLA2, lipoprotein associated phospholipase A2; hsCRP; high-sensitivity C-reactive protein; GGT, gamma glutamyltransferase; IL-6, interleukin-6; IL-2, interleukin-2; PR, prevalence ratio; CI, confidence interval

¹Number of study participants with CAC>100

²SD, standard deviation for each candidate biomarker; taken from the SD of the group as a whole (N=460)

[‡]**Model 1:** Multivariable log-binomial regression model adjusted for age, sex, ethnicity and MESA study site.

[§]**Model 2:** Model 1 + smoking history, and alcohol intake servings/day).

[†]**Model 3:** Model 2 + body mass index (BMI) and diabetes.

[†]**Model 4:** Model 3 + systolic blood pressure, use of anti-hypertensive medications, total cholesterol, high density lipoprotein (HDL) cholesterol, use of lipid-lowering medications and physical activity (continuous metabolic equivalent minutes [MET-MIN] per week)