Alcoholic and non-alcoholic fatty liver disease and associations with coronary artery calcification- Evidence from the Kangbuk Samsung Health Study

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Abstract

Objective

Recent evidence suggests that alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD) may differentially affect risk of cardiovascular mortality. To investigate whether early liver disease due to AFLD or NAFLD have similar or dissimilar effects on risk of early coronary artery atherosclerosis, we have investigated the associations between AFLD and NAFLD and coronary artery calcium (CAC).

Design

A cross-sectional study was performed in 105,328 Korean adults who attended a health checkup program. CAC score was assessed using computed tomography (CT), daily alcohol intake was recorded as grams/day and liver fat by ultrasound. Logistic regression model was used to calculate odds ratios (OR) with 95% confidence intervals (CIs) for prevalent CAC.

Results

Both NAFLD and AFLD were positively associated with CAC score. After adjusting for potential confounders, multivariable-adjusted OR (95% CIs) for CAC >0 comparing NAFLD and AFLD to the reference (absence of both excessive alcohol use and fatty liver disease) were 1.10 (1.05-1.16), and 1.20 (1.11-1.30), respectively. In post hoc analysis, OR (95% CI) for detectable CAC comparing AFLD to NAFLD was 1.09 (1.01-1.17). Associations of NAFLD and AFLD with CAC scores were similar in both non-obese and obese individuals without significant interaction by obesity (P for interaction=0.088). After adjusting for HOMA-IR and hsCRP, the associations between fatty liver disease and CAC scores remained statistically significant.

Conclusion

In this large sample of young and middle-aged individuals, early liver disease due to NAFLD and AFLD were both significantly associated with the presence of coronary artery calcification.

Key words: fatty liver, nonalcoholic fatty liver disease, alcoholic liver disease, coronary artery calcium, atherosclerosis

Significance of this study

What is already known on this subject?

- Previous studies have reported the association of non-alcoholic fatty liver disease (NAFLD) with increased risk of clinical and subclinical cardiovascular disease (CVD), but the impact of alcoholic fatty liver disease (AFLD) on CVD has received little attention.
- A recent study has reported that alcoholic liver disease requiring hospital admission was associated with a greater risk of CVD mortality than NAFLD.
- The impact of alcoholic fatty liver disease (AFLD) on early coronary atherosclerosis is largely unknown.

What are the new findings?

- In this large-scale study of 105,328 young and middle-aged adults, an increased risk of prevalent subclinical atherosclerosis was found not only in NAFLD but also in AFLD.
- These associations were observed in non-obese and obese individuals and with both low and intermediate/high fibrosis scores.
- The association of AFLD and NAFLD with prevalent CAC remained significant after adjustment for CVD risk factors.

How might it impact on clinical practice in the foreseeable future?

- AFLD and NAFLD are histologically similar liver diseases and clinicians need to be aware that both liver diseases are similarly associated with increased risk of subclinical early coronary atherosclerosis.
- Preventive measures are required to ameliorate CVD risk in both NAFLD and AFLD.

Introduction

Alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD) are two major types of fatty liver disease (FLD) with similar histologic features [1]. FLD ranges from simple steatosis to steatohepatitis that can progress to fibrosis, cirrhosis, liver failure, or hepatocellular carcinoma. With the global increase in obesity and type 2 diabetes, FLD is becoming one of the most common liver disorders worldwide [1, 2, 3]. While NAFLD and AFLD are each associated with significant morbidity, impaired health-related quality of life, and use of health care resources [4], most recent studies have focused on NAFLD and have excluded participants with AFLD.

Whilst many studies have reported the association of NAFLD with increased risk of clinical and subclinical cardiovascular disease (CVD) [5, 6], the impact of AFLD on CVD as an extrahepatic complication has received little attention [4, 7] and there are few studies comparing the association of NAFLD and AFLD with CVD risk [8, 9]. Recent evidence has suggested that in patients with severe AFLD or severe NAFLD, that necessitated hospital admission or was identified as the specific cause of death, there was a greater risk of CVD mortality with ALD than with NAFLD [10].

Coronary artery calcium (CAC) scoring using computed tomography (CT) is a useful and reliable marker of early coronary atherosclerosis, and CAC correlates well with total coronary atherosclerotic burden [11, 12]. CAC scores reflect the long-term impact of CVD risk factors and CAC scores predict future CVD events [11, 13].

To investigate whether subjects with early liver disease from AFLD and NAFLD, have similar (or dissimilar) risk of early coronary atherosclerosis, we have investigated the associations between AFLD and NAFLD, identified in subjects in a large Korean occupational cohort, and the presence of coronary artery calcium, measured by high

resolution computed tomography. Since it has been shown that even very modest alcohol consumption interacts with obesity to markedly increase the risk of cirrhosis [14, 15], we have also evaluated whether or not the association between FLD and CAC differs by the presence of obesity, severity of hepatic steatosis (assessed by ultrasonography), and degree of hepatic fibrosis (using non- invasive biomarkers for liver fibrosis). For comparison, we have also investigated associations between excess alcohol consumption (EAC) and CAC scores in the absence of FLD.

Methods

Study population

The Kangbuk Samsung Health Study (KSHS) is a cohort study of Korean men and women aged 18 years or over who underwent a comprehensive health examination annually or biennially at Kangbuk Samsung Hospital Total Healthcare Centers in Seoul and Suwon, South Korea [8]. This study population consisted of a subset of KSHS participants who underwent cardiac CT to measure CAC scores as part of a comprehensive health exam from 2011 to 2017 (N = 123,776). CAC scoring has become a common CVD screening test in Korea. Over 80% of participants were employees of various companies and local governmental organizations and their spouses. In South Korea, the Industrial Safety and Health Law requires annual or biennial health screening exams of all employees offered free of charge. The remaining participants were people voluntarily taking screening exams.

For the current cross-sectional study, we excluded 18,448 subjects for the following criteria: missing information on ultrasonography, alcohol consumption, and important covariates including body mass index (BMI), glucose, blood pressures, high density lipoprotein cholesterol (HDL-C), triglycerides, HOMA-IR, and high sensitivity C-reactive protein

(hsCRP) (n=9035), history of CVD (n=1,605), history of malignancy (n=3261), known liver disease or current use of medications for liver disease or positive serologic markers for hepatitis B or C virus (N = 5421), history of liver cirrhosis or findings of liver cirrhosis on ultrasound (N = 61), and use of steatogenic medications within the past year, such as valproate, amiodarone, methotrexate, tamoxifen, or corticosteroids (N=612) [2]. Some participants met more than one exclusion criteria, leaving 105,328 participants included in the final analysis (Figure 1).

The study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (IRB No. KBSMC 2018-01-018), which waived the requirement for informed consent as only de-identified data obtained as part of routine health screening exams were used.

Measurements

Data on demographic characteristics, lifestyle factors, education level, medical history, and family history of CVD were collected by standardized, self-administered questionnaires [8]. The questionnaire asked about the frequency of alcohol drinking and the amount of alcohol consumed per drinking day recorded in standard units [16]. Average alcohol consumption per day was calculated using the frequency and amount of beverages consumed per drinking day. Excessive alcohol consumption (EAC) was defined as average alcohol intake ≥30 g/day for men and ≥20 g/day for women [2]. Smoking status was categorized as never, former, or current smoker. Physical activity was assessed using the validated Korean version of the International Physical Activity Questionnaire (IPAQ) short form.[17] Participants were classified into inactive, minimally active, or health-enhancing physical activity (HEPA). HEPA was defined as physical activity that meets either of two criteria: (i) vigorous intensity activity on three or more days per week accumulating ≥1500 MET

min/week, or (ii) seven days with any combination of walking, moderate intensity, or vigorous intensity activities achieving at least 3000 MET min/week. History of CVD was defined as participants who reported physician-diagnosed CVD including angina/myocardial infarction and stroke (ischemic or hemorrhagic). Typical dietary consumption was assessed using a 103-item self-administered food frequency questionnaire (FFQ) designed and validated for use in Korea [18].

Height and weight were measured by trained nurses. Obesity was defined as BMI ≥25 kg/m^2 according to Asian-specific criteria [19]. Waist circumference was measured by trained personnel to the nearest 0.1 cm at the midpoint between the bottom of the rib cage and the top of the iliac crest with the subjects standing, their weight equally distributed on both feet, their arms at their sides, and head facing straight forward. We had waist circumference measurements in about 95 % (N=99,729) of participants (because one of the two study centers did not start measuring waist circumference until after 2012). Blood pressure (BP) was measured using an automated oscillometric device (53000, Welch Allyn, New York, USA) by trained nurses while examinees were in a sitting position with the arm supported at heart level. Three readings were recorded, and the average of the second and third readings was used in analysis. Hypertension was defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or the use of antihypertensive medications.

Blood specimens were sampled from the antecubital vein after at least 10 hours of fasting. Blood tests included total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides (TG), aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), serum albumin, platelet count, glucose, insulin and hsCRP. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (mg/dL) * fasting glucose (mg/dL) / 405. Diabetes mellitus was defined as

fasting serum glucose ≥126 mg/dL, A1c ≥6.5% (48mmol/mol), or use of blood glucose-lowering agents.

Ascertainment of fatty liver disease and non-invasive fibrosis indices

The diagnosis of fatty liver was based on abdominal ultrasound (US) operated by experienced radiologists who were blinded to the aim of the present study. Ultrasonographic diagnosis of fatty liver was determined based on standard criteria, including a diffuse increase of fine echoes in the liver parenchyma compared with kidney or spleen parenchyma, deep beam attenuation, and bright vessel walls [20]. Inter-observer and intra-observer reliability for fatty liver diagnosis was substantial (kappa statistic of 0.74) and excellent (kappa statistic of 0.94), respectively [21]. Severity of hepatic steatosis was also recorded as mild, moderate or severe steatosis on sonography. Degree of hepatic steatosis was categorized into mild and moderate/severe steatosis since the number of severe steatosis was small and combined with moderate steatosis. Of 42,701 participants with a diagnosis of fatty liver, 1.6% (N=685) did not have information available on severity of hepatic steatosis.

NAFLD was defined as the presence of fatty liver in the absence of EAC. AFLD was defined as the presence of FLD in the presence of EAC. Other identifiable causes of secondary hepatic steatosis other than alcohol were excluded, as described earlier in the exclusion criteria.

For further FLD categorization, two fibrosis scoring indices were used. The fibrosis-4 (FIB-4) index was calculated by the following formula: FIB-4 = (age (years) × AST (U/L)) / (platelet count ($\times 10^9$/L) × ALT (U/L)$^{1/2}$) [22]. Cut-off values for low, intermediate and high probability of advanced fibrosis were <1.30, 1.30-<2.67, and ≥2.67, respectively [23]. The FIB-4 index has been validated for use in assessing fibrosis stage in patients with both

alcoholic liver disease and NAFLD [7, 22]. For sensitivity analysis, the aspartate transaminase to platelet ratio index (APRI) was used as a noninvasive fibrosis index and was calculated by the following formula: APRI=100 × (AST/upper limit of normal) / platelet count ($\times 10^9$/L). Cut-offs for low and high probability of advanced fibrosis were 0.5 and 1.5, respectively [24, 25].

Measurement of CAC by multidetector CT

CAC was detected with a Lightspeed VCT XTe-64 slice MDCT scanner (GE Healthcare, Tokyo, Japan) in both Seoul and Suwon centers using the same standard scanning protocol of 2.5-mm thickness, 400-ms rotation time, 120-kV tube voltage, and 124-mAS (310 mA × 0.4 seconds) tube current under ECG-gated dose modulation. CAC scores were calculated as previously described by Agatston et al. [26]. The inter-observer reliability and intra-observer reliability for CAC scores were both excellent (intra-class correlation coefficient of 0.99) [8] . CAC scores were categorized as 0, 1–100, and >100 [27].

Statistical analysis

Participants were categorized into 4 groups: 1) no EAC and no FLD (reference category); 2) EAC and no FLD; 3) NAFLD; and 4) AFLD. Descriptive statistics were used to summarize the characteristics of participants by FLD categories.

To assess the relationship of the presence of CAC with FLD categories, a logistic regression model was used to estimate the odds ratios (OR) with a 95% confidence intervals (CI) for the presence of CAC comparing EAC and no FLD, NAFLD, and AFLD to the reference category (no EAC and no FLD). We used three models with progressive adjustments: model 1 was initially adjusted for age and sex and then model 2 was further

adjusted for study center (Seoul or Suwon), year of screening examination (one-year categories), BMI, smoking status (never, past, current, or unknown), physical activity (inactive, minimally active, HEPA or unknown), educational level (high school graduate or less, community college or university graduate, graduate school or higher, and unknown), total calorie intake (in quintile or missing), family history of CVD (yes, no or unknown), diabetes, hypertension, LDL-C and medication for dyslipidemia (yes, no or unknown). To assess whether the relationship between FLD categories and the presence of CAC is mediated by inflammation or insulin resistance, model 3 was further adjusted for hsCRP, and HOMA-IR in addition to the variables included in models 1 and 2. We evaluated whether or not the associations between FLD categories and the presence of CAC differ by the presence of obesity since the prognostic implications of non-obese FLD remains unclear [28].

Additionally, NAFLD and AFLD were further categorized into low and intermediate/high FIB-4 scores according to the degree of fibrosis based on FIB-4 index because fibrosis is the most important histologic predictor of liver and non-liver related mortality [29, 30]. Since few subjects were identified with FLD and high probability of advanced fibrosis, intermediate and high probability of advanced fibrosis were combined. The association of NAFLD and AFLD with the presence of CAC according to degree of fibrosis based on FIB-4 index was evaluated compared to the reference category. The association between fibrosis severity based on APRI and the presence of CAC was also evaluated. We also performed analysis on the association of FLD categories with presence of CAC by degree of hepatic steatosis on ultrasonography. Degree of hepatic steatosis was categorized into mild and moderate/severe steatosis since the number of severe steatosis was small and combined with moderate steatosis. Information on alcohol intake and physician-diagnosed CVD was collected before ultrasound and CAC measurements. When we categorized FLD into AFLD

and NAFLD, we assumed that persons with already recognized CVD may be more likely to abstain from alcohol as a result of their illness. Thus, in the main analysis, we excluded individuals who reported CVD. We also performed a further analysis in including participants with a history of CVD.

In sensitivity analyses, we also estimated the prevalence ratios and 95% CIs for CAC score 1 – 100 and >100 for EAC and no FLD, NAFLD, and AFLD compared to the reference category (no EAC and no FLD) using participants with CAC 0 as the reference group in multinomial logistic regression models. In another sensitivity analysis, to evaluate the association between FLD categories and CAC as a continuous variable, we used a Tobit regression model for natural log (CAC score +1) with Huber-White estimation of standard errors [8, 31]. Tobit models were used to estimate ratios and 95% CI of CAC score +1 by comparing EAC and no FLD, NAFLD, and AFLD to the reference category (no EAC and no FLD). Estimates of the Tobit models were presented as exponentiated Tobit regression coefficients (CAC score ratios) approximately representing the relative CAC score increment comparing EAC and no FLD, NAFLD and AFLD to the reference category (no EAC and no FLD). For example, a CAC ratio of 1.50 is interpreted as a 50% increase in the CAC score for a specific category compared to the reference category.

Subgroup analyses were conducted according to age group (<40 vs. ≥40 years of age), sex (men vs. women), current smoking (No vs. Yes), physical activity (no HEPA vs. HEPA), HOMA-IR (<2.5 vs. ≥ 2.5), and hs-CRP (<1.0 vs. ≥ 1.0 mg/l). Interactions by subgroups were tested using likelihood ratio tests comparing models with and without multiplicative interaction terms.

Finally, we evaluated a prospective association of NAFLD and AFLD with CAC progression. This analysis included all study participants who had baseline and at least one

follow-up cardiac CT to measure CAC scores between 2011 and 2017 (n = 23,320). Study participants have been recruited continuously into the study since 2011 and many of the participants recruited in more recent years did not have a second CAC score measurement included in the dataset we used. As a consequence, only 23,320 participants (21.1%) had a follow-up CAC score and were included in the investigation of the prospective association of FLD with CAC progression. We used linear mixed models with random intercepts and random slopes [32] to estimate CAC scores and their progression over time adjusting for baseline potential confounders. Since CAC scores were markedly right-skewed, we transformed the scores into $\log_e$ (CAC + 1) as the outcome. Annual progression rate with 95% CIs was estimated while comparing EAC and no FLD, NAFLD, and AFLD to the reference category (no EAC and no FLD).

Statistical analysis was performed using STATA version 15.0 (StataCorp LP, College Station, TX, USA). All reported P-values were two tailed, and comparisons with $P < 0.05$ were considered statistically significant.

Results

The mean age (standard deviation) and mean BMI (SD) of 105,328 participants were 40.8 years (7.8) and 24.4 kg/m^2 (3.3), respectively, and 77.5 percent of participants were male (Table 1). The prevalence of EAC and no FLD, NAFLD and AFLD were 9.6%, 32.6%, and 7.9%, respectively. EAC with no FLD and AFLD were positively associated with current smoking. NAFLD and AFLD were positively associated with diabetes, hypertension, obesity, and higher levels of BMI, BP, total cholesterol, LDL-C, glucose, triglycerides, AST, ALT, HOMA-IR, and hsCRP, and inversely associated with HDL-C. GGT level was higher in EAC and no FLD, NAFLD, and ALFD than in the reference category (no EAC and no FLD) with

the highest level of GGT in AFLD. The prevalence of CAC score >0 was 12.3% overall, and its prevalence was progressively higher across FLD categories.

Table 2 shows the relationship between FLD categories and the presence of detectable CAC (>0) overall and in the non-obese and obese groups separately. Both types of FLD, including NAFLD and AFLD, were positively associated with the presence of CAC. After adjusting for age, sex, screening center, year of screening examination, smoking status, physical activity, educational level, total calorie intake, BMI, family history of CVD, diabetes, hypertension, LDL-C and medication for dyslipidemia, multivariable-adjusted OR (95% CIs) for detectable CAC comparing EAC with no FLD, NAFLD, and AFLD to the reference category were 1.25 (1.16-1.35), 1.10 (1.05-1.16), and 1.20 (1.11-1.30), respectively. AFLD was associated with higher CAC than NAFLD. In post hoc analysis, OR (95% CI) for detectable CAC comparing AFLD to NAFLD was 1.09 (1.01-1.17) ($p = 0.021$). In analyses with adjustment for waist circumference instead of BMI, we found similar results (Supplementary table 1).

The associations between FLD categories and the presence of CAC tended to be slightly stronger in the non-obese than in the obese and although there was a trend towards there being a significant difference by obesity status, these associations did not reach significance (P for interaction=0.088, Table 2) even though obese FLD subjects showed unfavorable profiles of metabolic risk factors compared to non-obese FLD subjects (Supplementary Table 2). For the non-obese group, multivariable-adjusted OR (95% CIs) for detectable CAC comparing EAC with no FLD, NAFLD, and AFLD to the reference category were 1.31 (1.19-1.44), 1.10 (1.02-1.18) and 1.25 (1.10-1.43), respectively, while for the obese group, corresponding OR (95% CIs) were 1.11 (0.98-1.27), 1.06 (0.98-1.15), and 1.14 (1.02-1.26), respectively.

Similarly, in sensitivity analysis using multinomial regression model, the multivariable-

adjusted prevalence ratios comparing EAC with no FLD, NAFLD, and AFLD to the reference category were 1.24 (1.14-1.34), 1.11 (1.05-1.18), and 1.21 (1.11-1.31) for CAC score 1 – 100 and 1.32 (1.12-1.56), 1.07 (0.95-1.21), and 1.21 (1.03-1.43) for CAC score >100, respectively (Supplementary Table 3). In sensitivity analysis using Tobit regression model, multivariable-adjusted CAC score ratios (95% CIs) comparing EAC with no FLD, NAFLD, and AFLD to the reference category were 1.68 (1.42-1.98), 1.22 (1.09-1.37), and 1.54 (1.30-1.83), respectively (Supplementary Table 4).

To explore whether the association between FLD categories and the presence of CAC was mediated by inflammation and insulin resistance, additional analyses adjusting for hsCRP, and HOMA-IR were performed (Table 2, model 3). The association of both NAFLD and AFLD with the prevalent CAC remained statistically significant. When we performed a further analysis in including participants with a history of CVD, results were similar to those of the analyses excluding participants with a history of CVD (Supplementary table 5).

Table 3 shows the association of FLD categories with presence of CAC according to degree of fibrosis based on FIB-4 index. Compared with the reference category (no EAC and no FLD), multivariable adjusted OR (95% CIs) for detectable CAC in low and intermediate/high FIB-4 among NAFLD cases were 1.09 (1.03-1.15) and 1.14 (1.01-1.29), respectively, whereas corresponding OR (95% CIs) among AFLD cases were 1.17 (1.08-1.27) and 1.37 (1.16-1.63), respectively. After further adjustment for hsCRP, and HOMA-IR, the association between fibrosis scores and presence of CAC remained statistically significant in both NAFLD and AFLD groups. In a sensitivity analysis using the aspartate transaminase to platelet ratio index (APRI), the associations between FLD and presence of CAC were similarly observed (Supplementary Tables 6 and 7).

Table 4 shows the association of FLD categories with presence of CAC according to

severity of hepatic steatosis on ultrasonography. Compared with the reference category (no EAC and no FLD), multivariable adjusted OR (95% CIs) for detectable CAC in mild and moderate/severe steatosis among NAFLD cases were 1.09 (1.03-1.15) and 1.14 (1.01-1.29), respectively, whereas corresponding OR (95% CIs) among AFLD cases were 1.17 (1.08-1.27) and 1.37 (1.16-1.63), respectively.

In subgroup analyses other than obesity (Supplementary Table 8), the association between FLD categories and CAC scores was stronger in younger individuals (age <40 years) (vs. age ≥40 years; P for interaction < 0.001). Otherwise, the associations between FLD categories and CAC scores were similar across participant subgroups with no significant interactions by sex (men vs. women), current smoking (No vs. Yes), physical activity (no HEPA vs. HEPA), HOMA-IR (<2.5 vs. ≥ 2.5), and hs-CRP (<1.0 vs. ≥ 1.0 mg/l).

Finally, we evaluated a prospective association of NAFLD and AFLD with CAC progression among 23,320 participants with baseline and follow-up cardiac CT (Table 5). The median duration of follow-up was 3.0 years (interquartile range 2.0-4.2, maximum 6.7). The annual rates of CAC progression (95% CI) in no EAC and no FLD, EAC and no FLD, NAFLD, and AFLD were 5.1%, 8.2%, 9.2% and 12.3 %, respectively. The multivariable adjusted ratio of progression rates comparing EAC and no FLD, NAFLD, and AFLD to the reference category (no EAC and no FLD) were 1.03 (1.02-1.04), 1.04 (1.03-1.05) and 1.07 (1.06-1.08), respectively. These associations were similar in non-obese and obese individuals. Further adjustment for HOMA-IR and hsCRP did not change the result.

Discussion

In this large-scale study of 113,263 apparently healthy young and middle-aged men and women, both NAFLD and AFLD were significantly associated with a higher risk of prevalent

subclinical coronary atherosclerosis compared to the reference (no EAC and no FLD). This association was observed in non-obese individuals, indicating that non-obese NAFLD and AFLD are also associated with a higher risk of atherosclerosis. The risk of subclinical atherosclerosis in FLD was also observed with mild and moderate/severe hepatic steatosis and with both low and higher degrees of fibrosis. Our data suggest that there was a slightly stronger association between AFLD and CAC than between NAFLD and CAC [see Table 2, compared with NAFLD, OR (95% CIs) for AFLD and CAC was 1.09 (1.01-1.17) (p = 0.021)].

A slightly stronger risk of atherosclerosis with AFLD than with NAFLD seen in our study might reflect the fact that subjects in our cohort with AFLD have more advanced liver disease than subjects with NAFLD. Such speculation is supported by the recent evidence from a meta-analysis investigating the association between NAFLD and incident CVD [5]. In this meta-analysis, the OR (95%CIs) for the association between more severe NAFLD and incident CVD events was 2.58 (1.78, 3.75), compared with 1.64 (1.26, 2.13) for the association between overall NAFLD and incident CVD. Similarly, a long-term follow-up study of patients with biopsy-proven NAFLD demonstrated an increased risk of CVD death in those with advanced fibrosis [33]. In our study, there was limited power to study associations between liver fibrosis and CAC scores in subjects with AFLD and NAFLD, as very few subjects had advanced fibrosis. However, our data using FIB-4 or APRI scores show that there was a trend towards higher risk for prevalent atherosclerosis in subjects with evidence of liver fibrosis.

There are limited studies regarding the impact of AFLD on CVD although multiple studies have reported the association of NAFLD with clinical and subclinical CVD [4, 5, 7]. A recent cohort study reported that in patients with type 2 diabetes who had severe AFLD or severe

NAFLD (necessitating hospital admission or causing death), there was a greater risk of CVD mortality with ALD than with NAFLD [10]. An earlier cross-sectional study of 265 patients with early liver disease showed higher carotid intima-media thickness, in both AFLD and NAFLD patients compared with the reference (no FLD without alcohol history) but this study design was limited by lack of adjustment for confounders [34]. Another cross-sectional study of 10,710 participants involved in a health checkup program demonstrated that the estimated 10-year coronary heart disease risk based on Framingham risk scores was similarly higher in the AFLD and NAFLD groups compared to the no fatty liver group [8]. In our study, individuals with AFLD showed a higher prevalence of unhealthy behaviors and CVD risk factors but whether these behaviors or risk factor mediate an increase in risk of subclinical atherosclerosis is uncertain. Adjustment for those factors attenuated the association between AFLD and CAC scores, but these associations remained significant with AFLD, suggesting that AFLD, like NAFLD, is a metabolic liver disease that is associated with increased risk of CVD risk.

The mechanisms linking hepatic steatosis with atherosclerosis or CVD are not yet fully elucidated. Ectopic accumulation of fat in the liver can be an indicator of lipid overload [35] and has been strongly associated with both hepatic and systemic insulin resistance [36]. Hepatic steatosis has also been reported to be associated with individual CVD risk factors including diabetes, hypertension, impaired fasting glucose, low HDL-C, and hypertriglyceridemia, in accordance with our findings [37, 38]. However, the association of hepatic steatosis with subclinical atherosclerosis was not fully explained by those risk factors in our study. Indeed, hepatic steatosis is likely to be implicated in the interplay between insulin resistance, abnormal lipoprotein metabolism, low-grade inflammation, oxidative stress, and unfavorable adipokine profiles [37, 38]. Hepatic steatosis has also been closely

associated with altered secretory patterns of hepatokines and pro-atherogenic factors such as fibrinogen, plasminogen activator inhibitor-1, and other proinflammatory cytokines, all of which promote atherosclerosis [37].

In the present study, a positive association between FLD category and prevalent CAC was more evident in individuals younger than 40 years (Supplementary Table 6) than in the older age group. The reasons for this finding suggests that FLD may be more important contributor to subclinical atherosclerosis in younger than older populations. This is consistent with increasing prevalence of other CVD risk factors in older age groups. Due to the use of multiple comparisons, chance might be another possible explanation for the observed difference across subgroups.

We note that our study has some limitations. First, fatty liver was determined using US, which is less sensitive (60-90%) when hepatic fat infiltration is below approximately 30% [39], but is widely used both clinically and in population-based studies due to its non-invasive nature and acceptable degree of diagnostic accuracy for steatosis [39]. Additionally, in our study, there was limited power to study associations between liver fibrosis and CAC scores in subjects with AFLD and NAFLD, as very few subjects had evidence of advanced fibrosis from these scores. Second, behavioral factors such as smoking and alcohol use were assessed via a self-administered structured questionnaire used in health checkup programs in Korea as part of the National Health Insurance plan [40]. Measurement errors of these variables might introduce some degree of residual confounding, similar to most epidemiologic studies. Finally, our results derived from a sample of relatively healthy young and middle-aged educated Koreans who participated in a health check-up program and might not be generalizable to other ages and ethnic populations. However, our study population was mainly composed of healthy employees and their spouses without clinically manifest CVD,

minimizing the possibility of reverse causation and being less likely to be affected by biases related to comorbidities compared to studies conducted in higher risk populations.

Conclusion

In this large sample of young and middle-aged individuals, an increased risk of prevalent subclinical atherosclerosis was found not only in NAFLD but also in AFLD. These associations were observed in non-obese and obese individuals, with mild and moderate/severe steatosis and with both low and intermediate/high fibrosis scores. Our findings suggest that AFLD is also a metabolic liver disease associated with increased risk of subclinical coronary atherosclerosis.

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References

1 Mills SJ, Harrison SA. Comparison of the natural history of alcoholic and nonalcoholic fatty liver disease. Curr Gastroenterol Rep 2005;**7**:32-6.

2 Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K*, et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012;**142**:1592-609.

3 Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. J Hepatol 2013;**59**:160-8.

4 Younossi Z, Henry L. Contribution of Alcoholic and Nonalcoholic Fatty Liver Disease to the Burden of Liver-Related Morbidity and Mortality. Gastroenterology 2016;**150**:1778-85.

5 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**:589-600.

6 Sinn DH, Kang D, Chang Y, Ryu S, Gu S, Kim H*, et al.* Non-alcoholic fatty liver disease and progression of coronary artery calcium score: a retrospective cohort study. Gut 2017;**66**:323-9.

7 Toshikuni N, Tsutsumi M, Arisawa T. Clinical differences between alcoholic liver disease and nonalcoholic fatty liver disease. World J Gastroenterol 2014;**20**:8393-406.

8 Chang Y, Kim BK, Yun KE, Cho J, Zhang Y, Rampal S*, et al.* Metabolically-healthy obesity and coronary artery calcification. Journal of the American College of Cardiology 2014;**63**:2679-86.

9 Kim JH, Kim SY, Jung ES, Jung SW, Koo JS, Kim JH*, et al.* Carotid intima-media thickness is increased not only in non-alcoholic fatty liver disease patients but also in alcoholic fatty liver patients. Digestion 2011;**84**:149-55.

10 Wild SH, Walker JJ, Morling JR, McAllister DA, Colhoun HM, Farran B*, et al.* Cardiovascular Disease, Cancer, and Mortality Among People With Type 2 Diabetes and Alcoholic or Nonalcoholic Fatty Liver Disease Hospital Admission. Diabetes Care 2018;**41**:341-7.

11 Budoff MJ, Achenbach S, Blumenthal RS, Carr JJ, Goldin JG, Greenland P*, et al.* Assessment of coronary artery disease by cardiac computed tomography: a scientific statement from the American Heart Association Committee on Cardiovascular Imaging and Intervention, Council on Cardiovascular Radiology and Intervention, and Committee on Cardiac Imaging, Council on Clinical Cardiology. Circulation 2006;**114**:1761-91.

12 Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA*, et al.* Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. Journal of the American College of Cardiology 1998;**31**:126-33.

13 Keelan PC, Bielak LF, Ashai K, Jamjoum LS, Denktas AE, Rumberger JA*, et al.* Long-term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. Circulation 2001;**104**:412-7.

14 Hart CL, Morrison DS, Batty GD, Mitchell RJ, Davey Smith G. Effect of body mass index

and alcohol consumption on liver disease: analysis of data from two prospective cohort studies. BMJ 2010;**340**:c1240.
15 Liu B, Balkwill A, Reeves G, Beral V, Million Women Study C. Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ 2010;**340**:c912.
16 Park JT, Kim BG, Jhun HJ. Alcohol consumption and the CAGE questionnaire in Korean adults: results from the Second Korea National Health and Nutrition Examination Survey. J Korean Med Sci 2008;**23**:199-206.
17 Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE*, et al.* International physical activity questionnaire: 12-country reliability and validity. Medicine and science in sports and exercise 2003;**35**:1381-95.
18 Ahn Y, Kwon E, Shim JE, Park MK, Joo Y, Kimm K*, et al.* Validation and reproducibility of food frequency questionnaire for Korean genome epidemiologic study. European journal of clinical nutrition 2007;**61**:1435-41.
19 World Health Organization, Regional Office for the Western Pacific. The Asia-Pacific perspective: redefining obesity and its treatment. Sydney: Health Communications Australia, 2000.
20 Mathiesen UL, Franzen LE, Aselius H, Resjo M, Jacobsson L, Foberg U*, et al.* Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. Dig Liver Dis 2002;**34**:516-22.
21 Ryu S, Chang Y, Jung HS, Yun KE, Kwon MJ, Choi Y*, et al.* Relationship of sitting time and physical activity with non-alcoholic fatty liver disease. J Hepatol 2015;**63**:1229-37.
22 McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 2010;**59**:1265-9.
23 Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ*, et al.* Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;**7**:1104-12.
24 Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS*, et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;**38**:518-26.
25 Lombardi R, Buzzetti E, Roccarina D, Tsochatzis EA. Non-invasive assessment of liver fibrosis in patients with alcoholic liver disease. World J Gastroenterol 2015;**21**:11044-52.
26 Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. Journal of the American College of Cardiology 1990;**15**:827-32.
27 Martin SS, Blaha MJ, Blankstein R, Agatston A, Rivera JJ, Virani SS*, et al.* Dyslipidemia, coronary artery calcium, and incident atherosclerotic cardiovascular disease: implications for statin therapy from the multi-ethnic study of atherosclerosis. Circulation 2014;**129**:77-86.

28 Kim D, Kim WR. Nonobese Fatty Liver Disease. Clin Gastroenterol Hepatol 2017;**15**:474-85.
29 Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P*, et al.* Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2015;**149**:389-97 e10.
30 Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z*, et al.* Increased risk of mortality by fibrosis stage in non-alcoholic fatty liver disease: Systematic Review and Meta-analysis. Hepatology 2017.
31 Reilly MP, Wolfe ML, Localio AR, Rader DJ. Coronary artery calcification and cardiovascular risk factors: impact of the analytic approach. Atherosclerosis 2004;**173**:69-78.
32 Gassett AJ, Sheppard L, McClelland RL, Olives C, Kronmal R, Blaha MJ*, et al.* Risk Factors for Long-Term Coronary Artery Calcium Progression in the Multi-Ethnic Study of Atherosclerosis. Journal of the American Heart Association 2015;**4**:e001726.
33 Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stal P, Kechagias S*, et al.* Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;**61**:1547-54.
34 Kim JH, Kim SY, Jung ES, Jung SW, Koo JS, Kim JH*, et al.* Carotid intima-media thickness is increased not only in non-alcoholic fatty liver disease patients but also in alcoholic fatty liver patients. Digestion 2011;**84**:149-55.
35 Liu Q, Bengmark S, Qu S. The role of hepatic fat accumulation in pathogenesis of non-alcoholic fatty liver disease (NAFLD). Lipids in health and disease 2010;**9**:42.
36 Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. The Journal of clinical endocrinology and metabolism 2006;**91**:4753-61.
37 Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? Eur Heart J 2012;**33**:1190-200.
38 Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2015;**62**:S47-64.
39 Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E*, et al.* Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;**54**:1082-90.
40 Chang Y, Ryu S, Choi Y, Zhang Y, Cho J, Kwon MJ*, et al.* Metabolically Healthy Obesity and Development of Chronic Kidney Disease: A Cohort Study. Annals of internal medicine 2016;**164**:305-12.

Table 1. Baseline characteristics according to fatty liver categories

Characteristics	Overall	Categories of fatty liver			
		No excessive alcohol intake and no FLD	Excessive alcohol intake and no FLD	NAFLD	AFLD
Number	105,328	52,529	10,098	34,382	8,319
Age (years)*	40.8 (7.8)	40.3 (7.9)	40.8 (7.9)	41.1 (7.7)	42.0 (7.5)
Male (%)	77.5	64.7	88.6	89.1	97.4
Current smoker (%)	28.6	20.6	44.6	30.9	49.6
HEPA (%)	15.6	16.5	19.6	13.0	16.0
High education level (%)[c]	83.8	83.8	76.4	86.8	80.8
Obesity (%)[d]	39.5	19.6	31.4	64.3	72.2
Diabetes (%)	4.8	1.9	3.5	7.9	11.7
Hypertension (%)	15.3	9.1	17.0	20.5	30.9
Family history of CVD (%)	12.3	12.0	13.0	12.3	13.1
Body mass index (kg/m^2)	24.4 (3.3)	22.8 (2.7)	23.9 (2.6)	26.3 (3.1)	26.8 (3.0)
Waist circumference (cm) [e]	84.9 (9.2)	80.3 (7.8)	84.0 (7.3)	90.4 (7.8)	92.1 (7.6)
Systolic BP (mmHg)[a]	112.4 (12.4)	108.7 (11.8)	114.5 (11.8)	115.8 (11.7)	119.7 (11.9)
Diastolic BP (mmHg)[a]	73.0 (9.8)	70.0 (9.2)	75.0 (9.6)	75.4 (9.4)	79.0 (9.7)
Glucose (mg/dl)[a]	97.4 (15.9)	93.9 (10.9)	97.6 (13.2)	100.8 (19.1)	105.6 (23.0)
Total cholesterol (mg/dl)[a]	199.4 (34.6)	193.5 (32.6)	198.1 (33.4)	206.2 (35.7)	210.2 (36.8)
LDL-C (mg/dl)[a]	129.0 (32.2)	122.9 (30.5)	124.2 (31.3)	138.0 (32.3)	136.5 (33.2)
HDL-C (mg/dl)[a]	55.2 (14.5)	59.8 (14.7)	60.0 (14.9)	48.0 (10.9)	49.9 (12.0)
Albumin (g/dL)[a]	4.7 (0.2)	4.6 (0.2)	4.7 (0.2)	4.7 (0.2)	4.7 (0.2)
Platelet ($\times 10^9/L$)[a]	246.2 (50.2)	243.8 (50.4)	242.7 (48.3)	251.1 (50.6)	244.9 (47.5)
Triglycerides (mg/dl)[b]	111 (77-163)	88 (65-122)	109 (78-154)	145 (105-202)	166 (118-237)
AST (U/l)[b]	20 (17-25)	18 (16-22)	21 (18-25)	23 (19-29)	25 (20-32)
ALT (U/l)[b]	21 (15-32)	17 (13-23)	20 (15-27)	30 (21-45)	32 (23-46)
GGT (U/l)[b]	26 (17-44)	19 (14-28)	34 (22-56)	35 (24-54)	55 (36-88)
HOMA-IR[b,]	1.43 (0.95-2.14)	1.15 (0.79-1.64)	1.19 (0.82-1.73)	1.98 (1.38-2.86)	2.04 (1.41-2.95)

hsCRP (mg/l)[b,]	0.5 (0.3-1.0)	0.4 (0.2-0.7)	0.4 (0.3-0.8)	0.7 (0.4-1.4)	0.7 (0.4-1.4)
Fib4 [a]	0.81 (0.37)	0.82 (0.36)	0.86 (0.40)	0.76 (0.34)	0.87 (0.44)
APRI [a]	0.26 (0.18)	0.23 (0.15)	0.25 (0.17)	0.28 (0.20)	0.32 (0.23)
Total energy intake (kcal/d)[b,f]	1473.8 (1118.7-1865.1)	1415.6 (1065.8-1796.1)	1514.6 (1137.4-1926.0)	1512.8 (1169.0-1907.8)	1619.0 (1243.4-2034.6)
CAC score >0 (%)	12.3	8.5	14.7	15.3	20.7
CAC score 1-100 (%)	10.2	7.2	12.1	12.7	16.8
CAC score >100 (%)	2.1	1.4	2.6	2.5	3.9
CAC score [g]	19 (5-62)	18 (5-58)	21 (7-69)	18 (5-61)	22 (6-71)
FRS>10(%)	11.7	5.5	12.6	16.7	28.5

Data are expressed as [a]mean (standard deviation), [b]median (interquartile range), or percentage.

Abbreviations: AFLD, alcoholic fatty liver disease; ALT, alanine aminotransferase; APRI, aspartate transaminase to platelet ratio index; AST, aspartate aminotransferase; BP, blood pressure; FIB-4, fibrosis-4; GGT, gamma-glutamyl transferase ; FLD, fatty liver disease; FRS, Framingham risk score; HDL-C, high-density lipoprotein-cholesterol; HEPA, health-enhancing physical activity; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonal0.26coholic fatty liver disease.

[c]≥ College graduate; [d]BMI ≥25 kg/m^2;

[e] among 99,729 participants with available waist circumference; [f] among 71,521 participants with plausible estimated energy intake levels (within three standard deviations from log-transformed mean energy intake); [g]among 12,933 participants with CAC score >0

Table 2. Association between fatty liver categories and coronary artery calcification

	Categories of fatty liver			
	No excessive alcohol intake and no FLD	Excessive alcohol intake and no FLD	NAFLD	AFLD
Total				
Number	52,529	10,098	34,382	8,319
CAC score >0 (%)	4,479 (8.5)	1,484 (14.7)	5,249 (15.3)	1,721 (20.7)
Adjusted ORs (95% CIs)[a]				
Model 1	1.00 (reference)	1.40 (1.31-1.50)	1.56 (1.49-1.64)	1.90 (1.78-2.04)
Model 2	1.00 (reference)	1.25 (1.16-1.35)	1.10 (1.05-1.16)	1.20 (1.11-1.30)
Model 3	1.00 (reference)	1.25 (1.16-1.35)	1.10 (1.05-1.16)	1.20 (1.11-1.30)
Non-obese (BMI <25 kg/m^2)				
Number	50,954	8,465	16,279	3,051
CAC score >0 (%)	5,382 (10.6)	1,449 (17.1)	2,988 (18.4)	768 (25.2)
Adjusted ORs (95% CIs)[a]				
Model 1	1.00 (reference)	1.45 (1.33-1.58)	1.38 (1.29-1.48)	1.77 (1.57-2.00)
Model 2	1.00 (reference)	1.31 (1.19-1.44)	1.10 (1.02-1.18)	1.25 (1.10-1.43)
Model 3	1.00 (reference)	1.31 (1.19-1.44)	1.11 (1.03-1.20)	1.27 (1.11-1.45)
Obese (BMI ≥ 25 kg/m^2)				
Number	13,282	4,102	30,172	8,068
CAC score >0 (%)	2,178 (16.4)	909 (22.2)	6,336 (21.0)	2,108 (26.1)
Adjusted ORs (95% CIs)[a]				
Model 1	1.00 (reference)	1.19 (1.06-1.35)	1.30 (1.20-1.40)	1.50 (1.36-1.65)
Model 2	1.00 (reference)	1.11 (0.98-1.27)	1.06 (0.98-1.15)	1.14 (1.02-1.26)
Model 3	1.00 (reference)	1.11 (0.98-1.27)	1.05 (0.97-1.14)	1.13 (1.01-1.25)

P= 0.088 for overall interaction between obesity and by fatty liver category for coronary artery calcification (model 3).

Compared with NAFLD, ORs (95% CIs) in AFLD was 1.09 (1.01-1.17) (p = 0.021).

[a]Estimated from binomial logistic regression models. Multivariable model 1 was adjusted for age and sex; model 2: model 1 plus adjustment for center, year of screening exam, BMI, smoking status, physical activity, educational level, total calorie intake, family history of cardiovascular disease, diabetes,

hypertension, LDL-cholesterol, and medication for dyslipidemia; model 3 model 2 plus adjustment for hsCRP, and HOMA-IR.
Abbreviations: AFLD, alcoholic fatty liver disease; FLD, fatty liver disease; NAFLD, nonalcoholic fatty liver disease.

Table 3. Association of fatty liver categories and their severity based on FIB-4 with coronary artery calcification

	Reference	NAFLD		AFLD	
		Low	Intermediate/high	Low	Intermediate/high
Fibrosis severity based on FIB-4					
Number	52,529	32,512	1,865	7,527	791
CAC score >0 (%)	4.479 (8.5)	4,482 (13.8)	767 (41.1)	1,367 (18.2)	354 (44.8)
Adjusted ORs (95% CIs)[a]					
Model 1	1.00	1.55 (1.48-1.63)	1.65 (1.47-1.85)	1.87 (1.74-2.01)	2.17 (1.84-2.55)
Model 2	1.00	1.09 (1.03-1.15)	1.14 (1.01-1.29)	1.17 (1.08-1.27)	1.37 (1.16-1.63)
Model 3	1.00	1.09 (1.03-1.15)	1.14 (1.01-1.29)	1.17 (1.07-1.27)	1.37 (1.16-1.63)

Compared with low-Fib4 NAFLD, ORs (95% CIs) in intermediate/high FIB-4 NAFLD was 1.04 (0.93-1.18) (p = 0.477, model 3).

Compared with low-Fib4 AFLD, ORs (95% CIs) in intermediate/high FIB-4 AFLD was 1.18 (0.99-1.40) (p = 0.070, model 3).

[a]Estimated from binomial logistic regression models. Multivariable model 1 was adjusted for age and sex; model 2: model 1 plus adjustment for center, year of screening exam, BMI, smoking status, physical activity, educational level, total calorie intake, family history of cardiovascular disease, diabetes, hypertension, LDL-cholesterol, and medication for dyslipidemia; model 3 model 2 plus adjustment for hsCRP, and HOMA-IR.

Abbreviations: AFLD, alcoholic fatty liver disease; FLD, fatty liver disease; NAFLD, nonalcoholic fatty liver disease.

Table 4. Association of fatty liver categories and their severity of steatosis based on US with coronary artery calcification

	Reference	NAFLD		AFLD	
		Mild	Moderate / severe	Mild	Moderate / severe
Number	52,529	25,444	8,383	6,375	1,814
CAC score >0 (%)	4,479 (8.5)	3,864 (15.2)	1,278 (15.3)	1,337 (21.0)	348 (19.2)
Adjusted ORs (95% CIs)[a]					
Model 1	1.00	1.47 (1.39-1.54)	1.92 (1.78-2.06)	1.82 (1.69-1.97)	2.24 (1.96-2.55)
Model 2	1.00	1.09 (1.03-1.16)	1.12 (1.02-1.22)	1.20 (1.10-1.31)	1.18 (1.02-1.36)
Model 3	1.00	1.09 (1.03-1.16)	1.12 (1.02-1.22)	1.20 (1.10-1.31)	1.18 (1.02-1.36)

Compared with mild NAFLD, ORs (95% CIs) in moderate/severe NAFLD was 1.02 (0.94-1.11) (p = 0.617, model 3).

Compared with mild AFLD, ORs (95% CIs) in moderate/severe AFLD was 0.98 (0.85-1.14) (p = 0.801, model 3).

Of 42,701 participants with a diagnosis of fatty liver, 1.6% (N=685) did not have information available on severity of hepatic steatosis.

[a]Estimated from binomial logistic regression models comparing FLD and FIB-4 categories to reference category (no excessive alcohol use and no fatty liver). Multivariable model 1 was adjusted for age and sex; model 2: model 1 plus adjustment for center, year of screening exam, BMI, smoking status, physical activity, educational level, total calorie intake, family history of cardiovascular disease, diabetes, hypertension, LDL-cholesterol, and medication for dyslipidemia; model 3 model 2 plus adjustment for hsCRP, and HOMA-IR.

Abbreviations: AFLD, alcoholic fatty liver disease; CI, confidence intervals; FLD, fatty liver disease; NAFLD, nonalcoholic fatty liver disease.

Table 5. Ratio (95% CI) of annual progression rates of coronary artery calcium score by categories of fatty liver at baseline (n=23,320)

Ratio of annual progression rates [a]	Categories of fatty liver			
	No excessive alcohol intake and no FLD	Excessive alcohol intake and no FLD	NAFLD	AFLD
Number	9,854	2,406	8,678	2,382
Overall (N=23,320)				
Annual rate of CAC progression	1.0511 (1.0470-1.0552)	1.0821 (1.0719-1.0925)	1.0918 (1.0860-1.0977)	1.1231 (1.1101-1.1354)
Ratio of annual progression rates [a]				
Model 1	1.0 (reference)	1.0295 (1.0190-1.0402)	1.0388 (1.0320-1.0457)	1.0687 (1.0563-1.0811)
Model 2	1.0 (reference)	1.0297 (1.0191-1.0404)	1.0390 (1.0321-1.0459)	1.0688 (1.0565-1.0813)
Model 3	1.0 (reference)	1.0297 (1.0191-1.0404)	1.0390 (1.0321-1.0459)	1.0688 (1.0565-1.0813)
Non-obese (BMI < 25 kg/m^2) (N=13,038)				
Annual rate of CAC progression	1.0478 (1.0433-1.0523)	1.0701 (1.0587-1.0816)	1.0754 (1.0670-1.0838)	1.1101 (1.0882-1.1325)
Ratio of annual progression rates [a]				
Model 1	1.0 (reference)	1.0213 (1.0096-1.0331)	1.0264 (1.0172-1.0356)	1.0596 (1.0382-1.0814)
Model 2	1.0 (reference)	1.0213 (1.0096-1.0331)	1.0264 (1.0172-1.0356)	1.0596 (1.0382-1.0814)
Model 3	1.0 (reference)	1.0213 (1.0096-1.0331)	1.0264 (1.0172-1.0356)	1.0596 (1.0382-1.0814)
Obese (BMI ≥ 25 kg/m^2) (N=10,282)				
Annual rate of CAC progression	1.0645 (1.0545-1.0745)	1.1084 (1.0875-1.1298)	1.1008 (1.0930-1.1086)	1.1287 (1.1140-1.1435)
Ratio of annual progression rates [a]				
Model 1	1.0 (reference)	1.0413 (1.0193-1.0637)	1.0341 (1.0221-1.0464)	1.0604 (1.0435-1.0776)
Model 2	1.0 (reference)	1.0418 (1.0199-1.0643)	1.0344 (1.0223-1.0466)	1.0606 (1.0436-1.0778)
Model 3	1.0 (reference)	1.0418 (1.0199-1.0643)	1.0344 (1.0223-1.0466)	1.0606 (1.0436-1.0778)

[a] Estimated from linear mixed models with random intercept and random slopes used with natural log(CAC + 1) as the outcome and inverse probability weighting. Multivariable model 1 was adjusted for age and sex; model 2: model 1 plus adjustment for center, year of screening exam, BMI, smoking status, physical activity, educational level, total calorie intake, family history of cardiovascular disease, diabetes, hypertension, LDL-cholesterol, and medication for dyslipidemia; model 3 model 2 plus adjustment for hsCRP, and HOMA-IR.

Figure Legends

Figure 1. Flow chart of study participants.

Figure 1. Flow chart of study participants