



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

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

**Citation for published version:**

Chang, Y, Ryu, S, Sung, K-C, Cho, Y-K, Sung, E, Kim, H, Jung, H-S, Yun, KE, Ahn, J, Shin, H, Wild, S & Byrne, CD 2019, 'Alcoholic and non-alcoholic fatty liver disease and associations with coronary artery calcification- Evidence from the Kangbuk Samsung Health Study', *Gut*. <https://doi.org/10.1136/gutjnl-2018-317666>

**Digital Object Identifier (DOI):**

[10.1136/gutjnl-2018-317666](https://doi.org/10.1136/gutjnl-2018-317666)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Gut

**Publisher Rights Statement:**

This is the author's peer-reviewed manuscript as accepted for publication.

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



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

4 Yoosoo Chang<sup>1,2,3</sup>, Seungho Ryu<sup>1,2,3</sup>, Ki-Chul Sung<sup>4</sup>, Yong Kyun Cho,<sup>5</sup> Eunju Sung<sup>1,6</sup>, Han-Na  
5 Kim<sup>7</sup>, Hyun-Suk Jung<sup>1</sup>, Kyung Eun Yun<sup>1</sup>, Jiin Ahn<sup>1</sup>, Hocheol Shin<sup>1,6</sup>, Sarah H. Wild<sup>8</sup>,  
6 Christopher D Byrne<sup>9,10</sup>

7

8 <sup>1</sup>Center for Cohort Studies, Total Healthcare Center, Kangbuk Samsung Hospital,  
9 Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

10 <sup>2</sup>Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital,  
11 Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

12 <sup>3</sup>Department of Clinical Research Design & Evaluation, SAIHST, Sungkyunkwan University,  
13 Seoul, Republic of Korea

14 <sup>4</sup>Division of Cardiology, Department of Medicine, Kangbuk Samsung Hospital,  
15 Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

16 <sup>5</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kangbuk  
17 Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

18 <sup>6</sup>Department of Family Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University  
19 School of Medicine, Seoul, Republic of Korea

20 <sup>7</sup>Medical Research Institute, Kangbuk Samsung Hospital, Sungkyunkwan University School  
21 of Medicine, Seoul, Republic of Korea

22 <sup>8</sup>Usher Institute of Population Health Sciences and informatics, University of Edinburgh,  
23 Edinburgh, U.K.

24 <sup>9</sup>Nutrition and Metabolism, Faculty of Medicine, University of Southampton, Southampton,  
25 U.K.

26 <sup>10</sup>National Institute for Health Research Southampton Biomedical Research Centre,  
27 University Hospital Southampton, Southampton, U.K.

28

29 **Running title:** Fatty liver and atherosclerosis

1 **Financial Support:** This study was supported by the National Research Foundation of Korea  
2 funded by the Ministry of Science, ICT & Future (S20170622000).

3 **Disclosures:** The authors have no conflicts of interest to disclose.

4 **Word count:** Abstract 249; text 4,482

5 **Number of figures and tables:** 1 figure, 5 tables, and 8 supplementary tables

6  
7 **Address for correspondence:** Seungho Ryu, MD, PhD, Kangbuk Samsung Hospital,  
8 Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul, South Korea 04514

9 E-mail: [sh703.yoo@samsung.com](mailto:sh703.yoo@samsung.com). Telephone: 82-2-2001-5137. Fax: 82-2-757-0436.

10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24

1 **Abstract**

2 **Objective**

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

8 **Design**

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

13 **Results**

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

23 **Conclusion**

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

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

6

1 **Significance of this study**

2 **What is already known on this subject?**

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

11 **What are the new findings?**

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

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

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

## 1 **Introduction**

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

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

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

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

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

8

## 9 **Methods**

### 10 *Study population*

11 The Kangbuk Samsung Health Study (KSHS) is a cohort study of Korean men and women  
12 aged 18 years or over who underwent a comprehensive health examination annually or  
13 biennially at Kangbuk Samsung Hospital Total Healthcare Centers in Seoul and Suwon,  
14 South Korea [8]. This study population consisted of a subset of KSHS participants who  
15 underwent cardiac CT to measure CAC scores as part of a comprehensive health exam from  
16 2011 to 2017 (N = 123,776). CAC scoring has become a common CVD screening test in  
17 Korea. Over 80% of participants were employees of various companies and local  
18 governmental organizations and their spouses. In South Korea, the Industrial Safety and  
19 Health Law requires annual or biennial health screening exams of all employees offered free  
20 of charge. The remaining participants were people voluntarily taking screening exams.

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



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

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

11

## 12 ***Measurements***

13 Data on demographic characteristics, lifestyle factors, education level, medical history,  
14 and family history of CVD were collected by standardized, self-administered questionnaires  
15 [8]. The questionnaire asked about the frequency of alcohol drinking and the amount of  
16 alcohol consumed per drinking day recorded in standard units [16]. Average alcohol  
17 consumption per day was calculated using the frequency and amount of beverages consumed  
18 per drinking day. Excessive alcohol consumption (EAC) was defined as average alcohol  
19 intake  $\geq 30$  g/day for men and  $\geq 20$  g/day for women [2]. Smoking status was categorized as  
20 never, former, or current smoker. Physical activity was assessed using the validated Korean  
21 version of the International Physical Activity Questionnaire (IPAQ) short form.[17]  
22 Participants were classified into inactive, minimally active, or health-enhancing physical  
23 activity (HEPA). HEPA was defined as physical activity that meets either of two criteria: (i)  
24 vigorous intensity activity on three or more days per week accumulating  $\geq 1500$  MET

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

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

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

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

3

#### 4 *Ascertainment of fatty liver disease and non-invasive fibrosis indices*

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

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

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

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

6

### 7 ***Measurement of CAC by multidetector CT***

8 CAC was detected with a Lightspeed VCT XTe-64 slice MDCT scanner (GE Healthcare,  
9 Tokyo, Japan) in both Seoul and Suwon centers using the same standard scanning protocol  
10 of 2.5-mm thickness, 400-ms rotation time, 120-kV tube voltage, and 124-mAS (310 mA  $\times$   
11 0.4 seconds) tube current under ECG-gated dose modulation. CAC scores were calculated as  
12 previously described by Agatston et al. [26]. The inter-observer reliability and intra-observer  
13 reliability for CAC scores were both excellent (intra-class correlation coefficient of 0.99) [8].  
14 CAC scores were categorized as 0, 1–100, and >100 [27].

15

### 16 **Statistical analysis**

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

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

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

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

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

5 In sensitivity analyses, we also estimated the prevalence ratios and 95% CIs for CAC score  
6 1 – 100 and >100 for EAC and no FLD, NAFLD, and AFLD compared to the reference  
7 category (no EAC and no FLD) using participants with CAC 0 as the reference group in  
8 multinomial logistic regression models. In another sensitivity analysis, to evaluate the  
9 association between FLD categories and CAC as a continuous variable, we used a Tobit  
10 regression model for natural log (CAC score +1) with Huber-White estimation of standard  
11 errors [8, 31]. Tobit models were used to estimate ratios and 95% CI of CAC score +1 by  
12 comparing EAC and no FLD, NAFLD, and AFLD to the reference category (no EAC and no  
13 FLD). Estimates of the Tobit models were presented as exponentiated Tobit regression  
14 coefficients (CAC score ratios) approximately representing the relative CAC score increment  
15 comparing EAC and no FLD, NAFLD and AFLD to the reference category (no EAC and no  
16 FLD). For example, a CAC ratio of 1.50 is interpreted as a 50% increase in the CAC score  
17 for a specific category compared to the reference category.

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

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

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

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

15

## 16 **Results**

17 The mean age (standard deviation) and mean BMI (SD) of 105,328 participants were 40.8  
18 years (7.8) and 24.4 kg/m<sup>2</sup> (3.3), respectively, and 77.5 percent of participants were male  
19 (Table 1). The prevalence of EAC and no FLD, NAFLD and AFLD were 9.6%, 32.6%, and  
20 7.9%, respectively. EAC with no FLD and AFLD were positively associated with current  
21 smoking. NAFLD and AFLD were positively associated with diabetes, hypertension, obesity,  
22 and higher levels of BMI, BP, total cholesterol, LDL-C, glucose, triglycerides, AST, ALT,  
23 HOMA-IR, and hsCRP, and inversely associated with HDL-C. GGT level was higher in EAC  
24 and no FLD, NAFLD, and ALFD than in the reference category (no EAC and no FLD) with

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

3 Table 2 shows the relationship between FLD categories and the presence of detectable CAC  
4 (>0) overall and in the non-obese and obese groups separately. Both types of FLD, including  
5 NAFLD and AFLD, were positively associated with the presence of CAC. After adjusting for  
6 age, sex, screening center, year of screening examination, smoking status, physical activity,  
7 educational level, total calorie intake, BMI, family history of CVD, diabetes, hypertension,  
8 LDL-C and medication for dyslipidemia, multivariable-adjusted OR (95% CIs) for detectable  
9 CAC comparing EAC with no FLD, NAFLD, and AFLD to the reference category were 1.25  
10 (1.16-1.35), 1.10 (1.05-1.16), and 1.20 (1.11-1.30), respectively. AFLD was associated with  
11 higher CAC than NAFLD. In post hoc analysis, OR (95% CI) for detectable CAC comparing  
12 AFLD to NAFLD was 1.09 (1.01-1.17) ( $p = 0.021$ ). In analyses with adjustment for waist  
13 circumference instead of BMI, we found similar results (Supplementary table 1).

14 The associations between FLD categories and the presence of CAC tended to be slightly  
15 stronger in the non-obese than in the obese and although there was a trend towards there  
16 being a significant difference by obesity status, these associations did not reach significance  
17 ( $P$  for interaction=0.088, Table 2) even though obese FLD subjects showed unfavorable  
18 profiles of metabolic risk factors compared to non-obese FLD subjects (Supplementary Table  
19 2). For the non-obese group, multivariable-adjusted OR (95% CIs) for detectable CAC  
20 comparing EAC with no FLD, NAFLD, and AFLD to the reference category were 1.31 (1.19-  
21 1.44), 1.10 (1.02-1.18) and 1.25 (1.10-1.43), respectively, while for the obese group,  
22 corresponding OR (95% CIs) were 1.11 (0.98-1.27), 1.06 (0.98-1.15), and 1.14 (1.02-1.26),  
23 respectively.

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



1 adjusted prevalence ratios comparing EAC with no FLD, NAFLD, and AFLD to the  
2 reference category were 1.24 (1.14-1.34), 1.11 (1.05-1.18), and 1.21 (1.11-1.31) for CAC  
3 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  
4 >100, respectively (Supplementary Table 3). In sensitivity analysis using Tobit regression  
5 model, multivariable-adjusted CAC score ratios (95% CIs) comparing EAC with no FLD,  
6 NAFLD, and AFLD to the reference category were 1.68 (1.42-1.98), 1.22 (1.09-1.37), and  
7 1.54 (1.30-1.83), respectively (Supplementary Table 4).

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

14 Table 3 shows the association of FLD categories with presence of CAC according to degree  
15 of fibrosis based on FIB-4 index. Compared with the reference category (no EAC and no  
16 FLD), multivariable adjusted OR (95% CIs) for detectable CAC in low and intermediate/high  
17 FIB-4 among NAFLD cases were 1.09 (1.03-1.15) and 1.14 (1.01-1.29), respectively,  
18 whereas corresponding OR (95% CIs) among AFLD cases were 1.17 (1.08-1.27) and 1.37  
19 (1.16-1.63), respectively. After further adjustment for hsCRP, and HOMA-IR, the association  
20 between fibrosis scores and presence of CAC remained statistically significant in both  
21 NAFLD and AFLD groups. In a sensitivity analysis using the aspartate transaminase to  
22 platelet ratio index (APRI), the associations between FLD and presence of CAC were  
23 similarly observed (Supplementary Tables 6 and 7).

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

1 severity of hepatic steatosis on ultrasonography. Compared with the reference category (no  
2 EAC and no FLD), multivariable adjusted OR (95% CIs) for detectable CAC in mild and  
3 moderate/severe steatosis among NAFLD cases were 1.09 (1.03-1.15) and 1.14 (1.01-1.29),  
4 respectively, whereas corresponding OR (95% CIs) among AFLD cases were 1.17 (1.08-  
5 1.27) and 1.37 (1.16-1.63), respectively.

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

12 Finally, we evaluated a prospective association of NAFLD and AFLD with CAC  
13 progression among 23,320 participants with baseline and follow-up cardiac CT (Table 5). The  
14 median duration of follow-up was 3.0 years (interquartile range 2.0-4.2, maximum 6.7). The  
15 annual rates of CAC progression (95% CI) in no EAC and no FLD, EAC and no FLD,  
16 NAFLD, and AFLD were 5.1%, 8.2%, 9.2% and 12.3 %, respectively. The multivariable  
17 adjusted ratio of progression rates comparing EAC and no FLD, NAFLD, and AFLD to the  
18 reference category (no EAC and no FLD) were 1.03 (1.02-1.04), 1.04 (1.03-1.05) and 1.07  
19 (1.06-1.08), respectively. These associations were similar in non-obese and obese individuals.  
20 Further adjustment for HOMA-IR and hsCRP did not change the result.

21

## 22 **Discussion**

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

1 subclinical coronary atherosclerosis compared to the reference (no EAC and no FLD). This  
2 association was observed in non-obese individuals, indicating that non-obese NAFLD and  
3 AFLD are also associated with a higher risk of atherosclerosis. The risk of subclinical  
4 atherosclerosis in FLD was also observed with mild and moderate/severe hepatic steatosis  
5 and with both low and higher degrees of fibrosis. Our data suggest that there was a slightly  
6 stronger association between AFLD and CAC than between NAFLD and CAC [see Table 2,  
7 compared with NAFLD, OR (95% CIs) for AFLD and CAC was 1.09 (1.01-1.17) (p =  
8 0.021)].

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

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

1 NAFLD (necessitating hospital admission or causing death), there was a greater risk of CVD  
2 mortality with ALD than with NAFLD [10]. An earlier cross-sectional study of 265 patients  
3 with early liver disease showed higher carotid intima-media thickness, in both AFLD and  
4 NAFLD patients compared with the reference (no FLD without alcohol history) but this study  
5 design was limited by lack of adjustment for confounders [34]. Another cross-sectional study  
6 of 10,710 participants involved in a health checkup program demonstrated that the estimated  
7 10-year coronary heart disease risk based on Framingham risk scores was similarly higher in  
8 the AFLD and NAFLD groups compared to the no fatty liver group [8]. In our study,  
9 individuals with AFLD showed a higher prevalence of unhealthy behaviors and CVD risk  
10 factors but whether these behaviors or risk factor mediate an increase in risk of subclinical  
11 atherosclerosis is uncertain. Adjustment for those factors attenuated the association between  
12 AFLD and CAC scores, but these associations remained significant with AFLD, suggesting  
13 that AFLD, like NAFLD, is a metabolic liver disease that is associated with increased risk of  
14 CVD risk.

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

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

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

11 We note that our study has some limitations. First, fatty liver was determined using US,  
12 which is less sensitive (60-90%) when hepatic fat infiltration is below approximately 30%  
13 [39], but is widely used both clinically and in population-based studies due to its non-  
14 invasive nature and acceptable degree of diagnostic accuracy for steatosis [39]. Additionally,  
15 in our study, there was limited power to study associations between liver fibrosis and CAC  
16 scores in subjects with AFLD and NAFLD, as very few subjects had evidence of advanced  
17 fibrosis from these scores. Second, behavioral factors such as smoking and alcohol use were  
18 assessed via a self-administered structured questionnaire used in health checkup programs in  
19 Korea as part of the National Health Insurance plan [40]. Measurement errors of these  
20 variables might introduce some degree of residual confounding, similar to most  
21 epidemiologic studies. Finally, our results derived from a sample of relatively healthy young  
22 and middle-aged educated Koreans who participated in a health check-up program and might  
23 not be generalizable to other ages and ethnic populations. However, our study population was  
24 mainly composed of healthy employees and their spouses without clinically manifest CVD,

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

### 3 **Conclusion**

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

10

11 **Acknowledgements** CDB is supported in part by grants from the Southampton National  
12 Institute for Health Research Biomedical Research Centre.

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

## 1   **References**

- 2   1       Mills SJ, Harrison SA. Comparison of the natural history of alcoholic and nonalcoholic  
3 fatty liver disease. *Curr Gastroenterol Rep* 2005;**7**:32-6.
- 4   2       Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, *et al*. The diagnosis and  
5 management of non-alcoholic fatty liver disease: practice guideline by the American  
6 Gastroenterological Association, American Association for the Study of Liver Diseases, and  
7 American College of Gastroenterology. *Gastroenterology* 2012;**142**:1592-609.
- 8   3       Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. *J Hepatol*  
9 2013;**59**:160-8.
- 10  4       Younossi Z, Henry L. Contribution of Alcoholic and Nonalcoholic Fatty Liver Disease to  
11 the Burden of Liver-Related Morbidity and Mortality. *Gastroenterology* 2016;**150**:1778-85.
- 12  5       Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease  
13 and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol* 2016;**65**:589-600.
- 14  6       Sinn DH, Kang D, Chang Y, Ryu S, Gu S, Kim H, *et al*. Non-alcoholic fatty liver disease and  
15 progression of coronary artery calcium score: a retrospective cohort study. *Gut* 2017;**66**:323-9.
- 16  7       Toshikuni N, Tsutsumi M, Arisawa T. Clinical differences between alcoholic liver disease  
17 and nonalcoholic fatty liver disease. *World J Gastroenterol* 2014;**20**:8393-406.
- 18  8       Chang Y, Kim BK, Yun KE, Cho J, Zhang Y, Rampal S, *et al*. Metabolically-healthy obesity  
19 and coronary artery calcification. *Journal of the American College of Cardiology* 2014;**63**:2679-86.
- 20  9       Kim JH, Kim SY, Jung ES, Jung SW, Koo JS, Kim JH, *et al*. Carotid intima-media thickness is  
21 increased not only in non-alcoholic fatty liver disease patients but also in alcoholic fatty liver  
22 patients. *Digestion* 2011;**84**:149-55.
- 23  10      Wild SH, Walker JJ, Morling JR, McAllister DA, Colhoun HM, Farran B, *et al*. Cardiovascular  
24 Disease, Cancer, and Mortality Among People With Type 2 Diabetes and Alcoholic or Nonalcoholic  
25 Fatty Liver Disease Hospital Admission. *Diabetes Care* 2018;**41**:341-7.
- 26  11      Budoff MJ, Achenbach S, Blumenthal RS, Carr JJ, Goldin JG, Greenland P, *et al*. Assessment  
27 of coronary artery disease by cardiac computed tomography: a scientific statement from the  
28 American Heart Association Committee on Cardiovascular Imaging and Intervention, Council on  
29 Cardiovascular Radiology and Intervention, and Committee on Cardiac Imaging, Council on  
30 Clinical Cardiology. *Circulation* 2006;**114**:1761-91.
- 31  12      Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, *et al*.  
32 Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque  
33 burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying  
34 methodology. *Journal of the American College of Cardiology* 1998;**31**:126-33.
- 35  13      Keelan PC, Bielak LF, Ashai K, Jamjoum LS, Denktas AE, Rumberger JA, *et al*. Long-term  
36 prognostic value of coronary calcification detected by electron-beam computed tomography in  
37 patients undergoing coronary angiography. *Circulation* 2001;**104**:412-7.
- 38  14      Hart CL, Morrison DS, Batty GD, Mitchell RJ, Davey Smith G. Effect of body mass index

1 and alcohol consumption on liver disease: analysis of data from two prospective cohort studies.  
2 BMJ 2010;**340**:c1240.

3 15 Liu B, Balkwill A, Reeves G, Beral V, Million Women Study C. Body mass index and risk of  
4 liver cirrhosis in middle aged UK women: prospective study. BMJ 2010;**340**:c912.

5 16 Park JT, Kim BG, Jhun HJ. Alcohol consumption and the CAGE questionnaire in Korean  
6 adults: results from the Second Korea National Health and Nutrition Examination Survey. J Korean  
7 Med Sci 2008;**23**:199-206.

8 17 Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, *et al*.  
9 International physical activity questionnaire: 12-country reliability and validity. Medicine and  
10 science in sports and exercise 2003;**35**:1381-95.

11 18 Ahn Y, Kwon E, Shim JE, Park MK, Joo Y, Kimm K, *et al*. Validation and reproducibility of  
12 food frequency questionnaire for Korean genome epidemiologic study. European journal of clinical  
13 nutrition 2007;**61**:1435-41.

14 19 World Health Organization, Regional Office for the Western Pacific. The Asia-Pacific  
15 perspective: redefining obesity and its treatment. Sydney: Health Communications Australia, 2000.

16 20 Mathiesen UL, Franzen LE, Aselius H, Resjo M, Jacobsson L, Foberg U, *et al*. Increased liver  
17 echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in  
18 asymptomatic patients with mild/moderate abnormalities of liver transaminases. Dig Liver Dis  
19 2002;**34**:516-22.

20 21 Ryu S, Chang Y, Jung HS, Yun KE, Kwon MJ, Choi Y, *et al*. Relationship of sitting time and  
21 physical activity with non-alcoholic fatty liver disease. J Hepatol 2015;**63**:1229-37.

22 22 McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis  
23 scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver  
24 disease. Gut 2010;**59**:1265-9.

25 23 Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, *et al*. Comparison of  
26 noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol  
27 Hepatol 2009;**7**:1104-12.

28 24 Wai CT, Greenon JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, *et al*. A  
29 simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic  
30 hepatitis C. Hepatology 2003;**38**:518-26.

31 25 Lombardi R, Buzzetti E, Roccarina D, Tsochatzis EA. Non-invasive assessment of liver  
32 fibrosis in patients with alcoholic liver disease. World J Gastroenterol 2015;**21**:11044-52.

33 26 Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R.  
34 Quantification of coronary artery calcium using ultrafast computed tomography. Journal of the  
35 American College of Cardiology 1990;**15**:827-32.

36 27 Martin SS, Blaha MJ, Blankstein R, Agatston A, Rivera JJ, Virani SS, *et al*. Dyslipidemia,  
37 coronary artery calcium, and incident atherosclerotic cardiovascular disease: implications for statin  
38 therapy from the multi-ethnic study of atherosclerosis. Circulation 2014;**129**:77-86.



1 28 Kim D, Kim WR. Nonobese Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2017;**15**:474-85.  
2 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  
3 Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015;**149**:389-97 e10.  
4 30 Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, *et al.* Increased risk of mortality  
5 by fibrosis stage in non-alcoholic fatty liver disease: Systematic Review and Meta-analysis.  
6 *Hepatology* 2017.  
7 31 Reilly MP, Wolfe ML, Localio AR, Rader DJ. Coronary artery calcification and cardiovascular  
8 risk factors: impact of the analytic approach. *Atherosclerosis* 2004;**173**:69-78.  
9 32 Gasset AJ, Sheppard L, McClelland RL, Olives C, Kronmal R, Blaha MJ, *et al.* Risk Factors  
10 for Long-Term Coronary Artery Calcium Progression in the Multi-Ethnic Study of Atherosclerosis.  
11 *Journal of the American Heart Association* 2015;**4**:e001726.  
12 33 Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stal P, Kechagias S, *et al.* Fibrosis stage is  
13 the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up.  
14 *Hepatology* 2015;**61**:1547-54.  
15 34 Kim JH, Kim SY, Jung ES, Jung SW, Koo JS, Kim JH, *et al.* Carotid intima-media thickness is  
16 increased not only in non-alcoholic fatty liver disease patients but also in alcoholic fatty liver  
17 patients. *Digestion* 2011;**84**:149-55.  
18 35 Liu Q, Bengmark S, Qu S. The role of hepatic fat accumulation in pathogenesis of non-  
19 alcoholic fatty liver disease (NAFLD). *Lipids in health and disease* 2010;**9**:42.  
20 36 Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver  
21 disease. *The Journal of clinical endocrinology and metabolism* 2006;**91**:4753-61.  
22 37 Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and  
23 important cardiovascular risk factor? *Eur Heart J* 2012;**33**:1190-200.  
24 38 Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015;**62**:S47-64.  
25 39 Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, *et al.* Diagnostic accuracy  
26 and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology*  
27 2011;**54**:1082-90.  
28 40 Chang Y, Ryu S, Choi Y, Zhang Y, Cho J, Kwon MJ, *et al.* Metabolically Healthy Obesity and  
29 Development of Chronic Kidney Disease: A Cohort Study. *Annals of internal medicine*  
30 2016;**164**:305-12.  
31

32

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 (%) <sup>c</sup>	83.8	83.8	76.4	86.8	80.8
Obesity (%) <sup>d</sup>	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 <sup>2</sup> )	24.4 (3.3)	22.8 (2.7)	23.9 (2.6)	26.3 (3.1)	26.8 (3.0)
Waist circumference (cm) <sup>e</sup>	84.9 (9.2)	80.3 (7.8)	84.0 (7.3)	90.4 (7.8)	92.1 (7.6)
Systolic BP (mmHg) <sup>a</sup>	112.4 (12.4)	108.7 (11.8)	114.5 (11.8)	115.8 (11.7)	119.7 (11.9)
Diastolic BP (mmHg) <sup>a</sup>	73.0 (9.8)	70.0 (9.2)	75.0 (9.6)	75.4 (9.4)	79.0 (9.7)
Glucose (mg/dl) <sup>a</sup>	97.4 (15.9)	93.9 (10.9)	97.6 (13.2)	100.8 (19.1)	105.6 (23.0)
Total cholesterol (mg/dl) <sup>a</sup>	199.4 (34.6)	193.5 (32.6)	198.1 (33.4)	206.2 (35.7)	210.2 (36.8)
LDL-C (mg/dl) <sup>a</sup>	129.0 (32.2)	122.9 (30.5)	124.2 (31.3)	138.0 (32.3)	136.5 (33.2)
HDL-C (mg/dl) <sup>a</sup>	55.2 (14.5)	59.8 (14.7)	60.0 (14.9)	48.0 (10.9)	49.9 (12.0)
Albumin (g/dL) <sup>a</sup>	4.7 (0.2)	4.6 (0.2)	4.7 (0.2)	4.7 (0.2)	4.7 (0.2)
Platelet ( $\times 10^9/L$ ) <sup>a</sup>	246.2 (50.2)	243.8 (50.4)	242.7 (48.3)	251.1 (50.6)	244.9 (47.5)
Triglycerides (mg/dl) <sup>b</sup>	111 (77-163)	88 (65-122)	109 (78-154)	145 (105-202)	166 (118-237)
AST (U/l) <sup>b</sup>	20 (17-25)	18 (16-22)	21 (18-25)	23 (19-29)	25 (20-32)
ALT (U/l) <sup>b</sup>	21 (15-32)	17 (13-23)	20 (15-27)	30 (21-45)	32 (23-46)
GGT (U/l) <sup>b</sup>	26 (17-44)	19 (14-28)	34 (22-56)	35 (24-54)	55 (36-88)
HOMA-IR <sup>b</sup>	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) <sup>b</sup>	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 <sup>a</sup>	0.81 (0.37)	0.82 (0.36)	0.86 (0.40)	0.76 (0.34)	0.87 (0.44)
APRI <sup>a</sup>	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) <sup>b,f</sup>	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 <sup>g</sup>	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 <sup>a</sup>mean (standard deviation), <sup>b</sup>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, nonalcoholic fatty liver disease.

<sup>c</sup>≥ College graduate; <sup>d</sup>BMI ≥25 kg/m<sup>2</sup>;

<sup>e</sup> among 99,729 participants with available waist circumference; <sup>f</sup> among 71,521 participants with plausible estimated energy intake levels (within three standard deviations from log-transformed mean energy intake); <sup>g</sup>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
<b>Total</b>				
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) <sup>a</sup>				
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)
<b>Non-obese (BMI &lt;25 kg/m<sup>2</sup>)</b>				
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) <sup>a</sup>				
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)
<b>Obese (BMI ≥ 25 kg/m<sup>2</sup>)</b>				
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) <sup>a</sup>				
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).

<sup>a</sup>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
<b>Fibrosis severity based on FIB-4</b>					
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) <sup>a</sup>					
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).

<sup>a</sup>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) <sup>a</sup>					
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.

<sup>a</sup>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 <sup>a</sup>	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
<b>Overall (N=23,320)</b>				
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 <sup>a</sup>				
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)
<b>Non-obese (BMI &lt; 25 kg/m<sup>2</sup>) (N=13,038)</b>				
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 <sup>a</sup>				
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)
<b>Obese (BMI ≥ 25 kg/m<sup>2</sup>) (N=10,282)</b>				
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 <sup>a</sup>				
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)

<sup>a</sup> 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



