Fatty liver, insulin resistance and obesity: relationships with increase in coronary artery calcium over time

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Keywords

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Abbreviations list: NAFLD, nonalcoholic fatty liver disease; MetS, metabolic syndrome; ALT, alanine aminotransaminases; AST, aspartate transaminase; gGT, gamma-glutamyl transpeptidase; HDLc, high density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; BMI, body mass index; Coronary artery calcium (CAC) score, cardiovascular disease (CVD), IR (insulin resistant/resistance)

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Abstract

Background. Non alcoholic fatty liver disease (NAFLD), insulin resistance (IR) and obesity frequently co-exist with type 2 diabetes but it is uncertain whether these risk factors for vascular disease contribute to a change in atherosclerosis over time, independently of diabetes status. We tested whether the combination of fatty liver, IR and obesity was associated with an increase in coronary artery calcium (CAC) score over time, independently of diabetes status, other cardiovascular risk factors and medications.

Methods. Data were analysed from a South Korean occupational cohort of 2175 people. The outcome was increase in cardiac computed tomography (CT) CAC score between baseline and follow up. IR was defined by HOMA-IR≥75th centile and fatty liver by ultrasound. Hazard ratios (HR) [95% confidence intervals (CIs)] were estimated using Cox proportional hazards regression.

Results. There were 592 (27.2%) participants in whom CAC score increased from baseline (mean±SD age at baseline 44.8±5.5 years) and 1583 subjects in whom CAC did not change or improved during follow up (mean±SD age 41.6±5.6 years). Diabetes, HOMA-IR, fatty liver and obesity prevalence were all higher (all $p<0.001$), in participants in whom CAC score increased from baseline. Adjusting for diabetes and potential confounders, the combination of IR, obesity and fatty liver was independently associated with increase in CAC score over time [(HR 2.46, (95%CIs 1.50,4.03)].

Conclusions. The combination of fatty liver, IR and obesity is associated with progression of atherosclerosis over time independently of diabetes, cardiovascular risk factors, and all medications for cardiovascular disease and diabetes.

Introduction

Coronary artery calcium (CAC) scoring with cardiac computed tomography (CT) is a sensitive method to demonstrate the presence of early atherosclerosis and the use of CAC scores may improve CV risk prediction in asymptomatic individuals (1). The total volume of coronary artery calcium (CAC) deposits is a good indicator of overall plaque burden and of future coronary events. Therefore, CAC scores can be used as a marker of atherosclerotic disease and of cardiovascular risk. Although, localization of CAC does not correlate well with the severity or vulnerability of coronary lesions, particularly in older patients (2), estimation of the CAC score provides a useful non-invasive tool to assess risk of cardiovascular events (3). CAC scores also perform better in identifying high-risk individuals compared with an alternative non-invasive measurement, carotid intima-media thickness, CAC scans are associated with relatively low radiation exposure (0.9-1.1 mSv), and CAC scores provide information that can be used not only for risk stratification but also can be used to track the progression of atherosclerosis (4).

A recent meta-analysis of 49 studies with ultrasound and liver histology shows that ultrasound is an accurate, reliable imaging technique for the detection of fatty liver, as compared with histology, with a pooled sensitivity of 84.8%, a pooled specificity of 93.6% for detecting ≥20%-30% steatosis (5). Previously we have investigated relationships between fatty liver diagnosed by ultrasound, insulin resistance (IR) and obesity and the presence of CAC (6) in a cross sectional analysis of a large Korean cohort. These data showed that whereas fatty liver and IR were both independently associated with CAC, obesity was not (6). Several prospective studies have reported an increased incidence of cardiovascular events in people with non-alcoholic fatty liver disease (NAFLD) (7-18) but is still unclear whether NAFLD contributes independently to coronary artery plaque progression or whether NAFLD is simply a risk marker that co-exists with other recognised cardiovascular (CVD) risk factors such as type 2 diabetes (19, 20). IR co-exists very frequently with type 2 diabetes, obesity and NAFLD (21), and IR

has been shown to be associated with coronary artery calcium (CAC) (22) in cross sectional analysis, but it is uncertain whether IR also contributes to CAC progression over time, independently of diabetes, obesity and fatty liver.

Using data from an occupational cohort in Korea who had measurements of fatty liver and CAC score at baseline and who also had a repeat CAC score measured at follow up; we have investigated the relationship between fatty liver, IR and obesity with change in CAC score over time. Specifically, we tested whether the combination of fatty liver, IR and obesity was associated with an increase in coronary artery calcium (CAC) score (as a marker of early atherosclerosis) over time, independently of diabetes status, and other cardiovascular risk factors and medications used to treat cardiovascular risk factors, cardiovascular disease and diabetes.

Research Design and Methods

The study population consisted of individuals who had a comprehensive health examination and underwent coronary CT scanning to establish a CAC score in 2010 to 2012 and who were followed up in 2013 at Kangbuk Samsung Hospital, College of Medicine, Sungkyunkwan University in South Korea. For the purpose of this study an increase in CAC over time was defined as an increase in a subject's follow up CAC score, compared with their baseline CAC score. Initially 2623 participants were included and 379 individuals were excluded from the study if data were missing for key variables. 44 and 52 subjects were excluded due to past history of cancer and cardiovascular disease with some people meeting more than one exclusion criterion). Subsequently, data for the remaining 2175 participants were analyzed. In South Korea, employees are required to participate in annual or biennial health examinations by the Industrial Safety and Health Law. Some people pay for examinations themselves and in other instances employers pay for these health evaluations. The institutional review board at Kangbuk Samsung Hospital has approved the study, and no specific informed consent was considered necessary.

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Obesity was defined in this Asian population as BMI >25 kg/m^2. Blood samples were collected after an overnight fast. Waist circumference was measured according to a standardized operating procedure. Briefly, the mid-point between the lowest rib and the superior iliac crest was identified in the mid-axilliary line. At this point a measuring tape (SECA 200, circumference measuring tape) was placed around the abdomen, ensuring that the tape was horizontal to the floor. A measurement was taken to the nearest 0.1 cm, at the end of a normal expiration. If the two readings varied by more than 1%, there was a computer-generated prompt to take a third reading. Questionnaires were used to ascertain information regarding alcohol consumption (g/day), smoking (never, ex, current), and frequency of moderate activity each week. Moderate activity was defined as more than 30 min activity per

day that induced slight breathlessness. An enzymatic calorimetric test was used to measure total cholesterol (TC) and triglyceride (TG) concentrations. The selective inhibition method was used to measure HDL-C, and a homogeneous enzymatic calorimetric method was used to measure the concentration of LDL-C (Advia 1650 Autoanalyzer, Bayer Diagnostics, Leverkusen, Germany). Metabolic syndrome (MetS was defined by the 2009 joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention criteria, with waist circumference thresholds of ≥90cm for men and ≥80cm for women that are specific for Asian populations (23).

Questionnaires were used to ascertain information regarding alcohol consumption, smoking (never, ex, current), and frequency of moderate activity each week (defined as more than 30 min activity per day that induced slight breathlessness). Hypertension (HTN) was defined by self report, medication for hypertension or systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg, or self reported medication for hypertension. Diabetes was identified by self report, prescription of medication for diabetes, fasting glucose ≥126mg/dl or HbA1C≥6.5%. The homeostasis model assessment of IR (HOMA-IR) index was calculated by the following equation: HOMA-IR = (fasting insulin [mIU/mL] * fasting glucose [mmol/L])/22.5. Since there are no population-specific thresholds to define IR in a Korean population, we stratified the populations using the 75th percentile to establish an IR group (HOMA-IR ≥75^{th} percentile), as described previously in this population (6, 21) and as recommended by the European Group for the Study of Insulin Resistance (24). BMI≥25kg/m^2 was used to define overweight/obesity. Abdominal ultrasonography (Logic Q700 MR; General Electric, Milwaukee,WI) using a 3.5-MHz probe was performed in all subjects by experienced clinical radiologists, and fatty liver was diagnosed based on standard criteria, including hepatorenal echo contrast, liver brightness, and vascular blurring (25). All computed tomography scans were obtained with a Lightspeed VCT XTe-64 slice MDCT scanner (GE Healthcare, Tokyo, Japan) with the same standard scanning protocol using 2.5-mm section collimation, 400 ms

rotation time, 120 kV tube voltage, and 124 mAS (310 mA*0.4 second) tube current under ECG-gated dose modulation.The quantitative CAC scores were calculated according to the method described by Agatston et al (26) .

Statistical analysis

Statistical analyses were performed with Stata version 11.2 (StataCorp LP, College Station, Texas). All reported p values are 2-tailed, and comparisons with $p < 0.05$ were considered statistically significant. Continuous variables were expressed as mean(SD) for normally distributed variables or median (interquartile range) if not normally distributed.

Categorical variables were expressed as percentages and compared between groups using the chi-squared test. Cox proportional hazards models were used to estimate hazard ratios (HRs and 95% confidence intervals for CAC>0 change over time. We checked the proportional hazards assumption by examining graphs of estimated log (-log) CAC>0 change. HRs and 95% CIs were estimated for each individual risk factor from a multivariable model containing all risk factors. The models were adjusted for age and sex (model 1); age, sex center, year, alcohol consumption, smoking, exercise, education, diabetes status, hypertension, medication for lipid, medication for hypertension, medication for diabetes and LDL-C concentration (model 2); age, sex center, year, alcohol consumption, smoking, exercise, education, diabetes status, hypertension, medication for lipid, medication for hypertension, medication for diabetes, LDL-C concentration, eGFR and hsCRP concentration at baseline. Models were adjusted to test the independence of associations with the study outcome (increase in CAC score over time) and IR, fatty liver and obesity as single risk factors, combinations of any two of these three risk factors, and all three of these risk factors combined.

Results

A total of 2,175 subjects had CAC on baseline and follow-up scans performed approximately 2.3±0.6 years apart. Their mean age was 42.5 years, and 95.1% were men. Mean CAC scores were : 19.2(± 79.6) at baseline and 29.5(± 111.6) at follow-up.

During the median 2.3-year follow-up period, 592 subjects (27.2%) had an increased CAC score at follow-up examination compared with baseline. Table 1 shows the characteristics of the subjects in whom CAC increased from baseline during the follow up period, compared with 1583 subjects in whom CAC score was unchanged or improved during follow up. The age of subjects in whom CAC score increased during follow-up was 44.8±5.5 years and the age of subjects in whom CAC did not change or improved during follow up was 41.6±5.6 years (means±SDs). The proportion of people with diabetes, fatty liver and obesity were all higher in people with a CAC score that increased over time (all $p<0.001$) at 15.0%, 61.7%; and 56.9%, respectively, compared with 6.3%; 49.0%; and 45.4% among people whose CAC score did not change or improved over time. HOMA-IR was also higher in subjects in whom CAC increased compared to subjects in whom CAC score did not increase, or improved, from baseline (1.51 (0.97,2.36) (median, 95%CIs) versus 1.28 (0.82,1.90) (median, 95%CIs) ($p<0.001$).

Table 2 shows the baseline characteristics of the cohort by HOMA-IR quartile. Age was remarkably similar in each quartile and differed by <1 year between quartile group. The proportion of people with diabetes, obesity, fatty liver and MetS differed between quartiles, and in the highest quartile of HOMA-IR, fatty liver was present in 82.8%, obesity was present in 77.7% and diabetes was present in 21.0% of participants. Table 3 shows the numbers (%) of subjects with an improvement in CAC score between baseline and follow up, no change in

CAC score between baseline and follow up, and an increase in CAC score between baseline and follow up, according to HOMA-IR quartiles.

Table 4 shows the associations between individual key risk factors and increase in CAC score at follow up. IR (HOMA-IR quartile 4) was associated with increase in CAC score after adjusting for other risk factors (HR 1.79 (95%CIs 1.09, 2.95)). There were similar trends for the associations between an increase in CAC score over time and obesity, fatty liver and diabetes (HR 1.37 (95%Cis 0.96, 1.96) and (HR 1.28 (95%CIs 0.91, 1.80), 1.72 (0.91-3.22)]. respectively). Table 5 shows the associations between obesity, IR and fatty liver with an increase in CAC score during follow-up. Table 5 also shows associations for these exposures and an increase in CAC score during follow-up, when the factors were present in combinations of two risk factors, and for all three risk factors combined. Adjusting for diabetes status and all other covariates and potential confounders for an increase in CAC score, including age, sex, Center of study, year of study, alcohol consumption, smoking, exercise, education, hypertension, CVD, medication for hypertension, medications for diabetes, lipid lowering medications, LDL-C concentration, eGFR and hsCRP concentration at baseline; the combination of IR, obesity and fatty liver was associated with an increase in CAC score over time [(HR 2.46, (95%CIs 1.50,4.03)].

Among study subjects with baseline CAC = 0, the incidence of CAC>0 increased according to HOMA- IR quartiles. We conducted same analysis for table 4,5 using a cutoff point of 10 in CAC change, the results showed very similar tendency. (supplementary table 1,2).

Discussion

Our data show for the first time that the combination of fatty liver, IR and obesity is associated with progression of atherosclerosis during a median of 2.3 years follow-up in an occupational cohort whose median age was 42.0 years. This association was independent of diabetes status, lipid lowering medications (including statins), treatments for diabetes, and all measured cardiovascular risk factors including LDL-C concentration, eGFR and hsCRP concentration at baseline.

The proportions of people with diabetes, fatty liver, obesity, and the HOMA-IR values were all higher in people in whom CAC score increased during follow-up. However, adjustment for age, sex, diabetes, and other covariates and potential confounders for CVD, had little impact on the strength of the association for the combination of IR, obesity and fatty liver and increase in CAC score over time. We have previously shown in this cohort in a prospective study, that combining fatty liver, IR and obesity was associated with a very marked (~14 fold) increase in the risk of incident diabetes, during five years of follow up (21) . Although we have now shown that the combination of fatty liver, IR and obesity is associated with a comparatively smaller (~2.4 fold) increase in the risk of progression of CAC score over time, the presented data show that these three risk factors (fatty liver, IR and obesity) combined, are associated with a much greater hazard for CAC progression over time, than any single one of these three risk factors in isolation (Table 4). Fatty liver, insulin resistance and obesity all frequently cluster together in people with type 2 diabetes; and our data shows convincingly that fatty liver, IR and obesity combined are associated with an increased HR for CAC score over time, even after adjusting for diabetes status.

CAC progression over time is associated with future cardiovascular events (27, 28) and CAC progression predicts all cause mortality (29). Diabetes is strongly associated with all-cause mortality amongst persons with extensive CAC (30), and we have shown in this cohort that

fatty liver, IR and obesity occur in over 50% of people who develop diabetes (21). Since these three risk factors occur so frequently with diabetes, and diabetes is a strong risk factor for developing CAC, it has been uncertain to date whether the cluster of fatty liver, IR and obesity risk factors are associated with increased risk of CAC progression over time, independently of diabetes status. We adjusted our multivariable regression models not only for diabetes status, but also for LDL-C concentration and for all lipid lowering treatments, since it is known that statins can promote coronary artery plaque regression (specific data for statin medication alone was not available). Interestingly, it has been recently suggested that statins may stabilize coronary artery plaque by promoting coronary atheroma calcification, independent of their plaque-regressive effects (31), thus it is plausible that in subjects taking statins specifically, an increase in CAC may represent a stabilization of the plaque, rather than an increase in atheroma within an increasing coronary artery plaque burden.

The association between NAFLD and multiple complex metabolic and pro-inflammatory changes that have an effect on the vasculature (19, 20), mean that it is difficult to identify causality in assessing the relationship between fatty, IR, obesity and increase in CAC score over time. It is plausible that a predisposition towards fatty liver (and IR) with obesity and progression of the liver disease *per se,* (with increasing inflammation and fibrosis), could further worsen IR and inflammation and thereby increase cardiovascular disease risk. Non alcoholic steatohepatitis (NASH) is a more severe form of NAFLD and NASH is more strongly associated with cardiovascular disease and IR than simple steatosis (9, 32, 33). The hepatic inflammation that occurs with NASH is marked by macrophage activation (34) and it is possible that vascular inflammation and CAC is also more marked with NASH (and increased IR), compared to simple steatosis. Consequently, it seems plausible that altered liver fat metabolism and an inflammatory state in NASH are the important factors contributing to vascular disease in subjects who have the combination of fatty liver, IR and obesity.

There are a few limitations of our study. There was a relatively short period of follow-up, subjects were relatively young and most men mostly men) and there is not data on waist circumference and some secondary causes of chronic liver diseases (e.g., viral hepatitis markers). There were relatively few subjects who experienced an increase in CAC score >10 during follow up and therefore there was limited power to show independent associations between risk factors and an increase in CAC score >10. However, that said the results of these analyses were consistent with the data showing associations between risk factors and any increase in CAC score. Coefficients of variation for measurement of fatty liver and CAC within this cohort are not available. Fatty liver was assessed by liver ultrasound and ultrasonography has limited sensitivity, being unable to detect liver fat infiltration that is approximately <30% by liver weight. Ultrasonography was performed by experienced clinical radiologists who diagnosed fatty liver based on known standard clinical criteria that included hepatorenal echo contrast, liver brightness, and vascular blurring. We are therefore unable to include evidence of agreement between radiologists. However, in the presented analyses we used the clinical definition of fatty liver as a dichotomous exposure variable. It is unlikely that fatty liver status, IR or obesity would have been influenced by CAC score and consequently any random misclassification bias of fatty liver status would also bias our findings for the relationship between the combination of fatty liver, IR obesity with CAC progression, towards the null. With regard to fatty liver, we are also unable to comment on NAFLD severity as histological assessment of liver using the Kleiner score (35) (which is the gold standard for assessing hepatic inflammation and fibrosis), was not performed. Consequently, we are unable to examine whether the more severe forms of NAFLD such as NASH with fibrosis are associated with CAC progression over time. There is no established definition of IR as a categorical variable and we have used ≥75th centile of HOMA-IR to define IR, as we have described before in this cohort (6). Each of the individual risk factors i.e. obesity, IR and fatty liver was associated with an increased risk of increase in CAC score, albeit there was limited power to prove that each of these individual risk factors was independently associated with increase in

CAC score. For all three risk factors combined there was a greater cumulative risk conveyed by all three factors combined. Consequently, we were able to show a significant effect of all three risk factors combined, despite the limited power of the study.

In summary, we have shown that the combination of fatty liver, IR and obesity is associated with progression of atherosclerosis (as indicated by increase in CAC score over time) and this association was independent of diabetes status, lipid lowering and antihypertensive medications and all measured cardiovascular risk factors. Advice on effective approaches to primary prevention of cardiovascular disease should be offered to individuals with these risk factor patterns.

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Table 1. Baseline characteristics of study participants by change in CAC score at follow up

Characteristics	Overall	CAC change		P value
		No CAC change or improvement in CAC	CAC increased from baseline	
Number	2,175	1,583	592	
Age (years)[a]	42.5 (5.7)	41.6 (5.6)	44.8 (5.5)	<0.001
Male (%)	95.1	93.6	99.2	<0.001
Seoul center (%)	53.0	51.3	57.6	0.009
BMI (kg/m^2) [a]	25.1 (3.0)	24.9 (3.0)	25.8 (3.0)	<0.001
Obesity (%)	48.5	45.4	56.9	<0.001
Current smoker (%)	33.9	33.2	35.6	0.288
Alcohol intake (%)[b]	34.6	32.3	40.7	<0.001
High education level (%)[c]	85.9	85.5	87.0	0.404
Diabetes (%)	8.6	6.3	15.0	<0.001
Hypertension (%)	24.0	20.3	33.6	<0.001
Medication for dyslipidemia (%)	5.4	4.0	9.0	<0.001
Medication of Diabetes (%)	3.6	2.4	6.8	<0.001
Medication of Hypertension (%)	11.2	8.1	19.6	<0.001
Systolic BP (mmHg) [a]	119.2 (12.2)	118.5 (12.2)	121.3 (11.9)	<0.001
Diastolic BP (mmHg)[a]	76.5 (9.5)	75.9 (9.4)	78.1 (9.5)	<0.001
Glucose (mg/dL)[a]	100.1 (17.5)	98.6 (15.4)	103.9 (21.9)	<0.001
Total cholesterol (mg/dL)[a]	210.0 (37.2)	206.8 (36.4)	218.6 (38.0)	<0.001
LDL-C (mg/dL)[a]	133.0 (33.5)	130.0 (32.7)	141.1 (34.2)	<0.001
HDL-C (mg/dL)[a]	51.0 (12.1)	51.7 (12.4)	49.3 (11.1)	<0.001
Triglycerides (mg/dL)[d]	137 (94-197)	130 (89-190)	153 (110.5-215)	<0.001
ALT (U/l)[d]	25 (18-38)	25 (18-37)	28 (20-40)	<0.001
GTP (U/l)[d]	36 (23-56)	34 (22-54)	40 (27-65)	<0.001
HOMA-IR [d]	1.34 (0.86-2.01)	1.28 (0.82-1.90)	1.51 (0.97-2.36)	<0.001
Fatty liver (%)	52.5	49.0	61.7	<0.001
MetS (%)	26.3	22.2	36.1	<0.001
eGFR mls/min [a]	90.8 (13.8)	91.1 (13.4)	90.2 (14.7)	0.184
eGFR<90 mls/min (%)	55.6	55.1	57.1	0.401
eGFR<60 mls/min (%)	0.28	0.19	0.51	0.228
hsCRP (mg/dL)	0.06 (0.04-0.12)	0.06(0.03-0.11)	0.07(0.04-0.13)	0.022
CAC score [a]	72.7 (141.7)	33.2 (71.4)	81.3 (151.5)	<0.001
CAC score [d]	22 (6-74)	8 (3-21)	27 (8-81)	<0.001

Data are [a]means (standard deviation), [d]medians (interquartile range), or percentages. Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance, [b]≥20 g/day; [c]≥ College graduate.

CAC score [a] mean of cac score if cac>0

CAC score [d] median of cac score if cac>0…

Table 2. Baseline characteristics of study participants by HOMA-IR quartiles

Characteristics		HOMA-IR quartiles				P for trend
	Overall	Q1	Q2	Q3	Q4	
Number	2,175	544	544	544	543	
Age (years)[a]	42.5 (5.7)	42.7 (6.0)	42.9 (5.6)	42.2 (5.8)	42.1 (5.4)	0.014
Male (%)	95.1	93.8	94.7	94.9	97.1	0.016
Seoul center (%)	53.0	56.3	52.4	54.6	48.8	0.036
BMI(kg/m^2)	25.1(3.0)	23.1(2.3)	24.4(2.4)	25.6 (2.4)	27.3 (3.1)	<0.001
Obesity (%)	48.5	19.3	37.7	59.4	77.7	<0.001
Current smoker (%)	32.3	30.9	29.0	34.4	35.0	0.049
Alcohol intake (%)[b]	34.6	33.5	33.6	34.2	37.2	0.197
High education level (%)[c]	85.9	85.5	86.5	86.9	84.8	0.788
Diabetes (%)	8.6	2.4	4.8	6.4	21.0	<0.001
Hypertension (%)	24.0	14.5	20.0	27.2	34.1	<0.001
Medication of dyslipidemia	5.4	3.3	4.4	6.3	7.6	0.001
Medication of Diabetes	3.6	0.7	2.2	2.6	8.8	<0.001
Medication of Hypertension	11.2	6.1	9.6	14.0	15.3	<0.001
Systolic BP (mmHg) [a]	119.2 (12.2)	114.8 (11.1)	118.2 (11.9)	120.4 (12.1)	123.6 (12.0)	<0.001
Diastolic BP (mmHg)[a]	76.5 (9.5)	73.4 (8.8)	75.5 (9.2)	77.2 (9.2)	79.9 (9.5)	<0.001
Glucose (mg/dL)[a]	100.1 (17.5)	91.4 (8.7)	97.2 (10.4)	99.8 (11.4)	111.8 (26.4)	<0.001
Total cholesterol (mg/dL)[a]	210.0 (37.2)	203.7 (36.6)	209.5 (36.9)	211.8 (36.3)	215.0 (38.2)	<0.001
LDL-C (mg/dL)[a]	133.0 (33.5)	128.5 (34.4)	133.4 (33.6)	134.6 (32.1)	135.6 (33.5)	<0.001
HDL-C (mg/dL)[a]	51.0 (12.1)	56.9 (13.4)	51.2 (11.9)	49.1 (10.8)	46.0 (9.4)	<0.001
Triglycerides (mg/dL)[d]	137 (94-197)	95.5 (70-129.5)	129 (93.5-179)	156.5 (107-222)	183 (133-259)	<0.001
ALT (U/l)[d]	25 (18-38)	20 (15-27)	23 (17-32)	27 (20-40)	35 (25-50)	<0.001
GTP (U/l)[d]	36 (23-56)	25 (18-40)	32 (21-50)	39 (27-59.5)	48 (33-74)	<0.001
Fatty liver (%)	52.5	25.4	42.0	59.7	82.8	<0.001
MetS (%)	26.3	6.2	13.3	31.0	58.1	<0.001
eGFR mls/min [a]	90.8 (13.8)	90.1 (13.2)	89.8 (13.8)	91.4 (12.9)	91.9 (15.0)	0.008
eGFR<90 mls/min (%)	55.6	57.7	58.8	54.0	51.9	0.020
eGFR<60 mls/min (%)	0.28	0.00	0.74	0.37	0.00	0.716
hsCRP (mg/dL)	0.06(0.04-0.12)	0.05(0.02-0.09)	0.05(0.03-0.1)	0.06(0.04-0.12)	0.08(0.05-0.15)	<0.001
CAC score (if >0) [a]	72.7(141.7)	73.7(147.7)	53.4(87.6)	84.5(163.5)	75.8(148.8)	0.579
CAC score (if >0) [d]	22(6-74)	20(7-68)	22(5-64)	17(5-89)	24(6-75)	0.579

Data are [a]means (standard deviation), [d]medians (interquartile range), or percentages. Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein-cholesterol; [b] ≥20 g/day; [c] ≥ College graduate.

Table 3. Distribution of change in CAC score over time according to quartiles of HOMA-IR

	HOMA IR			
	Q1 (N=544)	**Q2 (N=544)**	**Q3 (N=544)**	**Q4 (N=543)**
	N (%)	**N (%)**	**N (%)**	**N (%)**
CAC score improved	18 (3.3)	20 (3.7)	27 (5.0)	19 (3.5)
No change in CAC score	412 (75.7)	387 (71.1)	373 (68.6)	327 (60.2)
CAC increased	114 (21.0)	137 (25.2)	144 (26.4)	197 (36.3)

HOMA-IR Quartiles 1 (Q1) ~0.856, Q2 0.858-1.337, Q3 1.338-2.007, Q4 2.008~

Table 4 Hazard ratios for an increase in CAC score over time for risk factors at baseline identified from a multi-variable model

	HR [95%CIs]
Age (per year)	1.11 [1.08, 1.15]
Male sex	18.35 [4.25, 79.23]
Center	1
Year of study	1.05 [0.77, 1.43]
Alcohol=0 g/day	1.00 (reference)
>0~20 g/day	0.62 [0.35, 1.09]
>=20 g/day	0.68 [0.38, 1.22]
Never smoking	1.00 (reference)
Ex-smoking	0.85 [0.58-1.24]
Smoking	0.92 [0.62-1.36]
Exercise	1.40 [0.91-2.14]
Education status	0.94 [0.56-1.58]
Diabetes	1.72 [0.91-3.22]
Hypertension	1.15 [0.74-1.80]
Medication for dyslipidemia	1.23 [0.71-2.14]
Medication for Diabetes	0.84 [0.35-2.04]
Medication for Hypertension	1.30 [0.73-2.30]
Fatty liver	1.28 [0.91-1.80]
Obesity	1.37 [0.96-1.96]
HOMA Q1	1.00 (reference)
Q2	1.45 [0.94-2.23]
Q3	1.05 [0.66-1.69]
Q4	1.79 [1.09-2.95]
hsCRP	1.14 [0.80-1.62]
eGFR	1.01[1.00-1.02]

Table 5. Associations between obesity, insulin resistance, fatty liver and increase in CAC score during follow up

		HR [95%CIs]		
Total	**Number with CAC score increase/number with risk factor(s) (%)**	**Model 1**	**Model 2**	**Model 3**
NONE OF 3 FACTORS	89/403 (22.1)	1	1	1
IR ALONE	11/26 (42.3)	2.60(1.09-6.19)	1.54(0.55-4.34)	1.70(0.59-4.91)
OBESITY ALONE	16/76 (21.1)	1.24(0.66-2.35)	1.31(0.66-2.61)	1.36(0.67-2.76)
FATTY LIVER ALONE	68/238 (28.6)	1.44(0.98-2.11)	1.27(0.83-1.93)	1.28(0.83-1.96)
IR + OBESITY	9/22 (40.9)	2.91(1.11-7.58)	3.14(1.10-8.96)	3.35(1.15-9.72)
IR + FATTY LIVER	21/54 (38.9)	2.60(1.40-4.82)	1.65(0.80-3.37)	1.62(0.78-3.34)
OBESITY + FATTY LIVER	61/173 (35.3)	1.98(1.31-2.99)	1.49(0.93-2.39)	1.51(0.93-2.44)
IR +OBESE +FATTY LIVER	66/159 (41.5)	3.04(2.01-4.62)	2.35(1.44-3.84)	2.46(1.50-4.03)

Insulin resistance (IR) = HOMA IR ≥75%.

MODEL 1 Adjusted for age and sex. MODEL 2 Adjusted for age, sex, center, year, alcohol consumption, smoking, exercise, education, diabetes status, hypertension, medication for lipid, medication for hypertension, medication for diabetes , LDL-C concentration. MODEL 3 Adjusted for age, sex, center, year, alcohol consumption, smoking, exercise, education, diabetes status, hypertension, medication for lipids, medication for hypertension, medication for diabetes , LDL-C concentration, eGFR and hsCRP concentration at baseline