Clinical Investigations

PREVENTION

Fatty Liver, Insulin Resistance, and Obesity: Relationships With Increase in Coronary Artery Calcium Over Time

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ABSTRACT

Background: Nonalcoholic fatty liver disease, insulin resistance (IR), and obesity frequently coexist with type 2 diabetes mellitus (DM), but it is uncertain whether these risk factors for vascular disease contribute to a change in atherosclerosis over time, independently of DM status.

Hypothesis: We hypothesized that the combination of fatty liver, IR, and obesity would be associated with an increase in coronary artery calcium (CAC) score over time, independently of DM status, other cardiovascular risk factors, and medications.

Methods: Data were analyzed from a South Korean occupational cohort of 2175 people. The outcome was increase in cardiac computed tomography CAC score between baseline and follow-up. Insulin resistance was defined by homeostatic model assessment of insulin resistance (HOMA-IR) $\geq$75th percentile and fatty liver by ultrasound.

Results: In 592 (27.2%) participants, CAC score increased from baseline (mean $\pm$ SD; mean age at baseline, 44.8 $\pm$ 5.5 years); and in 1583 subjects, CAC did not change or improved during follow-up (mean age, 41.6 $\pm$ 5.6 years). Diabetes mellitus, HOMA-IR, fatty liver, and obesity prevalence were all higher (all $P < 0.001$) in participants whose CAC score increased from baseline. Adjusting for DM and potential confounders, the combination of IR, obesity, and fatty liver was independently associated with increase in CAC score over time (hazard ratio: 2.46, 95% confidence interval: 1.50-4.03).

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

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 cardiovascular (CV) risk prediction in asymptomatic individuals.[1] The total volume of 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 CV 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 noninvasive tool to assess risk of CV events.[3] Coronary artery calcium scores also perform better in identifying high-risk individuals compared with an alternative noninvasive measurement, carotid intima-media thickness: CAC scans are associated with relatively low radiation exposure (0.9–1.1 mSv), and

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CAC scores provide information that can be used not only for risk stratification, but also 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% and a pooled specificity of 93.6% for detecting ≥20% to 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 CV events in people with nonalcoholic fatty liver disease (NAFLD),[7–18] but it is still unclear whether NAFLD contributes independently to coronary artery plaque progression or whether NAFLD is simply a risk marker that coexists with other recognized CV risk factors such as type 2 diabetes mellitus (DM).[19,20] Insulin resistance coexists very frequently with type 2 DM, obesity, and NAFLD,[21] and IR has been shown to be associated with CAC[22] in cross-sectional analysis, but it is uncertain whether IR also contributes to CAC progression over time, independently of DM, 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 CAC score (as a marker of early atherosclerosis) over time, independently of DM status, other CV risk factors and medications used to treat CV risk factors, and cardiovascular disease (CVD).

Methods

The study population consisted of individuals who had a comprehensive health examination and underwent coronary CT scanning to establish a CAC score from 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. Forty-four and 52 subjects were excluded due to past history of cancer and CVD, with some people meeting ≥1 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 was calculated as weight in kilograms divided by height in meters squared. Obesity was defined in this Asian population as body mass index >25 kg/m^2. Blood samples were collected after an overnight fast. Waist circumference was measured according to a standardized operating procedure. Briefly, the midpoint between the lowest rib and the superior iliac crest was identified in the mid-axillary line. At this point a measuring tape (Seca 200 circumference measuring tape; Seca, Birmingham, UK) 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 2 readings varied by >1%, there was a computer-generated prompt to take a third reading. Questionnaires were used to ascertain information regarding alcohol consumption (g/d), smoking (never, ex, current), and frequency of moderate activity each week. Moderate activity was defined as >30 minutes of activity per day that induced slight breathlessness. An enzymatic calorimetric test was used to measure total cholesterol and triglyceride concentrations. The selective inhibition method was used to measure high-density lipoprotein cholesterol (HDL-C), and a homogeneous enzymatic calorimetric method was used to measure the concentration of low-density lipoprotein cholesterol (LDL-C; Advia 1650 Autoanalyzer; Bayer Diagnostics, Leverkusen, Germany). Metabolic syndrome 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 ≥90 cm for men and ≥80 cm for women that are specific for Asian populations.[23]

Hypertension (HTN) was defined by self-report, medication for HTN, 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 DM, fasting glucose ≥126 mg/dL, or HbA_{1c} ≥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. Because 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 ≥75th percentile), as described previously in this population[6,21] and as recommended by the European Group for the Study of Insulin Resistance.[24] Body mass index ≥25 kg/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 CT scans were obtained with a LightSpeed VCT XTe 64-slice multidetector row CT 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 electrocardiogram-gated dose modulation. The quantitative CAC scores were calculated according to the method described by Agatston et al.[26]

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Statistical Analysis

Statistical analyses were performed with Stata version 11.2 (StataCorp LP, College Station, TX). 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 χ^2 test. Cox proportional hazards models were used to estimate hazard ratios (HRs and 95% confidence intervals (CIs) for CAC > 0 change over time. We checked the proportional hazards assumption by examining graphs of estimated log (−log) CAC > 0 change. Hazard ratios 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, DM status, HTN, medication for lipids, medication for HTN, medication for DM, and LDL-C concentration (Model 2); and age, sex center, year, alcohol consumption, smoking, exercise, education, DM status, HTN, medication for lipids, medication for HTN, medication for DM, LDL-C concentration, estimated glomerular filtration rate (eGFR), and high-sensitivity C-reactive protein (hsCRP) concentration at baseline (Model 3). 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 2 of these 3 risk factors, and all 3 of these risk factors combined.

Results

A total of 2175 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 male. 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 592 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 ± SD). The proportions of people with DM, 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. The HOMA-IR was also higher in subjects in whom CAC increased compared with subjects in whom CAC score did not increase or improved from baseline (median: 1.51, 95% CI: 0.97-2.36 vs median: 1.28, 95% CI: 0.82-1.90, respectively; $P < 0.001$).

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 DM, obesity, fatty liver, and metabolic syndrome 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 DM 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. Insulin resistance (HOMA-IR quartile 4) was associated with increase in CAC score after adjusting for other risk factors (HR: 1.79, 95% CI: 1.09-2.95). There were similar trends for the associations between an increase in CAC score over time and obesity, fatty liver, and DM (HR: 1.37, 95% CI: 0.96-1.96; HR: 1.28, 95% CI: 0.91-1.80; and HR: 1.72, 95% CI: 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 2 risk factors, and for all 3 risk factors combined. Adjusting for DM 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, HTN, CVD, medication for HTN, medications for DM, 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% CI: 1.50-4.03).

Among study subjects with baseline CAC = 0, the incidence of CAC > 0 increased according to HOMA-IR quartiles. We conducted the same analysis using a cutoff point of 10 in CAC change; the results showed a very similar tendency (see Supporting Information, Tables 1 and 2, in the online version of this article).

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 of follow-up in an occupational cohort whose median age was 42.0 years. This association was independent of DM status, lipid-lowering medications (including statins), treatments for DM, and all measured CV risk factors including LDL-C concentration, eGFR, and hsCRP concentration at baseline.

The proportions of people with DM, 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, DM, 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 DM during 5 years of follow-up.[21] Although we have now

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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 Change or Improvement in CAC	CAC Increased From Baseline	
N	2175	1583	592	
Age, y	42.5 (5.7)	41.6 (5.6)	44.8 (5.5)	<0.001
Male sex	95.1	93.6	99.2	<0.001
Seoul center	53.0	51.3	57.6	0.009
BMI, kg/m^2	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[a]	34.6	32.3	40.7	<0.001
High education level[b]	85.9	85.5	87.0	0.404
DM	8.6	6.3	15.0	<0.001
HTN	24.0	20.3	33.6	<0.001
Medications				
For dyslipidemia	5.4	4.0	9.0	<0.001
For DM	3.6	2.4	6.8	<0.001
For HTN	11.2	8.1	19.6	<0.001
SBP, mm Hg	119.2 (12.2)	118.5 (12.2)	121.3 (11.9)	<0.001
DBP, mm Hg	76.5 (9.5)	75.9 (9.4)	78.1 (9.5)	<0.001
Glucose, mg/dL	100.1 (17.5)	98.6 (15.4)	103.9 (21.9)	<0.001
TC, mg/dL	210.0 (37.2)	206.8 (36.4)	218.6 (38.0)	<0.001
LDL-C, mg/dL	133.0 (33.5)	130.0 (32.7)	141.1 (34.2)	<0.001
HDL-C, mg/dL	51.0 (12.1)	51.7 (12.4)	49.3 (11.1)	<0.001
TG, mg/dL	137 (94–197)	130 (89–190)	153 (110.5–215)	<0.001
ALT, U/L	25 (18–38)	25 (18–37)	28 (20–40)	<0.001
GGTP, U/L	36 (23–56)	34 (22–54)	40 (27–65)	<0.001
HOMA-IR	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, mL/min	90.8 (13.8)	91.1 (13.4)	90.2 (14.7)	0.184
eGFR <60 mL/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	19.2 (79.6)	2.2 (19.9)	64.9 (139.3)	<0.001
CAC score	0 (0–1)	0 (0–0)	2 (16–64)	<0.001

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CAC, coronary artery calcium; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; GGTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; HTN, hypertension; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglycerides.
Data presented as %, mean ± SD, or median (IQR).
[a] ≥20 g/d. [b] ≥ College graduate.

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Table 2. Baseline Characteristics of Study Participants by HOMA-IR Quartiles

Characteristics	Overall	HOMA-IR Quartiles				*P* for Trend
		Q1	Q2	Q3	Q4	
N	2175	544	544	544	543	
Age, y	42.5 (5.7)	42.7 (6.0)	42.9 (5.6)	42.2 (5.8)	42.1 (5.4)	0.014
Male sex	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[a]	34.6	33.5	33.6	34.2	37.2	0.197
High education level[b]	85.9	85.5	86.5	86.9	84.8	0.788
DM	8.6	2.4	4.8	6.4	21.0	<0.001
HTN	24.0	14.5	20.0	27.2	34.1	<0.001
Medications						
For dyslipidemia	5.4	3.3	4.4	6.3	7.6	0.001
For DM	3.6	0.7	2.2	2.6	8.8	<0.001
For HTN	11.2	6.1	9.6	14.0	15.3	<0.001
SBP, mm Hg	119.2 (12.2)	114.8 (11.1)	118.2 (11.9)	120.4 (12.1)	123.6 (12.0)	<0.001
DBP, mm Hg	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	100.1 (17.5)	91.4 (8.7)	97.2 (10.4)	99.8 (11.4)	111.8 (26.4)	<0.001
TC, mg/dL	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	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	51.0 (12.1)	56.9 (13.4)	51.2 (11.9)	49.1 (10.8)	46.0 (9.4)	<0.001
TG, mg/dL	137 (94–197)	95.5 (70–129.5)	129 (93.5–179)	156.5 (107–222)	183 (133–259)	<0.001
ALT, U/L	25 (18–38)	20 (15–27)	23 (17–32)	27 (20–40)	35 (25–50)	<0.001
GGTP, U/L	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, mL/min	90.8 (13.8)	90.1 (13.2)	89.8 (13.8)	91.4 (12.9)	91.9 (15.0)	0.008
eGFR <60 mL/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	19.2 (79.6)	16.4 (75.9)	12.7 (48.2)	24.0 (95.1)	24.0 (90.5)	0.579
CAC score if >0	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–2)	0 (0–4)	0.579

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CAC, coronary artery calcium; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; GGTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; HTN, hypertension; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; Q, quartile; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglycerides.
Data presented as %, mean ± SD, or median (IQR).
[a] ≥20 g/d. [b] ≥ College graduate.

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Table 3. Distribution of Change in CAC Score Over Time According to Quartiles of HOMA-IR

	HOMA-IR Quartiles[a]			
	Q1, n = 544	Q2, n = 544	Q3, n = 544	Q4, n = 543
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)

Abbreviations: CAC, coronary artery calcium; HOMA-IR, homeostatic model assessment of insulin resistance; Q, quartile.
Data are presented as n (%).
[a]HOMA-IR quartiles: Q1, ~0.856; Q2, 0.858–1.337; Q3, 1.338–2.007; Q4, 2.008 ~ .

Table 4. HRs for an Increase in CAC Score Over Time for Risk Factors at Baseline Identified From a Multivariable Model

	HR (95% CI)
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/d	1.00 (Ref)
>0–20 g/d	0.62 (0.35-1.09)
≥20 g/d	0.68 (0.38-1.22)
Never smoking	1.00 (Ref)
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)
DM	1.72 (0.91-3.22)
HTN	1.15 (0.74-1.80)
Medication	
For dyslipidemia	1.23 (0.71-2.14)
For DM	0.84 (0.35-2.04)
For HTN	1.30 (0.73-2.30)
Fatty liver	1.28 (0.91-1.80)
Obesity	1.37 (0.96-1.96)
HOMA-IR quartile	
Q1	1.00 (Ref)
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)

Abbreviations: CAC, coronary artery calcium; CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; HTN, hypertension; Ref, reference.

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 3 risk factors (fatty liver, IR, and obesity) combined are associated with a much greater hazard for CAC progression over time than any single 1 of these 3 risk factors in isolation (Table 4). Fatty liver, IR, and obesity all frequently cluster together in people with type 2 DM, and our data show convincingly that fatty liver, IR, and obesity combined are associated with an increased HR for CAC score over time, even after adjusting for DM status.

Coronary artery calcium progression over time is associated with future CV events,[27,28] and CAC progression predicts all-cause mortality.[29] Diabetes is strongly associated with all-cause mortality among persons with extensive CAC,[30] and we have shown in this cohort that fatty liver, IR, and obesity occur in >50% of people who develop DM.[21] Because these 3 risk factors occur so frequently with DM, and DM 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 is associated with increased risk of CAC progression over time, independently of DM status. We adjusted our multivariable regression models not only for DM status, but also for LDL-C concentration and for all lipid-lowering treatments, because it is known that statins can promote coronary artery plaque regression (specific data for statin medication alone were 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] means 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 toward 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 CVD risk. Nonalcoholic steatohepatitis (NASH) is a more severe form of NAFLD, and NASH is more strongly associated with CVD 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 with 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.

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Table 5. Associations Between Obesity, IR, Fatty Liver, and Increase in CAC Score During Follow-up

Total	No. With CAC Score Increase/No. With Risk Factor(s) (%)	HR (95% CI)		
		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 + obesity + fatty liver	66/159 (41.5)	3.04 (2.01-4.62)	2.35 (1.44-3.84)	2.46 (1.50-4.03)

Abbreviations: CAC, coronary artery calcium; CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; HTN, hypertension; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol.
Insulin resistance is defined as HOMA-IR ≥75%. Model 1 adjusted for age and sex. Model 2 adjusted for model 1 + center, year, alcohol consumption, smoking, exercise, education, DM status, HTN, medication for lipids, medication for HTN, medication for DM, and LDL-C concentration. Model 3 adjusted for model 2 + eGFR and hsCRP concentration at baseline.

Study Limitations

There are a few limitations to our study. There was a relatively short period of follow-up, subjects were relatively young and mostly male, and there is no data on waist circumference and some secondary causes of chronic liver diseases (eg, viral hepatitis markers). Relatively few subjects 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, and obesity with CAC progression toward the null. With regard to fatty liver, we are also unable to comment on NAFLD severity because 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 percentile of HOMA-IR to define IR, as we have described before in this cohort.[6] Each of the individual risk factors (eg, 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 3 risk factors combined, there was a greater cumulative risk conveyed by all 3 factors combined. Consequently, we were able to show a significant effect of all 3 risk factors combined, despite the limited power of the study.

Conclusion

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 DM status, lipid-lowering and antihypertensive medications, and all measured CV risk factors. Advice on effective approaches to primary prevention of CVD should be offered to individuals with these risk-factor patterns.

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