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Non-alcoholic steatohepatitis and progression of carotid atherosclerosis in patients with type 2 diabetes: a Korean cohort study

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Abstract

Background

There is increasing concern regarding cardiovascular risk in individuals with non-alcoholic fatty liver disease. This study was conducted to evaluate whether hepatic steatosis with or without fibrosis is associated with progression of carotid atherosclerosis in patients with type 2 diabetes.

Methods

From a longitudinal cohort, we enrolled 1,120 type 2 diabetes patients who underwent repeated carotid artery ultrasonography every 1–2 years. Ultrasonographic findings at baseline and after 6–8 years were compared. Presence of hepatic steatosis was mainly assessed by abdominal ultrasonography; patients with hepatic steatosis were further evaluated for hepatic fibrosis according to fibrosis-4 index. We investigated the association between liver status and atherosclerosis progression.

Results

Of 1,120 patients, 636 (56.8%) were classified as having hepatic steatosis at baseline. After 6–8 years, 431 (38.5%) showed atherosclerosis progression. Hepatic steatosis was significantly associated with atherosclerosis progression (adjusted odds ratio[AOR]: 1.370, 95% CI: 1.025–1.832; $p < 0.05$). Among patients with hepatic steatosis, only individuals with fibrosis showed significant association with atherosclerosis progression (AOR: 1.636, 95% CI: 1.024–2.612; $p < 0.05$). Furthermore, subjects with hepatic steatosis & fibrosis and ≥ 4 components of metabolic syndrome criteria showed markedly increased risk of atherosclerosis progression (AOR: 2.776, 95% CI: 1.276–6.039; $p < 0.05$). The association between hepatic fibrosis and atherosclerosis progression was significant in all metabolic subgroups, regardless of age, body mass index, presence of metabolic syndrome, or insulin sensitivity (all $p < 0.05$).

Conclusions

Hepatic steatosis with fibrosis is independently associated with progression of carotid atherosclerosis in patients with type 2 diabetes.

Background

The prevalence of non-alcoholic fatty liver disease (NAFLD) is rapidly rising relative to increased obesity and/or type 2 diabetes.[1] NAFLD is known to be associated with various complications such as chronic kidney disease (CKD), cancer, heart failure, or atherosclerosis,[2] and cardiovascular complications remain the leading cause of mortality of patients with NAFLD.[3–7] Non-alcoholic steatohepatitis (NASH),

one of several categories of NAFLD, produces more significant liver injury including lobular inflammation, hepatocyte ballooning, fibrosis, and cirrhosis compared to simple NAFLD,[8] and patients with NASH were reported to have much higher incidence of coronary artery disease-related mortality.[9–11] It is important to assess hepatic steatosis and fibrosis to identify those at high risk of cardiovascular disease and to optimally commence medical interventions.[12, 13]

This scenario is of special concern in patients with type 2 diabetes, which is known to be associated with higher risk of NAFLD.[12, 14] While NAFLD is an independent risk factor for cardiovascular complications, [15] when combined with type 2 diabetes, it further increases the risk of systemic atherosclerosis.[3] Insulin resistance, a characteristic feature of both type 2 diabetes and NAFLD, is known as the key pathophysiology linking type 2 diabetes, NAFLD, and atherosclerosis.[2, 16] However, little is known about longitudinal effects of NAFLD or NASH on systemic atherosclerosis in type 2 diabetes.

The aim of this study was to investigate the relationship between NAFLD with or without significant fibrosis and the risk of carotid atherosclerosis progression assessed by clinical, laboratory, and repeated imaging findings in type 2 diabetes patients.

Methods

Study participants

Participants were recruited from the Seoul Metabolic Syndrome Cohort, of which total 13,296 patients were diagnosed and treated for type 2 diabetes from November 1997 to September 2016 at Huh Diabetes Center as previously described.[3, 17] Enrollees were aged 19 years or older who had undergone repeated carotid artery ultrasonography at 1–2 year intervals for up to 8 years. Participants were diagnosed with type 2 diabetes according to the American Diabetes Association classification.[18] Patients were excluded for any one of the following criteria: 1) Under 19 years of age; 2) Diagnosed with type 1 diabetes; 3) Pregnant; 4) Diagnosed with liver disease other than NAFLD, such as viral or autoimmune hepatitis; 5) History of heavy alcohol consumption (> 140 g/week). Patients with baseline bilateral carotid artery plaque in whom presence of new-onset plaque was difficult to judge in repeat ultrasonography were also excluded. In total, we enrolled 1,120 type 2 diabetes patients who underwent repeat carotid artery ultrasonography at 6-8-year intervals and evaluations for presence of hepatic steatosis or fibrosis at baseline. All participants provided written informed consent, and the Ethics Committee of the Yonsei University College of Medicine approved this study (4-2019-0270).

Measurements and definitions of clinical and laboratory parameters

At baseline, we collected information from participants regarding medical and family history, smoking & alcohol history/consumption, and physical activity level/week. Medication history regarding aspirin, statin, and anti-diabetic drug (insulin, sulfonylurea, metformin, thiazolidinedione) usage was also reviewed. Anthropometrics including weight, height, and waist circumference were obtained by trained

nurses who were blinded to patients' clinical and laboratory data, and blood samples were collected from participants a) after ≥ 8 hours of fasting, and b) 2 hours after a meal. Metabolic parameters including HbA1c, lipid profiles (total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride), blood urea nitrogen (BUN), creatinine, total bilirubin, aspartate/alanine aminotransferase (AST/ALT), total protein, albumin, and platelet count were measured by routine laboratory methods on fresh samples at the same day of collection.

The estimated glomerular filtration rate (eGFR) was derived from Modification of the Diet in Renal Disease equation (MDRD).[19] Diagnosis and classification of CKD was based upon Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, and patients with eGFR < 60 mL/min/1.73 m^2 for > 3 months were diagnosed as CKD stage III-V accordingly.[20]

Insulin sensitivity was assessed by calculating rate constant for plasma glucose disappearance (KITT; %/min) in a short insulin tolerance test.[21] The test was performed at 8:00AM after an overnight fast, and venous blood samples were collected at 0, 3, 6, 9, 12, and 15 min after an intravenous bolus injection of regular insulin (Humulin; Eli Lilly, Indianapolis, IN, USA) at a dosage of 0.1U/kg. Plasma glucose concentrations were measured immediately after sampling using a Beckman glucose analyzer II (Beckman Coulter Inc., Brea, CA, USA), and KITT was determined by calculating the rate of the fall in log-transformed plasma glucose between 3 and 15 min. 100 mL of 20% dextrose solution was administered intravenously immediately after testing to prevent potential hypoglycemia. Insulin resistance was defined as KITT < 2.5%/min.[22]

The diagnosis of metabolic syndrome was made according to a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity published in 2009.[23] Hypertension was defined as systolic blood pressure (BP) ≥ 140 mmHg and/or a diastolic BP ≥ 90 mmHg, or current use of antihypertensive medications. Individuals who drank twice a month or more were defined as regular alcohol consumers, and participants who had ever smoked more than 5 packs of cigarettes were considered ever smokers. Regular exercise was defined as moderate to vigorous physical activity for over 30 min more than once a month. Overweight was defined as body mass index (BMI) ≥ 23 kg/m^2 according to scientific statement from World Health Organization.[24]

Liver status measurements

Among 1,120 participants, 1,086 underwent abdominal ultrasonography (iU22; Philips Healthcare, Andover, MA, USA) with a 3.5 MHz transducer after 8 hours of fasting. Ultrasound examinations were performed by trained radiologists who were blinded to patients' clinical and laboratory information. According to ultrasonographic findings, participants were assessed if they had hepatic steatosis or not. Presence of hepatic steatosis in 34 patients who did not undergo abdominal ultrasonography was determined by calculating the Comprehensive Non-Alcoholic Fatty Liver Disease Score (CNS),[25] in which a score ≥ 40 indicated hepatic steatosis. Those with hepatic steatosis were further evaluated for

presence of independent hepatic fibrosis by calculating the fibrosis-4 (FIB-4) index. Significant fibrosis was defined as FIB-4 index ≥ 1.45 in this study.[26]

Carotid atherosclerosis measurements

Every participant underwent repeated carotid ultrasonography every 1–2 years to evaluate carotid atherosclerosis status. We compared the rate of atherosclerosis progression at baseline and at 6–8 years. Both common carotid arteries were examined by high-resolution ultrasonography (LOGIQ7; GE Healthcare, Chicago, IL, USA) by trained technicians blinded to patients' clinical and laboratory data. The mid and distal common carotid artery was scanned by lateral longitudinal projection, and carotid intima-media thickness (IMT; mm) was measured at three points: far wall of mid; distal common carotid artery; and 1 cm proximal to the carotid bulb. Carotid IMT was defined as the distance between lumen-intima interface and media-adventitia interface, of which the mean value of 3 measurements on each side was used to represent carotid atherosclerosis status.

Carotid atherosclerosis progression was defined as the appearance of newly developed carotid plaque lesions on repeat ultrasonography. The presence of carotid plaque was defined as meeting any one of following criteria: 1) carotid IMT of 1.5 mm or higher; 2) protrusion of atherosclerosis into the lumen of artery with ≥ 50% thickness compared to the surrounding area; 3) presence of distinct area of hyperechogenicity.[27]

Statistical analysis

Baseline characteristics of study participants were analyzed according to liver status: no steatosis; steatosis only; and steatosis with fibrosis. Continuous variables were expressed as mean ± standard deviation (SD) and analyzed by one-way ANOVA for intergroup comparison. Bonferroni test or Dunn procedure was followed for post-hoc analysis. All categorical variables were expressed as number (proportion) and compared by χ^2 analysis.

The odds ratio (OR) of carotid atherosclerosis progression according to presence of hepatic steatosis with or without fibrosis was calculated using multivariable logistic regression analysis, and various confounding factors were adjusted in a stepwise manner to verify the independent association between liver status and carotid atherosclerosis progression. Age and gender were adjusted in model 2; duration of diabetes, HbA1c, LDL-cholesterol, HDL-cholesterol, and statin use were adjusted in model 3; alcohol/smoking consumption and exercise status in model 4; systolic BP, diastolic BP, KITT and CKD stage III-V in model 5; and BMI in model 6.

Study participants were divided into 9 subgroups according to liver status and metabolic syndrome criteria, and multivariable logistic regression analysis was performed to calculate OR of carotid atherosclerosis progression in each subgroup. Finally, logistic regression analysis was performed to detect the association between liver status and carotid atherosclerosis progression after dividing patients into 2 subgroups by age, BMI (overweight status), presence of metabolic syndrome, or KITT. *p* values <

0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics of study participants

Baseline characteristics are summarized in Table 1. Of 1,120 participants, 636 (56.8%) had hepatic steatosis; of these, 222 (19.8%) had significant fibrosis.

The mean age (years) of subjects with hepatic steatosis and fibrosis was 59.8 (± 7.8), which was significantly higher compared with the other subgroups ($p < 0.001$). BMI (kg/m^2) was higher in those with both hepatic steatosis and fibrosis (26.1 ± 3.2) or only steatosis (25.6 ± 3.0) compared to those without steatosis (23.2 ± 2.8) ($p < 0.001$). 282 (69.8%) participants with only hepatic steatosis and 150 (67.9%) participants with both hepatic steatosis and fibrosis had metabolic syndrome, while only 174 (37.3%) participants had metabolic syndrome among those without hepatic steatosis ($p < 0.001$). KITT (%/min) was 2.4 (± 1.0) in subjects with no hepatic steatosis, which was significantly higher than the subgroup with only steatosis (1.9 ± 0.8) or with both steatosis and fibrosis (1.8 ± 0.7) ($p < 0.001$), indicating that participants without hepatic steatosis were more insulin-sensitive than those in other 2 subgroups.

Participants with both steatosis and fibrosis displayed lower eGFR (88.0 ± 27.4) compared to those with no steatosis (94.5 ± 30.9) ($p = 0.018$), but it was not significantly lower than the steatosis only group (93.0 ± 28.0) ($p = 0.122$). There was no significant difference by sex or statin use between the three subgroups.

The mean carotid IMTs (mm) at baseline were 0.75 ± 0.15, 0.76 ± 0.15, and 0.81 ± 0.14 in patients with no hepatic steatosis, steatosis only, and steatosis with fibrosis, respectively ($p < 0.001$). The proportion of participants with carotid plaque at baseline was not significantly different between the 3 subgroups ($p = 0.365$).

Association between hepatic steatosis and progression of carotid atherosclerosis

The number (%) of patients with carotid plaque progression after 6–8 years was 166 (34.3%), 157 (37.9%), and 108 (48.6%), respectively among those with no hepatic steatosis, steatosis only, and steatosis with fibrosis (Table 2).

The presence of hepatic steatosis increased risk of carotid plaque progression (OR: 1.368, 95% CI: 1.071–1.748; $p = 0.012$). This result persisted after adjusting for age, gender, systolic BP, diastolic BP, duration of diabetes, HbA1c, KITT, CKD stage III-V, total cholesterol, statin use, and alcohol history (adjusted odds ratio[AOR]: 1.370, 95% CI: 1.025–1.832; $p = 0.034$) (Fig. 1).

Presence of hepatic fibrosis and carotid atherosclerosis progression in patients with NAFLD

To further investigate whether presence of hepatic fibrosis is associated with progression of carotid plaque in patients with NAFLD, we performed multivariable logistic regression analyses in a stepwise manner. With no adjustment (Model 1), hepatic steatosis with fibrosis was statistically significantly associated with carotid plaque progression (OR: 1.815, 95% CI: 1.314–2.507; $p < 0.001$), whereas steatosis only was not significant (OR: 1.170, 95% CI: 0.891–1.538; $p = 0.259$). Steatosis with fibrosis was still significantly associated with carotid plaque progression after adjusting for age, gender (Model 2. AOR: 1.494, 95% CI: 1.071–2.084; $p = 0.018$), duration of diabetes, HbA1c, LDL-cholesterol, HDL-cholesterol, statin use (Model 3. AOR: 1.657, 95% CI: 1.148–2.391; $p = 0.007$), alcohol/smoking consumption, exercise status (Model 4. AOR: 1.710, 95% CI: 1.129–2.590; $p = 0.011$), systolic BP, diastolic BP, KITT, CKD stage III-V (Model 5. AOR: 1.740, 95% CI: 1.111–2.723; $p = 0.015$), and BMI (Model 6. AOR: 1.636, 95% CI: 1.024–2.612; $p = 0.039$) (Table 3).

To investigate the synergistic effects of cardiometabolic risk factors and liver status, participants were divided into 9 subgroups according to liver status (no steatosis, steatosis only, steatosis with fibrosis) and number of metabolic syndrome criteria met (0–2, 3, 4–5). In each metabolic syndrome criteria subgroup, risk of carotid atherosclerosis progression was generally higher in subjects with hepatic steatosis, and far higher in those with both hepatic steatosis and fibrosis. Similarly, in each liver status subgroup, a higher number of metabolic syndrome criteria generally correlated with higher risk of carotid atherosclerosis progression. Compared to those with 0–2 metabolic syndrome criteria and no hepatic steatosis, subjects with 3 metabolic syndrome criteria and steatosis only and those with 4–5 metabolic syndrome criteria and both steatosis and fibrosis, respectively, were at significantly higher risk of carotid atherosclerosis progression (AOR: 1.982, 95% CI: 1.082–3.628, $p = 0.027$; AOR: 2.776, 95% CI: 1.276–6.039, $p = 0.010$) (Fig. 2).

Risk of carotid atherosclerosis progression according to metabolic profiles

To examine the presence of potential effect modification, we analyzed the risk of carotid atherosclerosis progression according to several metabolic factors. Overall, the analysis showed no difference between metabolic subgroups, whether they were divided by age, BMI, presence of metabolic syndrome, or insulin resistance (all p interaction > 0.05). In detail, hepatic steatosis without fibrosis was not associated with progression of carotid atherosclerosis in any metabolic subgroup. However, patients with combined hepatic steatosis & fibrosis showed statistically significantly higher risk of carotid atherosclerosis progression regardless of age (OR: 3.683, 95% CI: 1.036–13.100 in subgroup with age ≥ 70; OR: 1.653, 95% CI: 1.178–2.321 in subgroup with age < 70), BMI (OR: 1.531, 95% CI: 1.027–2.283 in subgroup with BMI ≥ 23; OR: 2.480, 95% CI: 1.113–5.527 in subgroup with BMI < 23), presence of metabolic syndrome (OR: 1.636, 95% CI: 1.051–2.548 in subgroup with metabolic syndrome; OR: 1.784, 95% CI: 1.051–3.026 in subgroup without metabolic syndrome), or insulin sensitivity (OR: 1.712, 95% CI: 1.164–2.518 in

subgroup with KITT < 2.5 (insulin resistant); OR: 1.972, 95% CI: 1.011–3.847 in subgroup with KITT ≥ 2.5 (insulin sensitive)). There was no effect modification by metabolic factors (*p* interaction = 0.224, 0.258, 0.815, and 0.889 for age, BMI, presence of metabolic syndrome, and insulin sensitivity, respectively).

Discussion

Ultrasonography is now widely accepted as a useful screening tool to detect carotid artery plaque and predict cardiovascular events.[27, 28] With serial carotid ultrasonography of patients with type 2 diabetes at intervals of 6–8 years, this study demonstrated that hepatic steatosis with significant fibrosis was strongly associated with progression of carotid artery atherosclerosis, even in relatively metabolically-healthy patients. It also showed that the association between hepatic fibrosis and risk of atherosclerosis progression increased with higher numbers of factors defining metabolic syndrome.

NAFLD was reported as an independent risk factor for cardiovascular disease in general population.[7, 29] In several efforts to prove this relationship via carotid ultrasonography, it was discovered that NAFLD was associated with increased carotid IMT in type 2 diabetes patients with insulin resistance[3] and a higher prevalence of carotid plaque.[30] In the present study, we focused on the long-term effect of NAFLD with or without significant fibrosis on atherosclerosis by repeated carotid ultrasonography. The results showed that progression of carotid atherosclerosis after 6–8 years occurred more frequently among patients with NASH. Like previous studies, the association between hepatic fibrosis and progression of carotid atherosclerosis became weaker as it was adjusted for metabolic factors including BMI,[31, 32] but it still remained significant after the adjustment.

To our knowledge, this is the first report demonstrating that hepatic fibrosis is significantly associated with progression of carotid artery atherosclerosis in subjects with type 2 diabetes. It indicates that not only presence of - but also severity of - metabolic liver disease can affect risk of cardiovascular complications. Previous long-term studies showed that risk of coronary artery disease-related mortality was much higher in patients with NASH (12–16%)[9, 33] compared to NAFLD (1–3%),[10, 34] and these findings are consistent with recent meta-analysis in which increased NAFLD severity produced higher risk of cardiovascular complications.[11]

NAFLD is considered a ‘hepatic manifestation of metabolic syndrome.’ It is very closely related with type 2 diabetes or metabolic syndrome, and the main pathophysiology underlying this relationship is known to be insulin resistance.[35, 36] NAFLD and metabolic syndrome can be thought to have similar effects on arteries, which accelerate atherogenesis via inflammation,[37, 38] increased oxidative stress,[39] atherogenic dyslipidemia,[40] imbalance of adipokines,[41] and hypercoagulable status.[42] In this study, we found that hepatic fibrosis accompanied by several metabolic syndrome factors synergistically increases the risk of atherosclerosis progression. Altered lipidomics and increased hepatic production of prothrombogenic factors, including fetuin-A in patients with fibrosing NASH, can be potential contributors to the link between NASH and cardiovascular diseases.[43]

In addition, the association between hepatic fibrosis and risk of atherosclerosis progression was significant in all metabolic subgroups, regardless of age, BMI, presence of metabolic syndrome, or insulin sensitivity. It indicates that hepatic fibrosis may serve as a predictive marker for increased susceptibility to atherosclerosis progression even with less evidence of systemic metabolic alterations, implicating the possible presence of systemic profibrogenic stimuli that accelerate atherogenesis in patients with hepatic fibrosis.[44] We suggest that hepatic fibrosis can be an independent risk factor for atherogenesis acceleration and its identification by clinical indicators may be helpful to predict the risk of atherosclerosis progression.

Conversely, there was no incremental risk of atherosclerosis progression in hepatic steatosis without fibrosis in type 2 diabetes patients. This finding is in similar with that of a previous study in which patients with hepatic steatosis and no additional feature of liver injury were found to follow a relatively benign clinical course, with mortality similar to the general population.[45] Although steatosis without fibrosis was not associated with increased risk of atherosclerosis progression in this study, repeat ultrasonography was not performed beyond 8 years, making it difficult to predict longer-term effect of steatosis without fibrosis on risk of atherosclerosis progression. Since high rates of fibrosis progression have been demonstrated in patients with steatosis,[46] it would be important to consider its clinical significance and to manage it appropriately without overlooking risk of cardiovascular complication.

This study has several distinguishing strengths. First, we analyzed long-term results of carotid ultrasonography in a large number of subjects with type 2 diabetes. Most previous studies using carotid ultrasonography were cross-sectional and were insufficient to determine a causal relationship. In addition, this study was a hospital-based cohort study conducted in a single institution, so participants were managed and evaluated under standardized conditions and practices.

A limitation of this study is the fact that a biochemical scoring system rather than liver biopsy was used to evaluate hepatic fibrosis. However, the FIB-4 index was initially validated by comparing results to that of liver biopsy,[26] and they were shown to have fairly high accuracy to predict hepatic fibrosis.[47, 48] This study analyzed the findings of carotid deterioration using ultrasonography, one of the major surrogate markers of cardiovascular disease. However, our methods did not allow for investigation of cardiovascular events that could represent a direct outcome of atherosclerosis progression. Finally, this was a cohort-based study of Koreans and is likely to include biases based on dietary patterns and characteristics of type 2 diabetes. Therefore, further study is needed to confirm whether the results of this study can be generalized to other ethnic populations.

Conclusions

In conclusion, hepatic steatosis with significant fibrosis was independently associated with progression of carotid atherosclerosis in patients with type 2 diabetes. The association was still significant in subgroups of patients who were metabolically healthy, and it became more prominent relative to criteria

for metabolic syndrome. Identification of hepatic steatosis with significant fibrosis may be helpful to predict and prevent risk of atherosclerosis progression in individuals with type 2 diabetes.

Abbreviations

ALT
Alanine aminotransferase
AST
Aspartate aminotransferase
BMI
Body mass index
BP
Blood pressure
BUN
Blood urea nitrogen
CKD
Chronic kidney disease
CNS
Comprehensive non-alcoholic fatty liver disease score
eGFR
Estimated glomerular filtration rate
FIB-4
Fibrosis-4
HDL-C
High-density lipoprotein cholesterol
IMT
Intima-media thickness
KITT
Rate constant for plasma glucose disappearance
LDL-C
Low-density lipoprotein cholesterol
MDRD
Modification of the Diet in Renal Disease
NAFLD
Non-alcoholic fatty liver disease
NASH
Non-alcoholic steatohepatitis

Declarations

Ethics approval and consent to participate

This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Yonsei University College of Medicine (4-2019-0270). All participants provided written informed consent in this study.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

HL conceptualized the study, provided methodology, curated/validated data, performed analysis, and wrote the manuscript. **YC** conceptualized the study, provided methodology, curated/validated data, performed analysis, and wrote the manuscript. **YJC** curated/validated data. **BWH** curated/validated data. **BL, ESK, SWP, BC, EJL, KBH** validated data. **YL** conceptualized the study, provided methodology, curated/validated data, and wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline Characteristics

Study Population N = 1,120	No Steatosis N = 484	Steatosis Only N = 414	Steatosis with Fibrosis N = 222	*p*-value
Age, years	55.4 ± 9.4	52.4 ± 9.7[a]	59.8 ± 7.8[ab]	< 0.001
Male, n (%)	216 (44.6%)	211 (51.0%)	104 (46.8%)	0.163
Weight, kg	61.1 ± 9.4	68.8 ± 11.8[a]	68.8 ± 10.7[a]	< 0.001
Height, cm	162.1 ± 8.6	163.6 ± 8.9[a]	162.4 ± 8.5	0.033
BMI, kg/m^2	23.2 ± 2.8	25.6 ± 3.0[a]	26.1 ± 3.2[a]	< 0.001
Waist Circumference, cm	79.2 ± 7.5	85.8 ± 8.0[a]	87.2 ± 7.8[a]	< 0.001
Metabolic Syndrome, n/total n (%)	174/466 (37.3%)	282/404 (69.8%)	150/221 (67.9%)	< 0.001
Regular alcohol consumption, n/total n (%)	182/437 (41.6%)	183/392 (46.7%)	70/204 (34.3%)	0.014
Smoking, ever, n/total n (%)	166/420 (39.5%)	174/384 (45.3%)	70/194 (36.1%)	0.072
Regular exercise, n/total n (%)	135/383 (35.2%)	172/360 (47.8%)	78/187 (41.7%)	0.002
Hypertension, n (%)	130 (26.9%)	135 (32.6%)	97 (43.7%)	< 0.001
SBP, mmHg	131.1 ± 16.7	135.0 ± 16.9[a]	138.3 ± 16.4[a]	< 0.001
DBP, mmHg	84.3 ± 10.6	88.3 ± 11.2[a]	87.4 ± 10.6[a]	< 0.001
Duration of diabetes, years	7.2 ± 6.9	5.5 ± 5.3[a]	6.1 ± 5.5	< 0.001
HbA1c, %	8.3 ± 2.1	8.7 ± 1.9[a]	8.0 ± 1.6[b]	< 0.001
HbA1c, mmol/mol	67.0 ± 23.0	72.0 ± 20.8[a]	64.0 ± 17.5[b]	< 0.001
KITT, %/min	2.4 ± 1.0	1.9 ± 0.8[a]	1.8 ± 0.7[a]	< 0.001
T.Cholesterol, mg/dL	188.6 ± 38.5	201.8 ± 45.1[a]	197.7 ± 39.3[a]	< 0.001
Triglyceride, mg/dL	115.7 ± 63.3	172.3 ± 140.0[a]	157.7 ± 89.2[a]	< 0.001
HDL-C, mg/dL	54.2 ± 14.8	48.4 ± 12.2[a]	50.6 ± 12.8[a]	< 0.001
LDL-C, mg/dL	110.1 ± 32.3	118.9 ± 35.7[a]	113.6 ± 35.3	0.002
BUN, mg/dL	17.5 ± 7.3	16.9 ± 5.0	18.7 ± 8.2[b]	0.005
Creatinine, mg/dL	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.2[a]	0.007
eGFR (MDRD), mL/min/1.73m^2	94.5 ± 30.9	93.0 ± 28.0	88.0 ± 27.4[a]	0.022
Total Bilirubin, mg/dL	0.9 ± 0.3	0.8 ± 0.3	1.0 ± 0.5[ab]	0.002
AST, IU/L	25.0 ± 12.6	24.4 ± 8.0	36.7 ± 17.6[ab]	< 0.001
ALT, IU/L	23.6 ± 15.9	30.5 ± 14.9[a]	37.0 ± 25.2[ab]	< 0.001
Total Protein, mg/dL	7.3 ± 0.4	7.3 ± 0.4	7.4 ± 0.5[ab]	0.006
Albumin, mg/dL	4.3 ± 0.4	4.4 ± 0.3	4.4 ± 0.3	0.068
Platelet, /uL	210.7 ± 59.2	240.0 ± 53.4[a]	183.3 ± 38.6[ab]	< 0.001
Insulin use, n (%)	47 (9.7%)	24 (5.8%)	15 (6.8%)	0.076
SU use, n (%)	240 (49.6%)	191 (46.1%)	140 (63.1%)	< 0.001
Metformin use, n (%)	172 (35.5%)	156 (37.7%)	93 (41.9%)	0.270
TZD use, n (%)	57 (11.8%)	25 (6.0%)	21 (9.5%)	0.012
Statin use, n (%)	63 (13.0%)	53 (12.8%)	25 (11.3%)	0.797
Aspirin use, n (%)	62 (12.8%)	58 (14.0%)	35 (15.8%)	0.568
Carotid IMT, mm	0.75 ± 0.15	0.76 ± 0.15	0.81 ± 0.14[ab]	< 0.001
Presence of plaque, n (%)	153 (31.6%)	127 (30.7%)	80 (36.0%)	0.365

Variables are shown as mean ± SD or n (%). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; KITT, rate constant for plasma glucose disappearance; LDL-C, low-density lipoprotein cholesterol; MDRD, modification of diet in renal disease equation; SBP, systolic blood pressure; SD, standard deviation; SU, sulfonylurea; T.Cholesterol, total cholesterol; TZD, thiazolidinedione.

[a] p values < 0.05 versus no steatosis.

[b] p values < 0.05 versus steatosis only.

Table 2. Carotid Atherosclerosis Progression at 6-8 Years by Hepatic Status

Atherosclerosis	No Steatosis	Steatosis Only	Steatosis with Fibrosis	*p*-value
Progression, n (%)	166 (34.3%)	157 (37.9%)	108 (48.6%)[a]	0.001
Non-Progression, n (%)	318 (65.7%)	257 (62.1%)	114 (51.4%)	

[a] p value < 0.001 versus no steatosis.

Table 3. Multiple Logistic Regression Analyses of the Association Between Hepatic Status and Carotid Atherosclerosis Progression

	Steatosis Only		Steatosis with Fibrosis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Model 1	1.170 (0.891-1.538)	0.259	1.815 (1.314-2.507)	< 0.001
Model 2	1.352 (1.018-1.796)	0.037	1.494 (1.071-2.084)	0.018
Model 3	1.235 (0.890-1.715)	0.207	1.657 (1.148-2.391)	0.007
Model 4	1.171 (0.813-1.687)	0.397	1.710 (1.129-2.590)	0.011
Model 5	1.141 (0.777-1.675)	0.500	1.740 (1.111-2.723)	0.015
Model 6	1.107 (0.747-1.642)	0.612	1.636 (1.024-2.612)	0.039

Model 1 = Crude OR with no adjustment

Model 2 = Model 1 + age, gender

Model 3 = Model 2 + Duration of diabetes, HbA1c, LDL-C, HDL-C, statin use

Model 4 = Model 3 + alcohol history, smoking history, exercise status

Model 5 = Model 4 + SBP, DBP, KITT, CKD stage III-V

Model 6 = Model 5 + BMI

Figures

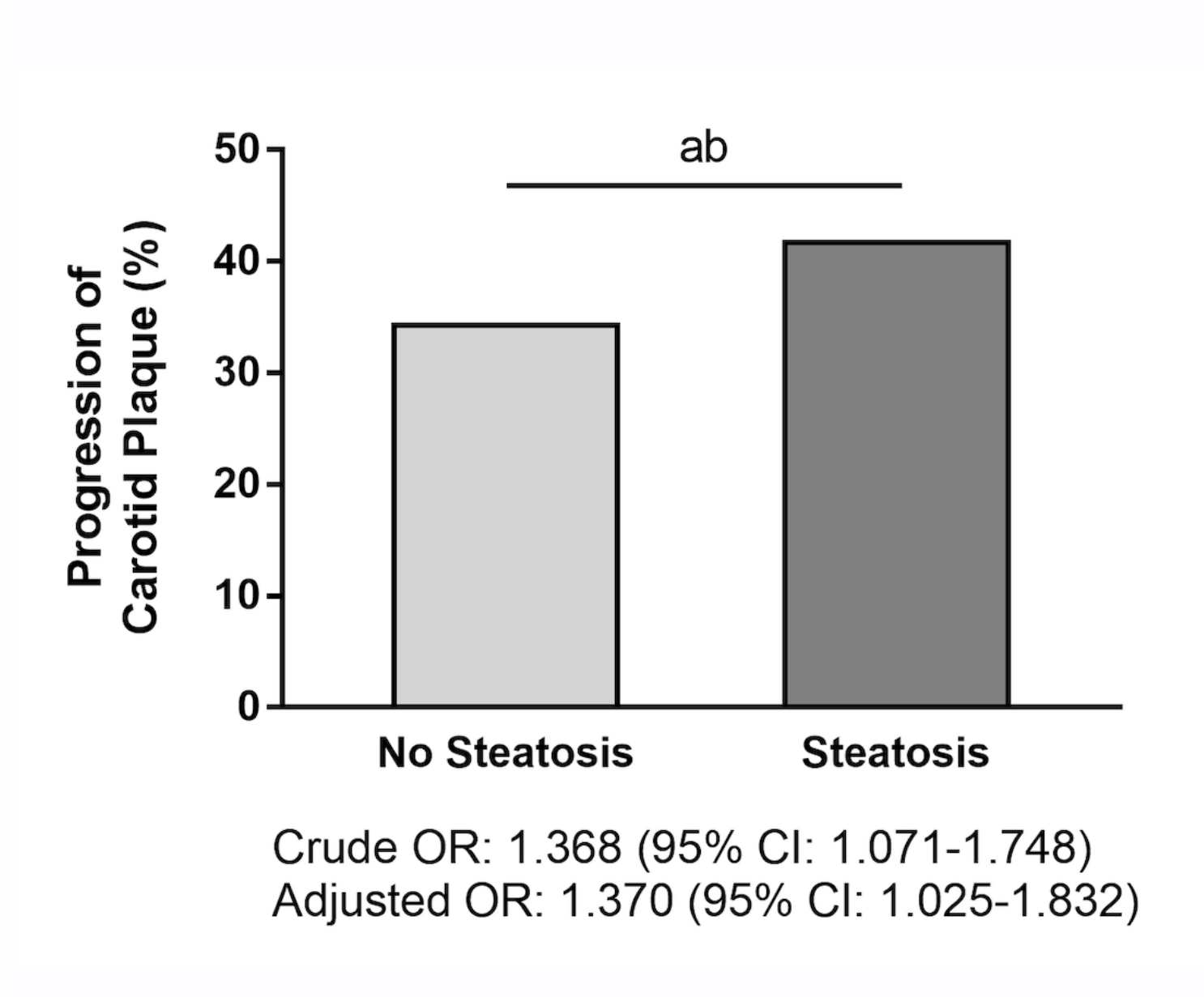

Figure 1

Progression of Carotid Atherosclerosis by Presence of Hepatic Steatosis. Odds ratio of carotid atherosclerosis progression according to the presence of hepatic steatosis. The result is adjusted for age, gender, systolic blood pressure, diastolic blood pressure, duration of diabetes, HbA1c, rate constant for plasma glucose disappearance (KITT), chronic kidney disease stage III-V, total cholesterol, statin use, and alcohol history. Levels of significance: a p = 0.012 (crude); b p = 0.034 (adjusted) (Logistic regression analysis)

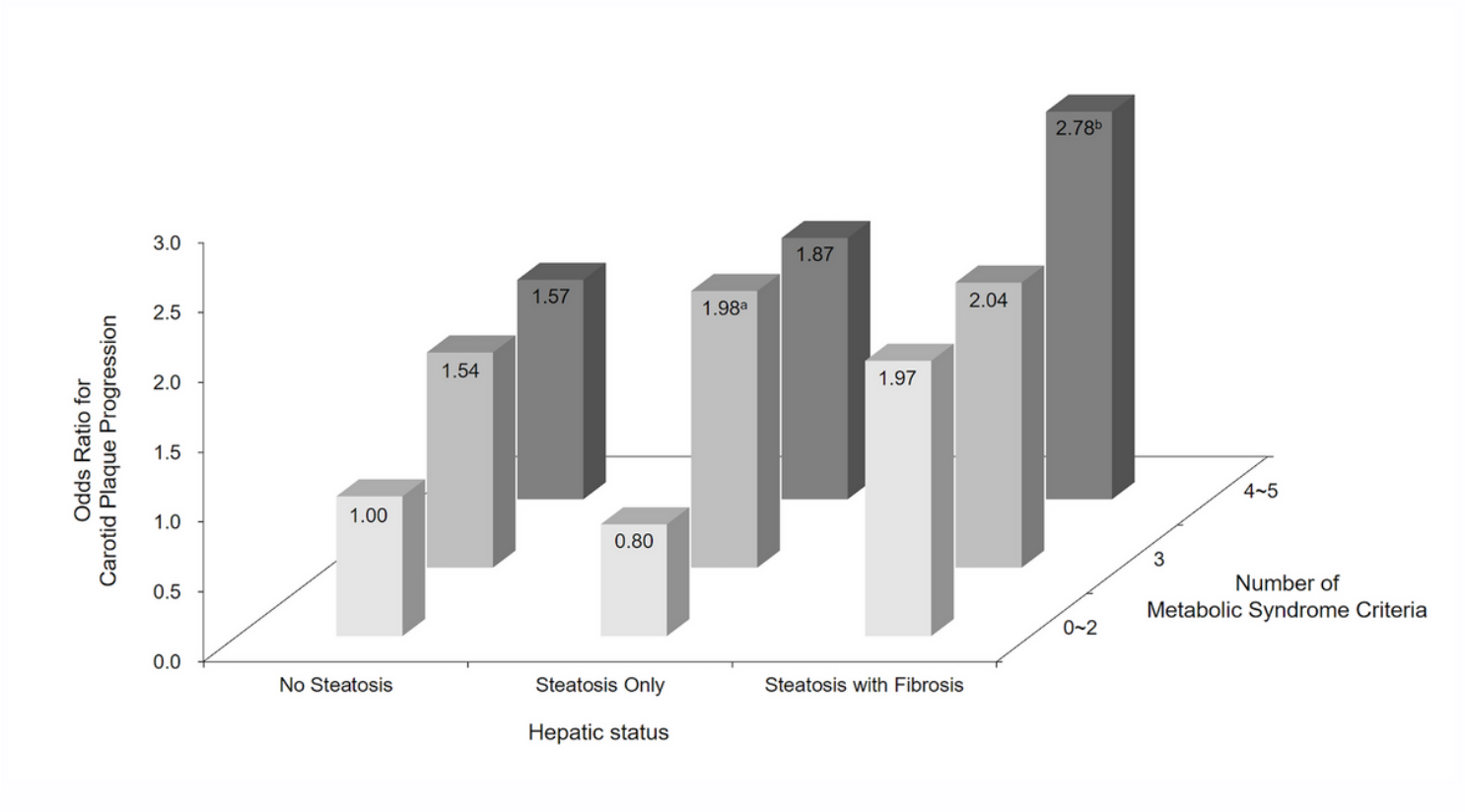

Figure 2

Risk of Carotid Atherosclerosis Progression by Subgroups (Hepatic Status and Metabolic Syndrome Criteria). Odds ratio of carotid atherosclerosis progression according to hepatic status and metabolic syndrome criteria. Odds ratio was calculated in each subgroup using the subgroup with 0-2 metabolic syndrome criteria with no hepatic steatosis as a reference. The result is adjusted for age, gender, duration of diabetes, HbA1c, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, statin use, alcohol/smoking consumption, exercise status, systolic blood pressure, diastolic blood pressure, rate constant for plasma glucose disappearance (KITT), chronic kidney disease stage III-V, and body mass index. Levels of significance: a p = 0.027; b p = 0.010 (Logistic regression analysis)

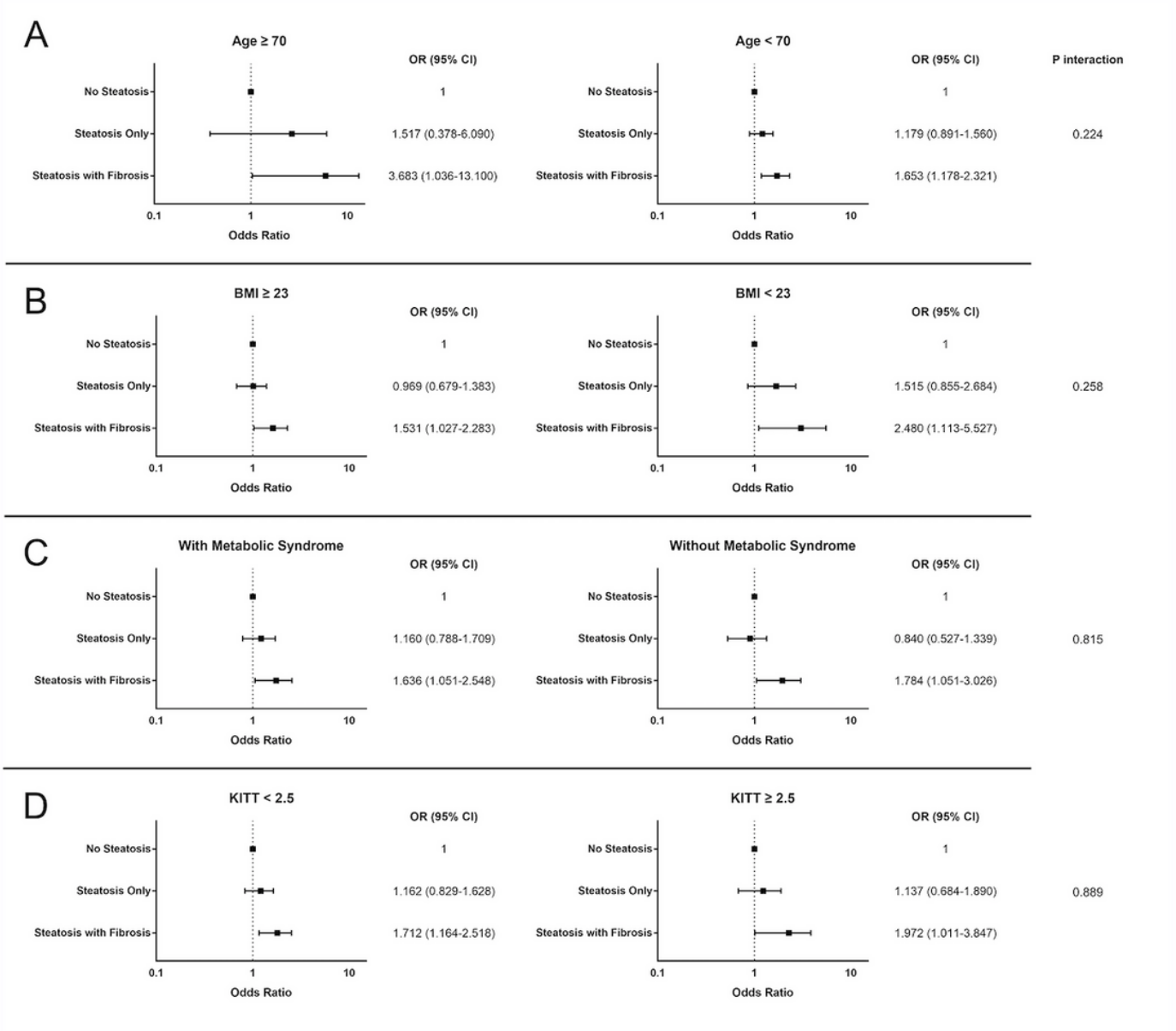

Figure 3

Hepatic Status and Carotid Atherosclerosis Progression by Metabolic Confounders. Odds ratio of carotid atherosclerosis progression according to hepatic status and (A) Age. Level of significance: p interaction = 0.224. (B) Body mass index. Level of significance: p interaction = 0.258. (C) Presence of metabolic syndrome. Level of significance: p interaction = 0.815. (D) Insulin sensitivity. Level of significance: p interaction = 0.889 (Logistic regression analysis). BMI, body mass index; KITT, rate constant for plasma glucose disappearance.