Abdominal aortic calcification is associated with Fibrosis-4 index and low body mass index in type 2 diabetes patients: A retrospective cross-sectional study

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Keywords
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ABSTRACT
Aims/Introduction: This study aimed to clarify the nature of the relationship between the abdominal aortic calcification (AAC) grade and the presence of cardiovascular diseases, and determine factors related to AAC grade in people with type 2 diabetes mellitus.

Materials and Methods: This retrospective cross-sectional study enrolled 264 inpatients with type 2 diabetes mellitus. The AAC score and length were measured using the lateral abdominal radiographs. Logistic regression models were used to assess the associations between AAC scores/lengths and the presence of coronary artery disease (CAD), cerebral infarction (CI) and peripheral artery disease (PAD). The correlation between AAC scores/lengths and other clinical factors were evaluated using linear regression models.

Results: The AAC score was significantly correlated with prevalent CAD and CI independent of age and smoking, but not with the prevalence of PAD. AAC length was not significantly correlated with the presence of CAD, CI or PAD; however, the sample size was insufficient to conclude, probably due to low prevalence. Both the AAC score and length were correlated inversely with body mass index (BMI) and, with the Fibrosis-4 (Fib-4) index >2.67; these correlations were significant after adjusting for cardiovascular risk factors and BMI, although AAC was not associated with ultrasonography-diagnosed fatty liver. There was a significant interaction between BMI and Fib-4 index; lower BMI and Fib-4 index >2.67 showed a synergistic association with high AAC grade.

Conclusions: AAC score is associated with CAD and CI morbidity in participants with type 2 diabetes mellitus. Low BMI and Fib-4 index >2.67 can be valuable indicators of AAC in people with type 2 diabetes mellitus.

INTRODUCTION
Type 2 diabetes mellitus accelerates atherosclerosis and increases cardiovascular disease (CVD) morbidity, including coronary artery disease (CAD), cerebral infarction (CI) and peripheral artery disease (PAD)¹-³.

Abdominal aortic calcification (AAC) is a marker of systemic atherosclerosis burden. AAC can be evaluated non-invasively using plain X-ray imaging or computed tomography (CT). Despite the high sensitivity of CT imaging, plain abdominal radiography is a valuable tool for evaluating AAC due to its low cost and simplicity. Additionally, the clinical significance of AAC has been detected by semiquantitative evaluation using this approach⁴,⁵.
Abdominal aortic calcification grade is an independent predictor of CVD mortality and mobility. The presence or extent of AAC is associated with diabetes. Independent associations between advanced AAC and a higher prevalence of CVD have also been shown in participants with type 2 diabetes mellitus. However, the risk of AAC is lower in Hispanic and African Americans, but not in Chinese Americans, than that in European Americans, which cannot be explained by differences in classical risk factors for CVD. Furthermore, white people and Chinese people have a different prevalence of coronary calcification, despite the similar prevalence of AAC.

Multiple studies have suggested age, smoking, hypertension, dyslipidemia and obesity as possible risk factors for the AAC development. Several studies showed that some of the factors have different correlations with AAC in people with type 2 diabetes mellitus. Although AAC was positively correlated with non-high-density lipoprotein-cholesterol (HDL-C), total cholesterol and the total cholesterol/HDL-C ratio, and negatively correlated with HDL-C in studies of participants without type 2 diabetes mellitus, a study involving participants with type 2 diabetes mellitus did not show a correlation between the lipid profile and AAC. Regarding associations with obesity, visceral fat was positively correlated with AAC in women. However, many studies have failed to show a correlation between BMI and AAC. In contrast, inverse associations between BMI, waist circumference and subcutaneous adipose tissue volumes and the severity of aortic calcification have been detected in African American and European American participants with type 2 diabetes mellitus. Thus, individuals with type 2 diabetes mellitus have different aspects of AAC risk factors, but they have not been sufficiently evaluated.

The present study aimed to investigate the cross-sectional association between AAC grade and characteristics, and laboratory data in Japanese people with type 2 diabetes mellitus.

**MATERIALS AND METHODS**

**Study design and participants**

This was a retrospective, cross-sectional study. We assessed the relationship between AAC grade and the presence of CVDs, such as CAD, CI and PAD, and researched which factors were related to AAC in Japanese people with type 2 diabetes mellitus. We included Japanese individuals with type 2 diabetes mellitus who had been hospitalized in the Department of Endocrinology and Metabolism at Yokohama City University Hospital between 1 January 2016 and 31 March 2018. All individuals aged ≥20 years who had undergone a standing lateral abdominal radiography examination for a routine screening ≥20 years who had undergone a standing lateral abdominal radiography examination for a routine screening ≥20 years who had undergone a standing lateral abdominal radiography examination for a routine screening ≥20 years who had undergone a standing lateral abdominal radiography examination for a routine screening ≥20 years who had undergone a standing lateral abdominal radiography examination for a routine screening. We excluded individuals with psychiatric disorders, cancers, severe ketosis, diabetic coma or precoma, severe infections (C-reactive protein ≥10), severe traumatic injuries, pancreatic exocrine diseases, hepatic cirrhosis and untreated endocrine diseases (pituitary, adrenal or thyroid disorders), and postoperative individuals.

**Ethics**

This study was carried out in accordance with the Declaration of Helsinki and was approved by the Clinical Ethics Committee of Yokohama City University, Yokohama, Japan (B180900053). An opt-out method was applied to obtain consent.

**Evaluation criteria**

Information on the duration of diabetes and history of CAD and cerebral infarction (CI), smoking, alcohol drinking, and medication was collected from interviews at the time of hospital admission. All participants underwent a physical examination at admission; their blood was drawn under fasted conditions (08.00 AM) and 2 h after breakfast (10.30 AM) to assess their general health and cardiovascular risk factors. Blood analysis included analysis of platelet, albumin, aspartate transaminase, alanine transaminase, γ-glutamyl transpeptidase, serum creatinine, estimated glomerular filtration rate (eGFR), calcium (Ca), phosphorus (P), high-sensitivity C-reactive protein (hsCRP), hemoglobin A1c (HbA1c), plasma glucose, low-density lipoprotein-cholesterol, HDL-C, triglyceride and plasma C-peptide reaction using blood samples drawn under fasting conditions and analysis of plasma glucose and C-peptide reaction at 2 h after breakfast. The corrected Ca level was calculated as Ca + 0.8 × (4 – albumin) in participants with hypoalbuminemia. The Fibrosis-4 (Fib-4) index was calculated using the formula: Fib-4 index = age (years) × aspartate transaminase (U/L)/(platelet [109/L] × alanine transaminase1/2 [U/L]). Participants were analyzed in three regions of the Fib-4 index based on the cutoff points (<1.30, 1.30–2.67 and ≥2.67). The Forns index was calculated using the formula: Forns index = 7.811–3.131 × log (platelet [109/L] + 0.781 × log (γ-glutamyl transpeptidase [IU/L] + 3.467 × log (age [years]) − 0.014 × (total cholesterol [mg/dL]). The standard deviations of blood glucose levels were calculated using values measured using a glucometer at 08.00 AM, 10.30 AM, 12.00 PM, 2.30 PM, 6.00 PM and 8.30 PM on hospitalization day 2. Urinary albumin excretion rate was measured using 24-h urine samples. Peripheral neuropathy was defined based on two symptoms – diminished deep tendon reflex and loss of vibration sense or nerve conduction velocity. Arterial stiffness was evaluated using brachial-ankle pulse wave velocity (baPWV). PAD was defined as an ankle–brachial index of <0.9. Autonomic neuropathy was defined as a reduction in the electrocardiographic RR interval variability below the reference values for each age. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg for two times during the hospitalization, or use of antihypertensive agent. Dyslipidemia was defined as low-density lipoprotein-cholesterol of ≥140 mg/dL, HDL-C <40 mg/dL, triglyceride ≥150 mg/dL or use of lipid-lowering medication. Fatty liver
was diagnosed using ultrasonography if the examination had been carried out within 6 months before or after admission.

**Quantification of abdominal aortic calcification**

Abdominal aortic calcification was evaluated using two methods — the AAC score and AAC length — based on the results of standing lateral abdominal radiography examinations carried out between 2 months before and after hospital admission. The AAC score is a validated grading system\(^30\); the extent of calcific deposits per vertebral segments L1–4 are graded on a scale of 0–3 for both the anterior and posterior wall of the aorta. The sum of these eight scores resulted in an AAC score of 0–24 points. AAC length is the total calcific deposits along the anterior and posterior aortic walls in the segments between L1 and L5. In the present study, we corrected the value using the ratio to the total spine length of L1–5 (approximated by the continuous distance connecting the upper end of L1, midpoint between L3 and L4, midpoint between L4 and L5, and lower end of L5). The scoring and measurement of AAC were carried out by an observer who was blinded to the participants’ background data. The reproducibility of the AAC score and AAC length measurement was assessed in 10 participants by two observers independently. The correlation coefficient for AAC score and AAC length were 0.98 and 0.88, respectively.

**Statistical analysis**

The results are expressed as the mean ± standard deviation or the median and the 25–75th percentiles. The sample size was determined from the number of cases during the study period. BMI was categorized by World Health Organization criteria. Because approximately one-third of the participants had AAC indices of 0, participants were categorized according to AAC tertiles. Participants were analyzed in three regions of the Fib-4 index based on the cut-off points (<1.30, 1.30–2.67 and >2.67). A multivariate logistic regression model was used to calculate age- and smoking history-adjusted odds ratio for CAD, CI, and PAD according to AAC tertiles. Spearman’s correlation coefficient was used to assess correlations between parameters. To assess how much type I errors are controlled by multiple comparison, a Bonferroni correction was applied. Multiple regression linear analysis was used to assess the independent associations between the AAC levels and BMI or between AAC levels and Fib-4 index, and the interaction between BMI and the Fib-4 index range for association with AAC levels was additionally included in the model. Participants with missing data were excluded from the analysis. The variables that were not normally distributed, including duration of diabetes, triglyceride and hsCRP levels, were logarithmic transformed. Statistical significance was set at \(P < 0.05\). Data processing and statistical analysis were carried out using JMP Pro 15 software (SAS Institute, Cary, NC, USA). To evaluate statistic power at \(\alpha = 0.05\), a post-hoc calculation was carried out using G*Power 3.1\(^{31}\) for a logistic regression model, and JMP Pro 15 for a multiple regression linear model.

**RESULTS**

The background characteristics of all participants are listed in Table 1. We estimated the prevalence of CAD, CI and PAD in participants in each tertile of AAC score or length. Representative images of AAC scores and lengths are shown in Figure 1 (see details in Materials and Methods). The approximate tertiles of AAC scores and lengths in both sexes are shown in Table S1. In the multivariate logistic regression model adjusted for age and smoking history, participants in tertile 3 of the AAC score had a higher odds ratio of CAD (adjusted odds ratio 5.14, 95% confidence interval 1.44–18.36; \(P = 0.012\)) and CI (adjusted odds ratio 3.40, 95% confidence interval 1.08–10.65; \(P = 0.036\)). Participants in tertile 3 of AAC length did not show a higher odds ratio of CAD or CI (Table 2). Tertiles of AAC score or length were not associated with PAD. In brief, the AAC score was positively associated with CAD and CI.

Subsequently, we next investigated the association between the AAC score and length, and various parameters (Table S2). Variables are statistically significant at \(P < 0.0008 \ (0.05/60)\) after Bonferroni corrections. In univariate linear regression analysis, AAC score and length were positively correlated with age, duration of diabetes, Fib-4 index and baPWV, and inversely correlated with BMI, alanine transaminase and eGFR. AAC score was negatively associated with waist circumference, but AAC length did not reach the significance. Among variables that showed relatively strong associations with both the AAC score and length — age, duration of diabetes, BMI, Fib-4 index, eGFR and baPWV — the results regarding the age, duration of diabetes, eGFR and baPWV were compatible with those reported in previous studies\(^{2,14,15,32,33}\). Because the association between AAC extent and both the BMI and Fib-4 index in participants with type 2 diabetes mellitus is obscure, we focused on the negative association between AAC and BMI, and the positive association between AAC and Fib-4 index.

The correlation between BMI and the AAC score or length was analyzed using multiple linear regression analysis after adjustments for potential arteriosclerosis risk factors (such as age, sex, duration of diabetes, smoking, hypertension, dyslipidemia, HbA1c, 2-h postprandial plasma glucose, eGFR and hsCRP) in 204 participants with no missing data (Table 3). BMI showed a significant inverse association with AAC score and length after adjustment for potential arteriosclerosis risk factors. In 169 participants with waist circumference data, the association between waist circumference, a surrogate measure of visceral fat accumulation, and AAC score and length was analyzed. Waist circumference also had a significant inverse association with AAC score and length after adjustment for potential arteriosclerosis risk factors (Table S3).

The associations between the cut-off range of the Fib-4 index and the AAC score and length was investigated using multiple linear regression analyses after adjusting for the above-mentioned atherosclerotic risk factors and BMI (Table 4). The Fib-4 index above the high cut-off value (>2.67) was
Table 1  | Background factors of the participants

|                          | No. participants (men/women) | Age (years) | Duration of diabetes (years) | BMI (kg/m²) | BMI classification, n (%) | Alcohol intake, yes/no (%) | History of smoking, yes/no (%) | Systolic BP (mmHg) | Diastolic BP (mmHg) | HbA1c, NGSP/IFCC (%/mmol/mol) | Dyslipidemia, yes/no (%) | Antihypertensive agents, yes/no (%) | Oral antidiabetic agent, yes/no (%) | GLP-1 receptor agonist, yes/no (%) |
|--------------------------|-----------------------------|-------------|-------------------------------|-------------|---------------------------|---------------------------|-----------------------------|---------------------|---------------------|-----------------------------------|-----------------------------------|-----------------------------------|----------------------------------|----------------------------------|
| No. participants (men/women) | 264 (155/109)              | 64.1 ± 13.6 | 13.2 ± 11.2                   | 26.5 ± 6.1 | Underweight (BMI <18.5) 11 (4.2) | Normal weight (BMI 18.5–24.9) 114 (43.2) | Overweight (BMI 25.0–29.9) 78 (29.5) | Obesity class I (BMI 30.0–34.9) 39 (14.8) | Obesity class II (BMI 35.0–39.9) 10 (3.8) | Obesity class III (BMI ≥40.0) 12 (4.5) | Alcohol intake, yes/no (%) | 116 (43.9)/148 (56.1) | History of smoking, yes/no (%) | 111(42.0)/153 (58.0) | Systolic BP (mmHg) | 132.0 ± 18.6 | Diastolic BP (mmHg) | 77.6 ± 13.8 | HbA1c, NGSP/IFCC (%/mmol/mol) | 9.4 ± 1.9/79 ± 21 | LDL-C (mg/dL) | 110.1 ± 30.5 | HDL-C (mg/dL) | 506 ± 15.3 | Triglyceride (mg/dL) | 1740 ± 150.7 | eGFR (mL/min/1.73 m²) | 68.5 ± 25.8 | Coronary artery disease, yes/no (%) | 35 (13.3)/229 (86.7) | Cerebral infarction, yes/no (%) | 35 (13.3)/229 (86.7) | Peripheral artery disease, yes/no/NA (%) | 16 (60)/243 (92.0)/5 (1.9) | Peripheral neuropathy, yes/no/NA (%) | 210 (79.5)/52 (19.7)/2 (0.8) | Retinopathy, NDR/SDR/PPDR | 164 (62.1)/38 (14.4)/31 (11.7) | and PDR/NA (%) | (11.7)/31 (11.7) | Nephropathy stage, 1/2/3/4 (%) | 172 (65.1)/53 (20.1)/218 (80.0)/18 (6.8) | Hypertension, yes/no (%) | 197 (74.6)/67 (25.4) | Dyslipidemia, yes/no (%) | 218 (82.6)/46 (17.4) | Hyperglycemic agents, yes/no (%) | 161 (61.0)/103 (39.0) | Statins, yes/no (%) | 125 (47.3)/139 (52.7) | Antplatelet agents, yes/no (%) | 65 (24.8)/199 (75.4) | Oral anti-diabetic agent, yes/no (%) | 213 (80.7)/51 (19.3) | Insulin treatment, yes/no (%) | 79 (29.5)/185 (70.1) | GLP-1 receptor agonist, yes/no (%) | 9 (3.4)/255 (96.6) |

Values are the mean ± standard deviation. BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; NA, not available; NDR, No diabetic retinopathy; PDR, proliferative diabetic retinopathy; PPDR, Pre-proliferative diabetic retinopathy; SDR, Simple diabetic retinopathy.

Figure 1  | Grading of abdominal aortic calcification (AAC). (a) The middle panel shows an example of the AAC score calculation. The anterior and posterior aortic walls are divided into eight segments corresponding to the L1–4 vertebral areas. Aortic calcification is scored as 0 (no calcification), 1 (<1/3 of the aortic wall in that segment is calcified), 2 (1/3–2/3 of the aortic area is calcified) or 3 (>2/3 of the aortic area is calcified). The total score ranges from 0 to 24. The right panel shows an example of the AAC length calculation. The AAC length is calculated using the ratio of the total length of calcific deposits along the anterior and posterior aortic wall in the segments between L1 and L5 to the total spine length of L1–5 (approximated by a + b + c, where a is the distance from the upper end of L1 to the midpoint between L3 and L4, b is the distance from the midpoint between L3 and L4 to the midpoint between L4 and L5, and c is the distance from the midpoint between L4 and L5 to the lower end of L5). (b) Examples of AAC images. The arrowheads point to calcification deposits. Upper left panel: AAC score = 0, AAC length = 0. Upper right panel: AAC score = 8, AAC length = 0.67. Lower left panel: AAC score = 16, AAC length = 1.33. Lower right panel: AAC score = 24, AAC length = 1.62.

DISCUSSION

The present cross-sectional study showed that the AAC score was correlated with the prevalence of CAD and CI, independent of age and smoking history, although not with PAD. A previous cross-sectional study of Veterans Affairs Diabetes Trial involving 309 participants showed a significant association between the AAC grade and the prevalence of not only CAD and CI, but also PAD, independent of cardiovascular risk factors, although the prevalence of PAD was less relevant to AAC than CAD. Participant characteristics, including age, duration of diabetes and HbA1c level, in the Veterans Affairs Diabetes Trial were similar to those in the present study. However, the prevalence of CAD and PAD in the Veterans Affairs Diabetes Trial was higher (27 and 13%, respectively) than those in the present study (13.8 and 6.1%, respectively). The number of participants with PAD (n = 16) in the present study might be small to detect a significance. A post-hoc power calculation showed that the statistical power of the results for PAD with AAC score was 9.7%. Those of CAD, CI and PAD with AAC significantly associated with the increasing AAC score and length values. The correlation between the Fib-4 index and the AAC score or length differed across BMI ranges (interaction P < 0.05; Table 4). The association between the Fib-4 index above the high cut-off value and a high AAC score and length was more pronounced in participants with a lower BMI (Figure 2a,b). People with type 2 diabetes mellitus frequently have non-alcoholic fatty liver disease (NAFLD), including non-progressive non-alcoholic fatty liver and non-alcoholic steatohepatitis (NASH) with progressive inflammation and liver fibrosis. Subsequently, we assessed the relationship between fatty liver and the AAC score and length in participants who had undergone an abdominal ultrasonography examination. The AAC score and length were not significantly different between participants with and without fatty liver (Figure S1a). An analysis of the AAC score and length for each Fib-4 index range (<1.3: no fibrosis, >2.67: fibrosis) in the group with and without fatty liver showed a more evident trend toward an increasing AAC score and length in participants with a high Fib-4 index in the group without fatty liver (Figure S1b). An analysis using the Forns index, another liver fibrosis scoring system, showed similar trend (Figure S1c).
AAC relates to Fib-4 index and low BMI
length were 8.0, 5.1 and 9.7%, respectively. Thus, the sample size was insufficient to lead to definitive conclusions for the association between AAC score and PAD, or AAC length and CVDs. Despite a small sample size, the present results suggest that the utility of AAC score as an independent predictor of CVD in people with type 2 diabetes mellitus is common across ethnicity. To determine the association between AAC score and PAD, or AAC length and CVDs, a study with larger sample size is required.

In the present study, there was inconsistency between the results of AAC score and AAC length in relation to CVDs. The difference in measurement range between the AAC score (L1–4 level) and the AAC length (L1–5 level) might lead to this inconsistency. Because aortic calcification in different zones develops asynchronously – appears first in the distal segment and spreads to the proximal14 – we consider that AAC length might tend to reflect an earlier stage of calcification, and which might attenuate its association with CVDs.

BMI was inversely correlated with both the AAC score and length after adjustments for conventional cardiovascular risk factors in participants with type 2 diabetes mellitus. Additionally, waist circumference, a surrogate measure of visceral fat accumulation, was also inversely correlated with AAC. The post-hoc calculation showed that the statistical power for the

| Table 2 | Adjusted odds† ratio for association between abdominal aortic calcification score/abdominal aortic calcification length tertiles and coronary artery disease, cerebral infarction and peripheral artery disease |
| --- | --- | --- |
| CAD | | | |
| n (events/participants) | Men | Women | Total |
| | 22/155 | 13/109 | 35/264 |
| AOR (95% CI) | | | |
| P | | | |
| AAC score | | | |
| Tertile 2 vs 1 | 2.61 (0.26, 26.04) | 0.414 | 1.06 (0.19, 5.81) | 0.945 |
| Tertile 3 vs 1 | 13.76 (1.46, 129.59) | 0.022 | 1.93 (0.34, 11.06) | 0.458 |
| AAC length | | | |
| Tertile 2 vs 1 | 0.90 (0.10, 3.44) | 0.566 | 2.19 (0.49, 9.83) | 0.307 |
| Tertile 3 vs 1 | 3.01 (0.65, 14.0) | 0.159 | 0.81 (0.13, 5.29) | 0.830 |
| CI | | | |
| n (events/participants) | Men | Women | Total |
| | 21/155 | 15/109 | 36/264 |
| AOR (95% CI) | | | |
| P | | | |
| AAC score | | | |
| Tertile 2 vs 1 | 0.90 (0.19, 4.24) | 0.895 | 2.65 (0.45, 15.42) | 0.279 |
| Tertile 3 vs 1 | 4.17 (0.89, 19.48) | 0.069 | 3.29 (0.56, 20.83) | 0.207 |
| AAC length | | | |
| Tertile 2 vs 1 | 1.25 (0.33, 4.79) | 0.741 | 1.78 (0.39, 8.24) | 0.459 |
| Tertile 3 vs 1 | 1.33 (0.32, 5.56) | 0.698 | 1.92 (0.36, 10.32) | 0.448 |
| PAD | | | |
| n (events/participants) | Men | Women | Total |
| | 8/155 | 8/109 | 16/264 |
| AOR (95% CI) | | | |
| P | | | |
| AAC score | | | |
| Tertile 2 vs 1 | 2.32 (0.21, 25.70) | 0.494 | 3.08 (0.28, 34.23) | 0.36 |
| Tertile 3 vs 1 | 2.87 (0.22, 36.8) | 0.417 | 4.23 (0.35, 51.49) | 0.258 |
| AAC length | | | |
| Tertile 2 vs 1 | 0.41 (0.03, 5.47) | 0.497 | 3.36 (0.31, 36.54) | 0.319 |
| Tertile 3 vs 1 | 1.79 (0.21, 15.12) | 0.592 | 5.91 (0.45, 77.84) | 0.177 |

†Adjusted for age and smoking. 95% CI, 95% confidence interval; AOR, adjusted odds ratio; CAD, coronary artery disease; CI, cerebral infarction; PAD, peripheral artery disease.
association between AAC score/length and BMI was 98 and 84%, and that for the association between AAC score/length and waist circumference was 91 and 84%, respectively, in model 2. These results differed from the observations showing positive or no correlations between AAC and obesity in previous studies that did not exclusively include participants with type 2 diabetes mellitus\(^{2,17}\). An inverse association between BMI, waist circumference, and subcutaneous adipose tissue volume and calcified aortic plaque was observed in a previous study including African Americans and European Americans with type 2 diabetes mellitus\(^{29}\). In the background data of another cross-sectional study in which the majority of participants were male and white, the fact that the BMI was significantly lower in the group with AAC than in the group without AAC is consistent with this previous report. The present results show that the inverse correlation between the obesity measurements and AAC found in people with type 2 diabetes mellitus is preserved in different ethnicities in which the prevalence of obesity and fat distribution differ. As appendicular lean mass was inversely correlated with AAC in Community-Dwelling Older Australians\(^{25,28}\), and subcutaneous fat thickness was inversely correlated with AAC in a study with a small proportion of participants with diabetes\(^{27}\), the inverse correlation between AAC and BMI or waist
circumference reflect those associations in lean Japanese partic-

ipants in the current study. Although there are sex differences
in fat distribution and contribution to AAC\(^27\), the sample size
was too small in our study to detect significance for sex sub-
division. A more detailed study of the relationship between

body composition and AAC in people with type 2 diabetes
mellitus is required with a larger sample size.

In the present study, Fib-4 index >2.67 was significantly
associated with AAC severity, independent of conventional car-
diovascular risk factors in participants with type 2 diabetes

Figure 2 | Abdominal aortic calcification (AAC) score and AAC length in participants within body mass index (BMI) classification and cut-off ranges of the Fibrosis-4 (Fib-4) index. (a) AAC score within cut-off ranges of the Fib-4 index among BMI categories. (b) AAC length within cutoff ranges of the Fib-4 index among BMI categories. BMI was classified by World Health Organization criteria. NW, normal weight; OB, obesity; OW, overweight; UW, underweight.
The correlation was more overt in participants with a lower BMI. The post-hoc statistical power for the association between AAC score/length and Fib-4 index (1.30–2.67 vs >2.67) in model 2 was 73 and 94%, respectively. The Fib-4 index is a scoring system developed using serum markers to assess liver fibrosis non-invasively in individuals with NAFLD. Shah et al. showed that Fib-4 index ≥2.67 had an 80% positive predictive value and Fib-4 index ≤1.30 had a 90% negative predictive value for advanced fibrosis. A recent study showed that a high Fib-4 index is associated with the severity of coronary artery calcification in individuals with NAFLD. However, the association between Fib-4 index and artery calcification in individuals with type 2 diabetes mellitus are unclear. The present study is the first to show the association between the Fib-4 index and AAC grade in individuals with type 2 diabetes mellitus.

NAFLD is closely associated to obesity and type 2 diabetes mellitus. NAFLD, defined using CT or ultrasonography imaging, is an independent risk factor for CVD and is associated with arterial calcification. Additionally, the histological stage of liver fibrosis, rather than NASH itself, is associated with cardiovascular mortality in individuals with NAFLD. The association between NAFLD and AAC might differ between ethnic groups. NAFLD was significantly associated with AAC in African Americans, but not in white people, Asian people and Hispanic people. In the study of 50% European Americans and 50% African Americans, NAFLD was not independently correlated with AAC, and the association decreased after adjustments for obesity, suggesting that obesity might mediate the association between NAFLD and AAC. In the present study, the AAC score and length were not altered by the presence of ultrasonography-diagnosed fatty liver (Figure S1a), suggesting that NAFLD might not be associated with AAC in Japanese individuals with type 2 diabetes mellitus. Notably, the correlation between the Fib-4 index >2.67 and severe AAC was enhanced with a lower BMI. Supporting this, it was shown that Fib-4 index was inversely correlated with BMI, low-density lipoprotein-cholesterol and eGFR in participants with type 2 diabetes mellitus, suggesting that differently from NAFLD, Fib-4 index increases by factors independent of insulin resistance. From these results, we hypothesize that the synergistic effect of a high Fib-4 index and low BMI on severe AAC might be mediated by advanced glycation end-products (AGEs). AGEs are produced through a non-enzymatic glycation reaction accelerated in hyperglycemia or diabetes, and correlate with diabetes progression in individuals with type 2 diabetes mellitus. Lower serum/plasma levels of pentosidine and carboxymethyllysine, commonly studied in AGEs, are associated with a high BMI or central obesity. A recent study showed that serum levels of AGEs positively correlated with AAC severity. Furthermore, AGEs, especially glycolaldehyde-derived AGEs, known as toxic AGEs, play a significant role in the pathogenesis of NASH, suggesting that a low BMI and high Fib-4 index might be a clinical manifestation reflecting the burden of AGEs in individuals with type 2 diabetes mellitus. Another possibility is that Fib-4 index >2.67 might not necessarily show liver fibrosis in the lean and no fatty liver groups, because the Fib-4 index is designed for individuals with fatty liver or hepatitis. The Fib-4 index failed to distinguish advanced fibrosis in lean and morbidly obese participants with NAFLD. Furthermore, the diagnostic accuracy changes with age, and increased cut-off values increase diagnostic accuracy in older age groups. Further research is required to understand the pathophysiology of lean individuals with type 2 diabetes mellitus and a high Fib-4 index.

The limitations of the present study are its retrospective cross-sectional design, small sample size and a possible selection bias, as individuals who had not undergone standing lateral abdominal radiography were not included. It should be noted that the significance of the multiple comparison depends on the correction method. Furthermore, a single investigator calculated the AAC score and length. Another limitation was the lack of validation of AAC grade using CT scanning. Additionally, the study participants were Japanese inpatients with type 2 diabetes mellitus who had relatively high HbA1c levels.

In summary, the present study showed that AAC severity was associated with CAD and CI, independent of age and smoking history in Japanese people with type 2 diabetes mellitus. A lower BMI and Fib-4 index >2.67 were synergistically associated with AAC severity, suggesting a type 2 diabetes mellitus-specific mechanism differing from that based on insulin resistance caused by metabolic syndrome. Low BMI and Fib-4 index >2.67 can be useful indicators of AAC risk in individuals with type 2 diabetes mellitus. Furthermore, the present data raised the possibility that AAC and liver fibrosis are associated. Further research involving liver biopsy or non-invasive diagnostic imaging of liver fibrosis is required to confirm this.

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DISCLOSURE
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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** | Abdominal aortic calcification score and abdominal aortic calcification length in participants with and without fatty liver as diagnosed using ultrasonography.

**Table S1** | Tertiles of abdominal aortic calcification score and abdominal aortic calcification length in men and women.

**Table S2** | Correlations between abdominal aortic calcification score or abdominal aortic calcification length and various other parameters.

**Table S3** | Multiple linear regression analysis using waist circumference for the determination of abdominal aortic calcification score and abdominal aortic calcification length.