Low-dose CT lung cancer screening has been reported to reduce mortality by 20% compared to single-view posteroanterior or chest radiography [1]. Also, the amount of coronary artery calcification (CAC) measured by electrocardiography (ECG)-gated CT reflects arteriosclerosis [2] and is a strong predictor of coronary artery disease (CAD), cardiovascular events, and all-cause mortality, above and beyond the Framingham risk score [3-5]. In a recent study, CAC on standard chest CT correlated well with that on ECG-gated CT and was linked to mortality risk [6]. In addition, in patients undergoing CT lung cancer screening, the assessment of coronary calcification has been successfully performed using low-dose chest CT. Results had excellent correlation with ECG-gated coronary calcium score [7-9]. The procedure had good sensitivity and specificity and showed overall accuracy [10]. The amount of CAC is an independent prognostic factor for heart disease [4,11-13]. However, the onset of CAC and the related factors in healthy individuals are not well understood. The aim of this study was to quantify CAC using low-dose chest CT, longitudinally performed for lung cancer screening.

Objective: Detection of coronary artery calcification (CAC) in healthy individuals and investigation of this risk factor are useful for preventing heart disease. One aim of this study was to quantify CAC using low-dose chest CT longitudinally performed for lung cancer screening. The other aim was to identify the risk factors related to CAC onset in healthy individuals.

Materials and Methods: Here, 203 individuals (mean age, 53 years) who underwent health screening and CT were enrolled. A total of 1108 CT examinations (mean follow-up period, 56 months) was analyzed. CAC (mm³) was defined as a high-density area with a CT value >130 Hounsfield units along coronary arteries. Subjects with a CAC >14 mm³ were defined as CAC onset; the others were defined as non-CAC.

Results: CAC onset and non-CAC were observed in 27 (13%) and 176 (87%) subjects, respectively. Age and hemoglobin A1c (HbA1c) were significantly higher and estimated glomerular filtration rate (eGFR) was significantly lower for CAC onset than for non-CAC (57.1 ± 4.3 years vs. 55.0 ± 5.2 years, 6.1% ± 1.5% vs. 5.6% ± 0.7%, and 70.6 ± 12.9 mL/min vs. 73.5 ± 11.5 mL/min, respectively). Multivariate logistic regression analysis revealed that CAC onset was significantly related to age [odds ratio (OR), 1.06; 95% confidence interval (CI), 1.03–1.10], male (OR, 1.59; 95% CI, 1.05–2.42), HbA1c level (OR, 1.77; 95% CI, 1.47–2.14), and eGFR (OR, 0.97; 95% CI, 0.95–0.99).

Conclusion: CAC occurred in about 10% of healthy middle-aged subjects and is associated with early glucose intolerance and a slight decline in renal function.

Key words: Atherosclerosis · Coronary arteries · Life style · Health care.
for lung cancer screening, and to identify the factors related to CAC onset in healthy individuals.

MATERIALS AND METHODS

Study population
All participants signed written informed consent for participation in the study, which was approved by the ethics committee of the Ehime General Health Care Association (approval number 2018-01). Then, 203 consecutive healthy individuals who underwent general health screening, including blood tests and low-dose chest CT for lung cancer screening, at one public interest foundation were enrolled in this study. This foundation conducts health checkups for local residents, and those enrolled in this study presented to the foundation between April 6, 2012 and May 17, 2018. The institute has conducted about 100000 lung cancer screenings for local residents over the past 20 years. During the enrolled period, 53 patients with a history of treatment for lifestyle-related diseases such as hypertension, diabetes, and hyperlipidemia were excluded. Forty-three of these patients had hypertension, 27 had diabetes, and 12 had hyperlipidemia. Some had multiple histories. In general health screening, age, sex, body height, body weight, blood pressure, smoking history (Brinkman index), and blood tests related to lifestyle-related diseases were conducted. The blood test consisted of triglycerides (TG), total cholesterol (T-CHO), low-density lipoprotein cholesterol (LDL-CHO), high-density lipoprotein cholesterol (HDL-CHO), hemoglobin A1c (HbA1c), estimated glomerular filtration rate (eGFR), creatinine (CRE), and uric acid (UA) and was performed concurrently with low-dose chest CT.

Low-dose CT scan
Low-dose chest CT for lung cancer screening was performed with a 16-channel multidetector CT scanner (ECLOS-16 slice, Hitachi, Ltd., Tokyo, Japan). The scan parameters were tube voltage 120 kV, tube flow 25 mA, rotation speed 0.75 seconds per rotation, and reconstructed slice thickness 5 mm. When a 0.014 conversion factor was used, the average CT dose index (CTDI) for males was 0.76 mGy, dose length product (DLP) was 29.9 mGy·cm, and effective dose was 0.42 mSv. The average for females was 0.73 mGy for CTDI, 26.2 mGy·cm for DLP, and 0.37 mSv for the effective dose.

Measurement of CAC
Data from a total of 1108 CT examinations for all 203 individuals were analyzed retrospectively. On CT images with a soft tissue condition, a high-density area with a CT value >130 Hounsfield units (HU) was defined as calcification. Those areas in the entire chest were extracted automatically by commercially available software (Ziostation2, Ziosoft, Inc., Tokyo, Japan). Among these areas, those along the coronary arteries were defined as coronary calcification and were measured manually on each slice throughout the whole heart. Last, the summed volume of coronary calcification throughout the heart was calculated as the CAC (mm$^3$) (Fig. 1).

Statistical analysis
Data are expressed as mean±standard deviation for normally distributed continuous variables, median (range) for skewed continuous variables, and count (percentage of total) for categorical variables. Testing of differences in demographic and clinical data based on CAC onset and non-CAC was accomplished with either the unpaired Student’s t test or Pearson’s chi-square test for categorical variables, as appropriate. Measures of the association between potential predictor variables and CAC onset were determined by univariate logistic regression. Covariates with p<0.05 during univariate testing were considered for inclusion in a multivariate model to identify factors independently associated with CAC onset. Covariates with p<0.05 in the final multivariate model were retained. Covariates showing evidence of significant confounding or effect modification also were retained. Testing of difference in annual CAC increase and risk score was accomplished with the Mann-Whitney U-test. Statistical significance was established using a two-tailed p-value<0.05. All statistical analyses were performed using the JMP statistical program package (version 9.0; JMP, Inc., Cary, NC, USA).

RESULTS
The initial screening age of the 203 participants in this study ranged from 43 years to 67 years, with a mean age of 53 years. Sex distribution was 118 (58%) males and 85 (42%) females. Of
the participants, 52 (26%) had a Brinkman index greater than 400 (51 males and one female).

**CAC onset vs. non-CAC**

Of the 1108 CT examinations, 874 with CAC of 0 mm³ accounted for 80% of the total. Among examinations, CAC distribution was not normal; the median value of CAC was 14 mm³ (Fig. 2). Subjects with a mean CAC greater than 14 mm³ on multiple CT scans were defined as CAC onset; the others were defined as non-CAC. In addition, those with a CAC greater than 0 mm³ and less than 14 mm³ were defined as micro-calcification and were included in the non-CAC group.

CAC onset was found in 27 (13%) of the 203 individuals. There were 24 (12%) subjects with micro-calcification, and no coronary calcification was observed in the other 152 individuals (75%). Figs. 3 and 4 show the distribution of CAC onset and micro-calcification by age and sex. The subjects with micro-calcification were classified into the non-CAC group as stated earlier. Male, age, and HbA1c were significantly higher in the CAC onset group than the non-CAC group. eGFR was significantly lower in the CAC onset group than in the non-CAC group. There was no difference in body mass index, Brinkman index, TG, T-CHO, LDL-CHO, HDL-CHO, CRE, or UA between the two groups (Table 1).

**Fig. 2.** Distribution of CAC. The median CAC amount is displayed as a black bar graph. CAC: coronary artery calcification.

**Fig. 3.** Distribution of age and sex in the coronary artery calcification-onset group.
Factors related to CAC onset

In the CAC onset group, age, sex, HbA1c, and eGFR were significantly related on univariate logistic regression analysis. Furthermore, as a result of multivariate logistic regression analysis using these four explanatory variables, CAC onset was significantly associated with increased age [odds ratio (OR) 1.06; 95% confidence interval (CI), 1.03–1.10; p=0.0010], HbA1c (OR 1.77; 95% CI, 1.47–2.14; p<0.0001), male (OR 1.59; 95% CI, 1.05–2.42; p=0.0258), and decreased eGFR (OR 0.97; 95% CI, 0.95–0.99; p=0.0009) (Table 2).

CAC risk score

The onset of CAC was found to be associated with three factors, age, HbA1c, and eGFR. Based on these results, we strati-
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Table 2. Results of univariate and multivariate logistic regression analyses in CAC onset and non-CAC groups

|                          | Univariate logistic regression analysis | Multivariate logistic regression analysis |
|--------------------------|----------------------------------------|------------------------------------------|
|                          | OR   | 95% CI | p value    | OR   | 95% CI | p value    |
| Age (years)              | 1.08 | 1.05–1.12 | <0.0001* | 1.06 | 1.03–1.10 | 0.0010* |
| Body mass index          | 1.04 | 0.99–1.09 | 0.080    |       |         |            |
| Sex                      |       |         |           |       |         |            |
| Male                     |       |         |           | 1.59 | 1.05–2.42 | 0.0258* |
| Female                   |       |         |           | 0.001* |         |            |
| Brinkman index           |       |         |           |       |         |            |
| ≤400                     |       |         |           |       |         | 0.337     |
| >400                     |       |         |           |       |         | 0.001      |
| Hemoglobin A1c, %        | 1.59 | 1.35–1.89 | <0.0001* | 1.77 | 1.47–2.14 | <0.0001* |
| Total cholesterol, mg/dL | 1.00 | 0.99–1.01 | 0.591    |       |         |            |
| High-density lipoprotein cholesterol, mg/dL | 1.01 | 0.99–1.02 | 0.081 |       |         |            |
| Low-density lipoprotein cholesterol, mg/dL | 1.00 | 0.99–1.00 | 0.329 |       |         |            |
| Triglycerides, mg/dL     | 1.00 | 0.99–1.00 | 0.346    |       |         |            |
| Creatinine, U/L          | 1.20 | 0.74–1.72 | 0.401    |       |         |            |
| Estimated glomerular filtration rate, mL/min | 0.98 | 0.96–0.99 | 0.007* | 0.97 | 0.95–0.99 | 0.0009* |
| Uric acid, mg/dL         | 0.99 | 0.87–1.13 | 0.872    |       |         |            |
| Systolic arterial pressure, mm Hg | 1.00 | 0.99–1.01 | 0.631 |       |         |            |
| Diastolic blood pressure, mm Hg | 1.00 | 0.99–1.02 | 0.507 |       |         |            |

*p<0.05. CI: confidence interval, CAC: coronary artery calcification, OR: odds ratio

We defined these three factors and proposed a new risk score for onset of CAC. The average age of those in the non-CAC group was 55 years (Table 1). In males, 1 point was given to those 55 years or older and 0 points to those 54 years or younger. In females, 1 point was given to those 60 years or older and 0 points to those 59 years or younger because menopause was considered a risk for cardiovascular events [15]. Based on HbA1c level, diabetes was identified at a value of 6.5% or more, borderline diabetes at 5.5% to 6.5%, and normal at less than 5.5%. Based on this criterion, 2 points were given for 6.5% or more, 1 point was given for 5.5% to 6.5%, and 0 points were given for less than 5.5%. The reference value of eGFR for middle-aged elderly was 60 mL/min. In this study, the average eGFR value of the CAC group was 70 mL/min (Table 1). Based on these two values, 2 points were given for less than 60 mL/min, 1 point was given for 60 mL/min to less than 70 mL/min, and 0 points were given for 70 mL/min or more. The sum of all scores was defined as the CAC risk score.

Risk factor for CAC increase

We defined those with a CAC greater than 0 mm³ and less than 14 mm³ as micro-calcification. The annual increase in CAC was significantly greater for the CAC onset group than for the micro-calcification group (30±34 mm³ vs. 3±4 mm³, p=0.0003) (Fig. 5). CAC risk score was significantly higher for the CAC onset group than the micro-calcification group (2.4±1.5 vs. 1.5±0.9, p=0.0306) (Fig. 6). In all 203 participants, the sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio of a positive result were 59%, 72%, 24%, 92%, and 2.06, respectively, when the criterion for the onset of CAC was a risk score of 3 or higher.

DISCUSSION

The present study investigated CAC onset and the factors of
lifestyle-related diseases in healthy individuals using low-dose CT without ECG gating. CAC is an important facet of CAD and a marker of overall disease burden. Early detection of CAC in younger subjects has an important prognostic impact in terms of predicting future coronary heart disease risk [16]. With no symptoms and non-CAC, the risk of cardiovascular disease is low [14]; in patients with a calcium score <100, the incidence of cardiac events is reported to be 0.4% per year [12]. These findings suggest that this study, which measures CAC of the entire heart precisely in healthy subjects and identifies the absence of CAC, is significant. In this study, we found that three factors, age, HbA1c level, and eGFR, were associated with the onset of CAC in healthy middle-aged and elderly adults. Based on these results, we propose a new risk score for the onset of CAC by stratifying the three factors. The negative predictive value of the risk score is 92%; therefore, the risk of CAC is low if the score is less than 2. A score of 3 or higher, however, more than doubles the risk of CAC. The risk score is useful in screening for potential CAD and can provide an opportunity for early intervention in cardiovascular disease prevention. Longitudinal analysis of low-dose CT contributes to lung cancer screening effectiveness as well as cardiovascular disease prevention. Itani et al. [17] reported that CAC identified by CT for lung cancer screening can be useful in predicting future cardiovascular disease. In a previous study of CAC using a mobile CT scanner, the mean effective radiation dose was 3.6 mGy [18]. The researchers measured CAC of only part of the heart. In contrast, the mean effective radiation dose in the present study was 0.76 mGy for males and 0.73 mGy for females, much lower than that in the previous study. Furthermore, CAC in the entire heart was measured, which is considered a more accurate evaluation. The frequency of CAC onset was reduced slightly to 13% in this study compared to 16% in a previous report [19]. This is probably because the definition of CAC was established at higher 110 HU or more. In addition, their study included a large number of people aged 60 and over (about 30%).

CAC onset in males was significantly higher than in females. This is consistent with studies in multiethnic individuals without cardiovascular disease [19]. Due to the anti-atherogenic effect of estrogen, females have less CAC before menopause compared to after [15]. Although the prevalence of CAC in females younger than 60 years is reported to be half that of males [20], the present study did not show such a strong trend. CAC increases with age, and diabetes is an independent related factor [20,21]. In this study, age and HbA1c were significantly higher in the CAC onset group than in the non-CAC group. Reduced eGFR, male status, and diabetes, all risk factors for cardiovascular disease, are consistent with our results [22,23]. However, hypertension, hyperlipidemia, and smoking, which were reported as independent factors [10], were not associated with CAC-onset in our study. In this study, the primary purpose of chest CT was to screen for lung cancer. Subjects with a Brinkman index greater than 400 accounted for 26% of participants who were considered as having high risk for lung cancer. The overall smoking rate for Japanese aged 40 years to 69 years is 23% according to the National Health and Nutrition Survey of Ministry of Health, Labor, and Welfare [17,18] and is almost equivalent to that for the present study subjects.

We acknowledge some limitations in the CAC measurement process using low-dose ungated CT, including partial manual operation. First, there is the possibility of missed small calcifications that can be detected by ECG-gated cardiac CT. The cause of this false negative prediction is thought to be cardiac motion, which occurs in low-dose CT scanning [8]. However, ghost calcification has been reported as a false positive prediction of low-dose CT. This is thought to be due to the higher noise level of low-dose CT than that of ECG-gated cardiac CT [8]. Convolutional neural networks using low-dose CT can cause decrease of Agatston scores directly from cardiac images without prior segmentation of CAC [24] and automatic detection of calcification [25]. For the detection of CAC by low-dose CT, verification of the accuracy and cost-effectiveness of the manual measurement method is necessary, as is comparison of the detection result to that of artificial intelligence. Ethnicity is important in progression of atherosclerosis. Since the subjects of this study are limited to Japanese, the results cannot be generalized globally.

In conclusion, CAC occurred in about 10% of healthy middle-aged people and is associated with early glucose intolerance and slight decline in renal function. Low-dose chest CT can contribute to detection of CAC onset and risk stratification for arteriosclerosis.
Conflicts of Interest
The authors have no potential conflicts of interest to disclose.

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