Oxidative Stress is Closely Associated with Increased Arterial Stiffness, Especially in Aged Male Smokers without Previous Cardiovascular Events: A Cross-Sectional Study

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Aim: Cigarette smoking is one of the major risk factors for cardiovascular diseases and induces deleterious vascular damage. Oxidative stress is involved in vascular inflammation, the process of atherosclerosis. The purpose of the present study was to investigate whether the effects of oxidative stress on the arterial wall differ between smokers and non-smokers.

Methods: Male smokers and non-smokers without physical deconditioning who visited Enshu hospital for an annual physical check-up were enrolled in the study. To assess oxidative stress, serum levels of derivative reactive oxygen metabolites (d-ROM) were measured. The radial augmentation index (RAI) was measured using an automated device and was used as an index for arterial stiffness.

Results: Univariate and multivariate linear regression analysis showed that RAI was independently associated with d-ROM levels only in smokers. Moreover, RAI was significantly higher in smokers than in non-smokers. Logistic regression analysis with the endpoint of a higher RAI than the mean revealed that older age (>65 years), hypertension, and smoking were independently associated with higher RAI. Similarly, logistic regression analysis with the endpoint of higher d-ROM levels than the mean showed that older age and smoking were independently associated with higher d-ROM levels.

Conclusions: Increased RAI is significantly associated with smoking and, in smokers, with increased d-ROM levels. These results suggest that the effects of oxidative stress on arterial properties differ between smokers and non-smokers and that oxidative stress is closely associated with arterial stiffness, especially in smokers.

Key words: Cigarette smoking, Oxidative stress, Arterial stiffness, Radial augmentation index

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stress in the cardiovascular system [7-9]. However, quantification of oxidative stress using several laboratory methods, such as oxidized low-density lipoprotein and 8-isoprostane, occasionally leads to different conclusions [10]. Therefore, for the maintenance of healthy vascular systems, it is important not only to measure oxidative stress markers, but also to assess the effects of oxidative stress on the vascular system.

Cigarette smoking increases serotonin levels in platelets [11]. Generally, serotonin synthesized by enterochromaffin cells in the gastrointestinal tract is incorporated into platelets and is released to the plasma upon platelet activation [12-14]. Released serotonin modulates vascular tone and thrombus formation, and it mediates the development and/or rupture of atherosclerotic plaques [12-14]. We have recently reported that plasma concentrations of serotonin were associated with endothelial damage in smokers and that 8 weeks smoking cessation failed to decrease plasma serotonin concentrations [14]. Thus, cigarette smoking is related to a disorder in vascular systems, and the effects last for at least a few months after smoking cessation.

In the present study, we tested the hypotheses that oxidative stress adversely affects the arterial wall, especially in smokers, and that the effects of oxidative stress on arterial stiffness are greater in smokers than in non-smokers. The aim of the present study was to investigate the effects of oxidative stress on arterial stiffness in smokers and non-smokers.

**Materials and Methods**

Male subjects without physical deconditioning who were either habitual smokers or not were enrolled in the present study. The study protocol was approved by the ethics committees of Nagoya City University Graduate School of Medical Sciences and Enshu Hospital. The study was performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant prior to the start of the study.

**Subjects**

Approximately half of all Japanese adults undergo a physical check-up every year in either public or private institutions. Enshu Hospital is one of the institutions that performs physical check-ups, which are medical examinations performed from the viewpoint

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**Table 1. Characteristics of the study subjects**

| Variable                        | All subjects (n = 909) | Smokers (n = 263) | Non-smokers (n = 646) |
|---------------------------------|-----------------------|-------------------|-----------------------|
| Age (years)                     | 58 ± 12               | 54 ± 12 ***       | 60 ± 12               |
| BMI (kg/m²)                     | 22.8 ± 3.0            | 22.8 ± 3.0        | 22.8 ± 3.0            |
| Systolic BP (mmHg)              | 124 ± 14              | 122 ± 13 *        | 125 ± 14              |
| Diastolic BP (mmHg)             | 75 ± 3                | 75 ± 9            | 75 ± 9                |
| Hemoglobin (g/dL)               | 14.5 ± 1.1            | 14.8 ± 1.1 ***    | 14.4 ± 1.1            |
| Creatinine (mg/dL)              | 0.87 ± 0.16           | 0.83 ± 0.13 ***   | 0.88 ± 0.17           |
| FPG (mg/dL)                     | 99 ± 19               | 96 ± 17 *         | 100 ± 19              |
| Total cholesterol (mg/dL)       | 193 ± 30              | 189 ± 31 *        | 194 ± 30              |
| HDL-C (mg/dL)                   | 58 ± 15               | 54 ± 14 ***       | 59 ± 16               |
| LDL-C (mg/dL)                   | 120 ± 27              | 119 ± 29          | 121 ± 27              |
| Triglycerides (mg/dL)           | 109 ± 59              | 118 ± 68 **       | 106 ± 54              |
| AST (U/L)                       | 20.8 ± 4.9            | 19.8 ± 5.0 ***    | 21.2 ± 4.8            |
| ALT (U/L)                       | 19.8 ± 7.4            | 19.6 ± 7.6        | 19.9 ± 7.3            |
| Uric acid (mg/dL)               | 5.9 ± 1.1             | 5.9 ± 1.2         | 5.8 ± 1.1             |
| eGFR (mL/min per 1.73 m²)       | 73.5 ± 13.5           | 78.5 ± 12.9 ***   | 71.5 ± 13.2           |
| Radial augmentation index (%)   | 79.4 ± 11.7           | 81.6 ± 13.1 **    | 78.6 ± 10.9           |
| d-ROM (Carratelli units)        | 340 ± 54              | 342 ± 54          | 339 ± 54              |
| Smoking duration (years)        | –                     | 27 ± 13           | –                     |
| Number of cigarettes smoked per day | –                  | 18.7 ± 8.3       | –                     |
| Brinkman index                  | –                     | 515 ± 345         | –                     |

Data are given as the mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.0001 compared with non-smokers.

BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; d-ROM, derivatives of reactive oxygen metabolites.
Table 2. Results of univariate regression analysis of factors possibly associated with the radial augmentation index in all subjects, smokers, and non-smokers

| Variable                        | All subjects (n=909) | Smokers (n=263) | Non-smokers (n=646) |
|---------------------------------|----------------------|-----------------|---------------------|
|                                 | Coefficient (r)      | p-value         | Coefficient (r)      | p-value         | Coefficient (r)      | p-value         |
| Age (years)                     | 0.34                 | <0.0001         | 0.38                | <0.0001         | 0.37                | <0.0001         |
| BMI (kg/m²)                     | -0.11                | <0.01           | -0.11               | 0.098           | -0.11               | <0.01           |
| Systolic BP (mmHg)              | 0.14                 | <0.001          | 0.15                | <0.05           | 0.16                | <0.001          |
| Diastolic BP (mmHg)             | 0.09                 | <0.01           | 0.09                | 0.17           | 0.10                | <0.05           |
| Hemoglobin (g/dL)               | -0.076               | <0.05           | -0.11               | 0.09           | -0.09               | <0.05           |
| Creatinine (mg/dL)              | -0.052               | 0.14           | -0.11               | 0.09           | -0.002              | 0.96           |
| FPG (mg/dL)                     | -0.061               | 0.09           | -0.14               | <0.05          | -0.012              | 0.77           |
| Total cholesterol (mg/dL)       | -0.063               | 0.08           | -0.049              | 0.46           | -0.056              | 0.18           |
| HDL-C (mg/dL)                   | -0.042               | 0.24           | 0.04                | 0.55           | 0.05                | 0.22           |
| LDL-C (mg/dL)                   | -0.086               | <0.05          | -0.12               | 0.07           | -0.060              | 0.16           |
| Triglycerides (mg/dL)           | 0.082                | <0.05          | 0.079               | 0.24           | 0.066               | 0.12           |
| AST (U/L)                       | 0.064                | 0.07           | 0.048               | 0.47           | 0.096               | <0.05          |
| ALT (U/L)                       | -0.072               | 0.06           | -0.087              | 0.19           | -0.070              | 0.096          |
| Uric acid (mg/dL)               | 0.011                | 0.75           | -0.050              | 0.46           | 0.036               | 0.40           |
| eGFR (mL/min per 1.73 m²)       | -0.050               | 0.16           | -0.040              | 0.55           | -0.098              | <0.05          |
| d-ROM (Carratelli units)        | 0.077                | <0.05          | 0.25                | <0.001         | -0.006              | 0.89           |
| Smoking duration (years)        | –                    | –              | 0.30                | <0.0001        | –                   | –              |
| Number of cigarettes smoked per day | –                   | –              | 0.033               | 0.47           | –                   | –              |
| Brinkman index                  | –                    | –              | 0.22                | <0.0001        | –                   | –              |

BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; d-ROM, derivatives of reactive oxygen metabolites.

of preventive medicine regardless of whether individuals are exhibiting subjective symptoms. Of those undergoing physical check-ups at Enshu Hospital between 2015 and 2016 (n=1800), 1175 male subjects were screened to determine their eligibility to be included in the study. Subjects who had a past smoking habit but had stopped smoking before the screening for the present study (n=160) were excluded from the study. Similarly, subjects taking any medications or those with renal insufficiency (creatinine ≥ 1.5 mg/dL), malignant neoplasm, active inflammatory disease, a history of obvious hepatic disease, or a history of cardiovascular events (stroke and myocardial infarction) were also excluded from the study (n=106). Thus, 909 male subjects were included in the present study. Subjects who never smoked were defined as non-smokers, whereas those who had a smoking habit at the time of the current examination were defined as smokers. Smokers were instructed not to smoke on the day of the physical check-up. Blood samples were taken early in the morning after an overnight fast. Blood pressure (BP) was measured using a standard mercury sphygmomanometer with subjects in a seated position. Three consecutive BP measurements were taken at 2-min intervals, and the mean of the second and third measurements was recorded as the BP. Subjects with systolic BP ≥ 140 mmHg and diastolic BP ≥ 90 mmHg were defined as having hypertension. Subjects with high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL, low-density lipoprotein cholesterol (LDL-C) ≥ 140 mg/dL, or triglycerides ≥ 150 mg/dL were defined as having dyslipidemia. Subjects with a fasting plasma glucose (FPG) level ≥ 126 mg/dL were defined as having diabetes mellitus, whereas subjects with FPG ≥ 111 and < 126 mg/dL were defined as having impaired glucose tolerance.

Biochemical Analysis

Biochemical tests, including serum total cholesterol, LDL-C, HDL-C, and triglyceride levels, were performed using standard laboratory assays. In terms of the various oxidative markers, a simple method of detecting hydroperoxide levels by measuring derivative reactive oxygen metabolites (d-ROM) has been reported to be useful for evaluating oxidative stress. Therefore, serum concentrations of d-ROM were measured in the present study to assess oxidative stress. Measurements of d-ROM levels were made as described previously.
where $P_2$ and $PP$ are the height of the late systolic shoulder/peak pressure and the pulse pressure of the radial arterial pressure contour, respectively \(^{21, 22}\).

### Statistical Analysis

Data were analyzed using IBM SPSS Statistics 19 (IBM Corp., Chicago, IL, USA) and are expressed as the mean ± SD. Histograms of d-ROM and RAI showed an approximately normal distribution for both parameters. Dichotomous variables were assigned values of 0 and 1. Comparisons of continuous variables were performed using paired or unpaired $t$-tests, as appropriate. Univariate and multivariate linear regression analyses associated with RAI were performed. Two-tailed $p < 0.05$ was considered statistically significant.

### Results

The characteristics of all of the study subjects, as well as for smokers and non-smokers separately, are

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Briefly, serum samples were mixed with a buffered solution, and a chromogenic substrate was then added to the mixture. Samples were immediately incubated in the analyzer for 5 min, after which absorbance was recorded at 505 nm, with d-ROM levels expressed in Carratelli units. The estimated glomerular filtration rate (eGFR) was calculated using a modified formula from the Modification of Diet in Renal Disease study for the Japanese population \(^{18}\).

**Measurement of Central BP and Radial Augmentation Index**

A fully automated device (HEM-9000AI) was used for the measurement of radial artery pressure waveforms and an estimation of central BP, as described previously \(^{19, 20}\). The radial augmentation index (RAI), which has been reported to be a marker of arterial stiffness and subclinical atherosclerosis, was calculated using the following equation:

$$
\text{RAI} (\%) = \left( \frac{P_2}{PP} \right) \times 100,
$$

where $P_2$ and $PP$ are the height of the late systolic shoulder/peak pressure and the pulse pressure of the radial arterial pressure contour, respectively \(^{21, 22}\).

**Fig. 1.** Association between the levels of derivatives of reactive oxygen metabolites (d-ROM) and the radial augmentation index (RAI).

Relationships between d-ROM levels and RAI in (A) all subjects, (B) smokers, and (C) non-smokers. There was a significant correlation between d-ROM levels and RAI across all subjects and in smokers, but not in non-smokers.
Table 3. Results of multivariate regression analysis\(^a\) for factors possibly associated with the radial augmentation index in all subjects, smokers, and non-smokers

| Variable                        | Standardized coefficient | Standard error | p-value  |
|---------------------------------|--------------------------|----------------|----------|
| **All subjects**                |                          |                |          |
| **Non-interaction model**       |                          |                |          |
| Age (years)                     | 0.40                     | 0.039          | <0.0001  |
| BMI (kg/m\(^2\))                | -0.12                    | 0.15           | <0.001   |
| Systolic BP (mmHg)              | 0.016                    | 0.040          | 0.74     |
| Diastolic BP (mmHg)             | 0.12                     | 0.058          | <0.01    |
| Hemoglobin (g/dL)               | 0.023                    | 0.39           | 0.52     |
| FPG (mg/dL)                     | -0.12                    | 0.022          | <0.001   |
| HDL-C (mg/dL)                   | -0.020                   | 0.028          | 0.59     |
| LDL-C (mg/dL)                   | -0.060                   | 0.014          | 0.07     |
| Triglycerides (mg/dL)           | 0.11                     | 0.007          | <0.01    |
| eGFR (mL/min per 1.73 m\(^2\))  | 0.082                    | 0.032          | <0.05    |
| d-ROM (Carratelli units)        | 0.023                    | 0.007          | 0.48     |
| Cigarette smoking               | 0.16                     | 0.89           | <0.0001  |
| **Interaction model**           |                          |                |          |
| Product term of smoking and d-ROM| 0.68                     | 0.015          | <0.001   |
| Age (years)                     | 0.41                     | 0.040          | <0.0001  |
| BMI (kg/m\(^2\))                | -0.12                    | 0.15           | <0.001   |
| Systolic BP (mmHg)              | 0.020                    | 0.040          | 0.67     |
| Diastolic BP (mmHg)             | 0.12                     | 0.058          | <0.01    |
| Hemoglobin (g/dL)               | 0.025                    | 0.39           | 0.61     |
| FPG (mg/dL)                     | -0.11                    | 0.022          | <0.001   |
| HDL-C (mg/dL)                   | -0.019                   | 0.028          | 0.61     |
| LDL-C (mg/dL)                   | -0.055                   | 0.014          | 0.10     |
| Triglycerides (mg/dL)           | 0.11                     | 0.007          | <0.01    |
| eGFR (mL/min per 1.73 m\(^2\))  | 0.082                    | 0.032          | <0.05    |
| d-ROM (Carratelli units)        | -0.057                   | 0.008          | 0.14     |
| Cigarette smoking               | -0.51                    | 5.2            | <0.05    |
| **Smokers**                     |                          |                |          |
| Age (years)                     | 0.37                     | 0.089          | <0.0001  |
| BMI (kg/m\(^2\))                | -0.044                   | 0.31           | 0.55     |
| Systolic BP (mmHg)              | 0.05                     | 0.092          | 0.95     |
| Diastolic BP (mmHg)             | 0.12                     | 0.12           | 0.15     |
| Hemoglobin (g/dL)               | 0.050                    | 0.85           | 0.48     |
| FPG (mg/dL)                     | -0.17                    | 0.045          | <0.01    |
| HDL-C (mg/dL)                   | 0.044                    | 0.069          | 0.59     |
| LDL-C (mg/dL)                   | -0.055                   | 0.029          | 0.39     |
| Triglycerides (mg/dL)           | 0.12                     | 0.13           | 0.09     |
| eGFR (mL/min per 1.73 m\(^2\))  | 0.11                     | 0.069          | 0.11     |
| d-ROM (Carratelli units)        | 0.17                     | 0.015          | <0.01    |
| **Non-smokers**                 |                          |                |          |
| Age (years)                     | 0.41                     | 0.044          | <0.0001  |
| BMI (kg/m\(^2\))                | -0.16                    | 0.17           | <0.001   |
| Systolic BP (mmHg)              | 0.016                    | 0.044          | 0.77     |
| Diastolic BP (mmHg)             | 0.12                     | 0.065          | <0.05    |
| Hemoglobin (g/dL)               | 0.031                    | 0.45           | 0.47     |
| FPG (mg/dL)                     | -0.088                   | 0.025          | <0.05    |
significant interaction of smoking in the association of d-ROM and RAI was indicated (Table 3).

To evaluate the effects of cardiovascular risk factors, including cigarette smoking, on RAI, logistic regression analysis was conducted using categorical data with the endpoint of higher RAI than the mean. The results indicated that increased age, hypertension, and cigarette smoking were independently associated with higher RAI (Table 4). Similarly, logistic regression analysis, with the endpoint of d-ROM levels higher than the mean, revealed that cigarette smoking was independently associated with higher d-ROM levels after an adjustment for increased age, hypertension, dyslipidemia, diabetes mellitus, and obesity (odds ratio 1.41; 95% confidence interval 1.05–1.90; \( P < 0.05 \)).

Discussion

The main findings of the present study are that: (i) RAI was independently associated with d-ROM levels only in smokers, as revealed by univariate and multivariate linear regression analyses; (ii) RAI was significantly higher in smokers than in non-smokers; (iii) older age (i.e., >65 years), hypertension, and smoking were independently associated with higher RAI; and (iv) older age (i.e., >65 years) and smoking were independently associated with higher d-ROM levels. These results indicate that oxidative stress is strongly associated with increased arterial stiffness in smokers.

Previously, we reported that oxidative stress, as assessed by d-ROM levels, was significantly associated with cardiovascular risk parameters in the general population\(^7\). In that study, d-ROM levels were associated with endothelial dysfunction and markers of vascular inflammation\(^7\). In the present study, we evaluated serum d-ROM concentrations and RAI, a parameter significantly associated with d-ROM and RAI was indicated (Tables 3, 3a, and 3b).
Table 3a. Results of multivariate regression analysis for factors possibly associated with the radial augmentation index adjusting for smoking intensity (Brinkman index) in all subjects, smokers, and non-smokers

| Variable                     | Standardized coefficient | Standard error | p-value |
|------------------------------|--------------------------|----------------|---------|
| **All subjects**             |                          |                |         |
| Non-interaction model        |                          |                |         |
| Age (years)                  | 0.38                     | 0.057          | <0.0001 |
| BMI (kg/m²)                  | -0.11                    | 0.19           | <0.05   |
| Systolic BP (mmHg)           | -0.001                   | 0.040          | 0.99    |
| Diastolic BP (mmHg)          | 0.15                     | 0.076          | <0.05   |
| Hemoglobin (g/dL)            | 0.022                    | 0.49           | 0.64    |
| FPG (mg/dL)                  | -0.15                    | 0.026          | <0.001  |
| HDL-C (mg/dL)                | -0.005                   | 0.037          | 0.91    |
| LDL-C (mg/dL)                | -0.020                   | 0.018          | 0.65    |
| Triglycerides (mg/dL)        | 0.093                    | 0.009          | <0.05   |
| eGFR (mL/min per 1.73 m²)    | 0.12                     | 0.041          | <0.05   |
| d-ROM (Carratelli units)     | 0.053                    | 0.010          | 0.21    |
| Cigarette smoking            | 0.13                     | 1.11           | <0.01   |
| Brinkman index               | 0.082                    | 0.002          | 0.084   |
| Interaction model            |                          |                |         |
| Product term of smoking and d-ROM | 0.87               | 0.018          | <0.01   |
| Age (years)                  | 0.38                     | 0.056          | <0.0001 |
| BMI (kg/m²)                  | -0.009                   | 0.19           | <0.05   |
| Systolic BP (mmHg)           | 0.007                    | 0.051          | 0.90    |
| Diastolic BP (mmHg)          | 0.14                     | 0.074          | <0.05   |
| Hemoglobin (g/dL)            | 0.029                    | 0.48           | 0.53    |
| FPG (mg/dL)                  | -0.15                    | 0.026          | <0.001  |
| HDL-C (mg/dL)                | -0.007                   | 0.036          | 0.89    |
| LDL-C (mg/dL)                | -0.018                   | 0.018          | 0.67    |
| Triglycerides (mg/dL)        | 0.095                    | 0.009          | <0.05   |
| eGFR (mL/min per 1.73 m²)    | 0.11                     | 0.041          | <0.05   |
| d-ROM (Carratelli units)     | -0.070                   | 0.013          | 0.22    |
| Cigarette smoking            | -0.73                    | 6.4            | <0.01   |
| Brinkman index               | 0.082                    | 0.002          | 0.080   |
| **Smokers**                  |                          |                |         |
| Age (years)                  | 0.37                     | 0.10           | <0.0001 |
| BMI (kg/m²)                  | -0.014                   | 0.31           | 0.85    |
| Systolic BP (mmHg)           | 0.011                    | 0.091          | 0.85    |
| Diastolic BP (mmHg)          | 0.11                     | 0.12           | 0.23    |
| Hemoglobin (g/dL)            | 0.079                    | 0.84           | 0.27    |
| FPG (mg/dL)                  | -0.20                    | 0.045          | <0.01   |
| HDL-C (mg/dL)                | 0.049                    | 0.069          | 0.50    |
| LDL-C (mg/dL)                | -0.052                   | 0.029          | 0.42    |
| Triglycerides (mg/dL)        | 0.065                    | 0.014          | 0.34    |
| eGFR (mL/min per 1.73 m²)    | 0.14                     | 0.071          | <0.05   |
| d-ROM (Carratelli units)     | 0.18                     | 0.015          | <0.01   |
| Brinkman index               | 0.078                    | 0.003          | 0.32    |
| **Non-smokers**              |                          |                |         |
| Age (years)                  | 0.41                     | 0.044          | <0.0001 |
| BMI (kg/m²)                  | -0.16                    | 0.17           | <0.0001 |
| Systolic BP (mmHg)           | 0.016                    | 0.044          | 0.77    |
of arterial stiffness, instead of endothelial function, and we confirmed that smoking was independently associated with increased arterial stiffness and increased oxidative stress. Classically, arterial stiffness, which is characterized by decreased arterial elasticity, with the loss of elastic fiber content in the vascular smooth muscle layer, is thought to have different features than vascular endothelial dysfunction, but both conditions are known to be surrogate markers of cardiovascular events. Meanwhile, oxidative stress has been shown to induce increased arterial stiffness in animal models and is associated with decreased arterial elasticity. Although the correlation coefficient between RAI and d-ROM was not so high, a significant association between arterial stiffness and oxidative stress demonstrated in smokers in the present study may indicate that smoking-related oxidative stress provokes arterial stiffness and accelerates arteriosclerosis. Indeed, RAI was greater in smokers than in non-smokers even though d-ROM concentrations were comparable in these two groups.

Oxidative stress can be measured using several biological markers in both smokers and non-smokers. However, caution should be taken when interpreting the results because cigarette smoking increases inflammatory molecules in addition to promoting lipid and protein oxidation products, and so may not be identified correctly using one of the markers. Although we had not evaluated oxidative stress in smokers using d-ROM levels, Kato et al. did evaluate d-ROM levels in 11 healthy male smokers and reported that smoking cessation for three months using varenicline significantly decreased d-ROM levels and ameliorated endothelial function. However, the sample in that study was too small, and the study lacked a comparative analysis with non-smokers. Hence, in the present study, we investigated the effects of oxidative stress on arterial stiffness in smokers and non-smokers and found that the effects of oxidative stress on arterial properties were not equivalent between these two groups. This may be the reason why a significant association between oxidative stress and arterial stiffness could not be observed when the analysis was performed in the study cohort as a whole, including smokers and non-smokers. Of note, d-ROM was significantly associated with smoking, but not with RAI, in all subjects. However, interaction of smoking in the association of d-ROM and RAI was significant, suggesting that smoking significantly modified the association between oxidative stress and arterial stiffness. In smokers, an increase in oxidative stress may indicate a parallel increase in levels of several other constituents of cigarette smoke that may directly affect arterial function and increase arterial stiffness. Increased plasma serotonin concentrations and decreased endothelial function are characteristics of smokers compared with non-smokers. In a previous study, we reported that plasma serotonin concentrations and the ratio of serotonin in platelet-poor plasma (PPP) to whole blood (WB) were independently associated with d-ROM levels in non-smoking subjects. Under physiological conditions, serotonin works in...
Table 3b. Results of multivariate regression analysis for factors possibly associated with the radial augmentation index adjusting for smoking duration and number of cigarettes smoked per day in all subjects, smokers, and non-smokers

| Variable                                | Standardized coefficient | Standard error | p-value  |
|-----------------------------------------|--------------------------|----------------|----------|
| **All subjects**                        |                          |                |          |
| **Non-interaction model**               |                          |                |          |
| Age (years)                             | 0.37                     | 0.064          | <0.0001  |
| BMI (kg/m²)                             | -0.11                    | 0.19           | <0.05    |
| Systolic BP (mmHg)                      | 0.002                    | 0.052          | 0.98     |
| Diastolic BP (mmHg)                     | 0.14                     | 0.075          | <0.05    |
| Hemoglobin (g/dL)                       | 0.023                    | 0.49           | 0.63     |
| FPG (mg/dL)                             | -0.15                    | 0.026          | <0.001   |
| HDL-C (mg/dL)                           | -0.006                   | 0.037          | 0.90     |
| LDL-C (mg/dL)                           | -0.020                   | 0.018          | 0.64     |
| Triglycerides (mg/dL)                   | 0.090                    | 0.009          | 0.054    |
| eGFR (mL/min per 1.73 m²)               | 0.11                     | 0.041          | <0.05    |
| d-ROM (Carratelli units)                | 0.049                    | 0.010          | 0.25     |
| Cigarette smoking                       | 0.12                     | 1.3            | <0.05    |
| Smoking duration (years)                | 0.080                    | 0.052          | 0.17     |
| Number of cigarettes smoked per day     | 0.042                    | 0.010          | 0.33     |
| **Interaction model**                   |                          |                |          |
| Product term of smoking and d-ROM       | 0.87                     | 0.018          | <0.01    |
| Age (years)                             | 0.36                     | 0.063          | <0.0001  |
| BMI (kg/m²)                             | -0.10                    | 0.19           | <0.05    |
| Systolic BP (mmHg)                      | 0.010                    | 0.051          | 0.87     |
| Diastolic BP (mmHg)                     | 0.14                     | 0.074          | <0.05    |
| Hemoglobin (g/dL)                       | 0.030                    | 0.49           | 0.52     |
| FPG (mg/dL)                             | -0.15                    | 0.026          | <0.001   |
| HDL-C (mg/dL)                           | -0.007                   | 0.036          | 0.88     |
| LDL-C (mg/dL)                           | -0.019                   | 0.018          | 0.66     |
| Triglycerides (mg/dL)                   | 0.093                    | 0.009          | <0.05    |
| eGFR (mL/min per 1.73 m²)               | 0.11                     | 0.041          | <0.05    |
| d-ROM (Carratelli units)                | -0.073                   | 0.013          | 0.20     |
| Cigarette smoking                       | -0.73                    | 6.4            | <0.01    |
| Smoking duration (years)                | 0.074                    | 0.051          | 0.20     |
| Number of cigarettes smoked per day     | 0.044                    | 0.058          | 0.29     |
| **Smokers**                             |                          |                |          |
| Age (years)                             | 0.15                     | 0.21           | 0.40     |
| BMI (kg/m²)                             | -0.013                   | 0.31           | 0.86     |
| Systolic BP (mmHg)                      | 0.019                    | 0.091          | 0.83     |
| Diastolic BP (mmHg)                     | 0.096                    | 0.12           | 0.27     |
| FPG (mg/dL)                             | -0.20                    | 0.045          | <0.01    |
| Hemoglobin (g/dL)                       | 0.080                    | 0.84           | 0.27     |
| HDL-C (mg/dL)                           | 0.033                    | 0.069          | 0.65     |
| LDL-C (mg/dL)                           | -0.056                   | 0.029          | 0.45     |
| Triglycerides (mg/dL)                   | 0.052                    | 0.014          | 0.45     |
| eGFR (mL/min per 1.73 m²)               | 0.13                     | 0.071          | 0.062    |
| d-ROM (Carratelli units)                | 0.17                     | 0.015          | <0.01    |
| Smoking duration (years)                | 0.009                    | 0.12           | 0.88     |
| Number of cigarettes smoked per day     | 0.20                     | 0.28           | 0.11     |
function may have resulted in decreased RAI in the diabetic subjects\(^\text{32}\).

Although the present study was designed to investigate the effects of oxidative stress on arterial stiffness in smokers and non-smokers, other important information may be obtained by analyzing the relationships among the RAI, d-ROM, and other factors in a future study focusing on factors other than smoking habit. Furthermore, it is important to determine whether smoking cessation influences oxidative stress and the RAI. An observational follow-up study that includes active smokers, non-smokers, and people who stopped smoking during the follow-up period is needed to address this issue.

The present study has several limitations and should be interpreted with caution. Specifically, the cross-sectional nature of the study and the relatively small number of subjects enrolled should be kept in mind. A selection bias cannot be completely excluded, because subjects in the present study were participants in a physical check-up program. Female subjects were not included in the present study because the number of female subjects eligible for inclusion was only half that of male subjects and the proportion of female smokers (\(n = 15\)) was extremely small. Moreover, the source of oxidative stress quantified by d-ROM was not identified and smoking-induced oxidative stress could not be evaluated directly. Biological assessments to demonstrate arterial response \textit{in vivo} and to elucidate the mechanisms underlying the differences between smokers and non-smokers were not performed. Finally, important indices representing ath-

### Table 3b

| Variable              | Standardized coefficient | Standard error | \(p\)-value |
|-----------------------|--------------------------|----------------|-------------|
| Non-smokers           |                          |                |             |
| Age (years)           | 0.41                     | 0.044          | <0.0001     |
| BMI (kg/m\(^2\))      | -0.16                    | 0.17           | <0.001      |
| Systolic BP (mmHg)    | 0.016                    | 0.044          | 0.77        |
| Diastolic BP (mmHg)   | 0.12                     | 0.065          | <0.05       |
| Hemoglobin (g/dL)     | 0.031                    | 0.45           | 0.47        |
| FPG (mg/dL)           | -0.088                   | 0.025          | <0.05       |
| HDL-C (mg/dL)         | -0.043                   | 0.032          | 0.33        |
| LDL-C (mg/dL)         | -0.064                   | 0.017          | 0.12        |
| Triglycerides (mg/dL) | 0.11                     | 0.009          | <0.05       |
| eGFR (mL/min per 1.73 m\(^2\)) | 0.076 | 0.038 | 0.08 |
| d-ROM (Carratelli units) | -0.060 | 0.008 | 0.13 |

\(^{a}\)The multivariate models included derivatives of reactive oxygen metabolites (d-ROM), major risk factors, and factors that were significantly correlated with the radial augmentation index on univariate regression analysis.

BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate.
Conclusions

Increased RAI is significantly associated with habitual smoking and, in smokers, with increased d-ROM levels. These results suggest that the effect of oxidative stress on arterial properties differs between smokers and non-smokers.

Table 4. Results of logistic regression analysis for factors possibly associated with higher levels of the radial augmentation index in all subjects ($n=909$)

| Variables                      | Category       | OR (95% CI)   | p-value |
|--------------------------------|----------------|---------------|---------|
| Elderly (>65 years old)        | Yes vs no      | 1.91 (1.37–2.66) | <0.001  |
| Cigarette smoking              | Yes vs no      | 1.74 (1.27–2.43) | <0.001  |
| Hypertension                   | Yes vs no      | 1.61 (1.15–2.26) | 0.01    |
| Dyslipidemia                   | Yes vs no      | 0.91 (0.68–1.22) | 0.34    |
| Diabetes mellitus              | Yes vs no      | 0.72 (0.44–1.20) | 0.21    |
| Obesity (BMI > 25 kg/m²)       | Yes vs no      | 0.70 (0.48–1.02) | 0.06    |

The endpoint for the radial augmentation index was a value higher than the mean value (79.4%).

OR, odds ratio; CI, confidence interval; BMI, body mass index.

erosclerosis and arterial stiffness, such as the carotid intima–media thickness, pulse wave velocity, plaque score, and stiffness parameter β, were not measured in the present study, even though these indices are the gold standard for obtaining accurate information regarding subclinical atherosclerosis. Further research with a larger study population and using a prospective approach is needed to clarify the effects of oxidative stress on arterial stiffness in smokers and non-smokers.

Fig. 2. Effects of different cardiovascular risk factors on the radial augmentation index (RAI).
Effects of (A) age (elderly being those aged > 65 years), (B) smoking, (C) hypertension, (D) dyslipidemia, (E) diabetes mellitus, and (F) obesity (where obesity is defined as a body mass index > 25 kg/m²) on RAI. Data are the mean ± SD.
closely associated with arterial stiffness, especially in smokers.

**Disclosure**

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