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ORIGINAL RESEARCH

Heart Rate Fragmentation, Ambulatory Blood Pressure, and Coronary Artery Calcification

A Population-Based Study

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ABSTRACT

BACKGROUND Little is known regarding whether ultra-rapid patterns of heart rate variability (eg, heart rate fragmentation [HRF]) are associated with coronary artery calcification (CAC) in a general population.

OBJECTIVES This study aimed to assess the association between HRF and CAC, and whether these associations are independent of systolic blood pressure (SBP) levels.

METHODS From SESSA (the Shiga Epidemiological Study of Subclinical Atherosclerosis), we used data from 24-hour ambulatory blood pressure monitoring to identify awake and asleep SBP levels, and data from concurrent 24-hour Holter monitoring to quantify HRF using the awake and asleep percentage of inflection points (PIP). CAC on computed tomography scanning was quantified using an Agatston score. We used multivariable binomial logistic regression to assess the associations of PIP and ambulatory SBP with the presence of CAC, as defined by Agatston score >0.

RESULTS Of the 508 participants in this study (mean age: 66.5 ± 7.3 years), 325 (64%) had CAC and 183 (36%) did not. In fully adjusted models of prevalent CAC that also included office SBP, the ORs with 95% CIs for awake PIP, awake SBP, asleep PIP, and asleep SBP were 1.23 (95% CI: 0.99–1.54), 1.40 (95% CI: 1.11–1.77), 1.31 (95% CI: 1.05–1.62), and 1.28 (95% CI: 1.02–1.60), respectively. There was no evidence of interaction between PIP and ambulatory SBP in association with CAC. Results were similar when other HRF indices instead of PIP were used.

CONCLUSIONS Higher HRF and SBP levels during sleep are each associated with the presence of CAC in a general male population. (JACC: Asia 2024;4:216–225) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Adaptive control of the heart's beating rate and the resulting variability in beat-to-beat intervals is a hallmark of health. Heart rate variability (HRV) is a physiological variable that reflects cardiac autonomic nervous system function. HRV in healthy subjects is primarily attributable to fluctuations in the sympathetic and parasympathetic nervous systems. Analyses of 24-hour HRV using Holter electrocardiography typically show circadian variation (eg, a nocturnal increase due to heightened vagal activity during sleep).[1,2]

Recently, Costa et al[3] introduced a novel dynamic biomarker of cardiovascular risk called heart rate fragmentation (HRF), which is derived from the measurement of ultra-fast, nonrespiratory HRV patterns. In the MESA (Multi-Ethnic Study of Atherosclerosis), the researchers demonstrated that HRF was independently associated with major adverse cardiovascular events,[4] atrial fibrillation,[5] and cognitive decline.[6] However, the following evidence remains to be established thus far: 1) whether HRF is associated with subclinical cardiovascular disease (CVD) (eg, coronary artery calcification [CAC]); 2) whether HRF while awake is associated with cardiovascular damage; and 3) whether these associations with HRF and cardiovascular damage is independent of blood pressure (BP) levels, which are also affected by the parasympathetic/sympathetic nervous system[7] and cause cardiovascular damage.[8-10]

Here, we expand these investigations by: 1) determining the relationship between HRF and CAC, during both awake and sleep periods; and 2) investigating whether those relationships are independent of ambulatory BP levels. In addition, we seek to determine the relationship between ambulatory BP and CAC, also during both awake and sleep periods.

METHODS

DATA SOURCE AND STUDY POPULATION. SESSA (The Shiga Epidemiological Study of Subclinical Atherosclerosis) is an ongoing prospective, population-based cohort of a sample of healthy Japanese men. The study design and recruitment details have been previously reported.[11] In brief, from May 2006 through March 2008, residents of Kusatsu City, Shiga, were randomly selected using the Basic Resident Registry of the city. We invited 2,379 Japanese men aged 40 to 79 years to participate in the study; a total of 1,094 men agreed to a baseline examination. Of the 1,094 participants, 853 underwent follow-up examination between October 2010 and August 2014. Furthermore, 542 of these 853 participants underwent concurrent 24-hour Holter monitoring and 24-hour ambulatory BP measurements between October 2014 and September 2015. In the present study, we used data from the follow-up examination for participants' characteristics and computed tomography (CT) images. All participants provided written informed consent. The study was approved by the Institutional Review Board of the Shiga University of Medical Science.

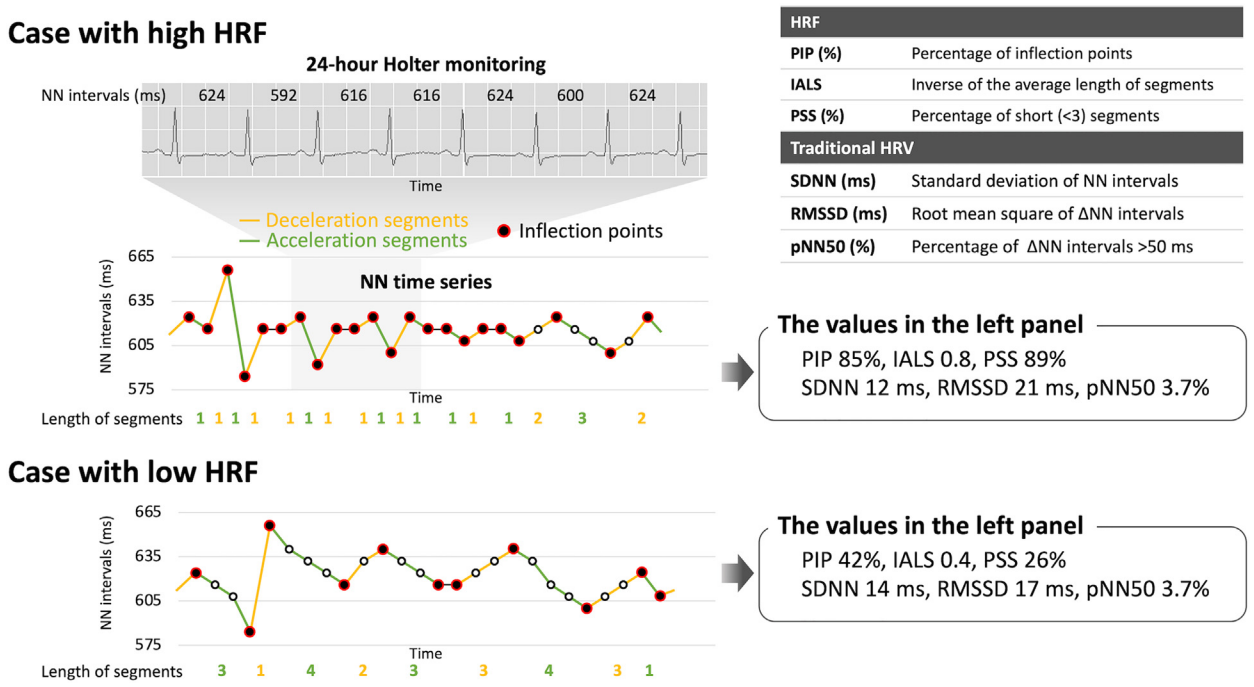
24-HOUR HOLTER MONITORING PROTOCOL. We used a 2-channel digital device (FM 800, Fukuda Denshi)[12] with a frequency response of 0.05 to 40.0 Hz, a sampling frequency of 125 Hz, and a resolution of 10 bits for 24-hour Holter monitoring. The detailed method for the measurement of 24-hour Holter monitoring in SESSA has been reported previously.[13] We reviewed automated beat annotations in the Holter data and manually judged whether sinus beating or non-sinus beating was occurring. Because non-sinus beats increase fragmentation, we then omitted RR segments including non-sinus beats from the whole 24-hour Holter monitoring. We evaluated data from periods of wakefulness and sleep separately, based on the participants' detailed diaries. The automated beat annotations were carefully examined by a skilled technician, who, under the guidance of a cardiologist, made any necessary adjustments.

ASSESSMENT OF HRF AND TRADITIONAL HRV. We quantified HRF using data from the 24-hour Holter monitoring by metrics derived from the analysis of the pulse interval time series.[3,6] This metric evaluates the acceleration, deceleration, or stability of heart rate (HR) for consecutive normal-to-normal intervals (NN intervals), allowing quantification of fragmented HR alteration. Specifically, the following 3 indices were assessed in this study: 1) the percentage of inflection points (PIP), calculated by the combined percentage of transitions from HR acceleration to HR deceleration or vice versa, and from HR acceleration/deceleration to stability (no change) or vice versa; 2) the inverse of the average length of the acceleration/deceleration segments (IALS), calculated by the inverse of the average number of ΔNN intervals in acceleration/deceleration segments; and 3) the percentage of short segments (PSS). We calculated the percentage of long segments (the number of ΔNN intervals in acceleration/deceleration segments with ≥3 ΔNN intervals over the total number of ΔNN intervals), and its complement was PSS. To visually

ABBREVIATIONS AND ACRONYMS

BP = blood pressure
CAC = coronary artery calcification
CT = computed tomography
CVD = cardiovascular disease
DBP = diastolic blood pressure
HR = heart rate
HRF = heart rate fragmentation
HRV = heart rate variability
IALS = inverse of the average length of the acceleration/deceleration segments
NN interval = normal-to-normal interval
PIP = percentage of inflection points
PNN50 = percentage of NN interval differences > 50 ms
PSS = percentage of short segments
RMSSD = root mean square of successive differences
SBP = systolic blood pressure
SDNN = standard deviation of normal-to-normal intervals

FIGURE 1 Schema of Cases With High and Low HRF



A higher heart rate fragmentation (HRF) means a higher frequency of inflection points and/or a shorter length of successive segments of acceleration or deceleration. IALS = inverse of the average length of the acceleration/deceleration segments; NN interval = normal-to-normal interval; PIP = percentage of inflection points; PNN50 = percentage of NN interval differences >50 ms; PSS = percentage of short segments; RMSSD = root mean square of successive differences; SDNN = standard deviation of normal-to-normal intervals.

understand the concept of HRF, we provide the schema of cases with high and low HRF in Figure 1.

We also quantified traditional HRV using the same pulse interval time series as that of HRF. In this study, we evaluated the following traditional time-domain HRV indices[14]: SDNN, calculated by SD of NN intervals; root mean square of successive differences (RMSSD), calculated by the square root of the mean of the squared differences between adjacent NN intervals; and percentage of NN interval differences >50 ms (pNN50), calculated by the percentage of differences between adjacent NN intervals that are >50 ms.

CT PROTOCOL AND IMAGE ANALYSIS. The detailed methodology for cardiac CT in SESSA has been previously published.[11,15] CT study was performed approximately 3 years (median [IQR]: 2.8 [2.4-3.1] years) before 24-hour Holter monitoring. We determined the presence of CAC based on CT images via a 16-channel multidetector-row CT using an Aquilion scanner (Canon Medical Systems). We acquired images at 70% of the cardiac cycle using an electrocardiogram triggering during a single breath-hold. The images were obtained from the level of the aortic root through the heart at a slice thickness of 3 mm and a scan time of 320 ms. The presence of CAC was defined as a minimum of 3 contiguous pixels with a density of at least 130 HU and was determined using AccuImage software (AccuImage Diagnostics); this software implements the widely accepted Agatston method.[16] The total CAC score was calculated by multiplying the pixel area (mm^2) by the density score (1: 130-199 HU; 2: 200-299 HU; 3: 300-399 HU; and 4: ≥400 HU) derived from the maximal Hounsfield units within this area. In the present study, we defined the presence of CAC as a dichotomous variable (Agatston score 0 or >0), consistent with prior studies.[8,17]

COVARIATE ASSESSMENT. Blood samples were obtained after participants had fasted for 12 hours. Samples were tested at a single laboratory (Shiga Laboratory, MEDIC, Shiga, Japan). Lipid measurements were standardized annually according to the protocol of the Centers for Disease Control and

Prevention/Cholesterol Reference Method Laboratory Network. Total cholesterol levels were measured using enzymatic assays, and high-density lipoprotein cholesterol levels were measured using a direct method. Plasma glucose levels were determined from sodium fluoride-treated plasma using a hexokinase glucose-6 phosphate-dehydrogenase enzymatic assay. Glycated hemoglobin (HbA1c) was measured using a latex agglutination assay according to the standardized method of the National Glycohemoglobin Standardization Program. Serum creatinine levels were measured using an enzymatic assay (Espa CRE-liquid II, NIPRO). The estimated glomerular filtration rate (mL/min/1.73 m^2) was calculated using serum creatinine levels: $194 \times \text{serum creatinine (mg/dL)}^{-1.094} \times \text{age}^{-0.287}$.[18]

Office BP was measured by a trained physician using an automated sphygmomanometer (BP-8800SF, Omron Healthcare Co Ltd)[19] after the participant rested for 5 minutes while sitting in a silent room without crossing the legs or speaking. In the present study, participants underwent 24-hour ambulatory BP measurement concurrently with Holter monitoring. The detailed method for the measurement of ambulatory BP in SESSA has been reported previously.[20] Ambulatory BP was measured with an appropriately sized cuff on the patient's nondominant arm using a fully automatic cuff-oscillometric method device (FM 800, Fukuda Denshi).[12] The device was set to measure BP every 30 minutes during the day and every 60 minutes during the night[21] in consideration of compliance and feasibility in obtaining measurements without excessively disrupting participants' sleep. Using the participants' detailed diaries, we then computed mean ambulatory systolic BP (SBP) and diastolic BP (DBP) levels during wakefulness and sleep.

Each participant provided data on their medical history and lifestyle factors using a self-administered questionnaire; trained technicians confirmed the accuracy of the completed questionnaires with the participants. Demographic characteristics, smoking status (eg, current, former, or never), alcohol drinking status (yes and no), medication use (eg, use of antihyperglycemic, antihypertensive, and antihyperlipidemic medications), and medical history (eg, stroke and myocardial infarction) were also recorded. Physicians additionally carried out an in-depth consultation with each participant to verify their medical history and establish if they had a history of CVD. Body mass index was calculated as weight (kg) divided by height squared (m^2). Diabetes mellitus was defined as HbA1c ≥6.5% and/or fasting plasma glucose level ≥126 mg/dL and/or taking antihyperglycemic medications.

STATISTICAL ANALYSIS. Summary statistics for demographic, clinical, and HR dynamical characteristics are provided for the study cohort and the subgroups of those with and without CAC. The summary statistics were mean ± SD for continuous variables with normal distributions, median and IQR for continuous variables with skewed distributions, and numbers and proportions for categorical variables. The differences in characteristics between the subgroups with and without CAC were assessed using chi-square tests for binary and ordinal variables and Student's *t*-tests and nonparametric Wilcoxon rank-sum tests for continuous variables with normal and non-normal distributions, respectively. The correlations among variables (HRF, traditional HRV indices, and ambulatory SBP and DBP levels) were quantified by Pearson correlation coefficients. We additionally examined the distribution of HRF, traditional HRV, and ambulatory SBP across consecutive age groups. The trend of each variable within these age categories was determined using the Jonckheere-Terpstra trend test.

We used binomial logistic regression models to assess the associations of HRF and ambulatory BP with CAC. ORs and 95% CIs for the presence of CAC were calculated with a 1-SD increment for each HRF index (eg, PIP, IALS, and PSS) and for each ambulatory BP level. The ORs were calculated in an unadjusted model (Model 1), and after adjustment for age, smoking status (current, former, or never), alcohol drinking (yes or no), body mass index, estimated glomerular filtration rate, total cholesterol level, high-density lipoprotein cholesterol level, diabetes mellitus (yes or no), antihypertensive medication use (yes or no), antihyperlipidemic medication use (yes or no), mean NN intervals (while awake or asleep according to analyses), and office BP (Model 2). In Model 3, we added both HRF and ambulatory BP measurements to the variables used in Model 2. Covariates were selected a priori because they have been shown to be associated with HRV, BP levels, and CAC.[4,8] In Model 3, we also tested for interaction in the association between each HRF index and CAC by ambulatory BP levels with the inclusion of multiplicative interaction terms. Stratified analyses were considered when an interaction was observed as $P < 0.05$. We also assessed traditional HRV indices (eg, SDNN, RMSSD, and pNN50) instead of HRF indices, and evaluated their association with the presence of CAC. RMSSD and pNN50 were log-transformed for analysis because of their skewed distributions. We computed the change in the Harrell concordance (C) statistic,[22]

comparing the base adjusted model (Model 2) against the new adjusted model (Model 3).

Sensitivity analyses were conducted by dividing CAC into 4 groups based on the Agatston score (0, >0 and <100, ≥100 and <300, and ≥300)[23,24] and examining their relationships with each HRF index and ambulatory SBP through a multivariable ordinal logistic regression model. Furthermore, CAC was treated as a continuous variable, specifically as ln (CAC+1),[8,25] and a multivariable Tobit regression model was used to accommodate the pronounced right-skewed distribution with numerous zero values. To investigate the possible effect of antihypertensive medication usage on the relationship between HRF or ambulatory SBP and CAC, we categorized our analysis according to this factor. In addition, we scrutinized the multiplicative interactions between antihypertensive medication usage and HRF or ambulatory SBP concerning their association with CAC. All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc.). A 2-sided *P* value of <0.05 was considered statistically significant.

RESULTS

CHARACTERISTICS OF PARTICIPANTS IN SESSA. Of 542 candidate participants, we excluded a total of 34 from analyses, including 23 with a history of stroke, 9 with a history of myocardial infarction, and 2 with missing 24-hour ambulatory BP measurements. The characteristics of the 508 men whose data were analyzed are shown in **Table 1**. The mean age was 66.5 ± 7.3 years, and 183 participants (36%) were taking antihypertensive medication.

Compared with participants without CAC (n = 183), those with CAC (n = 325) were older and more likely to have a higher prevalence of diabetes mellitus, dyslipidemia, antihyperlipidemic medication use, and antihypertensive medication use. Office SBP, awake and asleep SBP levels, and awake and asleep HRF indices (eg, PIP, IALS, and PSS) were significantly higher in participants with vs without CAC. HRV measures derived from the awake period were not associated with CAC. In contrast, sleep RMSSD, a measure of short-term (high-frequency) variability, was significantly lower in those with CAC. Sleep SDNN, a measure of overall variability, tended to be lower in those with CAC but statistical significance was not reached.

The HRF indices obtained from both the awake and sleep periods demonstrated strong correlations with each other (Pearson's correlation coefficients ≥0.9) (Supplemental Table 1). Ambulatory awake SBP was modestly correlated with PIP, IALS, and PSS (eg, Pearson's correlation coefficients 0.12 to 0.13).

CHANGES ACROSS AGE GROUPS IN HEART RATE DYNAMICS (HRF AND HRV METRICS) AND AMBULATORY BP. The summary statistics for the HRF and HRV indices, and ambulatory (systolic and diastolic) BP across age groups are provided in Supplemental Table 2. HRF metrics and SBP exhibited a positive relationship with age group. DBP exhibited a negative relationship. No linear trend existed for traditional HRV measures, indicating that in this study cohort the amplitude of traditional HRV did not monotonically decrease with cross-sectional age.

RELATIONSHIP BETWEEN HRF AND CAC. In unadjusted analyses, both awake and asleep HRF indices were significantly associated with CAC. The magnitude of these associations was similar to that of ambulatory SBP. After adjusting for the variables in Model 2, the associations between HRF indices and CAC were attenuated. Overall, the associations were stronger when the indices were derived from the sleep period. Specifically, in the most adjusted models (Model 3) that include ambulatory SBP levels, a 1-SD increase in asleep PIP (5.8%) was associated with a 31% (95% CI: 5%-62%) increase in the odds of CAC (**Table 2**). Of note, analyses in which CAC was modeled as an ordinal variable (Supplemental Table 3) as well as those in which CAC was modeled as a continuous variable (Supplemental Table 4) yielded results entirely consistent with those described previously.

The performance of Model 2 after the incorporation of awake PIP and awake SBP improved even though the model already included office SBP. The C statistic increased from 0.709 to 0.730 (delta C = 0.021; 95% CI: 0.0004 to 0.04). The increase in the performance of Model 2 after the inclusion of asleep PIP and asleep SBP was comparable to that with awake variables: delta C = 0.726 to 0.708 = 0.018 (95% CI: -0.001 to 0.04), but it did not reach statistical significance.

RELATIONSHIP BETWEEN TRADITIONAL HRV AND CAC. Traditional HRV tended to be lower in those with CAC. However, the associations between HRV metrics and CAC did not reach significance in any of the models, regardless of whether the metrics were derived from the awake or sleep periods (**Table 2**).

RELATIONSHIP OF AMBULATORY SBP AND DBP WITH CAC. Ambulatory SBP was significantly associated with CAC in all models (**Table 2**). The association of asleep SBP with CAC was more attenuated by including the covariates in Model 2 than the

TABLE 1 Characteristics of Participants With and Without CAC in SESSA

	Overall (N = 508)	With CAC (n = 325)	Without CAC (n = 183)	*P* Value
Age, y	66.5 ± 7.3	67.9 ± 6.3	64.1 ± 8.2	<0.001
Smoking status				0.199
Current	106 (21)	64 (20)	42 (23)	
Former	301 (59)	202 (62)	99 (54)	
Alcohol drinker	414 (82)	263 (81)	151 (83)	0.658
Body mass index, kg/m^2	23.3 ± 2.9	23.4 ± 2.9	23.2 ± 2.9	0.325
eGFR, mL/min/1.73 m^2	70.6 ± 13.2	70.0 ± 12.9	71.5 ± 13.7	0.239
Diabetes mellitus	108 (21)	89 (27)	19 (10)	<0.001
Total cholesterol, mg/dL	203.7 ± 33.1	204.4 ± 34.2	202.3 ± 31.0	0.483
HDL-C, mg/dL	60.8 ± 17.2	60.4 ± 17.8	61.5 ± 16.3	0.468
Antihyperlipidemic medication use	102 (20)	78 (24)	24 (13)	0.003
Antihypertensive medication use	183 (36)	138 (42)	45 (25)	<0.001
Office BP, mm Hg				
SBP	131.1 ± 16.7	133.0 ± 16.7	127.6 ± 16.3	<0.001
DBP	77.2 ± 10.2	77.2 ± 10.3	77.3 ± 10.1	0.939
Ambulatory BP, mm Hg				
Awake SBP	127.0 ± 13.8	129.0 ± 14.0	123.3 ± 12.6	<0.001
Awake DBP	79.0 ± 8.5	79.0 ± 8.4	79.1 ± 8.6	0.958
Asleep SBP	114.7 ± 14.9	116.6 ± 15.9	111.4 ± 12.5	<0.001
Asleep DBP	71.0 ± 9.2	70.9 ± 9.3	71.3 ± 9.0	0.619
HRF				
Awake PIP, %	70.6 ± 4.4	71.2 ± 4.2	69.4 ± 4.5	<0.001
Awake IALS	0.70 ± 0.06	0.71 ± 0.06	0.69 ± 0.06	<0.001
Awake PSS, %	84.9 ± 6.4	85.8 ± 5.9	83.1 ± 6.9	<0.001
Asleep PIP, %	66.1 ± 5.8	67.0 ± 5.4	64.6 ± 6.1	<0.001
Asleep IALS	0.64 ± 0.07	0.65 ± 0.07	0.62 ± 0.07	<0.001
Asleep PSS, %	79.6 ± 8.6	80.8 ± 8.1	77.4 ± 9.1	<0.001
Traditional HRV				
Awake SDNN, ms	108.5 ± 31.1	106.7 ± 31.6	111.8 ± 30.1	0.077
Awake RMSSD, ms	21.6 (16.8-29.3)	21.1 (16.7-28.8)	22.2 (17.6-31.6)	0.226
Awake pNN50, %	2.4 (0.8-6.5)	2.0 (0.8-6.1)	2.7 (0.8-7.6)	0.165
Asleep SDNN, ms	96.7 ± 34.5	94.7 ± 33.6	100.3 ± 35.8	0.079
Asleep RMSSD, ms	26.0 (19.3-38.2)	24.6 (18.8-35.0)	29.5 (20.7-39.3)	0.019
Asleep pNN50, %	4.4 (1.2-11.9)	3.2 (1.1-9.7)	6.6 (1.7-13.9)	0.005

Values are mean ± SD or median (IQR). The cohort included 508 men without a history of cardiovascular disease. A Student's *t*-test and a Wilcoxon rank-sum test were used for comparison. Values are number (proportion) of each categorical variable. Differences in characteristics were evaluated using a chi-square test. The *P* values were calculated between patients with CAC and those with CAC.

BP = blood pressure; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HDL-C = high-density lipoprotein cholesterol; HRF = heart rate fragmentation; HRV = heart rate variability; IALS = inverse of the average length of the segments; PIP = percentage of inflection points; pNN50 = percentage of normal-to-normal (NN) interval differences > 50 ms; PSS = percentage of short segments; RMSSD = root mean square of successive differences; SBP = systolic blood pressure; SDNN = standard deviation of NN intervals.

association with awake SBP. Notably, after further adjusting the analyses for HRF (Model 3), both awake and asleep SBP continue to be associated with CAC. Specifically, in fully adjusted models (Model 3), a 1-SD increase in awake SBP (13.8 mm Hg) was associated with a 40% (95% CI: 11%-77%) increase in the odds of CAC. A 1-SD increase in asleep SBP (14.9 mm Hg) was associated with an increase of 28% (95% CI: 2%-60%) in the odds of CAC. There was no evidence of interactions between awake PIP and awake SBP or between asleep PIP and asleep SBP in their associations with CAC (all *P* for interaction >0.30). Awake and asleep DBP were not significantly associated with CAC in any of the models, including the unadjusted one.

ASSOCIATION OF HRF AND AMBULATORY SBP WITH CAC, BY ANTIHYPERTENSIVE MEDICATION USE. In a stratified analysis involving 183 participants on antihypertensive medication and 325 not using such medication, we observed notable interactions between antihypertensive medication usage and HRF in relation to the presence of CAC. Conversely, we did not identify any significant interactions between antihypertensive medication usage and ambulatory SBP in relation to the presence of CAC (Supplemental Figure 1).

TABLE 2 ORs for CAC Associated With 1-SD Increase in HRF, Traditional HRV, or Ambulatory BP

	Awake			Asleep		
	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
PIP	1.50 (1.24-1.81)[c]	1.24 (0.99-1.54)[a]	1.23 (0.99-1.54)[a]	1.54 (1.27-1.87)[c]	1.28 (1.04-1.59)[b]	1.31 (1.05-1.62)[b]
PSS	1.53 (1.27-1.84)[c]	1.27 (1.02-1.58)[b]	1.26 (1.01-1.58)[b]	1.50 (1.24-1.81)[c]	1.30 (1.06-1.59)[b]	1.31 (1.07-1.61)[b]
SDNN	0.85 (0.71-1.02)[a]	0.83 (0.67-1.03)[a]	0.84 (0.68-1.05)	0.85 (0.71-1.02)[a]	0.84 (0.68-1.04)	0.83 (0.67-1.03)[a]
RMSSD	0.94 (0.78-1.12)	0.89 (0.73-1.10)	0.92 (0.75-1.14)	0.88 (0.74-1.05)	0.89 (0.73-1.09)	0.89 (0.73-1.09)
SBP	1.55 (1.28-1.89)[c]	1.41 (1.11-1.77)[c]	1.40 (1.11-1.77)[c]	1.45 (1.19-1.76)[c]	1.25 (1.00-1.57)[b]	1.28 (1.02-1.60)[b]
DBP	1.00 (0.83-1.19)	1.14 (0.91-1.44)	1.15 (0.91-1.45)	0.96 (0.80-1.15)	0.99 (0.80-1.23)	1.01 (0.81-1.25)

The presence of CAC is defined by Agatston score >0. Model 1 is unadjusted. Model 2 includes adjustment for age, smoking status (current, former, or never), alcohol drinking (yes or no), body mass index, eGFR, total cholesterol level, HDL-C level, diabetes mellitus (yes or no), antihypertensive medication use (yes or no), antihyperlipidemic medication use (yes or no), mean normal-to-normal intervals (while awake or asleep according to analyses), and office SBP. Model 3 includes the variables in Model 2 and mean ambulatory SBP for analysis of HRF or traditional HRV, and PIP for analysis of ambulatory SBP and DBP. [a]$0.05 \leq P < 0.10$. [b]$0.005 \leq P < 0.05$. [c]$P < 0.005$.

CAC = coronary artery calcification; other abbreviations as in Table 1.

DISCUSSION

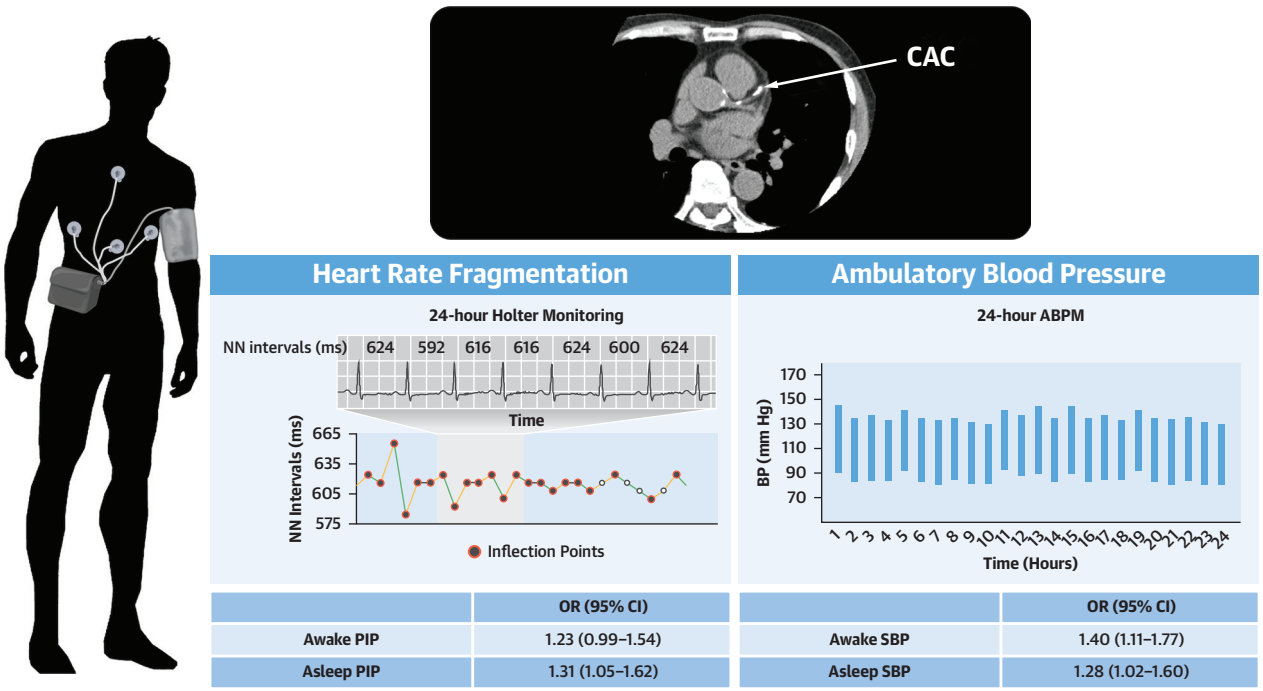
In this population-based study of Japanese men with a mean age of 67 years without a history of CVD, higher asleep HRF indices and ambulatory SBP were significantly associated with CAC even after adjusting for traditional atherosclerotic risk factors, including office SBP (**Central Illustration**). In addition, there was no evidence of interactions between PIP and ambulatory SBP in association with CAC. Furthermore, there was no statistically significant association between traditional HRV indices and CAC. These findings suggest that HRF is a dynamic biomarker of subclinical atherosclerosis.

The novel finding of our study is the positive association between HRF and CAC. Previously, 24-hour ambulatory SBP levels, per se, had a robust association with CAC, independent of office BP.[8,26] The present study also showed that higher ambulatory SBP levels had a robust relationship to CAC, which was still observed even after adjusting for HRF. However, it should be noted that we found a significant association between higher HRF levels and CAC, independently of office SBP and 24-hour ambulatory SBP levels, yet such a relationship between traditional HRV and CAC was not observed. Our findings align with earlier research conducted by Costa et al[4] and Berger et al,[27] which demonstrated that HRF, rather than traditional HRV measures, was associated with a heightened risk of major cardiovascular events. It is important to mention that we observed significant interactions between antihypertensive medication usage and HRF in relation to the presence of CAC. This finding is not readily explicable and needs to be confirmed by future studies. However, the result does show, importantly, that antihypertensive medication use cannot account for the observed relationship between HRF and CAC.

There was no significant association between traditional HRV and CAC in the present study. In comparison, Lovshin et al[28] studied 69 Canadian patients with type 1 diabetes (mean age: 66 years), and CAC was not associated with traditional HRV indices, including SDNN and RMSSD. In the CACTI (Coronary Artery Calcification in Type 1 Diabetes) study, however, Rodrigues et al[29] studied 915 participants (mean age: 39 years) and found that reduced SDNN predicted progression of CAC in adults with and without type 1 diabetes. The discrepancy in the results might be attributed to variations in the participants' age distribution and the limitations of the HRV metrics used. Rodrigues et al[29] assessed HRV through a 10-second electrocardiogram recording, which could be insufficient to capture its representative nature, as HR and HRV change over time. Importantly, a higher traditional HRV does not always imply better outcomes. In our study, traditional HRV did not exhibit a consistent decline with participants' age. Costa et al[3-5] demonstrated that 2 distinct mechanisms underlie short-term HRV: increased vagal tone modulation, primarily observed in healthy individuals, and heightened ultra-fast, nonrespiratory, beat-to-beat fluctuations (eg, HRF), which become more pronounced with aging and cardiovascular disease. Consequently, the lack of reduced traditional HRV in participants with CAC in the study by Lovshin et al,[28] which involved middle-aged and older individuals, could be due to the confounding impact of fragmented rhythms on HRV metrics. This hypothesis is also supported by the findings of Hayano et al[30] and Lensen et al.[31]

We found a robust relationship between HRF and CAC in the present study, especially during sleep.

CENTRAL ILLUSTRATION **Association of Heart Rate Fragmentation and Ambulatory Systolic Blood Pressure With Coronary Artery Calcification**



	OR (95% CI)
Awake PIP	1.23 (0.99–1.54)
Asleep PIP	1.31 (1.05–1.62)

	OR (95% CI)
Awake SBP	1.40 (1.11–1.77)
Asleep SBP	1.28 (1.02–1.60)

Sawayama Y, et al. JACC: Asia. 2024;4(3):216–225.

Using data from 24-hour Holter monitoring and concurrent 24-hour ambulatory blood pressure monitoring (ABPM), we assessed the association of heart rate fragmentation (HRF) and ambulatory systolic blood pressure (SBP) with coronary artery calcification (CAC), both for awake and sleep periods. Higher asleep HRF and ambulatory SBP were significantly associated with CAC even after adjusting for traditional atherosclerotic risk factors. The white arrow indicates CAC. BP = blood pressure; NN interval = normal-to-normal interval; PIP = percentage of inflection points.

Despite its multiple stages, sleep is considered a more stationary period than awake because physical activity has a major impact on HR dynamics. The fact that the association between HRF and CAC was weaker during awake may simply be because our analyses were not restricted to periods of rest or comparable degrees of physical activity. Another perspective includes that sleep is a different physiological condition from daytime awareness and is fundamental for an individual's health. Many biologic processes are active during sleep, particularly networks regulating the autonomic nervous system.[32] In addition, sleep or sleep-related mechanisms play a regulatory control role in the cardiovascular system.[33] Accordingly, poor sleep quality or sleep quantity has been linked with increases in atherosclerotic risk factors, including obesity, hypertension, and diabetes mellitus, resulting in an increased risk of CVD or death.[34]

STUDY LIMITATIONS. First, our results may not be widely generalizable, given that we only analyzed data from older men subjected to Holter monitoring within a sample obtained from a single area in Japan. Second, the number of variables assessed was limited, and measured and unmeasured confounders were present; however, we used a multivariable model by binomial logistic regression to reduce potential confounding. Third, the design of this study was cross-sectional, and the Holter monitoring was performed approximately 3 years after the CT scan, which does not allow for the direction of causality to be determined. As aforementioned, however, the logic behind the hypothesis that reduced vagal activity led to the development of CAC through the cholinergic anti-inflammatory pathway seems plausible. This hypothesis is also supported by the results of the longitudinal MESA study, in which higher HRF levels were observed preceding cardiovascular events.[4]

CONCLUSIONS

Higher HRF and SBP levels during sleep are each associated with the presence of CAC in apparently healthy men with a mean age of 67 years. We also found that there was no evidence of interactions between HRF and ambulatory SBP in association with CAC. These findings suggest that HRF is a dynamic biomarker of subclinical atherosclerosis. Further studies should investigate whether and how a higher magnitude of HRF should be managed to reduce the risk of CVDs.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The present study showed that higher HRF was significantly associated with CAC. This association between HRF and CAC was stronger during sleep than wakefulness. In addition, a significant association between higher HRF levels and CAC was independent of 24-hour ambulatory SBP levels. Ambulatory SBP levels also had a robust relationship to CAC, which was still observed even after adjusting for HRF.

TRANSLATIONAL OUTLOOK: The findings from the present study highlight the potential role of HRF in identifying a subset of individuals with coronary artery disease. Further studies are warranted to investigate whether and how a higher magnitude of HRF should be managed to reduce the risk of CVDs.

REFERENCES

1. Huikuri HV, Kessler KM, Terracall E, Castellanos A, Linnaluoto MK, Myerburg RJ. Reproducibility and circadian rhythm of heart rate variability in healthy subjects. *Am J Cardiol*. 1990;65:391-393. https://doi.org/10.1016/0002-9149(90)90308-n

2. Huikuri HV, Niemelä MJ, Ojala S, Rantala A, Ikäheimo MJ, Airaksinen KE. Circadian rhythms of frequency domain measures of heart rate variability in healthy subjects and patients with coronary artery disease. Effects of arousal and upright posture. *Circulation*. 1994;90:121-126. https://doi.org/10.1161/01.cir.90.1.121

3. Costa MD, Davis RB, Goldberger AL. Heart rate fragmentation: a new approach to the analysis of cardiac interbeat interval dynamics. *Front Physiol*. 2017;8:255. https://doi.org/10.3389/fphys.2017.00255

4. Costa MD, Redline S, Davis RB, Heckbert SR, Soliman EZ, Goldberger AL. Heart rate fragmentation as a novel biomarker of adverse cardiovascular events: the Multi-Ethnic Study of Atherosclerosis. *Front Physiol*. 2018;9:1117. https://doi.org/10.3389/fphys.2018.01117

5. Costa MD, Redline S, Soliman EZ, Goldberger AL, Heckbert SR. Fragmented sinoatrial dynamics in the prediction of atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis. *Am J Physiol Heart Circ Physiol*. 2021;320:H256-H271. https://doi.org/10.1152/ajpheart.00421.2020

6. Costa MD, Redline S, Hughes TM, Heckbert SR, Goldberger AL. Prediction of cognitive decline using heart rate fragmentation analysis: the Multi-Ethnic Study of Atherosclerosis. *Front Aging Neurosci*. 2021;13:708130. https://doi.org/10.3389/fnagi.2021.708130

7. Narkiewicz K, Winnicki M, Schroeder K, et al. Relationship between muscle sympathetic nerve activity and diurnal blood pressure profile. *Hypertension*. 2002;39:168-172. https://doi.org/10.1161/hy1201.097302

8. Zhang Y, Schwartz JE, Jaeger BC, et al. Association between ambulatory blood pressure and coronary artery calcification: the JHS. *Hypertension*. 2021;77:1886-1894. https://doi.org/10.1161/hypertensionaha.121.17064

9. Boggia J, Li Y, Thijs L, et al. Prognostic accuracy of day versus night ambulatory blood pressure: a cohort study. *Lancet*. 2007;370:1219-1229. https://doi.org/10.1016/s0140-6736(07)61538-4

10. Hansen TW, Jeppesen J, Rasmussen S, Ibsen H, Torp-Pedersen C. Ambulatory blood pressure and mortality. *Hypertension*. 2005;45:499-504. https://doi.org/10.1161/01.hyp.0000160402.39597.3b

11. Ueshima H, Kadowaki T, Hisamatsu T, et al. Lipoprotein-associated phospholipase A2 is related to risk of subclinical atherosclerosis but is not supported by Mendelian randomization analysis in a general Japanese population. *Atherosclerosis*. 2016;246:141-147. https://doi.org/10.1016/j.atherosclerosis.2015.12.027

12. Nakamura K, Kikuya M, Hara A, et al. Validation of the FM-800 ambulatory blood pressure monitor according to the Association for the Advancement of Medical Instrumentation criteria and the International Protocol. *Clin Exp Hypertens*. 2010;32:523-527. https://doi.org/10.3109/10641963.2010.496513

13. Hisamatsu T, Miura K, Fujiyoshi A, et al. Association between excessive supraventricular ectopy and subclinical cerebrovascular disease:a population-based study. *Eur J Neurol*. 2019;26:1219-1225. https://doi.org/10.1111/ene.13970

14. Tsuji H, Venditti FJ, Manders ES, et al. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. *Circulation*. 1994;90:878-883. https://doi.org/10.1161/01.cir.90.2.878

15. Hisamatsu T, Miura K, Fujiyoshi A, et al. Serum magnesium, phosphorus, and calcium levels and subclinical calcific aortic valve disease: a population-based study. *Atherosclerosis*. 2018;273:

145–152. https://doi.org/10.1016/j.atherosclerosis.2018.03.035

16. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827–832. https://doi.org/10.1016/0735-1097(90)90282-t

17. DeFilippis AP, Kramer HJ, Katz R, et al. Association between coronary artery calcification progression and microalbuminuria: the MESA study. *J Am Coll Cardiol Img*. 2010;3:595–604. https://doi.org/10.1016/j.jcmg.2010.01.008

18. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis*. 2009;53:982–992. https://doi.org/10.1053/j.ajkd.2008.12.034

19. Naschitz J, Gaitini L, Loewenstein L, et al. In-field validation of automatic blood pressure measuring devices. *J Hum Hypertens*. 2000;14:37–42. https://doi.org/10.1038/sj.jhh.1000937

20. Hisamatsu T, Ohkubo T, Hozawa A, et al. Association of self-measured home, ambulatory, and strictly measured office blood pressure and their variability with intracranial arterial stenosis. *J Hypertens*. 2021;39(10):2030–2039. https://doi.org/10.1097/hjh.0000000000002900

21. Li Y, Wei F-F, Thijs L, et al. Ambulatory hypertension subtypes and 24-hour systolic and diastolic blood pressure as distinct outcome predictors in 8341 untreated people recruited from 12 populations. *Circulation*. 2014;130:466–474. https://doi.org/10.1161/circulationaha.113.004876

22. Newson RB. Comparing the predictive powers of survival models using Harrell's C or Somers'. *D. Stata J*. 2010;10:339–358. https://doi.org/10.1177/1536867x1001000303

23. Budoff MJ, Young R, Burke G, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events:the multi-ethnic study of atherosclerosis (MESA). *Eur Heart J*. 2018;39:2401–2408. https://doi.org/10.1093/eurheartj/ehy217

24. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med*. 2008;358:1336–1345. https://doi.org/10.1056/nejmoa072100

25. Reilly MP, Wolfe ML, Localio AR, Rader DJ. Coronary artery calcification and cardiovascular risk factors: impact of the analytic approach. *Atherosclerosis*. 2004;173:69–78. https://doi.org/10.1016/j.atherosclerosis.2003.10.010

26. Sawayama Y, Hisamatsu T, Kadota A, et al. Association of ambulatory blood pressure with aortic valve and coronary artery calcification. *J Hypertens*. 2022;40:1344–1351. https://doi.org/10.1097/HJH.0000000000003147

27. Berger M, Pichot V, Solelhac G, et al. Association between nocturnal heart rate variability and incident cardiovascular disease events: the HypnoLaus population-based study. *Heart Rhythm*. 2022;19:632–639. https://doi.org/10.1016/j.hrthm.2021.11.033

28. Lovshin JA, Bjornstad P, Lovblom LE, et al. Atherosclerosis and microvascular complications: results from the Canadian Study of Longevity in Type 1 Diabetes. *Diabetes Care*. 2018;41:2570–2578. https://doi.org/10.2337/dc18-1236

29. Rodrigues TC, Ehrlich J, Hunter CM, Kinney GL, Rewers M, Snell-Bergeon JK. Reduced heart rate variability predicts progression of coronary artery calcification in adults with type 1 diabetes and controls without diabetes. *Diabetes Technol Ther*. 2010;12:963–969. https://doi.org/10.1089/dia.2010.0070

30. Hayano J, Kisohara M, Ueda N, Yuda E. Impact of heart rate fragmentation on the assessment of heart rate variability. *Applied Sciences*. 2020;10:3314.

31. Lensen IS, Monfredi OJ, Andris RT, Lake DE, Moorman JR. Heart rate fragmentation gives novel insights into non-autonomic mechanisms governing beat-to-beat control of the heart's rhythm. *JRSM Cardiovasc Dis*. 2020;9:2048004020948732. https://doi.org/10.1177/2048004020948732

32. Fink AM, Bronas UG, Calik MW. Autonomic regulation during sleep and wakefulness: a review with implications for defining the pathophysiology of neurological disorders. *Clin Auton Res*. 2018;28:509–518. https://doi.org/10.1007/s10286-018-0560-9

33. Zhang L, Wu H, Zhang X, Wei X, Hou F, Ma Y. Sleep heart rate variability assists the automatic prediction of long-term cardiovascular outcomes. *Sleep Med*. 2020;67:217–224. https://doi.org/10.1016/j.sleep.2019.11.1259

34. Kuehn BM. Sleep duration linked to cardiovascular disease. *Circulation*. 2019;139:2483–2484. https://doi.org/10.1161/circulationaha.119.041278

KEY WORDS ambulatory blood pressure, coronary artery calcification, heart rate fragmentation, heart rate variability

APPENDIX For a supplemental figure and tables, please see the online version of this paper.