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Original Article

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Longitudinal Associations between Alcohol Intake and Arterial Stiffness, Pressure Wave Reflection, and Inflammation

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Aims: This prospective observational study, which utilized repeated annual measurements performed over a 9-year period, applied mixed model analyses to examine age-related differences in longitudinal associations between alcohol intake and arterial stiffness, pressure wave reflection, and inflammation.

Methods: In 4016 middle-aged (43±9 years) healthy Japanese male employees, alcohol intake, brachial-ankle pulse wave velocity (baPWV), radial augmentation index (rAI), and serum C-reactive protein (CRP) levels were measured annually during a 9-year study period.

Results: The estimated marginal mean baPWV (non-drinkers=1306 cm/s, mild–moderate drinkers=1311 cm/s, and heavy drinkers=1337 cm/s, $P<0.01$) and that of rAI showed significant stepped increases in an alcohol dose-dependent manner in the entire cohort, but an increase in rAI was not observed in subjects aged ≥ 50 years. The estimated slope of the annual increase in baPWV, but not rAI, was higher for heavy drinkers than for non-drinkers (slope difference, 1.84; $P<0.05$), especially for subjects aged <50 years (slope difference, 2.84; $P<0.05$).

Conclusion: In middle-aged male Japanese employees, alcohol intake may attenuate inflammatory activity. While alcohol intake may exacerbate the progression of arterial stiffening in a dose-dependent manner without mediating inflammation, especially in subjects under 50 years of age, it may promote pressure wave reflection abnormalities with aging at earlier ages without further exacerbation at older ages.

Key words: Alcohol, Arterial stiffness, Inflammation

Introduction

While heavy alcohol intake has been reported as a risk factor for cardiovascular disease (CVD), some studies have reported that mild–moderate intake decreases this risk[1, 2]. The underlying mechanisms of the association between alcohol intake and the development of CVD have not been fully clarified. Arterial stiffness, abnormal pressure wave reflection, and inflammation are risk factors for CVD[3-5], and some studies have reported the association of alcohol intake with these three pathophysiological abnormalities related to CVD[6-8]. However, alcohol intake is also associated with conventional risk factors for CVD, such as hypertension, obesity, or abnormal glucose/lipid metabolism[9], and the significance of the effects of alcohol intake on the abovementioned pathophysiological abnormalities have not been fully examined because of the confounding effects of these risk factors. In addition, these three pathophysiological abnormalities are associated each other, and these associations may have age-related differences (i.e.,

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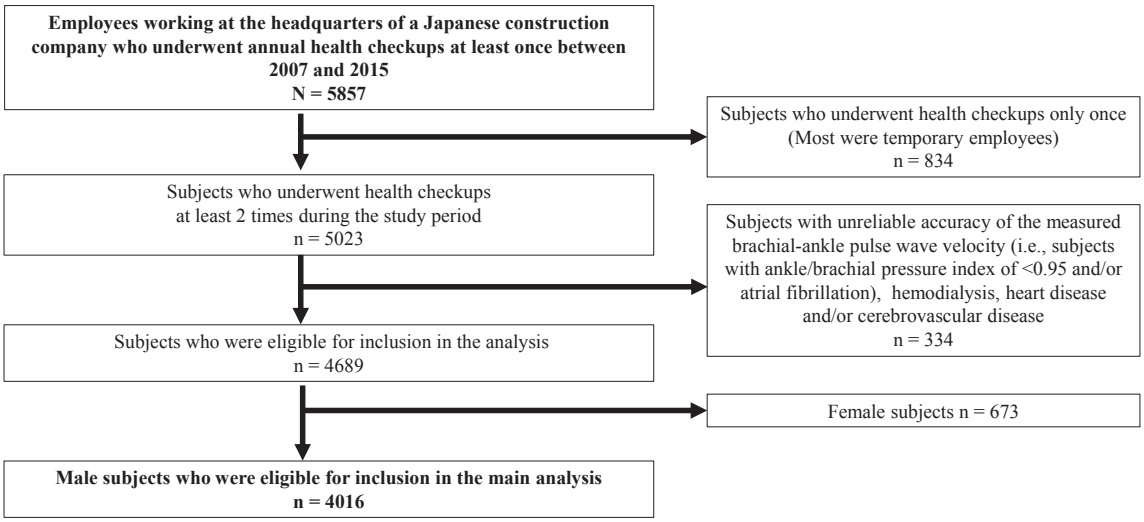

Fig. 1. Flow diagram of the subjects enrolled in the study

inflammation affects arterial stiffness and pressure wave reflection, and arterial stiffness and pressure wave reflection affect each other, especially in subjects aged <50 years)[3-6, 10-13]

This longitudinal observational study attempted to clarify these issues. Therefore, mixed model analyses using repeated measurement data[14] were applied to examine age-related differences in the longitudinal associations between the amount of alcohol intake (i.e., non, mild–moderate, and heavy) with arterial stiffness, pressure wave reflection, and inflammation simultaneously.

Methods

The data, analytical methods, and study materials will not be available to other researchers to avoidreproduce the results or replicating the procedure.

Design and Subjects

This study uses data from a previously reported prospective observational study, which examined the longitudinal association between arterial stiffness and cardiovascular risk status[7, 10, 11, 15]. This study population consisted of employees working at the headquarters of a single large Japanese construction company located in downtown Tokyo (all the study participants were office clerks). According to the Occupational Health and Safety Law in Japan, all company employees must undergo annual health checkups, and since 2007, annual measurements of the brachial-ankle pulse wave velocity (baPWV) and the radial augmentation index (rAI) have been conducted. Verbal informed consent was obtained from each of the study participants prior to their participation in this study. This study obtained approval from the Ethical Guidelines Committee of Tokyo Medical University (No. 209 and No. 210 in 2003).

The health checkup data obtained for the years 2007 through 2015 were used for the present study. Of the 5857 employees at the headquarters of the construction company who had undergone a baPWV (a marker of arterial stiffness) measurement, we excluded 834 subjects who underwent the measurement only once during the study period (most of these were temporary employees), 334 subjects whose baPWV measurements were thought to be unreliable or inaccurate, and 673 women. Finally, data for the remaining 4016 men were included in the present analyses. The subject selection flow diagram is shown in **Fig. 1**. In 2012, serum C-reactive protein (CRP) was not measured.

Assessment of Alcohol Intake

Habitual alcohol intake was assessed using a self-administered questionnaire. The level of alcohol intake was evaluated using two parameters: average drinking frequency (days/week) and average amount consumed each week (mL). The average daily alcohol intake (g/day, ethanol equivalent) was then calculated for each subject. The subjects were further categorized according to their daily alcohol intake as estimated from their responses to the questionnaire: 0 (non-drinker category), 1–19 g/day (mild–moderate alcohol intake category), and over 20 g/day (heavy alcohol intake category).

Measurement of baPWV and rAI

These measurements were offered as optional tests during the health checkups, which were conducted in the morning after the patients had fasted overnight. In addition, all the participants were instructed to abstain from smoking and caffeine intake but were allowed to take their regular medications (if applicable) with a small amount of water on the morning of their visit for a health checkup.

The baPWV was measured using a volume-plethysmographic apparatus (Form/ABI, Omron Healthcare Co., Ltd., Kyoto, Japan), as previously described[7, 9-11, 15, 16]. Briefly, occlusion cuffs connected to both the plethysmographic and oscillometric sensors were tied around both the upper arms and ankles of the subjects who were in supine position. Still in the supine position, measurements were conducted after the subjects had rested for at least 5 min in an air-conditioned room (maintained at 24℃) designated exclusively for this study.

Measurement of the rAI was conducted after the subjects had rested for at least 5 min in a seated position. The left radial arterial waveform was recorded using an arterial applanation tonometry probe equipped with an array of 40 micro piezoresistive transducers (HEM-9010AI; Omron Healthcare Co., Ltd.). Subsequently, the first and second peaks of the radial pressure waveform (SBP1 and SBP2, respectively) and brachial diastolic pressure (DP) were automatically detected using the fourth derivatives for each radial arterial waveform and then averaged. The rAI was calculated as follows: (SBP2 – brachial DP) / (SBP1 – brachial DP) × 100 (%)[11]. SBP1 minus SBP2 was calculated (deltaSBP1–2) as the marker of the peripheral pulse pressure amplification in the heart–arm segment.

The order in which the baPWV and the rAI measurements were performed was randomly determined.

The reproducibility and accuracy of these measurements have been reported to be acceptable elsewhere[12, 16].

Laboratory Measurements

Based on the results of the questionnaire, the smoking status (i.e., current smoker or not), medication history, and history of illness were confirmed. Serum concentrations of triglyceride (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and creatinine, as well as the plasma glucose concentration and glycohemoglobin A1c (HbA1c), were measured using standard enzymatic methods (Falco Biosystems Co. Ltd, Tokyo). Serum CRP was measured using the latex-aggregation method (Falco Biosystems Co Ltd, Tokyo)[10]. All the blood samples were obtained in the morning after the patients had fasted overnight.

Statistical Analysis

Data are expressed as the means ± SD. The differences in the measured values between the baseline and final examinations were assessed using the paired *t*-test for continuous variables and the McNemar non-parametric test for categorical variables. The minimum value of serum CRP was set at 0.5 mg/L[11]. In assessing age-related differences, subjects were divided by age 50 at the baseline of the study period into two groups.

The differences in the longitudinal status and the rate of change of the variables during the study period were assessed according to the three drinking categories using a mixed model linear regression (MML) analysis[17]. In the MML analysis, the three categories of alcohol intake (i.e., non, mild–moderate and heavy drinker) were entered as a fixed effect variable that interacted with time (time in years from the baseline); for the adjustments, age, body mass index, mean blood pressure, heart rate, serum levels of LDL, HDL, TG, HbA1c, uric acid, and creatinine, and smoking status plus a history of medication for hypertension, dyslipidemia, diabetes mellitus, and hyperuricemia (for each medication, not receiving medication = 0, receiving medication = 1) were entered as random effects variables. The differences in the longitudinal statuses of the variables among the three drinking categories were assessed using the estimated marginal mean of the variables. The differences in the rates of change of the variables during the study period among the three drinking categories were assessed using the difference in the estimated slopes of the annual changes in the variables during the study period.

All analyses were conducted using SPSS software (version 28.0; IBM/SPSS Inc., Armonk, NY, USA). A value of $P < 0.05$ was considered as being indicative of a statistically significant difference.

Results

Table 1 summarizes the clinical characteristics of the study participants. The amount of alcohol intake, baPWV, and rAI increased significantly over time. The mean follow-up period was 6.3 ± 2.5 (median = 7.0) years, and the measurements were repeated 5.2 ± 2.1 (median = 5.0) times. The clinical characteristics of the three drinking categories in all the study subjects are summarized in **Supplementary Table 1**.

Fig. 2 shows the means and error bars of the

Table 1. Clinical characteristics of the overall study population

Parameter	Baseline	Final
Age, years	43.2 ± 9.5	48.5 ± 9.6[a]
BMI, kg/m^2	24.0 ± 3.1	24.2 ± 3.2[a]
Smoking (current), *n* (%)	1269 (31.6)	1004 (25.0)[a]
ALC (Ethanol g/day)	12.8 ± 11.1	14.4 ± 11.9[a]
Systolic BP, mmHg	123.7 ± 14.2	123.4 ± 14.8
Diastolic BP, mmHg	75.3 ± 10.8	75.5 ± 10.9
Mean BP, mmHg	91.5 ± 11.2	91.5 ± 11.7
Heart rate, beats/min	64.9 ± 9.5	64.6 ± 9.7[b]
baPWV, cm/s	1289 ± 187	1342 ± 217[a]
AI, %	69.5 ± 13.4	72.5 ± 12.7[a]
HDL, mg/dL	62.2 ± 15.7	61.2 ± 15.3[a]
LDL, mg/dL	125.7 ± 30.5	123.9 ± 29.9[a]
TG, mg/dL	125.3 ± 87.7	121.3 ± 83.3[a]
UA, mg/dL	6.2 ± 1.2	6.0 ± 1.2[a]
HbA1c, %	5.3 ± 0.6	5.5 ± 0.6[a]
Creatinine, mg/dL	0.855 ± 0.114	0.861 ± 0.124[a]
CRP (mg/L)	1.04 ± 1.64	1.01 ± 1.54
Medications		
Receiving medication for hypertension, *n* (%)	304 (7.6)	615 (15.3)[a]
Receiving medication for diabetes mellitus, *n* (%)	96 (2.4)	172 (4.3)[a]
Receiving medication for dyslipidemia, *n* (%)	107 (2.7)	284 (7.1)[a]
Receiving medication for hyperuricemia, *n* (%)	186 (4.6)	245 (6.1)[a]

Abbreviations: BMI = body mass index; Smoking = number of current smokers; ALC = daily alcohol intake; BP = blood pressure; baPWV = brachial-ankle pulse wave velocity; AI = augmentation index; HDL = serum high-density lipoprotein cholesterol; LDL = serum low-density lipoprotein cholesterol; TG = serum triglyceride; UA = serum uric acid; HbA1c = glycosylated hemoglobin A1c; Creatinine = serum creatinine; CRP = serum C-reactive protein levels; Medications, number and percentage of subjects receiving medications;; [a] = $P < 0.01$ vs. baseline; [b] = $P < 0.05$ vs. baseline

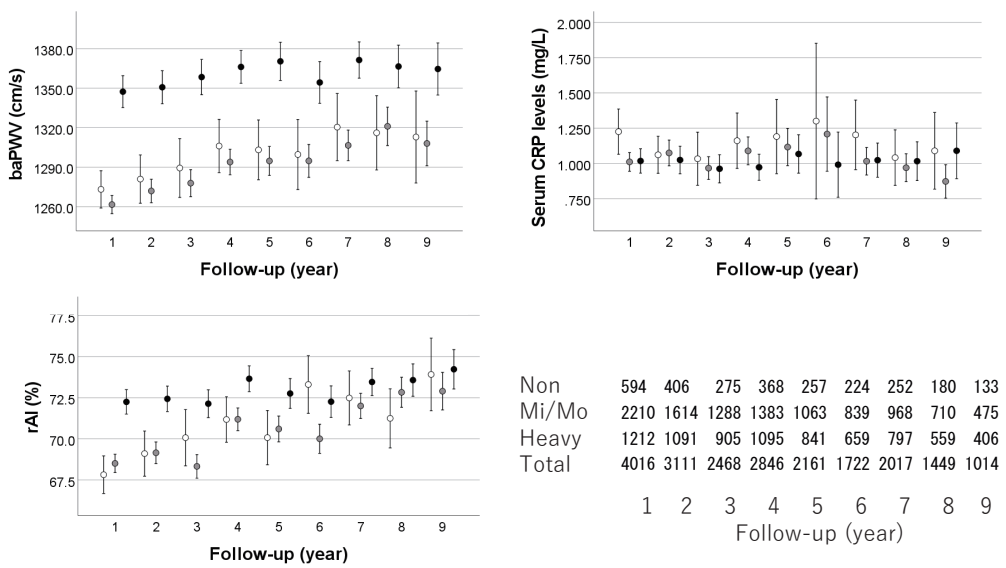

Fig. 2. Annual changes in brachial-ankle pulse wave velocity values, radial augmentation index values, and serum CRP levels among three drinking categories, and number of data points from the start to the end of the study period for three drinking categories

Abbreviations: baPWV = brachial-ankle pulse wave velocity, rAI = radial augmentation index, CRP = C-reactive protein; Heavy = number of heavy drinker in each year; Mi/Mo = number of mild–moderate drinker in each year; Non = number of non-drinker in each year; Total = total number of subjects in each year; open circle = non-drinker; gray-circle = mild–moderate drinker; black circle = heavy drinker

Table 2. Crude and adjusted values of the estimated marginal mean of the brachial-ankle pulse wave velocity, radial augmentation index, and serum C-reactive protein levels and differences in the annual increases in the brachial-ankle pulse wave velocity, radial augmentation index, and serum C-reactive protein levels during the study period among 3 drinking categories in all the study subjects

	EMM crude	95% CI	EMM adjust	95% CI	eSlope crude	95% CI	eSlope adjust	95% CI
For baPWV								
Non	1306	1297–1315	1272	1264–1281	ref	-	ref	-
Mi/Mo	1311	1306–1318	1280[a]	1274–1286	0.58	-1.29–2.46	0.91	-0.844–2.65
Heavy	1337[ab]	1330–1344	1302[ab]	1295–1309	1.33	-0.64–3.30	1.84[a]	0.00–3.68
For rAI								
Non	70.0	69.3–70.7	72.0	71.3–72.7	ref	-	ref	-
Mi/Mo	70.9[a]	70.4–71.3	72.9[a]	72.5–73.3	0.01	-0.13–0.14	0.00	-0.13–0.13
Heavy	72.5[ab]	72.0–72.9	74.5[ab]	74.0–75.0	-0.12	-0.26–0.03	-0.12	-0.26–0.02
For CRP								
Non	1.15	1.06–1.24	1.13	1.04–1.22	ref	-	ref	-
Mi/Mo	1.04[a]	0.99–1.08	1.02[a]	0.97–1.06	0.00	-0.03–0.03	0.00	-0.03–0.03
Heavy	1.01[a]	0.96–1.06	0.99[a]	0.94–1.05	0.01	-0.02–0.04	0.01	-0.02–0.04

Abbreviations: EMM crude and adjust = estimated marginal mean of the variable in a crude model and an adjusted model, respectively; eSlope crude and adjust = slope of annual changes in the variable during the study period in a crude model and an adjusted model, respectively; 95% CI = 95% confidence interval; Heavy = heavy drinker; Mi/Mo = mild-moderate drinker; Non = non-drinker; ref = reference. The covariates for the adjustment were age, body mass index, mean blood pressure, heart rate, serum levels of LDL, HDL, TG, HbA1c, uric acid, and creatinine, and smoking status plus history of medication for hypertension, dyslipidemia, diabetes mellitus and/or hyperuricemia (for each medication, not receiving medication = 0, receiving medication = 1); a = $P < 0.05$ vs. non-drinker; b = $P < 0.05$ vs. mild-moderate drinker.

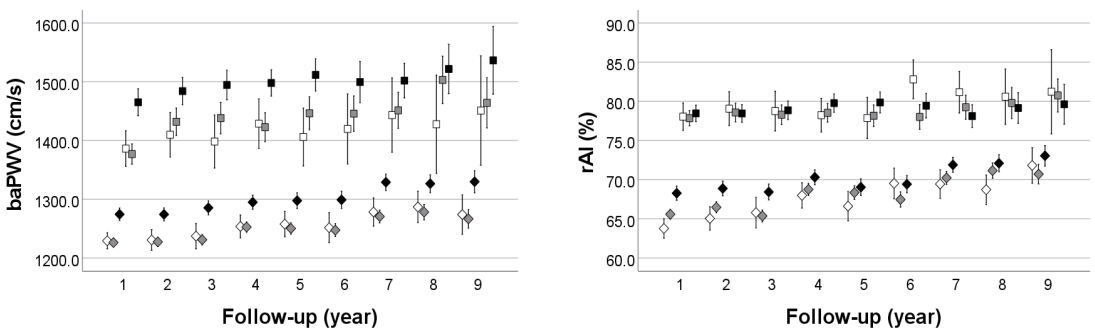

Fig. 3. Annual changes in the brachial-ankle pulse wave velocity values and radial augmentation index values among three drinking categories in subjects aged < 50 years and those aged ≥ 50 years

Abbreviations: square = subjects aged ≥ 50 years; rhombus = subjects aged < 50 years; open = non-drinker; gray = mild–moderate drinker; black = heavy drinker

annually measured values of baPWV, rAI, and serum CRP levels among the three drinking categories; **Fig. 2** also shows the number of available data points from the start to the end of the study period for three drinking categories in each year. The crude and adjusted values of the estimated marginal mean of the baPWV, rAI, and serum CRP levels and those values for the differences in the annual increases in the baPWV, rAI, and serum CRP levels during the study period among the three drinking categories in all the subjects are shown in **Table 2**. The estimated marginal mean of the baPWV and that of the rAI showed a significant stepped increase along with alcohol intake even after adjustment, while that for the serum CRP levels was lower in drinkers than in non-drinkers. After the adjustment, the estimated slope of the annual increase in baPWV was higher in heavy drinkers compared with non-drinkers.

Fig. 3 shows the means and error bars of the annually measured values of baPWV and rAI in subjects aged < 50 and ≥ 50 years. The crude and adjusted values of the estimated marginal mean of the baPWV and rAI and those values for the differences in the annual increases in baPWV and rAI among the

Table 3. Crude and adjusted values of the estimated marginal mean of the brachial-ankle pulse wave velocity and radial augmentation index and differences in the annual increases in the brachial-ankle pulse wave velocity and radial augmentation index during the study period among 3 drinking categories in subjects under 50 years of age and those over 50 years of age

	EMM crude	95% CI	EMM adjust	95% CI	eSlope crude	95% CI	eSlope adjust	95% CI
Age <50 years (n=2869)								
For baPWV								
Non	1251	1242–1260	1229	1220–1237	ref		ref	
Mi/Mo	1258[a]	1252–1263	1236[a]	1230–1241	1.02	-0.87–2.91	1.12	-0.65–2.90
Heavy	1279[ab]	1273–1285	1256[ab]	1250–1262	2.73[a]	0.73–4.74	2.84[a]	0.96–6.97
For rAI								
Non	66.7	66.0–67.4	69.1	68.3–69.9	ref		ref	
Mi/Mo	68.0[a]	67.5–68.4	70.3[a]	69.8–70.8	-0.03	-0.19–0.13	-0.05	-0.20–0.10
Heavy	69.5[ab]	69.0–70.0	71.7[ab]	71.1–72.2	-0.11	-0.28–0.07	-0.12	-0.28–0.04
For CRP								
Non	1.17	1.08–1.27	1.19	1.08–1.29	ref		ref	
Mi/Mo	1.04[a]	0.99–1.08	1.02[a]	0.97–1.07	0.01	-0.02–0.04	0.01	-0.02–0.04
Heavy	1.01[a]	0.95–1.07	0.99[a]	0.92–1.05	0.01	-0.03–0.05	0.01	-0.03-0.05
Age ≥ 50 years (n=1147)								
For baPWV								
Non	1434	1412–1457	1403	1381–1424	ref		ref	
Mi/Mo	1451	1437–1465	1420[a]	1407–1434	0.16	-4.32–4.94	2.01	-2.41–6.43
Heavy	1483[ab]	1469–1497	1447[ab]	1432–1461	-2.33	-7.22–2.55	0.55	-3.98–5.08
For rAI								
Non	78.4	77.2–79.5	80.4	79.2–81.5	ref		ref	
Mi/Mo	78.6	77.9–79.3	80.6	80.2–81.3	0.06	-0.21–0.32	0.07	-0.19–0.33
Heavy	79.2	78.5–79.9	81.2	80.5–81.8	-0.03	-0.30–0.24	-0.02	-0.29–0.24
For CRP								
Non	1.00	0.87–1.14	0.98	0.84–1.13	ref		ref	
Mi/Mo	1.00	0.92–1.07	1.01	0.92–1.09	-0.01	-0.06–0.05	-0.01	-0.06–0.05
Heavy	1.04	0.96–1.11	1.04	0.95–1.12	0.03	-0.02–0.09	0.03	-0.02–0.09

Abbreviations: Age <50 and >50 = subjects aged <50 and ≥ 50 years, respectively; other abbreviations and covariates for the adjustment are described in the footnote of Table 2; a = P<0.05 vs. non-drinker; b = P<0.05 vs. mild-moderate drinker.

three drinking categories in subjects aged <50 years and those aged ≥ 50 years are shown in Table 3. The estimated marginal mean of the baPWV and that of the rAI showed a significant stepped increase along with alcohol intake even after adjustment in subjects aged <50 years old. On the other hand, that of the baPWV, but not the rAI, showed a significant stepped increase along with alcohol intake even after adjustment in subjects aged ≥ 50 years old. Even after adjustment, the estimated slope of the annual increase in baPWV was significantly higher in subjects with heavy alcohol intake compared with that for non-drinkers, in subjects aged <50 years but not in those aged ≥ 50 years. However, the estimated slope of the annual change in rAI during the study period was not different between the heavy drinker and the non-drinker categories in both age-classified groups. The estimated marginal mean of the serum CRP levels was lower in drinkers than in non-drinkers, in subjects aged <50 years but not in those aged ≥ 50 years (Table 3).

Discussion

To the best of our knowledge, this study is the first to examine age-related differences in the association between the amount of alcohol intake and arterial stiffness, pressure wave reflection, and inflammation using a mixed model analysis conducted with repeated measurement data from a 9-year period. The longitudinal statuses of baPWV, rAI, and the serum CRP levels were assessed using the estimated marginal mean values during the study period, and the annual rates of change were assessed using the difference in the slopes of annual changes of variables during the study period among each of the drinking

categories in all the subjects. Then, these assessments were conducted in subjects aged <50 years and those aged ≥ 50 years since the rAI reportedly exhibits a phased increase with age until the age of 50 years, but not after 50 years[12, 13, 16].

Several cross-sectional studies as well as prospective studies have reported a significant association between heavy alcohol intake and increased arterial stiffness[6, 7, 18-20]. Nevertheless, whether heavy alcohol intake exacerbates the progression of arterial stiffening remains unclear. In this study, based on a comment in the JSH2019 [21], an alcohol intake of 1–19 g/day was defined as mild–moderate intake, while an intake of over 20 g/day was defined as heavy intake. Of note, alcohol intake affects conventional risk factors for CVD, and these risk factors act to increase arterial stiffness[3, 4]. To clarify the direct or indirect effect of alcohol intake on arterial stiffness, previous longitudinal studies assessed arterial stiffness at two points (baseline and at the end of the study period)[7, 18-20]; this strategy was even used in the O'Neill study, which examined the association of alcohol consumption trajectories with arterial stiffness[20]. Therefore, the influence of confounding factors on the association of alcohol intake with arterial stiffness was not fully excluded in these previous studies. This study analyzed repeated measurement data using mixed model analyses to reduce the influence of confounding variables[17]. In this study, the mild–moderate and heavy alcohol intake categories showed stepped increases in the estimated marginal mean of the baPWV during the study period; therefore, alcohol intake may longitudinally increase arterial stiffness in a dose-dependent manner. In addition, the slope of the annual change in baPWV, which may reflect the annual progression of baPWV, was higher in heavy drinkers than in non-drinkers, especially among those aged <50 years. Thus, heavy alcohol intake may exacerbate the progression of arterial stiffening. Because arterial stiffness reflects macro-vascular damage[22], heavy alcohol intake may exaggerate the progression of macro-vascular damage.

Age affects the progression of arterial stiffness[3, 4]. As shown in **Table 3**, the estimated marginal mean value of baPWV was 1279 in subjects aged <50 years and was 1483 those aged ≥ 50 years. Then, in the MML analysis, an age of ≥ 50 years affected the longitudinal association of alcohol intake with arterial stiffness (estimate=167, 95% confidence interval=152–183, $P<0.01$). Thus, aging may augment heavy alcohol intake-related increase of arterial stiffness. JSH 2019 recommends alcohol intake of less than 20–30 g of ethanol per day[21]. In this study, the estimated marginal mean of the baPWV was higher in subjects with an alcohol intake of over 30 g ethanol per day than in those with an alcohol intake of 20–30 g ethanol per day (data not shown). Thus, alcohol intake may longitudinally increase arterial stiffness in a dose-dependent manner.

The augmentation index is a marker of pressure wave reflection in the systemic arterial tree[3, 11-13, 22]; in addition to arterial stiffness (macro-vascular damage), other factors such as peripheral vascular damages (microvascular damage) are thought to contribute to abnormal pressure wave reflection[22]. Cross-sectional studies have already reported a significant association between heavy alcohol intake and abnormal pressure wave reflection[6, 23]. On the other hand, several studies have reported that the age-related increase in the augmentation index was blunted in subjects aged ≥ 50 years as a result of impedance mismatch[11-13, 24]. In this study, the mild–moderate and heavy alcohol intake categories showed stepped increases in the estimate marginal mean of the rAI during the study period in subjects aged <50 years, but not in those aged ≥ 50 years. However, the slope of the annual change in rAI during the study period was similar between the heavy drinker and the non-drinker categories in both age-classified groups. Based on these findings, alcohol intake may promote abnormalities in the pressure wave reflection with aging at an earlier stage in a dose-dependent manner, but they may not exacerbate this abnormality to further extent. In addition, when the baPWV, which reflects abnormality in large artery, was added as an additional covariate in the analyses, the stepped increases in the estimate marginal mean of the rAI were also significant. Thus, microvascular dysfunction might affect these changes at least in parts. As mentioned above, heavy alcohol intake may exacerbate the increase in arterial stiffness, and this exacerbation may shift the reflection point of the pressure wave to distal sites in the arterial tree, which may delay the pressure wave reflection as a result of impedance mismatch[24]. This might explain, at least in part, why heavy alcohol intake did not exacerbate the abnormalities of pressure wave reflection to a further extent.

Conflicting findings for the association between alcohol intake and inflammation have been reported[8, 25]. In the present study, the serum CRP levels were lower in drinkers than in non-drinkers, especially for subjects aged <50 years. The age-related dysregulation of interleukin-6 synthesis might affect this age-related difference in the association of alcohol intake and inflammation[26]. While the underlying mechanisms of this inverse association have not been clarified, one plausible mechanism might be that

ethanol downregulates interleukin-6 production[27]. Consistent with our previous report[10], in the present study, serum CRP levels had a significant longitudinal association with baPWV (data not shown). Taken together, inflammation might not have affected the increased arterial stiffness and abnormal pressure wave reflection related to alcohol intake in the participants of the present study. The precise mechanisms by which alcohol intake affects increased arterial stiffness and abnormal pressure wave reflection have yet to be clarified. Several factors other than conventional risk factors for CVD might affect arterial stiffness. Ethanol directly causes increased matrix protease activity[28], increased oxidative stress[29], the activation of NF-κ-β in vascular smooth muscle cells[30], and the activation of the sympathetic nervous system[31]; these factors affect increased arterial stiffness and abnormal pressure wave reflection.

Several studies have proposed a J-shaped relationship between alcohol intake and cardiovascular outcomes[1, 2]. Based on the findings of the present study, mild–moderate alcohol intake may provide a beneficial effect on cardiovascular outcomes via the attenuation of inflammatory activity, despite the mild exacerbation of increased arterial stiffness and abnormal pressure wave reflection. On the other hand, heavy alcohol intake may–have harmful effects on cardiovascular outcomes via the exacerbation of increased arterial stiffness and abnormal pressure wave reflection, despite the attenuation of inflammatory activity.

Study Limitations

This study had several limitations. 1) While the self-reporting of alcohol intake by participants has actually been validated in a previous study[32], this method may have led to some underestimation of the daily alcohol consumption of subjects. 2) The types of alcoholic beverages consumed by the subjects were not analyzed. 3) Habitual exercise and salt-intake were not assessed in the present study. Both of these factors are related to alcohol intake and also affect arterial stiffness and pressure wave reflection[33]. 4) The present study was conducted in middle-aged Japanese employees, and gender= and ethnic differences remain to be clarified.

Conclusion

In middle-aged male Japanese employees, alcohol intake may attenuate inflammatory activity. While alcohol intake may exacerbate the progression of arterial stiffening in a dose-dependent manner without mediating inflammation, especially in subjects under 50 years of age, it may promote pressure wave reflection abnormalities with aging at earlier ages without further exacerbation at older ages.

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Disclosures

Tomiyama Hirofumi: The sponsor (Omron Health Care Company) assisted in the data formatting (i.e., the brachial-ankle pulse wave velocity data stored in the hard disc of the equipment used to measure the brachial-ankle pulse wave velocity was transferred to an Excel sheet); however, the company played no other role in the design or conduct of the study, that is, in the data collection, management, analysis or interpretation of the data, or in the preparation, review, or approval of the manuscript. The remaining authors have no disclosures to make.

References

1) Holmes MV, Dale CE, Zuccolo L, Silverwood RJ, Guo Y, Ye Z, Prieto-Merino D, Dehghan A, Trompet S, et al. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ, 2014; 349: g4164
2) Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, de Gaetano G. Alcohol consumption and mortality in patients with cardiovascular disease: a meta-analysis. J Am Coll Cardiol, 2010; 55: 1339-1347
3) Tomiyama H, Yamashina A. Non-invasive vascular function tests: their pathophysiological background and clinical application. Circ J, 2010; 74: 24-33
4) Wilkinson IB, Mäki-Petäjä KM, Mitchell GF. Uses of Arterial Stiffness in Clinical Practice. Arterioscler Thromb Vasc Biol, 2020; 40: 1063-1067
5) Libby P, Loscalzo J, Ridker PM, Farkouh ME, Hsue PY, Fuster V, Hasan AA, Amar S. Inflammation, Immunity, and Infection in Atherothrombosis: JACC Review Topic of the Week. J Am Coll Cardiol, 2018; 72: 2071-2081
6) Sierksma A, Lebrun CE, van der Schouw YT, Grobbee DE, Lamberts SW, Hendriks HF, Bots ML. Alcohol consumption in relation to aortic stiffness and aortic wave reflections: a cross-sectional study in healthy postmenopausal women. Arterioscler Thromb Vasc Biol, 2004; 24: 342-348
7) Matsumoto C, Tomiyama H, Yamada J, Yoshida M, Shiina K, Nagata M, Yamashina A. Association of blood pressure levels with the effects of alcohol intake on the

vasculature in Japanese men. Hypertens Res, 2009; 32: 127-132
8) Imhof A, Froehlich M, Brenner H, Boeing H, Pepys MB, Koenig W. Ushape: Effect of alcohol consumption on systemic markers of inflammation. Lancet, 2001; 357: 763-767
9) Minzer S, Losno RA, Casas R. The Effect of Alcohol on Cardiovascular Risk Factors: Is There New Information? Nutrients, 2020; 12: 912
10) Tomiyama H, Shiina K, Vlachopoulos C, Iwasaki Y, Matsumoto C, Kimura K, Fujii M, Chikamori T, Yamashina A. Involvement of Arterial Stiffness and Inflammation in Hyperuricemia-Related Development of Hypertension. Hypertension, 2018; 72: 739-745
11) Tomiyama H, Yamazaki M, Sagawa Y, Teraoka K, Shirota T, Miyawaki Y, Yamashina A. Synergistic effect of smoking and blood pressure on augmentation index in men, but not in women. Hypertens Res, 2009; 32: 122-126
12) McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR; ACCT Investigators. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). J Am Coll Cardiol, 2005; 46: 1753-1760
13) Odaira M, Tomiyama H, Hashimoto H, Kojima I, Matsumoto C, Yoshida M, Shiina K, Nagata M, Miyawaki Y, Yamashina A. Increased arterial stiffness weakens the relationship between wave reflection and the central pressure indexes in men younger than 60 years of age. Am J Hypertens, 2011; 24: 881-886
14) Yang J, Zaitlen NA, Goddard ME, Visscher PM, Price AL. Advantages and pitfalls in the application of mixed-model association methods. Nat Genet, 2014; 46: 100-106
15) Fujii M, Tomiyama H, Nakano H, Iwasaki Y, Matsumoto C, Shiina K, Yamashina A, Chikamori T. Differences in longitudinal associations of cardiovascular risk factors with arterial stiffness and pressure wave reflection in middle-aged Japanese men. Hypertens Res, 2021; 44: 98-106
16) Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, Koji Y, Hori S, Yamamoto Y. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res, 2002; 25: 359-364
17) Raffield LM, Howard AG, Graff M, Lin DY, Cheng S, Demerath E, Ndumele C, Palta P, Rebholz CM, Seidelmann S, Yu B, Gordon-Larsen P, North KE, Avery CL. Obesity Duration, Severity, and Distribution Trajectories and Cardiovascular Disease Risk in the Atherosclerosis Risk in Communities Study. J Am Heart Assoc, 2021; 10: e019946
18) Hwang CL, Piano MR, Thur LA, Peters TA, da Silva ALG, Phillips SA. The effects of repeated binge drinking on arterial stiffness and urinary norepinephrine levels in young adults. J Hypertens, 2020; 38: 111-117
19) El Khoudary SR, Barinas-Mitchell E, White J, Sutton-Tyrrell K, Kuller LH, Curb JD, Shin C, Ueshima H, Masaki K, Evans RW, Miura K, Edmundowicz D, Sekikawa A; ERA JUMP Study Group. Adiponectin, systolic blood pressure, and alcohol consumption are associated with more aortic stiffness progression among apparently healthy men. Atherosclerosis, 2012; 225: 475-480
20) O'Neill D, Britton A, Brunner EJ, Bell S. Twenty-Five-Year Alcohol Consumption Trajectories and Their Association With Arterial Aging: A Prospective Cohort Study. J Am Heart Assoc, 2017; 6: e005288
21) Umemura S, Arima H, Arima S, Asayama K, Dohi Y, Hirooka Y, Horio T, Hoshide S, Ikeda S, Ishimitsu T, Ito M, Ito S, Iwashima Y, Kai H, Kamide K, Kanno Y, Kashihara N, Kawano Y, Kikuchi T, Kitamura K, Kitazono T, Kohara K, Kudo M, Kumagai H, Matsumura K, Matsuura H, Miura K, Mukoyama M, Nakamura S, Ohkubo T, Ohya Y, Okura T, Rakugi H, Saitoh S, Shibata H, Shimosawa T, Suzuki H, Takahashi S, Tamura K, Tomiyama H, Tsuchihashi T, Ueda S, Uehara Y, Urata H, Hirawa N.. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2019). Hypertens Res, 2019; 42: 1235-1481
22) Laurent S, Boutouyrie P. The structural factor of hypertension: large and small artery alterations. Circ Res, 2015; 116: 1007-1021
23) van Trijp MJ, Beulens JW, Bos WJ, Uiterwaal CS, Grobbee DE, Hendriks HF, Bots ML. Alcohol consumption and augmentation index in healthy young men: the ARYA study. Am J Hypertens, 2005; 18: 792-796
24) Sugawara J, Hayashi K, Tanaka H. Distal shift of arterial pressure wave reflection sites with aging. Hypertension, 2010; 56: 920-925
25) EIakunchykova O, Averina M, Kudryavtsev AV, Wilsgaard T, Soloviev A, Schirmer H, Cook S, Leon DA. Evidence for a Direct Harmful Effect of Alcohol on Myocardial Health: A Large Cross-Sectional Study of Consumption Patterns and Cardiovascular Disease Risk Biomarkers From Northwest Russia, 2015 to 2017. J Am Heart Assoc, 2020; 9: e014491
26) Young DG, Skibinski G, Mason JI, James K. The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGF-beta1) levels in normal healthy blood donors. Clin Exp Immunol, 1999; 117: 476-481
27) McCarty MF. Interleukin-6 as a central mediator of cardiovascular risk associated with chronic inflammation, smoking, diabetes, and visceral obesity: down-regulation with essential fatty acids, ethanol and pentoxifylline. Med Hypotheses, 1999; 52: 465-477
28) Partridge CR, Sampson HW, Forough R. Long-term alcohol consumption increases matrix metalloproteinase-2 activity in rat aorta. Life Sci, 1999; 65: 1395-1402
29) Kessova IG, Ho YS, Thung S, Cederbaum AI. Alcohol-induced liver injury in mice lacking Cu, Zn-superoxide dismutase. Hepatology, 2003; 38: 1136-1145
30) Altura BM, Gebrewold A, Zhang A, Altura BT. Ethanol induces rapid lipid peroxidation and activation of nuclear factor-kappa β in cerebral vascular smooth muscle: relation to alcohol-induced brain injury in rats. Neurosci Lett, 2002; 325: 95-98
31) Kawano Y, Abe H, Kojima S, Takishita S, Omae T. Effects of propranolol on cardiovascular and neurohumoral

actions of alcohol in hypertensive patients. Blood Press, 1999; 8: 37-42
32) Giovannucci E, Colditz G, Stampfer MJ, Rimm EB, Litin L, Sampson L, Willett WC. Assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol, 1991; 133: 810-817
33) Vamvakis A, Gkaliagkousi E, Lazaridis A, Grammatikopoulou MG, Triantafyllou A, Nikolaidou B, Koletsos N, Anyfanti P, Tzimos C, Zebekakis P, Douma S. Impact of Intensive Lifestyle Treatment (Diet Plus Exercise) on Endothelial and Vascular Function, Arterial Stiffness and Blood Pressure in Stage 1 Hypertension: Results of the HINTreat Randomized Controlled Trial. Nutrients, 2020; 12: 1326

Supplementary Table 1. Clinical characteristics of among 3 drinking categories in all the study subjects at the baseline of study

Parameter	Non	Mi/Mo	Heavy
Number of subjects	594	2210	1212
Age, years	42.9 ± 9.7	41.9 ± 9.3[a]	45.7 ± 9.2[ab]
BMI, kg/m^2	24.1 ± 3.6	23.9 ± 3.1	24.1 ± 2.9
Smoking (current), *n* (%)	165 (27.8)	621 (28.1)	483 (39.9)[ab]
Systolic BP, mmHg	122.0 ± 15.3	122.3 ± 13.9	129.1 ± 16.1[ab]
Diastolic BP, mmHg	74.3 ± 11.6	74.9 ± 11.2	80.0 ± 11.5[ab]
Mean BP, mmHg	90.3 ± 11.4	90.4 ± 10.9	94.0 ± 11.2[ab]
Heart rate, beats/min	65.1 ± 9.9	64.1 ± 9.2[a]	66.1 ± 9.7[b]
baPWV, cm/s	1273 ± 176	1262 ± 165[a]	1347 ± 214[ab]
AI, %	67.8 ± 13.9	68.5 ± 13.2	72.2 ± 13.0[ab]
HDL, mg/dL	57.4 ± 13.9	61.0 ± 14.6[a]	66.7 ± 17.3[ab]
LDL, mg/dL	129.8 ± 30.8	126.8 ± 30.6[a]	121.6 ± 29.9[ab]
TG, mg/dL	119.4 ± 78.5	120.4 ± 80.4	137.2 ± 102.4[ab]
UA, mg/dL	6.0 ± 1.2	6.2 ± 1.2[a]	6.4 ± 1.3[ab]
HbA1c, %	5.4 ± 0.7	5.2 ± 0.5[a]	5.3 ± 0.6[a]
Creatinine, mg/dL	0.86 ± 0.12	0.86 ± 0.11	0.83 ± 0.11[ab]
CRP (mg/L)	12.3 ± 20.0	10.1 ± 15.9[a]	10.2 ± 15.3[a]
Medications			
Receiving medication for hypertension, *n* (%)	43 (7.2)	131 (5.9)	130 (10.7)[ab]
Receiving medication for diabetes mellitus, *n* (%)	24 (4.0)	39 (1.8)[a]	33 (2.7)
Receiving medication for dyslipidemia, *n* (%)	24 (4.0)	51 (2.3)[a]	32 (2.6)
Receiving medication for hyperuricemia, *n* (%)	23 (4.0)	79 (4.0)	84 (7.0)[ab]

Abbreviations: BMI = body mass index; Smoking = number of current smokers;
BP = blood pressure; baPWV = brachial-ankle pulse wave velocity; AI = augmentation index; HDL = serum high-density lipoprotein cholesterol; LDL = serum low-density lipoprotein cholesterol; TG = serum triglyceride; UA = serum uric acid; HbA1c = glycosylated hemoglobin A1c; Creatinine = serum creatinine; CRP = serum C-reactive protein levels; Medications, number and percentage of subjects receiving medications; MEDhbp = medication for hypertension; MEDdm = medication for diabetes mellitus, MEDlipid = medication for dyslipidemia; MEDhu = medication for hyperuricemia; a = $P < 0.05$ vs. Non; b = $P < 0.05$ vs. Mi/Mo