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ORIGINAL ARTICLE

Effects of alcohol intake on ambulatory blood pressure, heart rate, and heart rate variability in Japanese men with different ALDH2 genotypes

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The effects of alcohol intake on haemodynamics and heart rate variability were investigated with relation to genotypes of aldehyde dehydrogenase 2 (ALDH2), which were determined in 33 male Japanese volunteers (mean ± s.e., 35.7 ± 1.4 years) using the PCR-RFLP method. On the alcohol intake day, they consumed 660 ml of beer containing 33 ml of ethanol (0.3–0.5 g/kg of body weight) from 18.00 to 18.30. On the control day, they ingested the same amount of non-alcoholic beer. Ambulatory blood pressure, heart rate, and ECG R-R intervals were measured during a 24-h period with a portable recorder. A power spectral analysis of R-R intervals was performed to obtain the low-frequency (LF) and high-frequency (HF) components. Sixteen subjects were homozygotes for the normal ALDH gene (active ALDH2), only one was a homozygote for the mutant ALDH2 gene (inactive ALDH2), and the remaining 16 were heterozygotes (inactive ALDH2). Alcohol intake did not change 24-h average blood pressure (BP) either in the active ALDH2 group or in the inactive ALDH2 group. However, during the time interval from 18.30 to 0.00, alcohol intake significantly decreased diastolic BP in the active ALDH2 group and both systolic and diastolic BPs in the inactive ALDH2 group. In the active ALDH2 group, alcohol intake did not change heart rate, while in the inactive ALDH2 group, alcohol intake significantly increased 24-h average heart rate by 5.3 ± 1.6 beats per minute ($P < 0.01$). In the active ALDH2 group, neither the LF nor the HF component was changed by alcohol intake, while in the inactive ALDH2 group, both the LF and the HF components were significantly decreased during the time interval from 18.30 to 0.00. These results demonstrate for the first time that ALDH2 genotypes modify the effects of intake of a small amount of alcohol on haemodynamics and heart rate variability in Japanese men.
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Keywords: alcohol; ALDH2 genotypes; ambulatory blood pressure; heart rate; heart rate variability; Japanese

Introduction

Inconsistent results have been shown regarding the effect of acute alcohol intake on blood pressure (BP).[1] Some investigators have reported a rise, others have found no change, and some have reported a fall. The disparity in the results can be in part explained by variations in the amount and type of alcohol ingested and the timing and method of BP measurement. In addition, racial background has been suggested to play a role in the acute BP response to alcohol intake.[2,3]

Aldehyde dehydrogenase (ALDH), the second enzyme on the ethanol metabolic pathway, converts acetaldehyde to acetic acid and consists mainly of two isozymes (ALDH1 and ALDH2).[4] Because acetaldehyde is highly active and toxic, increased blood acetaldehyde levels are responsible for the acute poisoning effects of alcohol such as flushing, headache, nausea, and even shock and chronic damage to many organs, particularly to the liver. At very low concentrations of aldehyde, ALDH with a low Km value (ALDH2) acts more efficiently than ALDH1. Deficiencies in enzyme activity have been recognised as genetic variations of ALDH in about 50% of individuals in Mongoloid populations including Japanese, resulting from the contribution of the inactive ALDH2*2 subunit to the isozymes.[5] The other

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allele, ALDH2*1, is monomorphic in Caucasians and Negroids and is prevalent in Mongoloids, conferring normal activity to isozymes. Two alternative codons, GAA and AAA, at amino acid position 487 in exon 12, are responsible for the allelic difference at the ALDH2 locus, coding for Glu for ALDH2*1 and Lys for ALDH2*2.

It is of interest how the effects of alcohol intake on haemodynamics are modified by ALDH2 genotypes. So far, however, few investigations have been done regarding this issue. We had the opportunity to investigate for the first time the effects of intake of a small amount of alcohol on ambulatory BP, heart rate (HR) and HR variability with relation to ALDH2 genotypes in Japanese men.

Methods

Subjects

A total of 33 Japanese male volunteers were enrolled (mean ± s.e., 35.7 ± 1.4 years) in the present study. All subjects had a history of alcohol intake. Twenty-one subjects were habitual drinkers, while the remaining 12 ingested alcoholic beverages socially. No subjects were receiving pharmacological therapy including antihypertensive medication. They all agreed to participate in the study after receiving a detailed explanation of its nature and purpose, and each subject gave written informed consent. The study protocol was approved by the Institutional Review Board of Dokkyo University School of Medicine.

Study protocol

On the alcohol intake day, each subject ingested 660 ml of beer (Heineken Lager Beer, Heineken Brouwerijen B.V., Amsterdam, Holland) containing 33 ml of ethanol (0.3–0.5 g/kg of body weight) from 18.00 to 18.30 in a quiet hospital room. On the control day, they consumed 660 ml of non-alcoholic beer (Buckler, Heineken Brouwerijen B.V.) containing 3 ml of ethanol from 18.00 to 18.30 in the same room. Forty-eight hour abstinence of alcohol drinking was required before the tests. They were seated in a chair during drinking. They ingested alcoholic or non-alcoholic beer without knowledge of exact ethanol content. After ingestion, they stayed in the room for about 1 h and then went home. They were instructed to eat an evening meal at home and abstain from alcoholic beverages until the next afternoon. They were also instructed to maintain their usual daily activities (except for exercise). The 2 days were separated by at least 7 days. The order of the 2 days was randomised.

ALDH2 genotyping[5]

Venous blood samples were obtained from each participant during the study protocol. Ten millilitres was used for the isolation of leukocyte DNA. Genomic DNA samples were recovered from buffy coat by SDS-proteinase K treatment followed by phenol-chloroform extraction and ethanol precipitation. The sequences of the two primers, one of which has an artificial single base mismatch introducing a Mbo II site into the ALDH2*1 sequence by polymerase chain reaction (PCR), were the same as those reported previously.[6] Amplification was performed under the conditions in the report with minor alterations as follows: reaction in a 50 μl scale; denaturation at 93°C for 90 sec; annealing at 58°C for 180 sec. Ten microlitres of crude PCR products was digested with the restriction enzyme Mbo II for 1 h, and just before electrophoresis, was incubated at 60°C for 5 min followed by chilling in ice water. The digests were separated in 7% polyacrylamide gels under 10 V/cm for 1 h. The gels were stained with ethidium bromide and the DNA bands were visualised under UV light.

Ambulatory monitoring and power spectral analysis of R-R Intervals[7,8]

Ambulatory BP and HR were monitored every 30 min by a cuff-oscillometric device, TM-2425 (A&D, Tokyo, Japan) on the alcohol intake and control days. The devices satisfied the criteria of the Association for the Advancement of Medical Instrumentation (AAMI) and the British Hypertension Society (BHS).[9] To minimise the effects of physical activities on data, the ambulatory monitoring was performed on the same day of the week. The monitoring was begun at 16.00 and ended at 18.00 the next afternoon. The same recorder was used for each subject to avoid having different BP readings obtained by different recorders. According to the BP circadian pattern, BP and HR were averaged for three time intervals: 18.30 to 0.00, 0.30 to 8.00, and 8.30 to 16.00.

The ambulatory BP recorder used in this study, the TM-2425, also monitored the R-R interval of the electrocardiogram. The procedures of the power spectral analysis of R-R intervals in this device were previously reported in detail by us.[7,8] Spectral R-R variability was computed as the low-frequency (LF) component (0.05–0.15 Hz) and the high-frequency (HF) component (0.15–0.40 Hz), using the autoregressive model from every 5-min block over a 24-h period. The LF component reflects both sympathetic and parasympathetic nerve activity, and the HF component reflects parasympathetic activity exclusively.

Statistical analysis

Values are expressed as means ± s.e. Comparisons of baseline characteristics of the two groups were made using Student's unpaired *t*-test and the χ^2 test as appropriate. Comparisons of haemodynamic and power spectral data between the two groups and

between the two days were analysed by two-way repeated measures ANOVA. Newman-Keuls tests were used as determined by the ANOVA results. For the comparisons of power spectral data, the naturally logarithmic values, ie ln (the LF component) and ln (the HF component) were used to normalise the skewness of the data. Statistical significance was accepted at the level of $P < 0.05$.

Results

In the present study, 16 subjects were determined to be normal homozygotes for ALDH2*1, 16 subjects as heterozygotes for ALDH2*2, and only one subject as a mutant homozygote for ALDH2*2. The gene frequency of ALDH2*1 to ALDH2*2 allele was 0.73/0.27, which was similar to those reported previously in Japanese populations.[5,10] We defined homozygotes for ALDH2*1 as active ALDH2 ($n = 16$), while both heterozygotes and a homozygote for ALDH2*2 together were defined as inactive ALDH2 ($n = 17$).

Baseline characteristics of the active and inactive ALDH2 groups

Table 1 shows the baseline characteristics of the active and inactive ALDH2 groups. Age, height, body weight, body mass index (BMI), systolic and diastolic BPs, and pulse rate did not differ significantly between the two groups. Although no subjects had a history of antihypertensive treatment, 15 subjects had a clinic systolic BP ⩾140 mm Hg or a diastolic BP ⩾90 mm Hg, or both. Daily ethanol intake was insignificantly higher in the active ALDH2 group than in the inactive ALDH2 group ($P = 0.06$), while one of its biochemical markers, serum γ-glutamyl transpeptidase (γ-GTP) did not differ significantly between the two groups. There were more current smokers in the active ALDH2 group than in the inactive ALDH2 group ($P < 0.05$).

Table 1 Baseline characteristics of the active and inactive ALDH2 groups

	Active ALDH2 (n = 16)	*Inactive ALDH2 (n = 17)*
Age (years)	33.6 ± 1.2	37.6 ± 2.4
Height (cm)	173.0 ± 1.1	171.9 ± 1.4
Body weight (kg)	71.3 ± 2.5	75.0 ± 2.3
Body mass index (kg/m²)	23.8 ± 0.8	25.5 ± 1.0
Systolic BP (mm Hg)	139.1 ± 3.4	133.6 ± 3.8
Diastolic BP (mm Hg)	84.3 ± 2.7	83.5 ± 2.1
Pulse rate (beats/min)	82.9 ± 2.9	76.0 ± 3.2
Serum γ-GTP (IU/l)	52.6 ± 6.8	45.9 ± 8.0
Daily ethanol intake (ml/day)	39.1 ± 7.1	23.1 ± 5.8*
Current smokers (*n*)	14/16	8/17†

BP = blood pressure; GTP = glutamyl transpeptidase. Values are mean ± s.e. $0.05 < {}^*P < 0.1$, ${}^\dagger P \pm 0.05$, *vs* the active ALDH2 group.

Effect of alcohol intake on ambulatory BP and HR in the active and inactive ALDH2 groups

Circadian patterns of BP and HR for each day are shown in Figure 1 for the active ALDH2 group and in Figure 2 for the inactive ALDH2 group, respectively.

As shown in Table 2, on the control day, BP levels of 24-h and each time interval were similar between the active ALDH2 and inactive ALDH2 groups. Alcohol intake did not change 24-h average BP either in the active ALDH2 group or in the inactive ALDH2 group. However, during the time interval from 18.30 to 0.00, alcohol intake significantly decreased diastolic BP by 5.7 ± 1.3 mm Hg

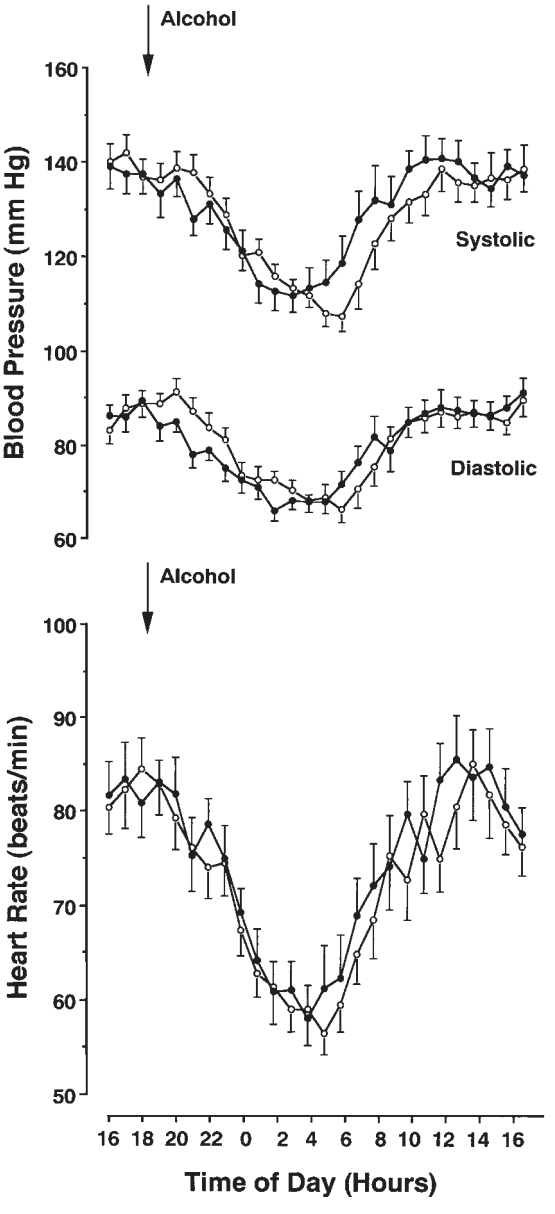

Figure 1 Circadian patterns of systolic and diastolic blood pressures (upper panel) and heart rate (lower panel) for alcohol intake day (●) and control day (○) in the active ALDH2 group. Values are mean ± s.e.

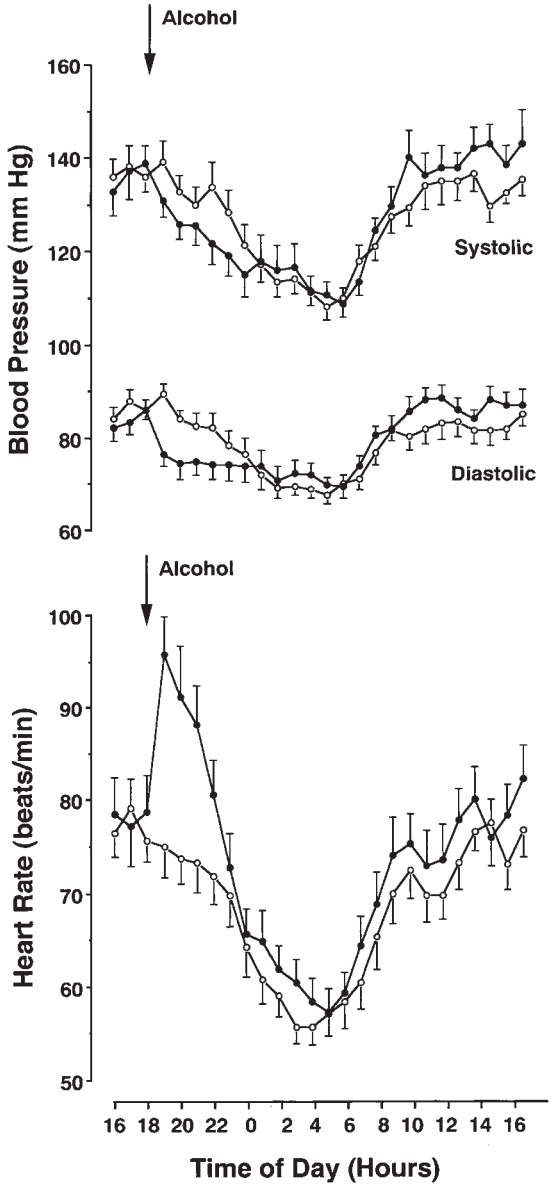

Figure 2 Circadian patterns of systolic and diastolic blood pressures (upper panel) and heart rate (lower panel) for alcohol intake day (●) and control day (○) in the inactive ALDH2 group. Values are mean ± s.e.

($P < 0.05$) in the active ALDH2 group and both systolic and diastolic BPs by 7.2 ± 2.5 mm Hg systole ($P < 0.05$) and by 7.7 ± 1.9 mm Hg diastole ($P < 0.001$) in the inactive ALDH2 group. During the time intervals from 0.30 to 8.00 and from 8.30 to 16.00, BP did not differ significantly between the alcohol intake and control days either in the active ALDH2 group or in the inactive ALDH2 group.

As shown in Table 2, on the control day, 24-h average HR was significantly lower in the inactive ALDH2 group than in the active ALDH2 group ($P < 0.01$). In the active ALDH2 group, alcohol intake did not change HR during a 24-h period, while in the inactive ALDH2 group, alcohol intake significantly increased HR by 5.3 ± 1.6 beats per minute (bpm) during a 24-h period ($P < 0.01$) and by 11.5 ± 2.8 bpm during the time interval from 18.30 to 0.00 ($P < 0.001$).

Effect of alcohol intake on power spectral components of HR variability in the active and inactive ALDH2 groups

Table 3 lists the average values of the LF and the HF components for the 24-h period and the three time intervals on the alcohol intake and control days. On the control day, the LF component was significantly higher in the inactive ALDH2 group than in the active ALDH2 group during a 24-h period ($P < 0.01$), the time interval from 0.30 to 8.00 ($P < 0.001$), and the time interval from 8.30 to 16.00 ($P < 0.01$). Alcohol intake did not change the LF or the HF component in the active ALDH2 group, whereas alcohol intake decreased the LF component significantly during a 24-h period ($P < 0.05$) and the time interval from 18.30 to 0.00 ($P < 0.001$) and the HF component during the time interval from 18.30 to 0.00 ($P < 0.001$).

Discussion

Our results demonstrate for the first time that ALDH2 genotypes modify the effects of intake of a small amount of alcohol on ambulatory BP, HR, and HR variability in Japanese men. Alcohol intake significantly decreased only diastolic BP in the active ALDH2 group during about 6 h after drinking, while it significantly decreased both systolic and diastolic BPs in the inactive ALDH2 group during the same time interval. Regarding HR and HR variability responses to alcohol intake, more prominent differences were observed. In the active ALDH2 group, alcohol intake did not change HR during a 24-h period, whereas in the inactive ALDH2 group, alcohol intake significantly increased 24-h average HR and a marked HR increase was observed during several hours after drinking. In the active ALDH2 group, neither the LF nor the HF component was changed by alcohol intake, whereas in the inactive ALDH2 group, both the LF and HF components were significantly decreased during about 6 h after drinking and a decrease in the LF component was significant even for the 24-h average value.

Earlier studies have provided inconsistent results about the effects of acute alcohol intake on BP, especially from the point of view of office BP measurement.[1] Twenty-four hour ambulatory BP monitoring is now widely used in various clinical researches.[7,8] Using ambulatory BP monitoring, Kawano *et al*[11] reported that a single moderate dose of alcohol (0.8 g/kg) acted to lower BP in 16 Japanese male patients with essential hypertension who were habitual drinkers, and the significant depressor effect of alcohol lasted for up to 8 h after drinking. Rosito *et al*[12] also showed that alcohol intake elicited a biphasic response on BP, causing at first, vaso-

Table 2 Ambulatory blood pressure (BP) and heart rate of the alcohol intake and control days in the active and inactive ALDH2 groups

	Active ALDH2		*Inactive ALDH2*	
	Control	*Alcohol*	*Control*	*Alcohol*
Systolic BP (mm Hg)				
24-hour	126.4 ± 2.7	127.9 ± 3.1	124.9 ± 2.8	125.6 ± 3.2
18.30 to 0.00	133.2 ± 3.0	129.2 ± 3.4	131.0 ± 3.7	123.9 ± 3.5†
0.30 to 8.00	112.7 ± 2.4	116.2 ± 4.1	112.6 ± 2.5	112.8 ± 3.4
8.30 to 16.00	132.8 ± 3.6	136.1 ± 3.7	130.6 ± 3.3	136.0 ± 3.7
Diastolic BP (mm Hg)				
24-hour	79.3 ± 1.9	78.5 ± 2.0	77.2 ± 1.9	77.6 ± 1.8
18.30 to 0.00	84.9 ± 2.2	79.2 ± 2.1†	82.0 ± 2.2	74.3 ± 2.6§
0.30 to 8.00	68.9 ± 2.1	69.4 ± 2.0	69.0 ± 1.7	71.1 ± 2.0
8.30 to 16.00	83.7 ± 2.2	84.4 ± 2.8	80.5 ± 2.5	84.4 ± 2.2
Heart Rate, bpm				
24-hour	71.7 ± 2.6	73.4 ± 2.8	67.5 ± 1.8*	72.8 ± 2.5‡
18.30 to 0.00	76.6 ± 2.7	78.1 ± 2.9	72.0 ± 2.7	83.5 ± 3.6§
0.30 to 8.00	60.6 ± 2.1	62.5 ± 3.2	58.1 ± 2.0	61.2 ± 2.2
8.30 to 16.00	77.5 ± 3.4	79.8 ± 3.7	71.6 ± 2.0	74.7 ± 2.7

Values are mean ± s.e. *$P < 0.01$, *vs* the control day in the active ALDH2 group. $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$, $^{\S}P < 0.001$ *vs* the control day in the same group.

Table 3 Power spectral components of heart rate variability of the alcohol intake and control days in the active and inactive ALDH2 groups

	Active ALDH2		*Inactive ALDH2*	
	Control	*Alcohol*	*Control*	*Alcohol*
LF, ln (msec^2)				
24-hour	5.23 ± 0.20	5.16 ± 0.19	5.66 ± 0.14*	5.40 ± 0.17‡
18.30 to 0.00	5.04 ± 0.19	4.89 ± 0.24	5.37 ± 0.18	4.55 ± 0.18§
0.30 to 8.00	5.32 ± 0.24	5.30 ± 0.20	5.86 ± 0.17	5.85 ± 0.20
8.30 to 16.00	5.27 ± 0.24	5.20 ± 0.20	5.72 ± 0.15*	5.58 ± 0.18
HF, ln (msec2)				
24-hour	4.71 ± 0.18	4.53 ± 0.21	4.81 ± 0.26	4.55 ± 0.23
18.30 to 0.00	4.24 ± 0.20	4.01 ± 0.21	4.23 ± 0.28	3.58 ± 0.24§
0.30 to 8.00	5.49 ± 0.22	5.38 ± 0.24	5.57 ± 0.29	5.47 ± 0.31
8.30 to 16.00	4.40 ± 0.20	4.18 ± 0.24	4.62 ± 0.30	4.46 ± 0.22

LF = low-frequency component; HF = high-frequency component. Values are mean ± s.e. *$P < 0.01$, $^{\dagger}P < 0.001$, *vs* the control day in the active ALDH2 group. $^{\ddagger}P < 0.05$, $^{\S}P < 0.001$ *vs* the control day in the inactive ALDH2 group.

dilatation, and afterwards a pressor effect in 40 non-Japanese male medical students by the use of ambulatory BP monitoring. In the present study, a depressor response to alcohol intake was observed as well, although the response was less apparent in the active ALDH2 group than in the inactive ALDH2 group.

So far, only a few studies have been done to compare haemodynamic responses between subjects with and without flushing after alcohol intake. Kupari *et al*[3] compared the BP responses with low doses of acute alcohol intake (0.5 g/kg) between 10 Finnish and nine Japanese subjects who were normotensive. In five of the Japanese subjects, post-drinking facial flush was associated with marked BP reduction and tachycardia. Although the other four Japanese and Finnish subjects without facial flush showed fewer haemodynamic changes, alterations were similar in direction. The findings of the present study were almost in line with the earlier observations. To the best of our knowledge, the present study is the first to investigate the effects of intake of alcohol on haemodynamic parameters by the use of ambulatory monitoring with relation to ALDH2 genotypes.

Regarding mechanisms involved in different haemodynamic changes, specifically during several hours after drinking, it has been suggested that blood acetaldehyde plays a major role. Acetaldehyde is known to dilate peripheral blood vessels.[13] It is suggested that acetaldehyde-mediated angiotensin-converting enzyme (ACE) inhibition may play a contributory role in the development of vasodilatation and facial flush reaction consequent to alcohol

intake.[14] In Kuparis' study,[3] acetaldehyde concentration of blood was elevated after alcohol intake in five Japanese subjects with facial flushing, while such a change was not significant in the remaining four Japanese and Finnish subjects. Moreover, Hatake *et al*[15] reported that after intake of a small amount of alcohol (0.4 g/kg), a deficient ALDH group without a low Km isozyme of ALDH for acetaldehyde showed high levels of blood acetaldehyde with various cardiovascular symptoms such as facial flushing and tachycardia, while a normal ALDH group with a low Km isozyme of ALDH for acetaldehyde did not manifest these changes. In their study, there were no significant differences in alcohol levels at any of the measurement points between the two groups, indicating that not alcohol itself but higher blood acetaldehyde levels after alcohol intake contribute to production of cardiovascular symptoms in a deficient ALDH group. In the present study, we did not measure blood acetaldehyde levels. However, in the previous study, we reported that blood acetaldehyde levels scarcely increased in the subjects homozygous for ALDH2*1 after intake of a small amount of alcohol (0.4 g/kg), while the acetaldehyde levels in the subjects with the ALDH2*1/*2 heterozygote increased on average to 23.4 μM, and those in the subjects with the ALDH2*2 homotygote increased to 79.3 μM on average.[16] Taken together, it is thought that differences in blood acetaldehyde levels were intimately related to different haemodynamic responses after alcohol intake between the active ALDH2 and inactive ALDH2 groups.

In accordance with different BP and HR responses to alcohol intake between the active ALDH2 and inactive ALDH2 groups, responses of power spectral components of HR variability also differed in some respects. In the active ALDH2 group, neither the LF nor the HF component was changed significantly by alcohol intake, whereas in the inactive ALDH2 group, both the LF and HF components were significantly decreased during about 6 h after drinking and a decrease in the LF component was significant even for the 24-h average value. In the present study, significant relationships were not found between changes in power spectral data and changes in haemodynamic data. So far, a number of investigators have reported an association between reduced HR variability and risk for various cardiovascular morbidity and mortality.[17] For example, measures of HR variability including the spectral LF and HF components were shown to be significantly associated with risk for a cardiac event in participants in the Framingham Heart Study.[18] Therefore, it is thought that attention should be paid to excessive drinking especially in cardiovascular disease patients with deficient ALDH2 activity.

In the present study, daily alcohol intake was more in the active ALDH2 group than in the inactive ALDH2 group, although the difference was not statistically significant ($P = 0.06$). There is a little possibility that this factor might be related to different haemodynamic responses to alcohol intake between the two groups, although 48 h of abstinence from alcohol drinking was required before the tests.

In conclusion, we have demonstrated for the first time that ALDH2 genotypes modify the effects of intake of a small amount of alcohol on ambulatory BP, HR, and HR variability in Japanese men. The present findings highlight a major role of ALDH2 genotypes in responses of haemodynamics and HR variability to alcohol intake. Further studies are needed to clarify whether ALDH2 genotypes have an influence on a well-known relationship between chronic alcohol consumption and BP elevation.

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