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Effect of ALDH2 Genetic Polymorphism on the Adaptive Change in Alcohol Metabolism Due to Continuous Moderate Alcohol Consumption in Humans

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Abstract

Few studies have assessed the difference in adaptive changes of alcohol metabolism in the case of chronic alcohol consumption pertaining to the genetic polymorphism of aldehyde dehydrogenase 2 (ALDH2) in humans. To evaluate the influences of ALDH2 genotypes on changes in alcohol metabolism due to continuous alcohol intake, we conducted an intervention study by setting a continuous drinking period between two abstinence periods. The subjects in this study comprised 20 - 25-year-old males, including 15 males carrying *ALDH21/*1 and 16 carrying *ALDH2**1/*2 genotypes. Following the abstinence period of 4 weeks (from day 1 to day 28), all subjects drank commercially available beer (500 ml) every evening for 6 weeks (from day 30 to day 71) and subsequently abstained from drinking again for 4 weeks (from day 73 to day 100). The next morning, after the end of each period, drinking tests (DTs) were performed on each subject (DT1 on day 29, DT2 on day 72, and DT3 on day 101) to examine alcohol metabolism. The subjects drank shochu (a distilled alcoholic beverage), with an ethanol dose of 0.32 g/kg, within 20 minutes after overnight fasting. The alcohol elimination rate in subjects with *ALDH2**1/*1 genotype was significantly higher during DT2 after the drinking period as compared with those at both DT1 and DT3 after the abstinence periods, whereas the elimination rate in subjects with *ALDH2**1/*2 genotype did not change significantly during 3 DTs. However, blood acetaldehyde levels significantly decreased in subjects with both ALDH2 genotypes during DT2 as compared with those during DT1. The physiological subjective responses to alcohol also significantly decreased during DT2 in subjects with *ALDH2**1/*2 genotype. Moreover, serum lipids, gamma-glutamyltransferase (GGT), and uric acid**

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concentrations also varied between subjects with different ALDH2 genotypes due to continuous drinking. These results suggested that ALDH2 polymorphism modified adaptive changes in alcohol metabolism and physiological responses to continuous moderate alcohol consumption.

Keywords

Alcohol Metabolism, ALDH2 Polymorphism, Continuous Alcohol Consumption

1. Introduction

Previous studies have reported a U-shaped relationship between alcohol consumption and mortality [1]-[5] that indicates health benefits of moderate alcohol consumption as compared with its complete abstinence or heavy alcohol use. The basic knowledge about the effects of chronic alcohol consumption in different amounts on alcohol metabolism is also essential to understand physiological effects of alcohol consumption. Moreover, numerous animal studies have demonstrated an increase in alcohol metabolism following chronic alcohol intake [6]-[12]. Some human studies also advocate the results of these animal studies. Misra *et al*. [13] reported that chronic consumption of ethanol for one month under specific dietary conditions results in acceleration in the clearance of ethanol from blood in both alcoholics and nonalcoholics. Ethanol is rapidly oxidized to acetaldehyde and acetate in a two-step process involving alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Furthermore, in alcoholics, an elevation of blood acetaldehyde levels was often observed [14], because of the acceleration of alcohol metabolism or the decrease of low-Km aldehyde dehydrogenase (ALDH2) activity in the liver following excessive alcohol intake [15] [16]. Also in healthy subjects, the activity of ALDH2 is important to regulate the effects of alcohol consumption. Asians, who genetically lack ALDH2 activity, show facial flushing or a sense of discomfort due to remarkably high concentrations of blood acetaldehyde after drinking. Phenotype of ALDH2 deficiency (known as Asian flush) has been related to *ALDH2**1/*2 and *ALDH2**2/*2 genotypes, and leads to reduced alcohol consumption in these subjects. Therefore, *ALDH2**2 allele is one of the important factors that prevents humans from excessive drinking and hence from developing alcoholism. However, there has been only limited information about the influences of continuous moderate consumption of alcohol on adaptive changes in ethanol and acetaldehyde metabolisms in subjects with different ALDH2 genotypes.

In this study, we aimed to clarify how ALDH2 polymorphism affects alcohol metabolism during continuous moderate alcohol consumption. For this purpose, we conducted an intervention study that sets continuous beer drinking and abstinence periods as well as examining blood samples of the subjects.

2. Subjects and Methods

2.1. Subjects and Protocols

This study was proposed and approved by the Shiba Palace Clinic Ethics Committee. Informed consent was obtained from 82 adult Japanese men aged 20 - 25 years, who participated in baseline screening. ALDH2 genotypes included *ALDH2**1/*1 in 46 subjects, *ALDH2**1/*2 in 26 subjects, and *ALDH2**2/*2 in 10 subjects. Inclusion criteria for the intervention study were as follows: 1) 20 - 25 years of age, 2) healthy males, free of chronic illness such as alcoholism, and 3) the ability to drink beer and conduct abstinence according to the protocol. Subsequently, 72 men were recruited, and 16 subjects were randomly selected from *ALDH2**1/*1 and *ALDH2**1/*2 groups, respectively. *ALDH2**2/*2 subjects were excluded because they have a genetic intolerance to alcohol. Analyses were finally performed using data from 15 *ALDH2**1/*1 and 16 *ALDH2**1/*2 carriers because one *ALDH2**1/*1 subject dropped out before the beginning of the study.

2.2. Study Design

All subjects abstained from alcohol for 4 weeks (from day 1 to day 28) in period 1 (**Figure 1**). During the subsequent period (period 2), subjects drank 500 ml of commercially available beer every day at dinnertime for 6 weeks (from day 30 to day 71). Finally, subjects abstained again from alcohol consumption for 4 weeks (from day 73 to day 100) (period 3). Drinking tests (DTs) were conducted on days 29 (DT1), 72 (DT2), and 101 (DT3)

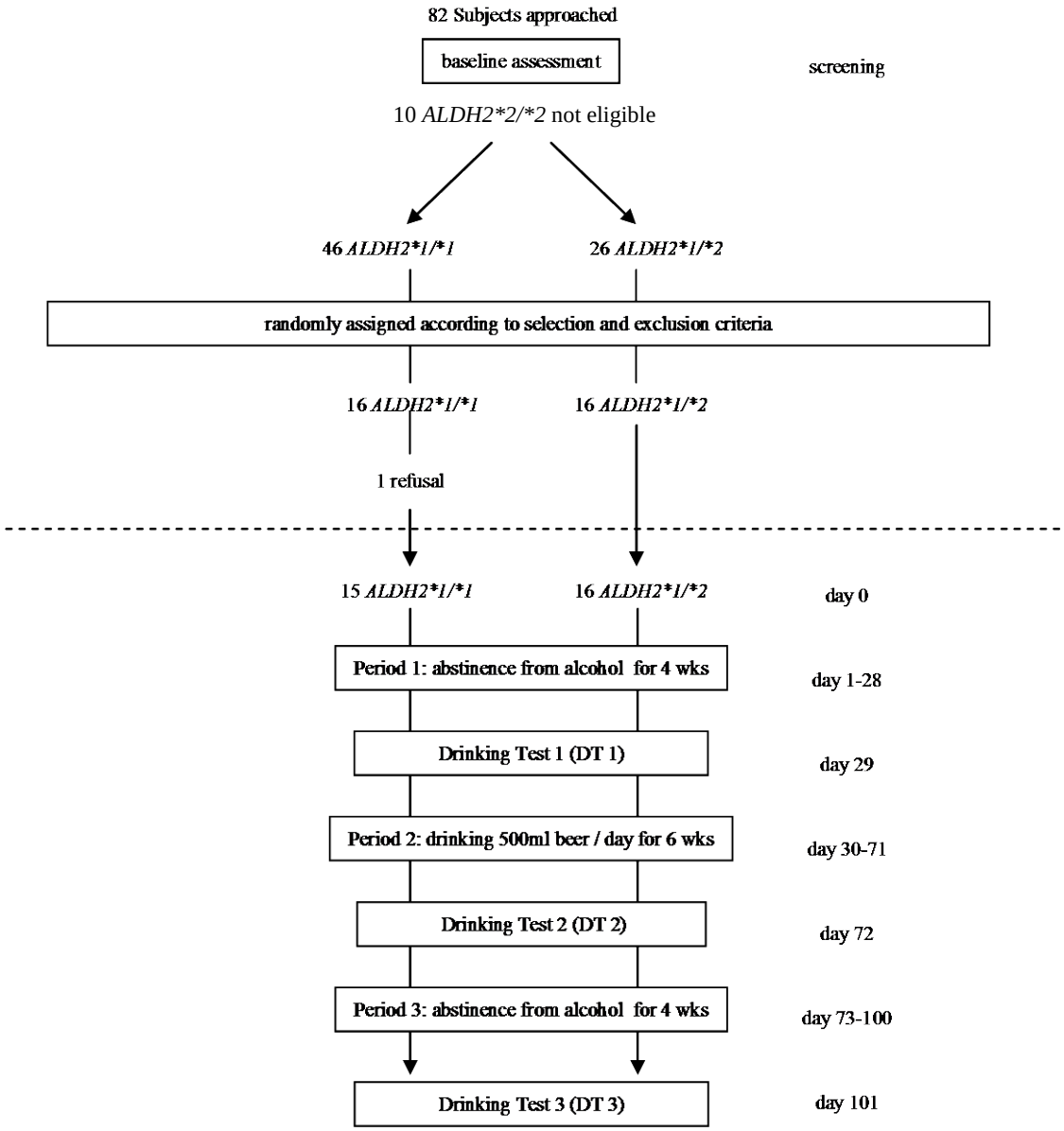


Figure 1. Flowchart of participant selection and the intervention study.

and changes in alcohol metabolism and physiological situations due to continuous drinking or abstinence were assessed. Subjects refrained from eating for at least 10 h prior to each DT, and after measurements of weight, water content of the body, blood pressure and heart rate, they were given 0.32 g of ethanol/kg body weight in the form of commercially available shochu (distilled alcoholic beverage), which was diluted to 16% (v/v) alcohol in water. Blood samples were then collected from the cubital vein before and after alcohol ingestion on each experimental day.

2.3. Analytical Techniques

Blood samples were mixed with 0.5 N perchloric acid and the supernatants were prepared by centrifugation. Subsequently, blood ethanol and acetaldehyde concentrations were measured using a head space gas chromatography according to the procedures described by Okada and Mizoi [17], and blood acetate concentration was measured using a high-performance liquid chromatography (Shimadzu organic acid determination system; Shi-

madzu, Japan) fitted with an ion-exclusion column and a conductivity detector [18]. Blood ethanol elimination rate (β value; mg/ml/h) was calculated from the slope of pseudolinear decline in the blood ethanol concentration curve.

Serum gamma-glutamyltransferase (GGT, U/l), uric acid (mg/dl), and lipid concentrations (mg/dl) (triglyceride, total-cholesterol, LDL-cholesterol, HDL-cholesterol) were determined by the routine measurements in a clinical laboratory after fasting and before the start of each DT. Atherogenic index was calculated using the following formula: (total-cholesterol − HDL-cholesterol)/HDL-cholesterol [19]. To assess subjective sensations to alcohol, subjects were required to select a score from 0 - 10 grading the sensations of face flush or changed heart beats before and after alcohol consumption in each DTs.

2.4. Statistical Analysis

Statistical analyses were conducted using Dr. SPSS II software (SPSS Inc.). Data were presented as mean ± SD. Maximum blood acetaldehyde and acetate levels at DTs 2 and 3 were expressed relative to those of DT1. Differences between DTs were identified using repeated measures ANOVA and the Scheffe's *post-hoc* test, and were considered significant when $p < 0.05$. Different letters in the figures indicate statistically significant differences between means (Scheffe's test, $p < 0.05$). Differences between *ALDH**1/*1 and *ALDH*2*1/*2 groups were identified using Student's t-test and were considered significant when $p < 0.05$.

3. Results

3.1. Subject Characteristics

Recruited subjects who completed the whole process (periods 1 - 3 and DTs 1 - 3) included 15 males with *ALDH*2*1/*1 genotype and 16 with *ALDH*2*1/*2 genotype. As shown in **Table 1**, BMI and water content of the body did not differ between 3 DTs in *ALDH*2*1/*1 subjects, whereas BMI was greater at DTs 2 and 3 than at DT1 in *ALDH*2*1/*2 subjects. BMI and body water contents did not differ between the genotype groups (**Table 1**). Blood pressure (systolic, diastolic) did not differ between DTs in subjects of either ALDH2 genotype (data not shown).

3.2. Blood Ethanol Concentration and Ethanol Elimination Rate

Maximal blood ethanol concentration (BAC max) and the blood elimination rate (β value) were calculated using blood ethanol concentration curves at each DT (**Table 2** and **Figure 2**). BAC max showed no differences between *ALDH*2*1/*1 and *1/*2 groups, as well as between DTs. However, whereas β value was significantly higher during DT2 than at DTs 1 and 3 in subjects with *ALDH*2*1/*1, it did not vary between DTs in subjects carrying *ALDH*2*1/*2 genotype. Particularly, β value was higher in *ALDH*2*1/*1 than in *ALDH*2*1/*2 subjects during DT2.

Table 1. Changes in body composition status during the abstinence and the drinking periods.

		***ALDH*2*1/*1 (n = 15)**	***ALDH*2*1/*2 (n = 16)**	***p* by Student's t-test (*1/*1 vs *1/*2)**
BMI (kg/m^2)	Day 29 (after period 1)	21.2 (3.8)	21.4 (2.6)[a]	0.857
	Day 72 (after period 2)	21.2 (3.8)	21.7 (2.7)[b]	0.645
	Day 101 (after period 3)	21.1 (3.9)	21.7 (2.7)[b]	0.627
	p by repeated ANOVA	0.522	0.003	
Body water content (liter)	Day 29	35.9 (4.4)	37.7 (4.2)[a]	0.267
	Day 72	35.7 (4.4)	38.1 (4.2)[b]	0.131
	Day 101	35.7 (4.7)	38.0 (4.2)[a,b]	0.159
	p by repeated ANOVA	0.395	0.025	

Values are means (standard deviation). Means with different subscripts are significantly different at $p < 0.05$ in the each index.

Table 2. The maximal blood ethanol concentrations (BAC max) after ethanol ingestion (0.32 g/kg) at each drinking test (DT).

		***ALDH2**1/*1 (n = 15)**	***ALDH2**1/*2 (n = 16)**	***p* by Student's t-test (*1/*1 vs *1/*2)**
BAC max (mg/ml)	DT1	0.57 (0.15)	0.60 (0.22)	0.710
	DT2	0.58 (0.09)	0.54 (0.13)	0.377
	DT3	0.60 (0.11)	0.56 (0.11)	0.342
	p by repeated ANOVA	0.567	0.433	

Values are means (standard deviation). No significant difference among DTs in each ALDH2 genotype.

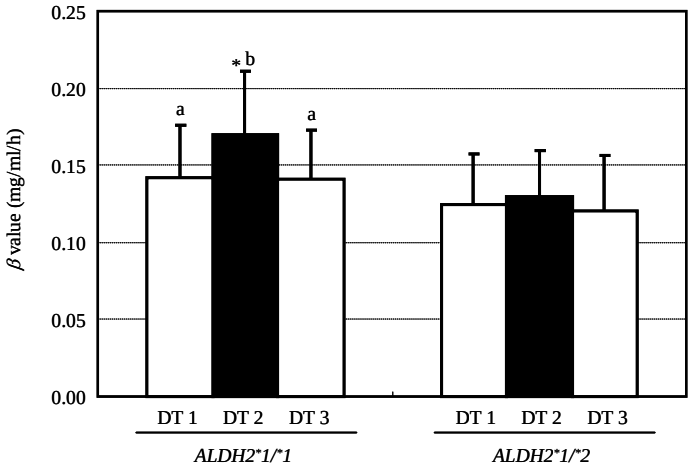


Figure 2. Changes in ethanol disappearance rate from the blood (β value) after ethanol ingestion (0.32 g/kg) at each drinking test (DT). Results are expressed the means and standard deviation (Mean ± SD). Means with different subscripts are significantly different at $p < 0.05$ by repeated ANOVA in each DT. *$p < 0.05$ by Student's t-test between two ALDH2 genotypes at each DT.

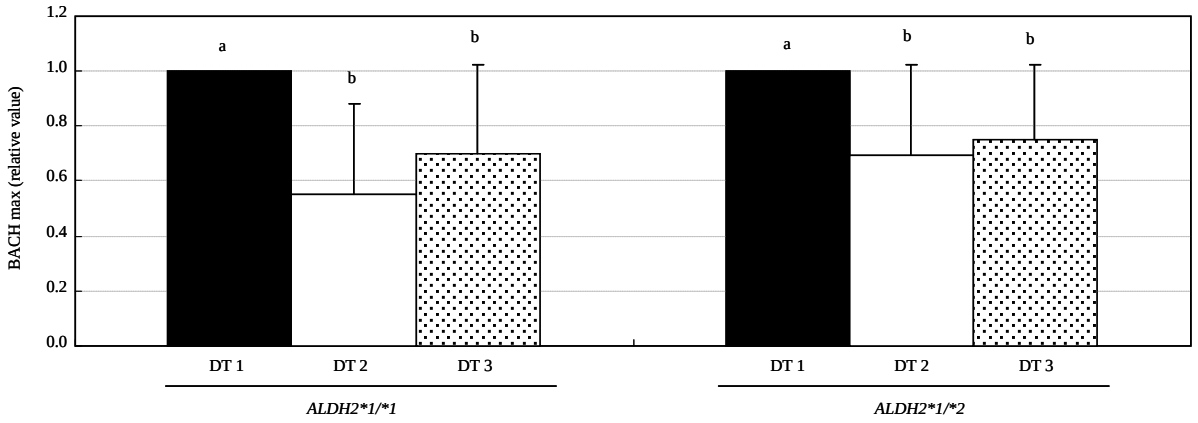
3.3. Blood Acetaldehyde and Acetate Concentrations

In accordance with the results of previous reports [20], blood acetaldehyde levels were significantly higher in subjects with *ALDH2**1/*2 than in subjects with *ALDH2**1/*1 during all DTs (Data not shown). However, the maximal blood acetaldehyde concentrations (BACH max) were significantly less during DTs 2 and 3 than during DT1 in both ALDH2 genotype groups (**Figure 3**). Consequently, the maximal blood acetate concentrations (BACE max) were significantly increased during DTs 2 and 3 in subjects with *ALDH2**1/*2, and were similarly but insignificantly increased in subjects with *ALDH2**1/*1 (repeated ANOVA; $p = 0.069$). Moreover, BACE during DT3 was significantly lower in subjects with *ALDH2**1/*2 than in subjects with *ALDH2**1/*1 (Data not shown).

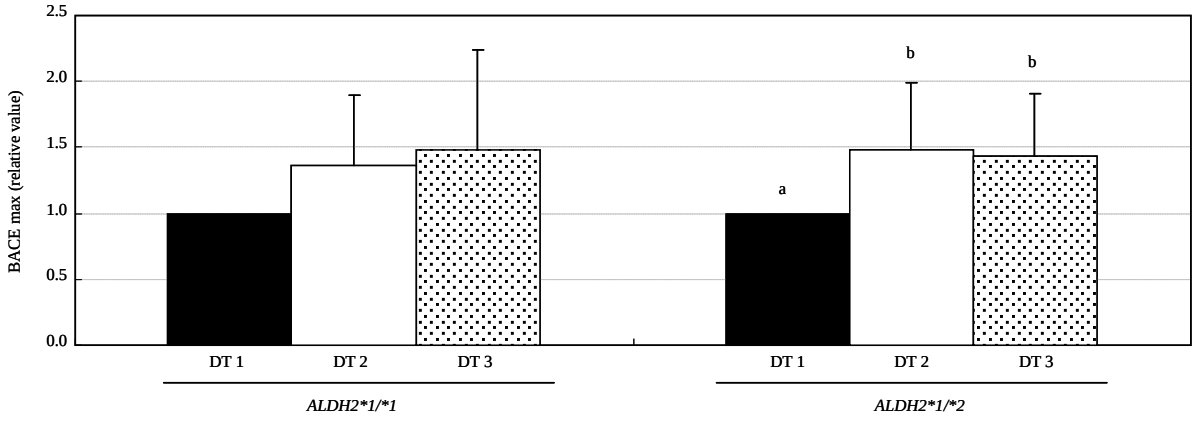
3.4. Subjective Physiological Responses to Alcohol and Blood Biochemistry Markers

Sensations of face flush and change in heart beat were recorded after 1 h post alcohol consumption at each DT (**Figure 4**), because blood ethanol, acetaldehyde and acetate levels were close to maximal at this time. Sensation scores were very low in the *ALDH2**1/*1 group and did not differ significantly between DTs. In comparison, both sensation scores were very high in subjects with *ALDH2**1/*2 and were significantly lower at DTs 2 and 3 than at DT1. "Face numbness", "dizzy" and "warm cheeks" also indicated similar tendencies in subjects with *ALDH2**1/*2 (data not shown).

To examine the influence of continuous drinking and abstinence on physiological conditions, the blood biochemistry markers (serum GGT, uric acid, triglyceride, total-cholesterol, LDL-cholesterol, HDL-cholesterol) and the atherogenic index were analyzed under fasting conditions before the start of each drinking test (**Table 3**).

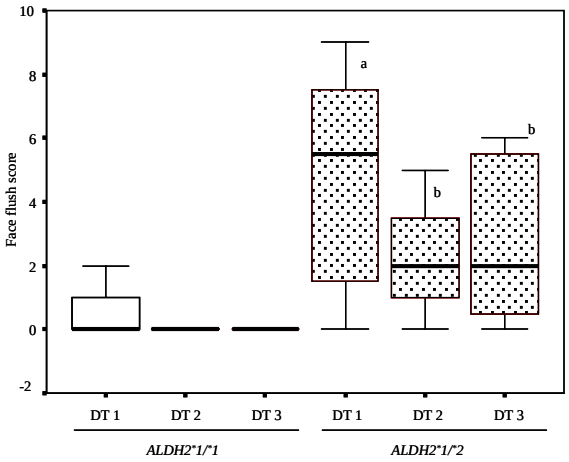


(A)

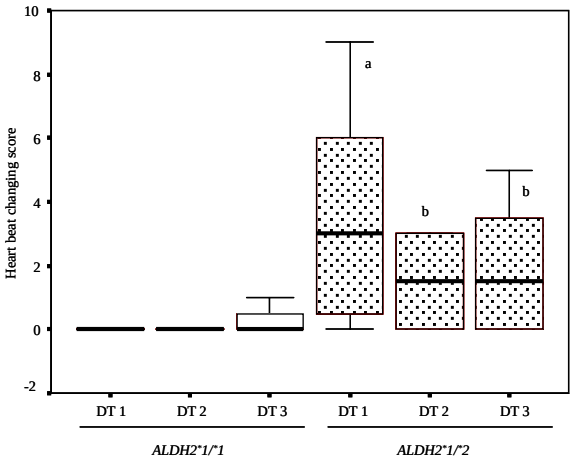


(B)

Figure 3. The maximal blood acetaldehyde (BACH max) or acetate concentrations (BACE max) after ethanol ingestion (0.32 g/kg) at each drinking test. (A) BACH max; (B) BACE max. Results are expressed the means (relative value) and standard deviation. Relative value is the ratio of the measured value at DT2 or 3 to the measured value at DT1. Means with different subscripts are significantly different at $p < 0.05$ by repeated ANOVA at each DT.



(A)



(B)

Figure 4. Changes in face flush or heart beat after ethanol ingestion (0.32 g/kg) at each drinking test (DT). (A) Face flush scores; (B) Heart beat changing scores. Results are expressed the median, quartiles or the lowest and highest data in the box plots. Means with different subscripts are significantly different at $p < 0.05$ in the each DT.

Table 3. Changes in serum GGT, uric acid, lipid concentrations and atherogenic index during the abstinence and the drinking period.

		*ALDH2**1/*1 (n = 15)	*ALDH2**1/*2 (n = 16)	*p* by Student's t-test (*1/*1 vs *1/*2)
GGT (U/l)	DT1	21.8 (11.2)[a,b]	20.7 (7.3)	0.743
	DT2	23.7 (12.4)[b]	23.3 (12.4)	0.923
	DT3	20.2 (9.0)[a]	21.8 (10.2)	0.644
	p by repeated ANOVA	0.010	0.110	
Uric acid (mg/dl)	DT1	5.8 (1.1)[a]	5.7 (0.9)	0.771
	DT2	6.3 (1.1)[b]	5.9 (1.1)	0.261
	DT3	5.6 (0.9)[a]	5.8 (0.9)	0.677
	p by repeated ANOVA	< 0.001	0.407	
Triglyceride (mg/dl)	DT1	66.5 (27.6)	71.1 (22.3)	0.608
	DT2	62.4 (21.5)	73.9 (28.1)	0.211
	DT3	67.3 (50.6)	85.1 (49.3)	0.328
	p by repeated ANOVA	0.811	0.292	
LDL-cholesterol (mg/dl)	DT1	101.7 (24.1)[a]	95.8 (21.4)	0.474
	DT2	90.0 (21.6)[b]	96.3 (26.9)	0.479
	DT3	90.3 (20.7)[b]	98.7 (25.1)	0.318
	p by repeated ANOVA	0.009	0.610	
Total-cholesterol (mg/dl)	DT1	181.3 (23.2)[a]	167.5 (21.3)	0.096
	DT2	169.5 (20.5)[b]	169.1 (23.9)	0.954
	DT3	168.1 (24.0)[b]	169.7 (24.2)	0.853
	p by repeated ANOVA	0.010	0.787	
HDL-cholesterol (mg/dl)	DT1	61.3 (13.8)	54.4 (6.5)[a]	0.082
	DT2	63.5 (12.9)	57.8 (6.0)[b]	0.121
	DT3	60.6 (15.1)	53.3 (5.2)[a]	0.104
	p by repeated ANOVA	0.275	0.002	
Atherogenic index ((TC − HDL-C)/HDL-C)	DT1	2.1 (0.8)[a]	2.1 (0.5)[a,b]	0.897
	DT2	1.8 (0.7)[b]	2.0 (0.6)[a]	0.379
	DT3	1.9 (0.8)[a,b]	2.2 (0.6)[b]	0.245
	p by repeated ANOVA	0.001	0.012	

Values are means (standard deviation). Means with different subscripts are significantly different at $p < 0.05$ in the each index.

Although no abnormal values in biochemical markers were observed in any of the subjects, significant differences were identified between DTs in subjects with *ALDH2**1/*1, whose GTT and uric acid levels increased during DT2, compared with those at DTs 1 and 3, whereas total-cholesterol and LDL-cholesterol decreased during DTs 2 and 3. In *ALDH2**1/*2 subjects, only HDL-cholesterol levels increased during DT2. These changes of blood lipids resulted in falls of atherogenic index during DT2 compared with other DTs in both ALDH genotypes, though the baselines were different between two genotypes (repeated ANOVA). No blood biochemistry markers differed significantly between ALDH2 genotypes during all DTs.

4. Discussion

The mitochondrial enzyme ALDH2 is subjected to the genetic polymorphism of ALDH2 in Mongoloid popula-

tions. The variant *ALDH*2*2 gene produces inactive ALDH2 with a Glu487Lys substitution, and is responsible for flushing symptoms after alcohol intake [21]. Thus, the *ALDH*2*2 allele is an important factor that prevents from drinking excessively and hence from developing alcoholism [22]. However, the influences of ALDH2 polymorphism on adaptations of ethanol and acetaldehyde metabolism to chronic moderate alcohol consumption remain poorly understood in normal subjects. Thus, in the present interventional study, alcohol metabolism was monitored across continuous beer drinking and abstinence periods, and the effects of ALDH2 polymorphism were investigated. ALDH2 polymorphism is known to affect the rate of alcohol metabolism [20] and the daily consumption of alcohol [23], which also in turn affects the rate of alcohol metabolism [24]. Thus, alcohol metabolism was compared between subjects with *ALDH*2*1/*1 and *ALDH*2*1/*2 genotypes after a 4-week abstinence period, a subsequent 6-week drinking period, and then another 4-week abstinence period.

Previous studies showed that chronic alcohol consumption causes dose-dependent increase in alcohol elimination rates, reflecting the acceleration of alcohol metabolism [6]-[13]. In this study, the consumption of 500 ml beer per day for 6 weeks increased β value in subjects with *ALDH*2*1/*1, indicating that continuous intake of alcohol induces adaptive increases in alcohol metabolism even when the consumption is moderate. In contrast, no such adaptive increases were observed in subjects with *ALDH*2*1/*2. The suppression of ADH activity by acetaldehyde has been demonstrated to result in partial inhibition of alcohol metabolism in both animal and kinetic studies [25] [26]. Because ADH1 accounts for about 70% of systemic alcohol metabolism [27], resistance to adaptive changes in alcohol metabolism in *ALDH*2*1/*2 subjects may be due to their high levels of blood acetaldehyde, even under conditions of moderate drinking. Moreover, previous studies showed faster alcohol metabolism in subjects with *ALDH*2*1/*1 than in subjects with *ALDH*2*1/*2 [20]. β values in this study were also greater in the subjects with *ALDH*2*1/*1 than in the subjects with *ALDH*2*1/*2 after continuous drinking. However, no difference was observed between the two genotypes after each abstinence period. It is also known that humans with the *ALDH*2*1/*1 genotype drink more in daily life than those with *ALDH*2*1/*2 genotype [28]. Thus, the present data may suggest that differences in the rate of alcohol metabolism between two genotypes reflect differences in daily consumption of alcohol. Further studies are needed to assess the influence of ALDH2 polymorphism on alcohol metabolism.

Blood acetaldehyde concentrations were found to decrease following continuous moderate intake of alcohol in both subjects with *ALDH*2*1/*1 and *ALDH*2*1/*2. Moreover, these low levels were maintained after a second abstinence, leading to maintenance of high blood acetate levels. In agreement with these metabolic changes, subjective physiological responses to alcohol, as indicated by sensation scores on face flush and change in heart beats, decreased following continuous drinking, particularly in *ALDH*2*1/*2 carriers. These observations further indicate that the continuous moderate intake of alcohol increases the rate of acetaldehyde metabolism to lower blood acetaldehyde levels. In agreement, Tomita *et al.* [29] showed that although both low- and high-Km mitochondrial ALDHs were decreased by liquid diets containing 4% ethanol for one week in mice, they were adaptively increased after 4 weeks.

Few studies have assessed the influence of ALDH2 polymorphism on changes in body composition following continuous moderate consumption of alcohol. Our present data demonstrated that BMI and body water contents are increased after continuous beer consumption in subjects with *ALDH*2*1/*2, but not in subjects with *ALDH*2*1/*1. The increase in BMI in subjects with *ALDH*2*1/*2 may not reflect an increase in caloric intake because body water contents were also elevated in these subjects during the study. Persistent pharmacological stresses caused by higher acetaldehyde levels might trigger such adaptive phenomena in subjects with *ALDH*2*1/*2, although detailed mechanisms remain unknown.

Positive influences of moderate drinking on health remain controversial, although it is accepted that chronic excessive alcohol consumption increases various disease risks. We evaluated the health influences of continuous moderate beer consumption by analyzing serum GGT, uric acid, and lipid contents and the atherogenic index in healthy subjects in this study. Interestingly, changes in these markers following continuous beer consumption differed between subjects with *ALDH*2*1/*1 and *ALDH*2*1/*2 genotypes. GGT and uric acid were more affected in subjects with *ALDH*2*1/*1 than *ALDH*2*1/*2 by the continuous drinking. Although the atherogenic index (TC − HDL-C)/(HDL-C) decreased following continuous drinking in both ALDH2 genotypes, the causality of the decrease of atherogenic index differed between the genotype groups. LDL-cholesterol decreased together with TG contents in subjects with *ALDH*1/*1, whereas HDL-cholesterol increased in subjects with *ALDH*2*1/*2. It cannot be denied that the difference in the baseline may have reflected differences in caloric intake between the genotype groups during the study period, because no calorie surveys were performed in the

present study. Nonetheless, our study demonstrated that influences of continuous moderate drinking are different between *ALDH*2*1/*1 and *ALDH*2*1/*2 carriers, not only on alcohol metabolism but also on blood biochemistry markers. The differences of these adaptive changes between two genotypes are mainly due to the differences in their blood acetaldehyde levels. It is considered that the increases in BMI and HDL-cholesterol observed only in *ALDH*2*1/*2 subjects are the results of adaptation to the higher levels of blood acetaldehyde. On the other hand, acetaldehyde metabolism was adaptively accelerated, regardless of the different genotype of ALDH2, and consequently, subjective physiological responses to acetaldehyde decreased especially in *ALDH*2*1/*2 subjects. However, alcohol metabolism is accelerated only in *ALDH*2*1/*1 subjects, but not in *ALDH*2*1/*2 subjects. Several blood biochemistry markers, such as GGT, uric acid, LDL-cholesterol and total-cholesterol, were also adaptively changed only in *ALDH*2*1/*1 subjects. These adaptive changes observed only in *ALDH*2*1/*1 subjects may be disturbed and become unclear by the high levels of blood acetaldehyde in *ALDH*2*1/*2 subjects. Further investigations are required to assess the influence of ALDH2 polymorphisms on physiological and pathological effects of chronic alcohol consumption.

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