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Original Article

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Additive Effects of Drinking Habits and a Susceptible Genetic Polymorphism on Cholesterol Efflux Capacity

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Aims: High levels of high-density lipoprotein cholesterol (HDL-C) are not necessarily effective in preventing atherosclerotic cardiovascular disease, and cholesterol efflux capacity (CEC) has attracted attention regarding HDL functionality. We aimed to elucidate whether drinking habits are associated with CEC levels, while also paying careful attention to confounding factors including serum HDL-C levels, other life style factors, and rs671 (*2), a genetic polymorphism of the *aldehyde dehydrogenase 2* (*ALDH2*) gene determining alcohol consumption habit.

Methods: A cross-sectional study was performed in 505 Japanese male subjects who were recruited from a health screening program. Associations of HDL-C and CEC levels with drinking habits and *ALDH2* genotypes were examined.

Results: The genotype frequencies of *ALDH2* *1/*1 (homozygous wild-type genotype), *1/*2 and *2/*2 (homozygous mutant genotype) were 55%, 37% and 8%, respectively. Both HDL-C and CEC levels were higher in *ALDH2* *1/*1 genotype carriers than in *2 allele carriers. Although HDL-C levels were higher in subjects who had a drinking habit than in non-drinkers, CEC levels tended to be lower in subjects with ≥ 46 g/day of alcohol consumption than in non-drinkers. Furthermore, CEC levels tended to be lower in *ALDH2* *1/*1 genotype carriers with a drinking habit of ≥ 46 g/day than non-drinkers, while for *2 allele carriers, CEC levels tended to be lower with a drinking habit of 23-45.9 g/day compared to no drinking habit.

Conclusions: Our results suggest that heavy drinking habits may tend to decrease CEC levels, and in the *ALDH2* *2 allele carriers, even moderate drinking habits may tend to decrease CEC levels.

Key words: Alcohol consumption, Drinking habit, Cholesterol efflux capacity, High-density lipoprotein cholesterol, Aldehyde dehydrogenase 2

Introduction

Recent epidemiological studies have shown that higher levels of high-density lipoprotein cholesterol (HDL-C) are not necessarily effective in preventing atherosclerotic cardiovascular disease (ASCVD) and extremely high levels of HDL-C lead to greater ASCVD risk[1-3]. Moreover, a meta-analysis of randomized controlled trials showed that niacin, fibrates and cholesteryl ester transfer protein (CETP) inhibitors, which increase HDL-C levels, did not reduce all-cause mortality, coronary heart disease

mortality, myocardial infarction, or stroke in patients treated with statins[4]. Meanwhile, a Mendelian randomization study showed that genetic polymorphisms associated with lower levels of HDL-C are not related with increased risk of ASCVD[5]. Thus, in recent years, not the quantity, but the functionality of HDL particles has attracted increased attention for verifying the beneficial effects of HDL[6]. HDL plays an important role in cholesterol efflux from peripheral cells to serum, the first step in reverse cholesterol transport, and decreased cholesterol efflux capacity (CEC) has been reported to be associated with the risk of ASCVD[7-9]. Although CEC is positively correlated with the amount of HDL-C, CEC varies substantially even with similar HDL-C levels[6, 10]. Therefore, improving CEC has recently been considered as a potential target for ASCVD prevention, rather than increasing HDL-C levels[6-10]. Furthermore, elucidating the factors associated with inter- and intra-individual variability in CEC should provide useful information for efficiently preventing the development of ASCVD.

The risk of ASCVD was reported to decrease with alcohol intake, primarily in relation to increased HDL-C levels, but the decreasing effect was attenuated by heavy alcohol consumption[11-13]. However, the effect of alcohol intake on CEC remains controversial[14-18]. An *in vitro* study showed that the addition of ethanol to rat embryo fibroblasts suppressed cholesterol efflux from the fibroblasts to HDL[14]. In humans *ex vivo*, long-term alcohol intake was found to impair CEC among individuals with chronic alcoholism[15]. On the other hand, other clinical studies have reported that alcohol intake is associated with an increase or no change in CEC[16-18]. We speculated that the inconsistency in associations between alcohol intake and CEC may be attributable, at least in part, to the frequency, duration and/or amount of alcohol consumed. Drinking behavior and ethanol metabolism are influenced by various factors (e.g., age, sex, genetic background and race)[19-21] and alcohol consumption is also connected with other individual factors (e.g., cigarette smoking and obesity), all of which may affect CEC levels[10]. Furthermore, an alcohol-induced quantitative increase in HDL-C levels may obscure the direct effect of alcohol intake on CEC. Therefore, the above confounding factors should be carefully considered in clarifying the relationship between alcohol intake and CEC levels.

Ethanol is metabolized to acetaldehyde mainly by alcohol dehydrogenase (ADH), which is subsequently metabolized to acetic acid by mitochondrial aldehyde dehydrogenase 2 (ALDH2)[22]. An allelic variant at rs671 of the *ALDH2* gene (i.e., *ALDH2* *2) affects drinking behavior by reducing an individual's alcohol tolerance through a lack of ALDH2 enzyme activity[21, 22]. Blood levels of acetaldehyde after alcohol consumption were higher in carriers of the *ALDH2* *2 allele than in those of the homozygous wild-type genotype, i.e., *1/*1 genotype[23], and thus, *2 allele carriers are more susceptible to alcohol-induced damage[21]. Meanwhile, ALDH2 also plays a key role in detoxifying endogenous toxic aldehydes derived from lipid peroxidation under oxidative stress, including 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) as well as environmental aldehydes[21]. Epidemiological studies have shown that the *ALDH2* *2 allele was associated with increased risk of coronary artery disease (CAD)[24], coronary spastic angina[25], ST-segment elevation myocardial infarction[25] and diabetic retinopathy[26]. Furthermore, HDL-C levels have been reported to be lower in carriers of the *ALDH2* *2 allele than in those of the *1/*1 genotype[24, 27]. Based on these observations, the *ALDH2* rs671 polymorphism may affect CEC levels through decreased acetaldehyde metabolism and/or drinking behavior but the relationship between *ALDH2* rs671 polymorphism and CEC levels remains unclear.

Aim

The present study aimed to determine factors of CEC that could potentially be adjusted to reinforce prevention of ASCVD, by investigating relationships of drinking habits and/or *ALDH2* rs671 polymorphism with levels of CEC among Japanese subjects, while also paying careful attention to confounding factors.

Methods

Study Subjects

We retrospectively investigated 865 Japanese subjects who participated in the health screening program. Among them, 360 subjects were excluded for the following reasons: female subjects (n=298) to eliminate gender differences in drinking habits and levels of HDL-C and CEC[10], no plasma available for measuring cholesterol efflux capacity (n=49), no

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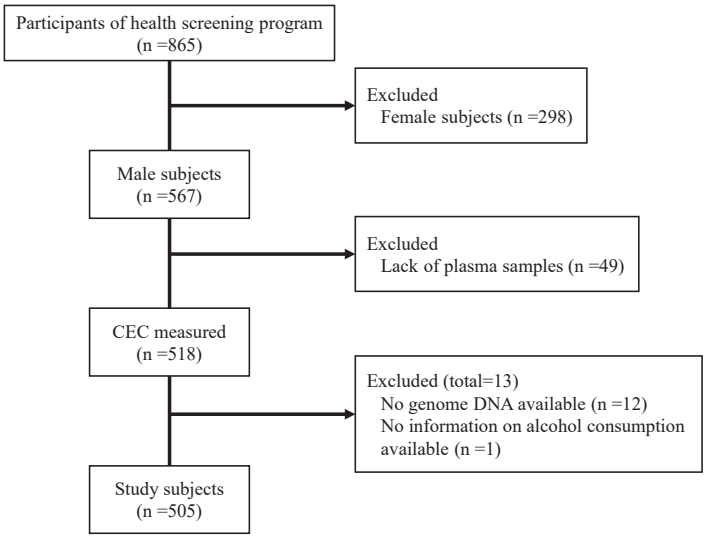

Fig. 1. Flow chart showing the enrolment of the study subjects
CEC, cholesterol efflux capacity

genome DNA available for determining *ALDH2* rs671 polymorphism (n=12) and no information available on daily alcohol consumption (n=1). The remaining 505 male subjects (mean age, 62.3±12.6 years) were enrolled in the study **(Fig. 1)**.

The sample size of the associations of CEC levels with alcohol consumption and drinking habit categories at a significance (alpha) level of 0.05 (two-tailed) using the expected effect size based on the findings from previous studies[16-18]. A power analysis estimated that at least 448 to 500 male subjects would be needed to detect changes in high or low CEC levels due to differences in alcohol consumption or drinking habit categories, and the power of this study were 81 to 84%, exceeding the required power limit (i.e., 80%), and therefore, we included 505 Japanese male subjects in the present study.

This study complied with the principles of the Declaration Helsinki, and the study protocol was approved by the ethics committees of the Faculty of Life Science, Kumamoto University and the National Cerebral and Cardiovascular Center, and the Japanese Red Cross Kumamoto Health Care Center. All study subjects provided written informed consent prior to participating in the study.

Measurements

After approximately 12 hours of fasting, lipid profiles and other biomedical parameters were measured in blood using the standard methods of the Japan Society of Clinical Chemistry at the Japanese Red Cross Kumamoto Hospital Health Center. Information regarding drinking habits and smoking status was obtained via face-to-face interviews with health care providers using a structured questionnaire as described in a previous study[28]. The ethanol equivalent of alcohol consumption (g/day) was calculated based on the total weekly volume of alcohol intake. In the present study, study subjects were categorized into 4 groups based on alcohol consumption: 1) non-drinkers (who do not drink alcohol even once a week), 2) subjects consuming less than 23 g/day of alcohol, which approximates 2 US standard drinks 3) subjects consuming 23-45.9 g/day of alcohol and 4) subjects consuming 46 g/day or more of alcohol[29]. Overweight and normal-weight were defined as body mass index (BMI) ≥ 25 kg/m^2 and BMI <25 kg/m^2, respectively[30].

Genotyping

We extracted genomic DNA from the subjects' whole blood using a DNA purification kit (Flexi Gene DNA kit; QIAGEN, Hilden, Germany), and detected the genetic polymorphism of *ALDH2* (rs671) using a real-time TaqMan allelic discrimination assay (assay no. C_11703892_10). We included a positive control (samples that had an already known genotype) and a negative control (water) in each assay to ensure quality.

Assessment of CEC Levels

CEC levels in fasting plasma samples were quantified using the same method as in our previous studies[31, 32]. To correct for inter-assay variation across wells, a pooled plasma control sample obtained from healthy volunteer was included in the assessment of

CEC levels, and levels for plasma samples from study subjects were normalized to the level of the pooled control sample. In the present study, the CEC level of the pooled control sample was defined as 100.

Assessment of Oxidized Human Serum Albumin

Since the redox state of human serum albumin (HSA) is a sensitive indicator of the progression of chronic diseases related to oxidative stress[33, 34], the current study adopted it as a systemic oxidative stress marker. The redox status of HSA was measured by high-performance liquid chromatography using the same method as in a previous study[35]. The proportion of the oxidized form of HSA to total HSA was defined as oxidized HSA (%).

Statistical Analyses

Data are expressed as the mean ± standard deviation or proportion for categorical variables. Student's *t*-test or the Tukey-Kramer method were used to compare the means of continuous variables between groups. Fisher's exact test was used for comparisons of categorical variables. Pearson correlation analysis was used to detect correlations between two continuous variables. Associations of the HDL-C and CEC levels with the alcohol consumption and drinking habit categories were examined using multiple linear regression analysis with calculation of adjusted unstandardized regression coefficients (B) and standard error (SE) and trend test. Bs were adjusted by age, BMI ≥ 25 kg/m^2, low-density lipoprotein cholesterol (LDL-C), log-transformed triglycerides (log-TGs), smoking status and *ALDH2* genotype for the multiple regression models regarding the levels of HDL-C and CEC (Model 1) or by age, BMI ≥ 25 kg/m^2, LDL-C, log-TGs, smoking status, HDL-C and *ALDH2* genotype only regarding the level of CEC (Model 2). *P* values for trend tests were adjusted by age, BMI ≥ 25 kg/m^2, LDL-C levels, log-TGs, smoking status and *ALDH2* genotype for the HDL-C levels or by age, BMI ≥ 25 kg/m^2, LDL-C, log-TGs, smoking status, HDL-C and *ALDH2* genotype for the CEC levels. The variables in the bi-variable model were HDL-C and drinking habit or CEC and drinking habit. A *P* value of <0.05 was statistically significant. Multiple comparisons were corrected using Bonferroni's method, and *P* values $<$ 0.05/n were considered to be statistically significant after correcting for the number of comparisons made. All statistical analyses were performed using the SPSS software package (version 23.0, IBM Japan Inc., Tokyo, Japan).

Results

Subject Characteristics

The clinical characteristics of the subjects are shown in **Table 1**. The frequencies of the *ALDH2* *1/*1, *1/*2 and *2/*2 genotypes were 55%, 37% and 8%, respectively. The frequency distribution of the *ALDH2* genotypes was consistent with the Hardy–Weinberg equilibrium ($P>0.05$). The amount of alcohol consumption (g/day) and the proportions of drinking habit categories differed between the *ALDH2* rs671 genotypes **(Table 1)** as in the case of a previous study[36]. The values for aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), systolic blood pressure (SBP), uric acid (UA) and total bilirubin were higher, and those for TGs and frequency of smokers were lower in carriers of the *ALDH2* *1/*1 genotype than in those of the *2 allele **(Table 1)**.

Associations of ALDH2 rs671 Polymorphism with Levels of HDL-C and CEC

A positive correlation between HDL-C and CEC levels was observed **(Supplemental Fig. 1)**. In the analyses stratified by the *ALDH2* genotype, positive correlations between HDL-C and CEC levels were also observed in both *ALDH2* *1/*1 genotype carriers and *2 allele carriers **(Supplemental Fig. 1)**. The levels of both HDL-C and CEC were higher in carriers of the *ALDH2* *1/*1 genotype than in those of the *2 allele **(Table 1, Fig. 2)**. Since drinking habits were affected by the *ALDH2* rs671 polymorphism **(Table 1)**, we assessed associations of the *ALDH2* genotype with HDL-C and CEC levels stratified according to drinking habits **(Fig. 2)**. Among non-drinkers, the levels of both HDL-C and CEC were higher in carriers of the *ALDH2* *1/*1 genotype than in those of the *2 allele **(Fig. 2)**. In contrast, this association was not observed among drinkers **(Fig. 2)**. These associations of the *ALDH2* gennotype with HDL-C and CEC levels were also observed in the multivariable analyses **(Supplemental Table 1)**.

Associations of Drinking Habits with Levels of HDL-C and CEC

Since we had found that the levels of HDL-C and CEC were lower in carriers of the *ALDH2* *2 allele than in those of the *1/*1 genotype among non-drinkers only **(Table 1, Fig. 2, Supplemental Table 1)**, we next analyzed the influence of drinking habits on the levels of HDL-C and CEC, considering the *ALDH2* genotypes and other subject factors. Alcohol consumption was positively correlated with HDL-C levels by Pearson correlation analysis **(Fig. 3)** and

Table 1. Clinical characteristics of study subjects

	All (n=505)	*ALDH2* genotype		P value
		*1/*1 (n=277)	*1/*2 or *2/*2 (n=228)	
Age (years)	62.3 ± 12.6	62.9 ± 12.7	61.6 ± 12.3	0.252
BMI (kg/m^2)	23.4 ± 2.8	23.7 ± 2.8	23.2 ± 2.7	0.055
CEC	81.9 ± 14.2	83.1 ± 14.3	80.5 ± 14.0	0.040
HDL-C (mg/dL)	62.3 ± 15.4	64.6 ± 15.3	59.5 ± 15.1	<0.001
LDL-C (mg/dL)	119.6 ± 26.8	117.8 ± 26.6	121.7 ± 26.9	0.097
TGs (mg/dL)	97.0 (26-520)	93.0 (30-508)	100.5 (26-520)	0.046[†]
FBG (mg/dL)	102.8 ± 18.9	103.6 ± 17.9	101.8 ± 20.2	0.284
AST (U/L)	25.4 ± 10.8	26.7 ± 12.7	23.9 ± 7.6	0.002
ALT (U/L)	25.4 ± 15.5	26.8 ± 15.3	23.6 ± 15.6	0.018
GGT (U/L)	43.7 ± 50.5	50.1 ± 62.4	35.8 ± 28.6	0.001
SBP (mmHg)	121.7 ± 16.0	123.1 ± 16.4	120.0 ± 15.5	0.026
DBP (mmHg)	73.6 ± 10.8	74.4 ± 11.2	72.7 ± 10.3	0.078
UA (mg/dL)	5.8 ± 1.3	6.0 ± 1.3	5.6 ± 1.2	0.005
Cr (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.929
Total bilirubin (mg/dL)	1.0 ± 0.4	1.0 ± 0.4	0.9 ± 0.3	0.039
CRP (mg/dL)	0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.3	0.180
Albumin (g/dL)	4.5 ± 0.3	4.5 ± 0.3	4.5 ± 0.3	0.239
Oxidized HSA (%)	45.2 ± 3.8	45.0 ± 3.7	45.4 ± 4.0	0.204
Alcohol consumption (g/day)	16.1 ± 18.9	21.5 ± 19.7	9.7 ± 15.6	<0.001
Drinking habit categories				
No drinking habit (%)	159 (31.5)	46 (16.6)	113 (49.6)	<0.001[‡]
<23 g/day (%)	205 (40.6)	124 (44.8)	81 (35.5)	
23-45.9 g/day (%)	92 (18.2)	70 (25.3)	22 (9.6)	
≥ 46 g/day (%)	49 (9.7)	37 (13.4)	12 (5.3)	
Smoking status (%)				
Non-smoker (%)	413 (81.8)	240 (86.6)	173 (75.9)	0.002[§]
Smoker (%)	92 (18.2)	37 (13.4)	55 (24.1)	

Values are means ± SD, median (range) or number of subjects (%).
[†]Mann-Whitney U test. [‡]Chi-square test. [§]Fisher's exact test (otherwise, Student's t-test was used).
ALDH2, aldehyde dehydrogenase 2; BMI, body mass index; CEC, cholesterol efflux capacity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides; FBG fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; SBP, systolic blood pressure; DBP, diastolic blood pressure; UA, uric acid; CRP, C-reactive protein; Cr, creatinine; HSA, human serum albumin; SD, standard deviation.

multiple regression analysis (Table 2). However, alcohol consumption was not were not correlated with CEC levels (Fig. 3 and Table 2). In the analyses stratified by the *ALDH2* genotype, alcohol consumption was positively correlated with HDL-C levels but not with CEC levels in both carriers of the *ALDH2* *1/*1 genotype and the *2 allele (Fig. 3 and Table 2).

We further analyzed associations of drinking habit categories with levels of HDL-C and CEC (Table 3). The levels of HDL-C were higher in subjects with <23 g/day, 23-45.9 g/day and ≥ 46 g/day of alcohol consumption than in the non-drinkers in the bi-variable analysis and this association was also observed in multivariable model 1 (Table 3). On the other hand, although no association between drinking habit categories and levels of CEC was observed in the bi-variable model and multivariable model 1 (Table 3), the level of CEC was lower in subjects with ≥ 46 g/day of alcohol consumption than in non-drinkers in multivariable model 2 (Table 3). However, this significant association between CEC levels and drinking habit categories was disappeared after Bonferroni's correction (Table 3). The trend tests showed that there was significant association of drinking habit categories with HDL-C levels, while there was a tendency in the association with CEC levels (Table 3). Since there is an interactive effect of drinking habit categories and *ALDH2* genotype on CEC levels (P=0.021), we performed the stratified analyses by the *ALDH2* genotype (Table 3). Higher HDL-C and lower CEC levels were observed in the *ALDH2* *1/*1 genotype carriers with a drinking habit

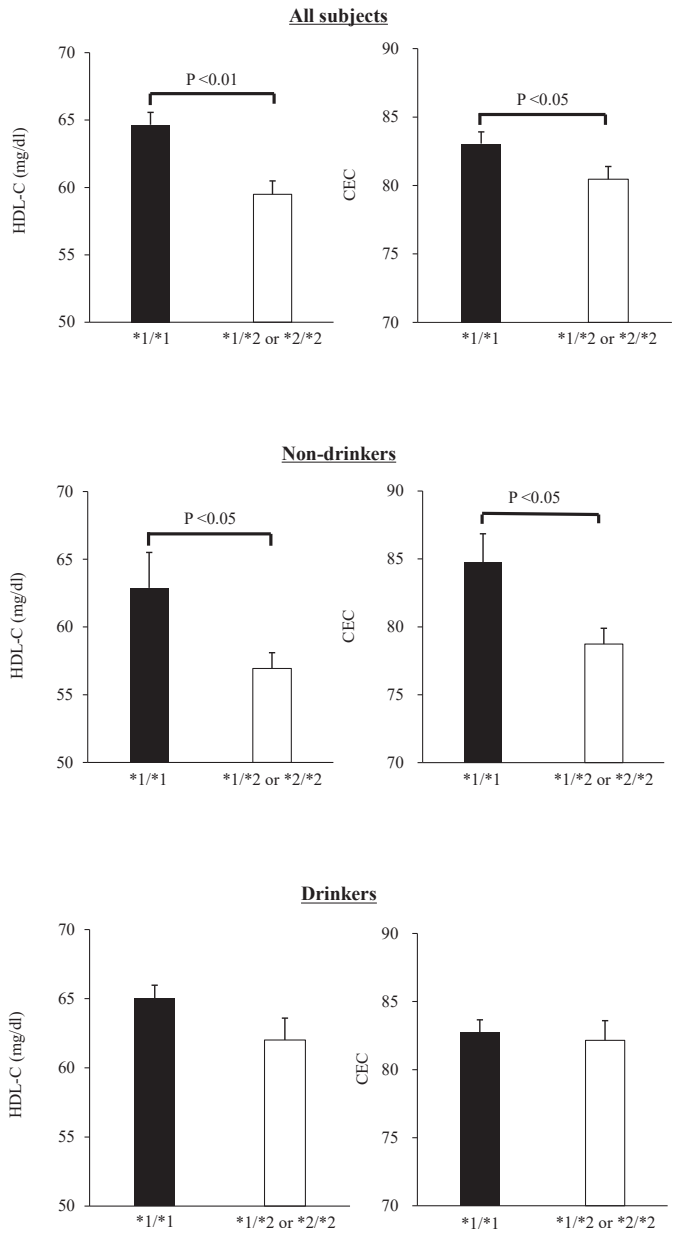

Fig. 2. Levels of HDL-C and CEC between *ALDH2* genotypes among all subjects, non-drinkers and drinkers

Data shown are mean ± SE. *P* values were calculated by Student's *t*-test. HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; SE, standard error.

of ≥ 46 g/day of alcohol consumption than in those with no drinking habit **(Table 3)**. Meanwhile, lower CEC levels were observed in the *ALDH2* *2 allele carriers with a drinking habit of ≥ 23 g/day of alcohol consumption than in the *1/*1 genotype carriers with no drinking habit **(Table 3)**. However, these significant associations between CEC levels and drinking habit categories in both carriers of the *ALDH2* *1/*1 genotype and the *2 allele were disappeared after Bonferroni's correction **(Table 3)**. The trend tests showed that there were significant associations of drinking habit categories with HDL-C levels in both carriers of the *ALDH2* *1/*1 genotype and the *2 allele **(Table 3)**. In contrast, the trend tests showed that there was no association of drinking habit categories with CEC levels in both carriers of the *ALDH2* *1/*1 genotype and the *2 allele.

Since we had found that the *ALDH2* genotype was tended to be associated with the association of

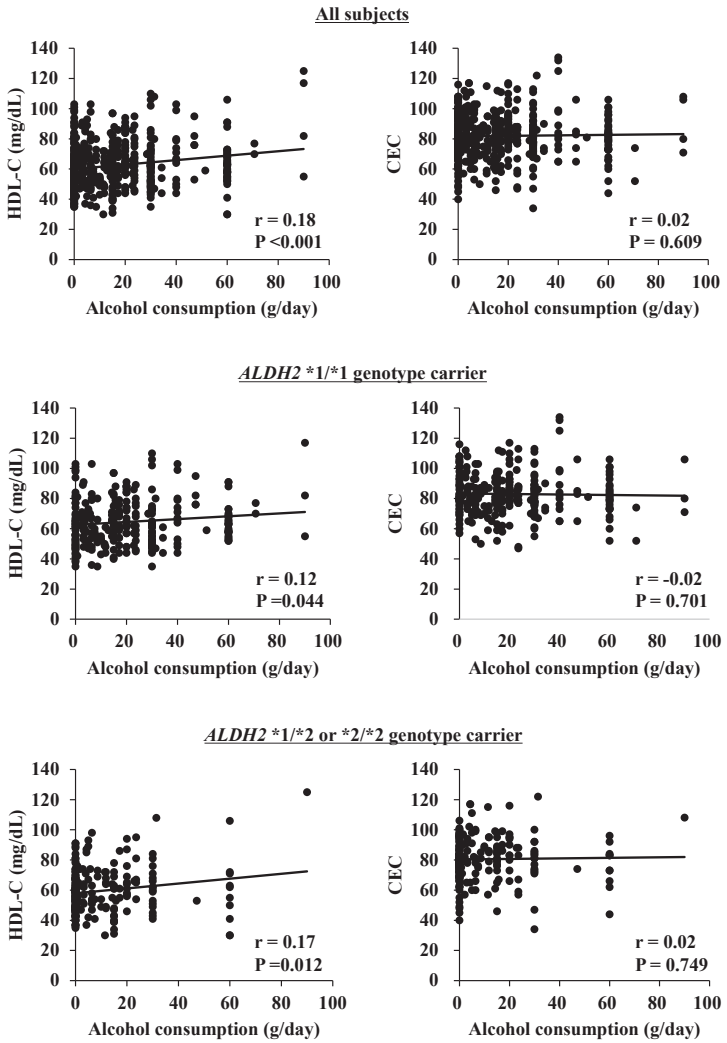

Fig. 3. The correlations of alcohol consumption with the levels of HDL-C and CEC

Pearson correlation analysis. CEC, cholesterol efflux; HDL-C, high-density lipoprotein cholesterol.

Table 2. Associations of levels of HDL-C and CEC with alcohol consumption using regression analysis

	HDL-C (mg/dl)				CEC					
	Bi-variable model		Multivariable model 1		Bi-variable model		Multivariable model 1		Multivariable model 2	
	B (SE)	*P* value	B (SE)†	*P* value	B (SE)	*P* value	B (SE)†	*P* value	B (SE)‡	*P* value
All subjects										
Alcohol consumption (g/day)	0.15 (0.04)	＜0.001	0.17 (0.03)	＜0.001	0.02 (0.03)	0.609	0.03 (0.04)	0.396	-0.06 (0.03)	0.063
ALDH2 *1/*1 genotype										
Alcohol consumption (g/day)	0.09 (0.05)	0.044	0.15 (0.04)	＜0.001	-0.02 (0.04)	0.701	0.02 (0.04)	0.702	-0.06 (0.04)	0.162
ALDH2 *1/*2 or *2/*2 genotype										
Alcohol consumption (g/day)	0.16 (0.06)	0.012	0.20 (0.06)	＜0.001	0.02 (0.06)	0.749	0.05 (0.06)	0.501	-0.07 (0.05)	0.141

†Adjusted by age, BMI ≥ 25 kg/m², LDL-C, Log-TGs, smoking status and *ALDH2* genotype. ‡Adjusted by HDL-C, age, BMI ≥ 25 kg/m², LDL-C, Log-TGs, smoking status and *ALDH2* genotype.

HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; B, unstandardized regression coefficients; SE, standard error; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides.

Table 3. Associations of levels of HDL-C and CEC with drinking habit categories using multiple regression analysis

	HDL-C (mg/dl)				CEC					
	Bi-variable model		Multivariable model 1		Bi-variable model		Multivariable model 1		Multivariable model 2	
	B (SE)	*P* value	B (SE)†	*P* value	B (SE)	*P* value	B (SE)†	*P* value	B (SE)‡	*P* value
Drinking habit categories										
No drinking habit	0		0	<0.001§	0		0		0	0.060§
<23 g/day	4.47 (1.61)	0.006	2.28 (1.50)	0.129	2.80 (1.50)	0.063	1.03 (1.55)	0.506	-0.19 (1.33)	0.889
23-45.9 g/day	5.57 (1.99)	0.005	4.85 (1.90)	0.011	1.52 (1.86)	0.414	1.42 (1.96)	0.468	-1.16 (1.69)	0.493
≥ 46 g/day	8.61 (2.49)	0.001	9.51 (2.34)	<0.001	0.04 (2.32)	0.987	0.63 (2.41)	0.795	-4.43 (2.10)	0.035‖
Combination of the *ALDH2* genotype and drinking habit categories										
*1/*1 & no drinking habit	0		0	§§	0		0		0	§§§
*1/*1 & <23 g/day	1.69 (2.61)	0.519	1.12 (2.33)	0.632	-1.93 (2.44)	0.428	-2.41 (2.39)	0.315	-3.01 (2.04)	0.142
*1/*1 & 23-45.9 g/day	0.94 (2.87)	0.745	2.56 (2.59)	0.324	-1.38 (2.68)	0.606	0.01 (2.66)	0.997	-1.36 (2.27)	0.549
*1/*1 & ≥ 46 g/day	5.70 (3.34)	0.089	8.44 (3.01)	0.005	-3.47 (3.12)	0.266	-1.78 (3.09)	0.565	-6.30 (2.66)	0.018‖
*2 allele & no drinking habit	-5.96 (2.65)	0.025‖	-4.07 (2.38)	0.089	-6.00 (2.47)	0.015‖	-4.79 (2.45)	0.051	-2.61 (2.10)	0.213
*2 allele & <23 g/day	-2.00 (2.80)	0.474	-1.39 (2.52)	0.582	-0.76 (2.60)	0.769	-0.92 (2.59)	0.722	-0.18 (2.21)	0.937
*2 allele & 23-45.9 g/day	2.61 (3.93)	0.507	4.59 (3.54)	0.196	-7.10 (3.66)	0.053	-5.11 (3.64)	0.161	-7.57 (3.11)	0.015‖
*2 allele & ≥ 46 g/day	0.28 (4.91)	0.955	5.30 (4.50)	0.239	-6.57 (4.57)	0.151	-2.76 (4.61)	0.550	-5.60 (3.95)	0.156

†Adjusted by age, BMI ≥ 25 kg/m^2, LDL-C, Log-TGs, smoking status and *ALDH2* genotype. ‡Adjusted by HDL-C age, BMI ≥ 25 kg/m^2, LDL-C, Log-TGs, smoking status and *ALDH2* genotype. §*P* values for trend. §§*P* values for trend in HDL-C levels with drinking habit categories among the carriers of *ALDH2* *1/*1 genotype and those of *2 allele were 0.010 and 0.002, respectively. §§§*P* values for trend in CEC levels with drinking habit categories among the carriers of *ALDH2* *1/*1 genotype and those of *2 allele were 0.134 and 0.086, respectively. ‖ Significance disappeared after Bonferroni's correction.

HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; B, unstandardized regression coefficients; SE, standard error; ALDH2, aldehyde dehydrogenase; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides.

drinking habit categories with HDL-C and CEC levels **(Table 3)**, we next analyzed an association of *ALDH2* genotype with antioxidant and oxidative stress markers **(Supplemental Table 2)**. Carriers of the *ALDH2* *1/*2 or *2/*2 genotype had lower albumin and UA levels and tended to be higher oxidized HSA levels compared to carriers of the *1/*1 genotype **(Supplemental Table 2)**. Among drinkers, the level of albumin was lower and that of oxidized HSA was higher in carriers of the *ALDH2* *1/*2 or *2/*2 genotype than in those of the *1/*1 genotype **(Supplemental Table 2)**. In contrast, among the non-drinkers, no association of *ALDH2* genotypes with antioxidant and oxidative stress markers was observed **(Supplemental Table 2)**.

It has been reported that weight status is associated with the level of CEC[10], and in the present study, the levels of HDL-C and CEC were lower in overweight subjects than in normal-weight subjects **(Supplemental Fig. 2)**. Therefore, we assessed the associations of drinking habits with levels of CEC and HDL-C stratified by weight status **(Supplemental Table 3)**. The trend tests showed that there were significant associations of drinking habit categories with HDL-C level among both overweight and normal-weight subjects **(Supplemental Table 3)**. Moreover, the multivariable model showed that the level of HDL-C was higher in subjects with <23 g/day, 23-45.9 g/day or ≥ 46 g/day of alcohol consumption than in those with no drinking habit among both overweight and normal-weight subjects **(Supplemental Table 3)**. On the other hand, the trend tests showed that there was a significant association between CEC level and drinking habit categories among only normal-weight subjects **(Supplemental Table 3)**. Moreover, the multivariable model also showed that the levels of CEC were lower in subjects with alcohol consumption of ≥ 46 g/day than in those with no drinking habit only among normal-weight subject, but this significant association was disappeared after Bonferroni's correction **(Supplemental Table 3)**.

Discussion

This is the first study to show that a heavier drinking habit (more than 46 g/day of alcohol consumption) tend to be associated with a decreased level of CEC despite an increase in HDL-C level. Therefore, high HDL-C levels in heavy drinkers do not necessarily mean that CEC is also high. Moreover, CEC levels were lower in carriers of the *ALDH2* *2

allele than in those of the *1/*1 genotype, and the decreasing effect of drinking habit on CEC levels tended to be pronounced in carriers of the *2 allele. This indicates that the amount of CEC may be affected not only by intake of alcohol but also a susceptible genetic background. Additionally, our data suggest that drinking should not be recommended to drinkers with low alcohol tolerance to increase HDL-C levels.

In the present study, the bi-variable analyses showed that drinking habits were associated with increased HDL-C levels, but not CEC levels **(Table 2-3)**. Interestingly, the multivariable analyses adjusted for confounding factors including HDL-C showed that alcohol consumption of >46 g/day tended to be associated with decreased CEC levels **(Table 3)**. Based on these results, we suggest that a heavy drinking habit increases HDL-C levels but may decrease CEC per unit of HDL-C. HDL particles are divided into subclasses by size, density, charge, and composition. The lower molecular weight, higher density and relatively cholesterol-poor form is classified as HDL_3, and the higher molecular weight, lower density and relatively cholesterol-rich form as HDL_2 [37]. HDL_3 is the major HDL subclass involved in cholesterol efflux from peripheral cells[38], and sphingosine-1-phosphate and its carrier protein, APOM, contained in HDL_3 are directly associated with CEC[39]. On the other hand, several enzymes are involved in HDL metabolism and remodeling, phospholipid transfer protein (PLTP) appears to convert HDL_3 into HDL_2, and CETP promotes the formation of small HDL[40]. It has been reported that alcohol intake increases HDL_2 levels primarily through a decrease in CETP activity and an increase in PLTP activity[40, 41]. Therefore, alcohol intake is associated with an increase in HDL-C levels by increasing HDL_2 through increased PLTP activity, but, conversely, an increase in PLTP activity may lead to a decrease in HDL_3, resulting in reduced CEC for HDL-C.

It has also been reported that the vasodilatory activity of HDL is inversely correlated with its TG content[42]. Alcohol intake (i.e., ethanol exposure) increases hepatic uptake of exogenous fatty acids (FAs) and subsequent incorporation of FAs (e.g., palmitate) into TGs in the liver, thereby increasing the release of TGs in the form of VLDL-loaded into the blood[13, 43]. Elevated blood levels of TGs cause the formation of TG-rich and cholesterol-ester-depleted HDL particles that are rapidly removed from the circulation, primarily by uptake into the liver, some of which are converted to HDL_3 by hepatic triglyceride lipase (HTGL)[44]. In addition, HDL_2 has been reported to be inversely correlated with HTGL activity[45], so an increase in HDL_2 level due to alcohol intake may be associated with a decrease in HTGL activity. Therefore, a heavy drinking habit may increase TG-rich HDL particles, which have lower cholesterol efflux capacity, by promoting TG synthesis and/or inhibiting the conversion of TG-rich HDL to HDL_3, resulting in decreased CEC levels.

A previous epidemiological study has shown that HDL-C levels were lower in carriers of the *ALDH2* *2 allele than in carriers of the *1/*1 genotype[24], and a similar association was observed even in subjects with no drinking habit[27]. In the present study, both levels of HDL-C and CEC were lower in carriers of the *ALDH2* *2 allele than in those of the *1/*1 genotype among all subjects and non-drinkers **(Fig. 2 and Supplemental Table 1)**. ALDH2 detoxifies not only acetaldehyde derived from alcohol but also other reactive aldehydic products including malondialdehyde (MDA), methylglyoxal and 4-hydroxynonenal, which induce protein carbonylation and mitochondrial dysfunction[21]. A previous *in vitro* study showed that MDA impaired CEC for HDL by modification of apolipoprotein (APO) A-I, a major protein component of HDL particles[46]. Therefore, ALDH2 dysfunction derived from the ALDH2 rs671 polymorphism can accelerate the accumulation of reactive aldehydes, leading to a decrease in CEC for HDL.

In the multiple regression analyses with adjustment for HDL-C level, the association between alcohol intake and a decrease in CEC levels tended to be more pronounced in the *ALDH2* *2 allele carriers than in the 1/* 1 genotype carriers **(Table 3)**. Among the *ALDH2* *2 allele carriers, CEC levels tended to be lower in subjects with an alcohol consumption of 23-45.9 g/day compared to those with no drinking habit, whereas CEC levels tended to be lower in subjects with a greater amount of alcohol consumption, i.e., >46 g/day, compared to those with no drinking habit among the *1/*1 genotype carriers **(Table 3)**. Furthermore, among the drinkers, levels of albumin were lower and those of oxidized HSA were higher in carriers of the *ALDH2* *2 allele than in those of the *1/*1 genotype, suggesting that * 2 allele carriers are more likely to be exposed to oxidative stress derived from alcohol consumption **(Supplemental Table 2)**. Therefore, *ALDH2* *2 allele carriers may be more susceptible to a decrease in CEC caused by alcohol intake, even if the amount of alcohol is low, as compared with the *1/*1 genotype, due to low detoxification capacity for ethanol-derived acetaldehyde.

Alcohol consumption is associated with several individual factors (e.g., cigarette smoking, obesity, sex), which may also be associated with lower CEC

levels[10]. Cigarette smoking has been reported to reduce CEC as well as HDL-C, and smokers have been shown to have reduced ABCA1-mediated cholesterol efflux compared with non-smokers[10]. The multivariable analyses adjusted for HDL-C levels indicated that drinking habits were associated with decreased levels of CEC independently of smoking status **(Table 3)**. Therefore, we suggest that acetaldehydes derived from alcohol intake may be associated with a decrease in CEC for HDL particles regardless of smoking habits. Increased BMI was reported to be associated with decreased APOA-I and HDL-C levels[10], and obesity to be associated with reduced CEC levels due to a decrease in genes related to cholesterol metabolism (e.g., *ABCA1*, *ABCG1*)[10]. In our analysis stratified by weight status, an association between drinking habits and CEC levels was observed in the normal weight subjects, but not in the overweight subjects **(Supplemental Table 3)**. Since CEC levels in overweight subjects were lower than normal-weight subjects **(Supplemental Fig. 2)**, there may be loss of the effects of alcohol consumption on CEC levels in overweight subjects. Furthermore, a previous study showed that both HDL-C and CEC levels were higher in pre-menopausal female subjects than in male subjects[47], but the impact of menopause on CEC levels is not well understood[10]. Since the present study was conducted in male subjects only, further studies in female subjects are needed to investigate the association between drinking habits and CEC levels, including the effects of menopause.

In the current study, we found that drinking habits tended to be associated with lower CEC levels, independently of HDL-C levels **(Table 3)**. In contrast, a previous cross-sectional study conducted in 1,932 Caucasians showed that alcohol consumption was associated with increased CEC levels, without any relation to HDL-C levels[16]. Moreover, a previous cross-over trial with a 4-week intervention period showed that moderate beer consumption (30 g for men and 15 g for women in terms of ethanol) increased CEC levels[17]. Another cross-sectional study found no association between drinking habits and CEC levels[18]. We speculate that the differences in the results among the present study and these previous studies may result from differences in races and genetic polymorphisms. It is well-known that the frequency of *ALDH2* *2 allele varies among races, with *2 allele carriers common among East Asians but not other racial groups, and thus, racial differences may also be important in associations between drinking habits and CEC levels. In addition, differences in genotypes other than the *ALDH2* genotype might also affect CEC levels[22, 48]. The frequency of *ADH1B* rs1229984 polymorphisms associated with reduced ability to convert alcohol to acetaldehyde differs between races[22], and they are more common in Caucasians than in East Asians[22]. Furthermore, a recent genome-wide association study has identified genetic variations at the *APOE/C1/C2/C4* locus associated with CEC levels independent of HDL-C levels[48]. Although further studies on diverse populations incorporating several genotypes are needed, simultaneous measurement of CEC levels and the *ALDH2* rs671 genotype may contribute to appropriate lifestyle modification (e.g., moderation in alcohol intake) and precision/personalized medicine.

Several limitations of the present study should be noted. APOA-I is a small sized particle in HDL and has been reported to be more closely related to CEC levels than HDL-C levels[49, 50]. Therefore, it is important to examine the relationship between drinking habits, APOA-1 and CEC levels in order to clarify the effects of drinking habits on CEC levels, but we were unable to measure APOA1 levels in our study. Further studies are needed to comprehensively examine the relationship between drinking habits, *ALDH2* genotype, APO-AI and CEC levels. Information regarding the subjects' alcohol consumption and smoking status may have lacked reliability because it was evaluated through face-to-face interviews (e.g., there might have been bias related to under-evaluation of alcohol consumption). Moreover, there is the possibility that the association between drinking habits and CEC levels may be influenced by not only current drinking habits but also past drinking habits, but we could not collect information on past drinking habits. Furthermore, the present study has a retrospective cross-sectional design, and the number of subjects was small. Although the statistical power of this study were above the required power limit (i.e., 80%), alcohol consumption and drinking habit categories did not reach the statistically significant effects on CEC levels, because the actual effect size was smaller than the estimated effect size. Further investigations in larger populations and/or according to a prospective design are needed to verify our findings.

Conclusion

A heavy drinking habit is associated with quantitative increases in HDL-C levels but may tend to decrease CEC for HDL particles. Additionally, the decreasing tendency effect of drinking habits on CEC for HDL particles may be more pronounced in carriers of the *ALDH2**2 allele than in those of the *

1/*1 genotype. In the future, simultaneous measurement of both CEC levels and the *ALDH2* rs671 polymorphism may be useful for lifestyle modification, such as moderation in alcohol intake, as well as precision/personalized medicine.

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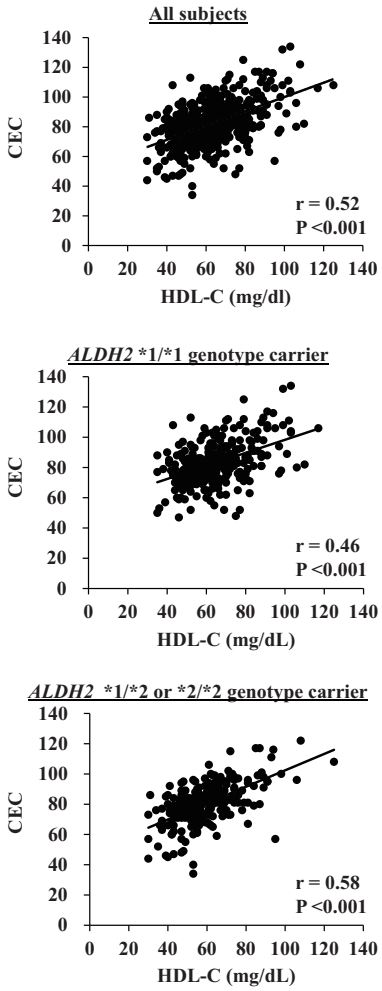


Supplementary Fig. 1. Correlation between HDL-C and CEC levels
HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity.

Supplemental Table 1. Associations of *ALDH2* rs671 polymorphism with levels of HDL-C and CEC

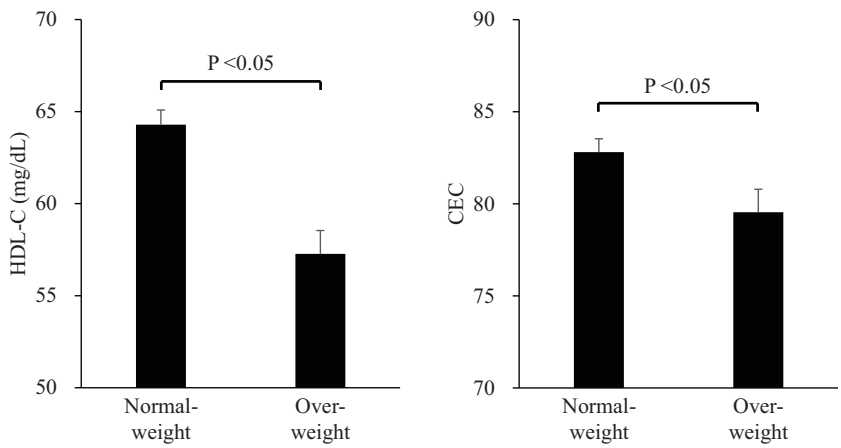
	HDL-C (mg/dl)				CEC			
	Bi-variable model		Multivariable model 1		Bi-variable model		Multivariable model 1	
	B (SE)	*P* value	B (SE)†	*P* value	B (SE)	*P* value	B (SE)†	*P* value
All subjects								
ALDH2 *1/*1 genotype	0		0		0		0	
ALDH2 *1/*2 or *2/*2 genotype	-5.15 (1.36)	<0.001	-4.19 (1.24)	0.001	-2.60 (1.27)	0.040	-2.02 (1.26)	0.109
Subjects with no drinking habit								
ALDH2 *1/*1 genotype	0		0		0		0	
ALDH2 *1/*2 or *2/*2 genotype	-5.96 (2.47)	0.017	-4.82 (2.24)	0.033	-6.00 (2.26)	0.009	-4.75 (2.24)	0.035
Subjects with drinking habit								
ALDH2 *1/*1 genotype	0		0		0		0	
ALDH2 *1/*2 or *2/*2 genotype	-2.99 (1.78)	0.094	-2.15 (1.64)	0.192	-0.60 (1.67)	0.733	-0.30 (1.68)	0.858

†Adjusted by age, BMI ≥ 25 kg/m^2, LDL-C, Log-TGs and smoking status.
ALDH2, aldehyde dehydrogenase 2; HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; B, unstandardized regression coefficients; SE, standard error; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides.

Supplemental Table 2. Associations of *ALDH2* rs671 polymorphism with antioxidant or oxidative stress markers

	Albumin (g/dL)		UA (mg/dL)		Total bilirubin (mg/dL)		Oxidized HSA (%)	
	B (SE)†	*P* value	B (SE)†	*P* value	B (SE)†	*P* value	B (SE)†	*P* value
All subjects								
ALDH2 *1/*1 genotype	0		0		0		0	
ALDH2 *1/*2 or *2/*2 genotype	-0.04 (0.02)	0.043	-0.38 (0.11)	0.001	-0.04 (0.03)	0.170	0.60 (0.34)	0.076
Subjects with no drinking habit								
ALDH2 *1/*1 genotype	0		0		0		0	
ALDH2 *1/*2 or *2/*2 genotype	0.03 (0.04)	0.467	-0.33 (0.22)	0.138	0.01 (0.06)	0.855	-0.45 (0.82)	0.587
Subjects with drinking habit								
ALDH2 *1/*1 genotype	0		0		0		0	
ALDH2 *1/*2 or *2/*2 genotype	-0.08 (0.03)	0.003	-0.23 (0.14)	0.096	-0.05 (0.04)	0.241	0.75 (0.38)	0.048

†Adjusted by age, BMI ≥ 25 kg/m^2, LDL, log TG, smoking status and HDL-C.
ALDH2, aldehyde dehydrogenase 2; UA, uric acid; HSA, human serum albumin; B, unstandardized regression coefficients; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol.



Supplementary Fig. 2. The association of weight status with levels of HDL-C and CEC

Data shown are mean ± SE. *P* values were calculated by Student's *t*-test. HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; SE, standarderror.

Supplemental Table 3. Associations of levels of CEC and HDL-C with drinking habit categories among normal weight or overweight subjects

	HDL-C (mg/dl)				CEC					
	Bi-variable model		Multivariable model		Bi-variable model		Multivariable model 1		Multivariable model 2	
	B (SE)	*P* value	B (SE)[†]	*P* value	B (SE)	*P* value	B (SE)[†]	*P* value	B (SE)[‡]	*P* value
Subjects with normal weight										
No drinking habit	0		0	0.003[§]	0		0		0	0.034[§]
0.1-22.9 g/day	2.51 (1.89)	0.186	1.43 (1.76)	0.415	2.15 (1.73)	0.215	0.94 (1.78)	0.600	0.21 (1.55)	0.893
23-45.9 g/day	3.35 (2.37)	0.156	3.70 (2.33)	0.113	-0.99 (2.17)	0.648	-0.65 (2.36)	0.784	-2.53 (2.05)	0.220
>46 g/day	6.60 (2.97)	0.027	8.97 (2.86)	<0.001	-1.35 (2.72)	0.619	-0.83 (2.90)	0.775	-5.39 (2.55)	0.035[‖]
Overweight subjects										
No drinking habit	0		0	0.003[§]	0		0		0	0.545[§]
0.1-22.9 g/day	6.84 (2.91)	0.020	4.87 (2.94)	0.101	2.83 (2.97)	0.342	0.92 (3.15)	0.769	-2.01 (2.64)	0.449
23-45.9 g/day	9.77 (3.45)	0.005	7.64 (3.44)	0.028[‖]	6.96 (3.52)	0.050	5.17 (3.68)	0.163	0.57 (3.11)	0.854
>46 g/day	12.48 (4.26)	0.004	11.42 (4.17)	0.007	2.84 (4.34)	0.515	2.90 (4.46)	0.518	-3.98 (3.81)	0.298

[†]Adjusted by age, BMI ≥ 25 kg/m^2, LDL-C, Log-TGs, smoking status and *ALDH2* genotype.
[‡]Adjusted by HDL-C, age, BMI ≥ 25 kg/m^2, LDL-C, Log-TGs, smoking status and *ALDH2* genotype.
[§]*P* values for trend.
[‖]Significance disappeared after Bonferroni's correction.
HDL-C, high-density lipoprotein cholesterol; CEC, cholesterol efflux capacity; B, unstandardized regression coefficients; SE, standard error; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides; ALDH2, aldehyde dehydrogenase 2.