PO Box 2345, Beijing 100023, China
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CLINICAL RESEARCH

Improved method of plasma 8-Isoprostane measurement and association analyses with habitual drinking and smoking

Soichi Kitano, Hisashi Hisatomi, Nozomu Hibi, Katsumi Kawano, Shoji Harada

Soichi Kitano, Hisashi Hisatomi, Nozomu Hibi, Katsumi Kawano, Technology Development Department, SRL Inc., Hachioji, Tokyo, Japan
Shoji Harada, Center for Molecular Biology and Cytogenetics, SRL Inc. , Hino, Tokyo, Japan
Correspondence to: Soichi Kitano, Development Planning Section, Technology Development Department, SRL Inc., 5-6-50 Shinmachi, Hino-shi, Tokyo, 191-0002, Japan. kitano@srl.srl-inc.co.jp
Telephone: +81-42-6484076 Fax: +81-42-6484094
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Abstract

AIM: To develop a simple and accurate method for quantifying 8-isoprostane in plasma by employing a combination of two-step solid-phase extraction of samples and a commercially available ELISA kit, and by this method to examine the effects of drinking and smoking habits against the levels of plasma 8-isoprostane in healthy Japanese volunteers.

METHODS: Plasma 8-isoprostane was extracted with ODS gel suspension followed by NH_2 Sep-Pak column. The 8-isoprostane fractions were assayed using a commercially available ELISA kit. We measured plasma 8-isoprostane levels in 157 healthy Japanese volunteers divided into three groups (64 non-habitual drinkers, 56 moderate drinkers and 37 habitual drinkers) according to their alcohol consumption per week. Genotypes of aldehyde dehydrogenase 2 (ALDH2) were also determined to investigate the plasma 8-isoprostane levels with reference to drinking habits. In addition, the plasma 8-isoprostane levels of 96 non-smokers and 61 smokers from the same subjects were compared.

RESULTS: Our method fulfilled all the requirements for use in routine clinical assays with respect to sensitivity, intra- and inter-assay reproducibility, accuracy and dynamic assay range. Significant increases of plasma 8-isoprostane levels were observed in female habitual drinkers when compared with those of non-habitual drinkers (t = 5.494, P < 0.0001) as well as moderate drinkers (t = 3.542, P < 0.005), and 8-isoprostane levels were also significantly different between *ALDH2*2/1* and *ALDH2*1/1* in the female habitual drinkers (t = 6.930, P < 0.0001), suggesting that excessive drinking of alcohol may increase oxidization stress, especially in females. On the contrary, no significant difference of the plasma 8-isoprostane levels was observed between non-smokers and smokers.

CONCLUSION: Our present method was proved to be a simple and accurate tool for measuring plasma 8-isoprostane. However, the clinical utility of plasma 8-isoprostane for drinking and smoking habits was limited since elevated 8-isoprostane levels were observed in female heavy drinkers, and no association was found between smokers and nonsmokers.

Key words: 8-Isoprostane; ELISA; Lipid peroxidation; Drinking; Smoking

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INTRODUCTION

A number of studies have revealed that oxidative stress plays important roles in the pathogenesis of various diseases, such as cancer, diabetes and atherosclerosis[1,2]. The 8-isoprostane present in biological fluids is produced from arachidonic acid by a non-enzymatic, free radical-catalyzed reaction, and has been proposed as a reliable marker for lipid peroxidation and oxidative stress *in vivo*[3]. 8-Isoprostane is chemically stable, in contrast to other conventional markers of oxidative stress, and its levels in either plasma or urine are elevated in subjects who smoke[4-8] and ingest alcohol[9,10], as well as in patients with diabetes mellitus[11], heart disease[12-15], hypertension[16], preeclampsia[17] and asthma[18]. Urinary 8-isoptostane levels increase during the progression of alcohol-induced liver disease[9] and are decreased by abstinence[10]. However, accurate measurement of 8-isoptostane is not easy and requires special instruments, such as GC/MS or LC/MS, since various types of analogues and metabolites are present in biological fluids. For instance, the plasma and urinary 8-isoprostane levels determined by a recently developed immunoassay were much higher than those obtained by GC/MS or LC/MS assays[19,20]. This could be attributed to cross-reaction of the 8-isoprostane analogues and metabolites in the samples with the 8-isoprostane antibody used in the immunoassay.

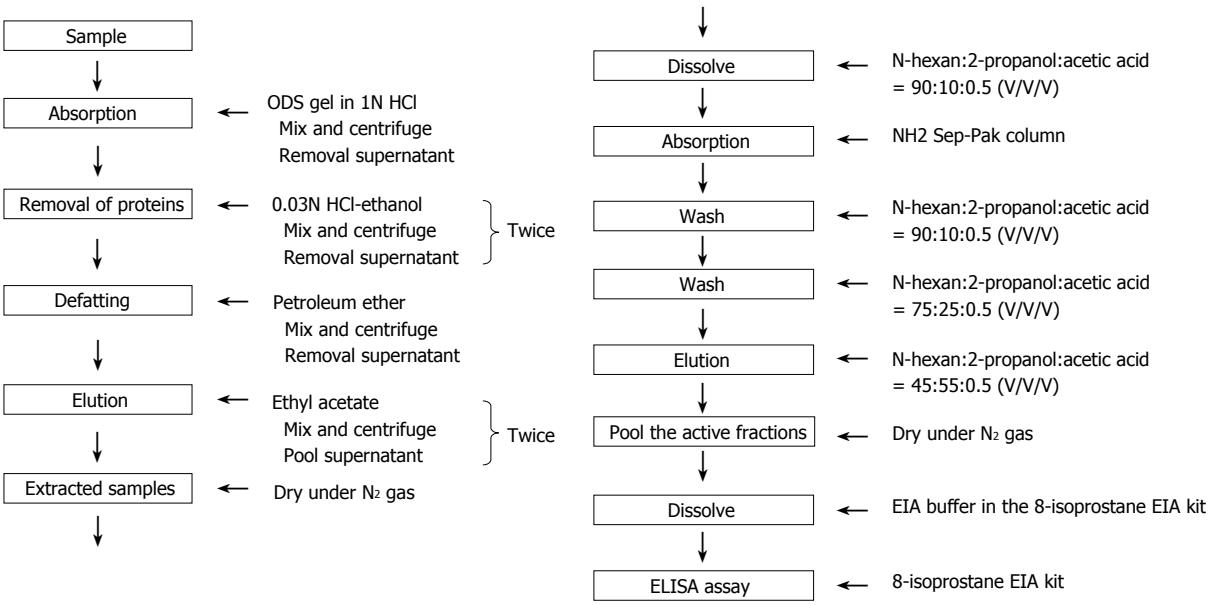

Figure 1 Flow chart of the improved ELISA for plasma 8-isoprostane.

In this study, we developed a new method for pretreatment before analyzing by the commercially available ELISA kit, and performed various examinations for accurate assay of 8-isoprostane. Using this method, we examined the effects of drinking and smoking habits against the levels of plasma 8-isoprostane in healthy Japanese volunteers.

MATERIALS AND METHODS

Samples

After informed consent was obtained, 157 healthy volunteers (83 males and 74 females; age 36.2 ± 8.4 years) were enrolled in this study. The information on drinking and smoking habits was collected by questionnaire. Volunteers were asked the frequency of drinking (nondrinker, rare drinking, 1-2 times/wk, 3-5 times/wk, almost every day), and smoker or nonsmoker. Various lipid parameters (TC, TG, LDL-C, HDL-C, ApoA-I, apoB, apoE and Lp (a)) were measured by EDTA•2Na, and hepatic functions (AST, ALT and γ-GTP) were measured by serum. Blood samples for plasma 8-isoprostane assay were collected in the specific tubes containing 10 mmol/L EDTA•3Na, 20 kU/L Trasylol and 0.1 mmol/L indomethacin, and were separated within 4 h in an ice cooling both. Among the 157 subjects, plasma (heparin) samples were also collected from 3 healthy volunteers to test whether these samples could be used interchangeably for the 8-isoprostane assay. Furthermore, another 3 healthy volunteers as control subjects (2 males, 1 female; age 36.2 ± 8.4 years) were given alcohol (0.5-1.3 g/kg), and their plasma 8-isoprostane, serum AST, ALT, and γ-GTP were measured on D1 (ca. 12 h) and 2 (ca. 36 h) after drinking to investigate the influence of alcohol on these levels. All plasma and serum samples were stored at -80℃ until analysis.

Extraction of 8-isoprostane from plasma samples

A two-step solid-phase extraction procedure that was previously used for quantifying plasma TXA2[21] was modified for the purification of plasma 8-isoprostane. In principle, the first step of the extraction was performed to remove proteins and lipids using ODS gel (ODS-Q3; Fuji Gel, Tokyo, Japan) and the second step was used to separate 8-isoprostane from its analogues and related compounds using an NH_2 Sep-Pac column (Sep-Pak Vac NH_2; Waters, MA, USA). To optimize the extraction conditions, ^{3}H-labeled 8-isoprostane and other related compounds including PGF2α, TXB2, 6-keto-PGF1, PGE2 and PGD2 (Cayman Chemical, MI, USA) were used as spiked tracers.

Extraction and measurement of 8-isoprostane

The detailed procedure for plasma 8-isoprostane extraction is shown in Figure 1. One milliliter of ODS gel suspension (80 mg silica gel ODS-Q3 in 0.1 mol/L HCl containing 40 mL/L ethanol) was mixed with 0.5 mL of plasma and allowed to stand at room temperature for 5 min. The gel was collected by centrifugation and washed twice with 1 mL of 0.03 mol/L HCl containing 150 mL/L ethanol and once with 1 mL of petroleum ether to remove proteins and lipids. 8-isoprostane was eluted from the gel twice using 1 mL of ethyl acetate for each elution. The eluates were combined, transferred to another test tube and dried under N_2 gas. The residue containing 8-isoprostane was dissolved in 1 mL of solution A (hexane: 2-propanol: acetic acid = 90:10:0.5, *V*/*V*/*V*), and applied to an NH_2 Sep-Pak column pre-equilibrated with solution A. The column was washed once with 5 mL of solution A, followed by another wash with 5 mL of solution B (hexane: 2-propanol: acetic acid = 75:25:0.5, *V*/*V*/*V*). Finally, 8-isoprostane was eluted from the column with solution C (hexane: 2-propanol: acetic acid = 45:55:0.5, *V*/*V*/*V*) and dried under N_2 gas. The residue was dissolved in 1 mL of the assay buffer included in the 8-isoprostane ELISA kit (Cayman Chemical). The ELISA was performed according to the manufacturer's instructions without further

purification of the samples, and the absorbance was measured with a plate reader (V-Max; Molecular Dynamics, NJ, USA). The 8-isoprostane standards included in the ELISA kit were extracted in the same way as the samples to obtain a calibration curve, which was used to estimate the 8-isoprostane levels in the samples.

Effect of interfering substances on the assay

Interference with the 8-isoprostane assay was tested before and after the addition of free and conjugated-bilirubin (up to 342 μmol/L), hemoglobin (up to 5 g/L) and triacylglycerol (up to 55 mmol/L) to each plasma sample. A concentrated reagent set of the interfering substances was purchased from International Reagents Co. Ltd. (Hyogo, Japan).

Storage stability of plasma 8-isoprostane under various conditions

Plasma samples were collected from the 3 control subjects using special tubes as described above. Aliquots of the samples were separately stored at -80℃, 4℃ and 25℃. The 8-isoprostane levels were tested within 4 h after the blood collection and on d 1, 3, 7, 14, 21, 28 and 120 using fresh aliquots of the samples at each time point.

Determination of other lipid profiles and hepatic functions in blood samples

The concentration of TC, TG, LDL-C and HDL-C were determined by an enzymatic method (Kyowa Medics Co. Ltd, Tokyo, Japan). ApoA-I, apoB, apoE and Lp (a) were measured by using immunoturbidimetric assay kits (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan). AST and ALT were determined using UV method[22] and γ-GTP were determined by L-γ-glutamyl-3-carboxy-4-nitroanilid substrate method[23].

Genotyping of ALDH2

DNA was extracted from blood samples collected with EDTA•2Na using a commercially available kit (Sanko Pure Chemicals Co. Ltd., Tokyo, Japan). Mismatched PCR primers for determining the ALDH2 genotypes were designed with reference to the ALDH2 gene sequence (GenBank Accession No. AH002599). The wild-type allele (*ALDH2*1*) of ALDH2 was amplified using the forward and reverse primers CAAATTACAGGGTCAACTGCTATG and CCACACTCACAGTTTTCACTTC, respectively. The mutant type (*ALDH2*2*) of ALDH2 was amplified using the forward and reverse primers CAAATTACAGGGTCAACTGCTATG and CCACACTCACAGTTTTCACTTT, respectively. Amplification was performed in 25 μL of 1 × Qiagen PCR buffer containing 0.2 μmol/L of each ALDH2 primer, 200 μmol/L of each dNTP, and 2 U of HotStartTaq DNA polymerase (Qiagen, Hilden, Germany). The PCR conditions were denaturation at 95℃ for 10 min, followed by 35 cycles of amplification (30 s at 94℃, 30 s at 58℃ and 30 s at 72℃). After electrophoresis in a 25 g/L agarose gel, the 135-bp PCR products were stained with ethidium bromide and visualized under UV light.

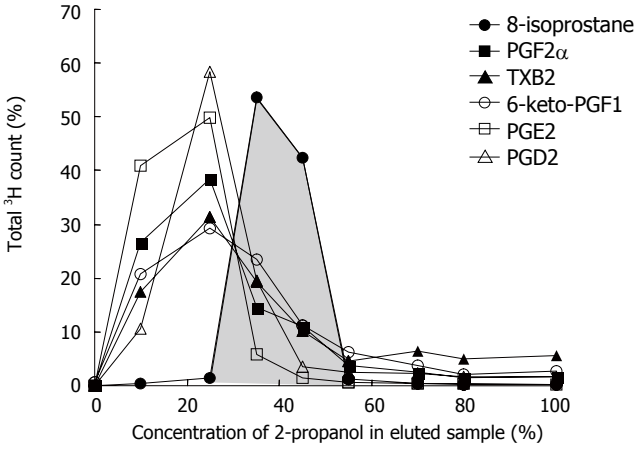

Figure 2 Results of the second extraction step using an NH_2 Sep-Pac column to isolate the plasma 8-isoprostane. Spiked plasma samples containing ^{3}H-labeled 8-isoprostane, PGF2α, TXB2, 6-keto-PGF1, PGE2 or PGD2 were used. Samples extracted with ODS gel were used to assess the absorption by, washing and elution from the NH_2 Sep-Pac column.

Statistical analysis

Statistical analyses of the data were performed by the paired *t*-test using the In Stat computer software (version 3.06; GraphPad Software Inc.). The correlation between two variables was calculated by the non-parametric Spearman rank coefficient test. A corrected value of $P < 0.05$ was considered statistically significant.

RESULTS

Extraction of plasma 8-isoprostane

Using ^{3}H-labeled 8-isoprostane and its related compounds as spiked tracers, optimal conditions for the two-step solid-phase extraction of plasma 8-isoprostane were carefully determined. The method was based on a procedure used in a radioimmunoassay for serum TXA2[21]. First, the plasma samples were treated with reverse-phase ODS gel to remove proteins and lipids. The 8-isoprostane bound to the gel was then eluted and separated from its related compounds on a Sep-Pac NH_2 column by stepwise elution with increasing concentrations of 2-propanol in the eluent. As shown in Figure 2, most of the 8-isoprostane eluted from the column at 55% 2-propanol, whereas the other compounds eluted at 10%-25% 2-propanol. The average yield of plasma 8-isoprostane in the overall extraction was estimated to be 67.1% by counting the ^{3}H-labeled 8-isoprostane in 5 spiked plasma samples.

Quantification of extracted 8-isoprostane by ELISA

Since it has been shown that plasma 8-isoprostane can be accurately measured using a commercially available ELISA kit after the two-step extraction, we next evaluated the analytical performance and accuracy of the overall ELISA for plasma 8-isoprostane. The detection limit of the ELISA kit was 2.2 ng/L, and this was defined as the concentration corresponding to the optical density of the zero calibrator plus 2SD. The reproducibility of this method estimated using the plasma samples from 3 control subjects was

Table 1 Plasma 8-isoprostane in three groups with different drinking habits (means ± SD)

	Plasma 8-isoprostane (ng/L)			Group Ⅲ *vs* Group Ⅰ	Group Ⅲ *vs* Group Ⅰ
	Group Ⅰ	Group Ⅱ	Group Ⅲ		
Total	20.3 ± 6.1 (n = 64)	20.9 ± 5.7 (n = 56)	26.6 ± 9.5 (n = 37)	t = 4.059 P < 0.0001 (CI: 3.222-9.387)	t = 3.587 P < 0.001 (CI: 2.523-8.783)
Male	22.5 ± 6.3 (n = 32)	21.1 ± 4.9 (n = 31)	23.7 ± 8.0 (n = 20)	t = 0.589 N.S. (CI: -2.832-5.181)	t = 1.464 N.S. (CI: -0.981-6.252)
Female	18.1 ± 5.0 (n = 32)	20.8 ± 6.6 (n = 25)	30.0 ± 10.3 (n = 17)	t = 5.494 P < 0.0001 (CI: 7.572-16.321)	t = 3.542 P < 0.005 (CI: 3.954-14.466)

CI: 95% Confidence Interval.

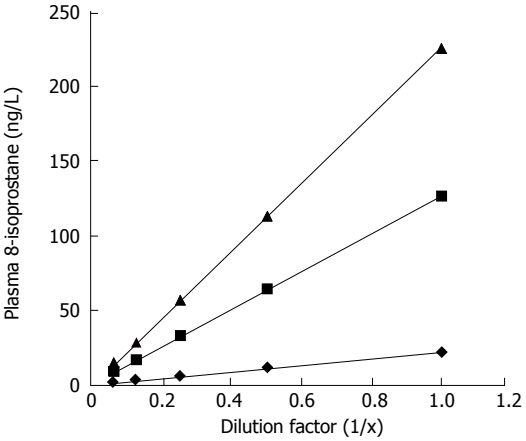

Figure 3 Dilution curves of plasma 8-isoprostane in 3 different plasma samples (▲ y = 227.6x-2.34, r = 0.999; ■ y = 126.3x + 0.06, r = 1.000; ◆ y = 21.8x-0.04, r = 0.999).

4.2%-6.3% for the within-run (8 repeats) and 0.8%-8.1% for the between-run (5 repeats) , respectively. To assess the linearity of the assay, the above mentioned plasma samples were diluted serially, extracted and measured for their 8-isoprostane levels. A good dilution linearity was obtained for the assay, as shown in Figure 3. Analytical recovery studies were carried out using 3 plasma samples containing 3 different concentrations of 8-isoprostane, revealing that the recovery rate ranged from 95.9%-97.8%. There were no significant differences between the 8-isoprostane levels in serum and those of the plasma collected with heparin, EDTA or EDTA + trasirol + indomethacin. All of the samples were freshly prepared and subjected to assay. No interference in the assay was observed for hemoglobin (up to 5 g/L), free bilirubin (up to 342 μmol/L), conjugated bilirubin (up to 342 μmol/L) or triacylglycerol (up to 55 mmol/L).

Storage stability of plasma 8-isoprostane

The 8-isoprostane in the plasma samples was stable for at least 120 d at -80℃, as long as freezing and thawing were avoided. However, 2 cycles of freezing at -80℃ and thawing at room temperature decreased the apparent 8-isoprostane levels by 30%. It was previously reported that plasma 8-isoprostane was stable for 6 mo at -80℃, and increased approximately 1.4-fold after 3 cycles of freeze and thawing[4]. Although the reason for this disagreement is not clear, it may be attributed to the method of blood collection, since we used blood collection tubes containing 10 mmol/L EDTA•3Na, 20 kU/L trasylol and 0.1 mmol/L indomethacin, while they used common evacuated tubes containing EDTA•2Na (2.5 mmol/L). The EDTA and indomethacin could function to prevent the induction of new synthesis of 8-isoprostane in plasma samples during storage. When the plasma samples were stored at 4℃, 8-isoprostane levels increased rapidly after 1 wk and were 3-4-fold higher after 28 d. When the samples were stored at 25℃, 8-isoprostane levels reached 15-50-fold the original values after 28 d.

Association of plasma 8-isoprostane levels with drinking habits

The mean level of the plasma 8-isoprostane in 157 healthy subjects using our method was 20.9 ± 9.3 ng/L and no age or gender differences were observed. For the drinking habits obtained by questionnaire, the subjects of each gender were divided into three groups according to their alcohol consumption, namely non-habitual drinkers (nondrinker, rare drinking ,Group I, n = 64), moderate drinkers (1-2 times drinking/wk Group II, n = 56), and habitual drinkers (3-5 times drinking /wk, Group III, n = 37), and the plasma 8-isoprostane levels were compared among these groups. In females, the plasma 8-isoprostane levels were significantly higher in Group Ⅲ (30.0 ± 10.3 ng/L) than in Group I (18.1 ± 5.0 ng/L, P < 0.0001) and Ⅱ (20.8 ± 6.6 ng/L, P < 0.005), but not males (Table 1). The serum γ-GTP levels in Group Ⅲ were elevated in both genders (males: 488 ± 335 nkat/L at 37℃; females: 298 ± 185 nkat/L at 37℃) compared with those in Group I (males: 343 ± 253 nkat/L at 37℃; females: 220 ± 70 nkat/L at 37℃; P < 0.05 each). No significant differences in AST and ALT were observed among the three groups. The plasma 8-isoprostane levels showed no correlations with AST, ALT, γ-GTP and various lipid parameters (TC, TG, LDL-C, HDL-C, ApoA-I, apoB and Lp(a)) in the subjects (Table 2).

Next, the subjects in each group were further divided into 3 groups according to their ALDH2 genotypes, and the plasma 8-isoprostane levels were compared along with those of AST, ALT and γ-GTP. For both the *ALDH2*1/1* and *ALDH2*2/1* genotypes, the plasma 8-isoprostane level was significantly higher in Group Ⅲ than that in Groups I and Ⅱ (Table 3) in both genders. This tendency was more prominent in females with the *ALDH2*21/1* genotype. Especially, the 8-isoprostane level was significantly higher in female habitual drinkers with the *ALDH2*2/1* than those with the *ALDH2*1/1* genotype (41.2 ± 12.3 *vs* 26.9 ± 7.7 ng/L, P < 0.0001). The mean level of AST in subjects with the *ALDH2*1/2* genotype was significantly higher in Group Ⅲ than in Group Ⅱ (22.3 ± 9.2 *vs* 17.1 ± 2.5 nkat/L at 37℃, P < 0.05). The γ-GTP level was significantly higher in subjects with the *ALDH2*1/1* genotype than those with the *ALDH2*1/2* genotype (478 ± 396 *vs* 303 ± 198 nkat/L at 37℃, P < 0.005) . Three healthy subjects (2 males, 1 female) were given alcohol (0.5-1.3 g/kg), and the changes in plasma

Table 2 Spearman correlation coefficients between biochemical parameters (n = 157)

	TC	TG	HDL-C	LDL-C	apoA I	apoB	Lp(a)	AST	ALT	γ-GTP	8-isoprostane
Age	0.373	0.122	0.012	0.324	0.088	0.381	0.055	0.116	0.139	0.436	-0.062
TC	-	0.356	0.272	0.818	0.233	0.799	0.211	0.091	0.123	0.273	-0.042
TG	-	-	-0.215	0.446	-0.037	0.565	0.128	0.182	0.247	0.325	-0.020
HDL-C	-	-	-	-0.209	0.849	-0.242	-0.001	-0.068	-0.250	-0.059	-0.101
LDL-C	-	-	-	-	-0.169	0.965	0.264	0.122	0.248	0.235	0.080
apoA I	-	-	-	-	-	-0.155	-0.038	0.022	-0.121	0.083	-0.118
apoB	-	-	-	-	-	-	0.295	0.171	0.296	0.335	0.069
Lp(a)	-	-	-	-	-	-	-	0.057	0.060	-0.029	0.124
AST	-	-	-	-	-	-	-	-	0.897	0.411	-0.034
ALT	-	-	-	-	-	-	-	-	-	0.509	0.002
γ-GTP	-	-	-	-	-	-	-	-	-	-	-0.085

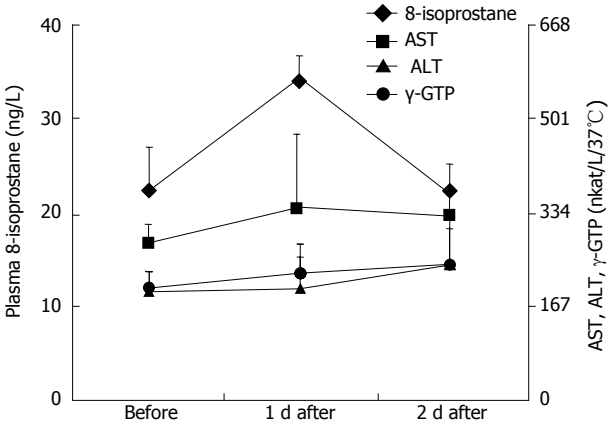

Figure 4 Changes in AST, ALT, γ-GTP and plasma 8-isoprostane after alcohol intake. The levels of plasma 8-isoprostane in the 3 individuals increase significantly on d 1 after drinking, but return to their original levels on d 2. No significant changes are observed in AST, ALT and γ-GTP.

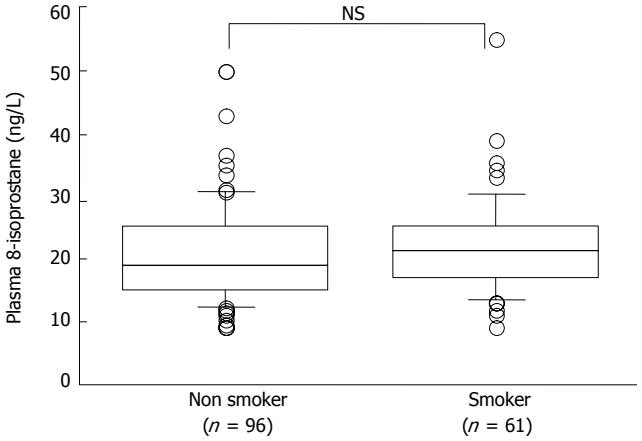

Figure 5 Effect of smoking habit on plasma 8-isoprostane levels. The levels of plasma 8-isoprostane were not significantly different between non smokers and smokers (21.5 ± 7.3 *vs* 22.8 ± 7.4 ng/L).

8-isoprostane were assayed together with the serum AST, ALT and γ-GTP. Plasma 8-isoprostane significantly increased on d 1, and returned to its original level on d 2 (Figure 4).

Association of plasma 8-isoprostane with smoking habits

The same population of 157 healthy subjects were divided into two groups: non-smokers (n = 96; age 36.7 ± 8.2 years) and smokers (n = 61; age 35.3 ± 8.7 years), and their plasma 8-isoprostane levels were compared. As shown in Figure 5, no significant difference of plasma 8-isoprostane level was observed between these two groups (21.5 ± 7.3 *vs* 22.8 ± 7.4 pg/mg).

DISCUSSION

We have developed a simple and accurate method for quantifying plasma 8-isoprostane by employing a combination of two-step solid-phase extraction of samples and a commercially available ELISA kit.

When the plasma 8-isoprostane level was measured by using our method, the mean value of 157 healthy volunteers was 20.9 ± 9.3 ng/L, which is almost equal to the reported values using GC/MS or LC/MS[5,24,25]. The level of plasma 8-isoprostane in 4 healthy subjects among these samples measured directly by ELISA without extraction was more than 20-fold higher than those obtained by the combination of the two-step extraction and ELISA (651.5 ± 149.2 *vs* 24.4 ± 4.1 ng/L). According to 8-Isoprostane EIA kit booklet, although the cross-reactivity of the anti 8-isoprostane antibody employed in the ELISA kit is reported to be low, the extraction of plasma 8-isoprostane from plasma prior to the assay is indispensable. In fact, it has been reported that various 8-isoprostane analogues and related compounds are present at dozens to hundreds of times than the concentration of 8-isoprostane in biological fluids[26].

In an association study of plasma 8-isoprostane with drinking habits, heavy drinkers were higher than non-habitual drinkers and moderate drinkers. However, the plasma 8-isoprostane level was not significantly different when compared among the 3 male groups. Also, the plasma 8-isoprostane levels were significantly higher in heavy drinkers than in non-habitual and moderate drinkers. This tendency was more prominent in females, and with the *ALDH2*21/1* genotype. In other words, the 8-isoprostane level was significantly higher in female habitual drinkers with the *ALDH2*2/1* than with the *ALDH2*1/1* genotype. These results suggest that excessive drinking may increase oxidative stress, especially in females with the *ALDH2*2/1* genotype. When the same amount of alcohol is ingested, females tend to show

Table 3 Plasma 8-isoprostane analyzed by ALDH2 genotype in three groups with different drinking habits (means ± SD)

		Plasma 8-isoprostane (ng/L)			Group Ⅲ *vs* Group Ⅰ	Group Ⅲ *vs* Group Ⅰ
		Group Ⅰ	Group Ⅱ	Group Ⅲ		
*ALDH2*1/1*	Male	20.2 ± 5.8	19.8 ± 5.0	22.8 ± 5.4	t = 1.165, N.S. (CI: -1.988-7.137)	t = 1.664, N.S. (CI: -0.684-6.758)
	Female	19.0 ± 5.8	21.6 ± 6.6	26.9 ± 7.7	t = 2.754, P < 0.05 (CI: 1.926-13.808)	t = 1.897, N.S. (CI: -0.447-10.887)
	Total	19.6 ± 5.7 (n = 22)	20.6 ± 5.8 (n = 33)	24.6 ± 6.7 (n = 27)	t = 2.776, P < 0.01 (CI: 1.375-8.616)	t = 2.471, P < 0.05 (CI: 0.757-7.219)
*ALDH2*1/2*	Male	23.7 ± 7.5 (n = 11)	22.6 ± 4.4 (n = 12)	28.5 ± 14.8 (n = 4)	t = 0.834, N.S. (CI: -7.517-16.976)	t = 1.281, N.S. (CI: -3.934-15.600)
	Female	16.0 ± 4.0 (n = 15)	19.9 ± 7.4 (n = 8)	41.2 ± 12.3 (n =3)	t = 6.930, P < 0.0001 (CI: 17.459-32.848)	t = 3.622, P < 0.01 (CI: 8.017-34.683)
	Total	19.3 ± 6.8 (n = 26)	21.5 ± 5.8 (n = 20)	33.9 ± 14.4 (n = 7)	t = 3.898, P < 0.001 (CI: 6.973-22.276)	t = 3.267, P < 0.005 (CI: 4.586-20.221)
*ALDH2*2/2*	Male	22.2 ± 5.5 (n = 6)	-	-	-	-
	Female	21.8 ± 2.0 (n = 3)	-	-	-	-
	Total	22.0 ± 4.7 (n = 9)	-	-	-	-

CI: 95% confidence interval.

a higher concentration of blood alcohol than males due to their lower body weight and higher ratio of adipose tissue into which alcohol shows poor penetration. In addition, female hormones, such as estradiol, inhibit the activity of ADH, contributing to the increased concentration of blood alcohol[27].

Changes in plasma 8-isoprostane levels after alcohol (0.5-1.3 g/kg) intake for 3 d, showed 8-isoprostane significantly increased on d 1, and returned to its original level on d 2. It was previously reported that alcohol consumption induced lipid peroxidation in healthy volunteers, and urinary 8-isoprostane increased in a dose- and time-dependent manner reaching its peak at 0-6 h after ingestion[9], and through induction of the CYP450 2E1 isozyme, alcohol intake may increase the generation of reactive oxygen intermediates that have the potential to peroxidize lipids[28,29]. Similarly, our results may suggest that alcohol ingestion induced oxidative stress in a relatively short time after drinking.

It has been reported that smoking induces oxidative stress and increases urinary 8-isoprostane[30]. Therefore, we compared plasma 8-isoprostane levels in non-smokers and smokers. In contrast to urinary 8-isoprostane, no difference in plasma 8-isoprostane was observed between these 2 groups This is consistent with a previous report measured by LC/MS[5]. The plasma 8-isoprostane may be rapidly metabolized since 75% of plasma 8-isoprostane is excreted in the urine within 4.5 h[31].

In conclusion, we have developed a simple and accurate method for quantifying plasma 8-isoprostane by employing a combination of two-step solid-phase extraction of samples and a commercially available ELISA kit. Our method fulfilled all the requirements for use in routine clinical assays with respect to sensitivity, intra- and inter-assay reproducibility, accuracy and dynamic assay range. However, the clinical utility of plasma 8-isoprostane for drinking and smoking habits was limited. Further studies using a large population is required for a final conclusion for an association of plasma 8-isoprostane with drinking and smoking habits.

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