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Correlation of urinary furan with plasma γ-glutamyltranspeptidase levels in healthy men and women

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Abstract

Furan is a colorless, volatile compound that is found in heat-treated foods, such as canned and jarred foods, at levels up to 100 ppb. When animals ingest high doses, furan metabolites, such as *cis*-2-butene-1,4-dial, cause severe hepatotoxicity and carcinogenicity. However, the levels and effects of furan on humans are not known. Therefore, we measured urinary furan in 100 healthy individuals consumed normal diet (49 men, 51 women) using solid phase micro-extraction–gas chromatography–mass spectrometry (SPME–GCMS). Urinary furan was detected in 56 subjects (31 males, 25 females) and ranged up to 3.14 ppb. In individuals with detectable urinary furan, the level of γ-glutamyltranspeptidase (γ-GT), a marker for liver damage, was strongly correlated with the urinary furan concentration ($r = 0.56$, $p < 0.0001$). Linear regression analysis indicated that the urinary furan level was significantly associated with γ-GT in both univariate ($p < 0.0001$) and multiple ($p = 0.0001$) models including age, sex, body weight, and blood pressure as covariates. To our knowledge, this is the first study to measure detectable levels of furan in human urine. These levels of urinary furan, which may be dietary origin, could be hepatotoxic in humans; therefore; the metabolic fates and potential toxicity of dietary furan in humans should be investigated further.

Keywords: Urinary furan; Human; Hepatotoxicity; γ-Glutamyltranspeptidase

1. Introduction

Furan is a colorless, volatile, heterocyclic organic compound with a boiling point close to room temperature (31.4 °C). It has been classified as a possible carcinogen by the International Agency for Research on Cancer (IARC) since 1995 (McGregor et al., 1995). In 2004, the formation of furan in foods during heat treatment, including cooking, jarring, and canning (FDA, 2004a,b,c), was confirmed by research at the US food and drug administration (FDA), and interest has focused on the potential health risks of dietary furan. The primary source of furans in foods is the thermal degradation and rearrangement of carbohydrates, such as glucose, lactose, and fructose (Maga, 1979). Ascorbic acid derivatives and polyunsaturated fatty acids could be converted into furan (Becalski and Seaman, 2005). Perez-Locas et al. (Perez Locas and Yaylayan, 2004) proposed a pyrolysis-GC–MS (gas chromatography–mass spectrometry) model system to confirm whether furan could form from ^{13}C-labled sugars in the presence of amino acids and ascorbic acid.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotrasferase; FDA, food and drug administration; GC–MS, gas chromatography–mass spectrometry; γ-GT, γ-glutamyltranspeptidase; HDL, high density lipoprotein; LDL, low density lipoprotein; SIM, selective-ion monitoring mode; SPME–GCMS, solid phase micro-extraction–gas chromatography–mass spectrometry.

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Furan levels measured in a number of foods, such as baby foods, coffee, stew, and canned meat (FDA, 2004a; Hasnip et al., 2006) were as high as 100 ppb. Based on these results, the US FDA estimated that the respective average furan exposure in adults and infant foods was 0.3 and 0.4 μg/kg body weight per day. The 90th percentile of furan exposure in adult and infant foods was 0.6 and 1.0 μg/kg body weight per day, respectively (FDA, 2004b,c). To date, however, urinary furan levels in humans have not been reported.

Furan is hepatocarcinogenic in rats and mice (NTP, 1993), inducing hepatocellular carcinomas and cholangiocarcinomas. Furan may be hepatotoxic in rats by increasing plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Fransson-Steen et al., 1997; Hamadeh et al., 2004). *cis*-2-Butene-1,4-dial, which is hepatocarcinogenic and hepatotoxic, forms from furan in the liver and is transformed by cytochrome P450 in the liver (Chen et al., 1995; Peterson et al., 2005).

Since these toxicity studies examined laboratory animals given extremely high doses of furan, we do not know whether exposure to dietary furan, mainly via the intake of heat-treated foods, is hepatotoxic. Therefore, we measured urinary furan, which may be a surrogate marker for furan levels *in vivo*, and then investigated the association between urinary furans and liver enzyme levels in humans.

2. Materials and methods

2.1. Chemicals

Furan [CAS 110-00-9] and d4-furan [CAS 6142-90-1] were purchased from Sigma–Aldrich (St. Louis, MO, USA). Water (HPLC grade) and methanol were obtained from J.T. Baker (Phillipsburg, NJ, USA). Working solutions of furan and d4-furan (4 μg/ml), prepared from the 2 mg/ml methanolic standard solution by dilution in water, were stored at 4 °C. Kits for triglyceride and high-density lipoprotein (HDL) measurements were purchased from Asan Pharm. Co. (Kyeonggi-do, Korea).

2.2. Plasma and urine samples

Between June and September 2005, plasma and urine samples were obtained from 100 healthy subjects (49 males, 51 females) between 30 and 70 years old. None of the individuals was being treated for any disease at the time of the study and all had unremarkable clinical histories. Plasma and urine samples were collected at the Korea University Anam Hospital (Seoul, Korea) according to a protocol approved by the Institutional Review Board. All participants gave informed consent, and the procedure followed institutional guidelines. All the volunteers were confirmed by dietician to intake regular Korean diet and did not consume large amount of furan containing food items. Volunteers rarely consume smokes salmon, powdered milk (<1 serving/month); apple juice, spaghetti (<2 servings/wk); potato and peas (<3 servings/wk); coffee (<1 cup/day); canned or jarred food (<1 serving/wk). In order to minimize the loss of furan in the urine samples, we collected fasting urine samples on ice, then immediately placed on dry ice after collection, and stored the samples in −80 °C. Also, when handling the samples, the experimenters wore cryoglobes cooled down on ice and try to minimize the time exposed on room temperature. To validate the procedure, we performed recovery test by adding 32 ng of internal standard (d4-furan) in the random samples. The recovery ratio was 101.6% and the limit of quantification (LOQ) was 1.0 ppb. Blood was collected after 12-h fasting and the fasting blood samples (10 ml) were collected in EDTA containing glass tubes on ice and centrifuged to separate plasma. The clinical samples were stored at –80 °C until the furan levels were measured.

2.3. Clinical analyses of blood samples

Blood glucose, total cholesterol, AST, ALT, and γ-GT levels in whole blood were measured immediately after collecting the blood samples using standard automated methods at the Laboratory for Clinical Tests of Korea University Anam Hospital. Blood triglyceride and HDL cholesterol levels were measured from plasma using a colorimetric enzymatic method. LDL (low-density lipoprotein) cholesterol was calculated using the Friedewald equation (Friedewald et al., 1972).

2.4. Measurement of urinary furan levels

2.4.1. Calibration curve of furan

Standard samples at appropriate concentrations were injected with each analytical series to produce a calibration curve. The calibration standards were prepared by injecting various volumes of aqueous furan solutions (1–8 μl) and a fixed amount (4 μl) of d4-furan into a 20-ml headspace vial. This gave furan solutions ranging from 0.4 to 3.2 ppb in the vial. The vial was sealed immediately after injection.

For recovery test of furan, furan (1.6 ppb) and d4-furan (1.6 ppb) were spiked to sample which has not furan. The sample was prepared and analyzed by SPME–GC–MS as described above.

2.4.2. Sample preparation for furan analysis

To avoid vaporizing the furan, urine samples were cooled on ice immediately after collection and stored at –80 °C until analysis. In a 20-ml headspace vial, 10 ml of water were added to 1 ml of urine. d4-Furan was used as an internal standard into a sample vial. A d4-furan is almost same as furan except for having deuterium instead of hydrogen. This internal standard was used to validate the recovery of urinary furan. We calculated furan concentration in urine based on PAR (peak area ratio) of furan and d4-furan. Though furan (analyte) could be loused during sample preparation, the PAR is constant after GC/MS analysis. For analysis, a fixed amount (4 μl) of internal standard (4 μg/ml: d4-furan) was mixed in the vial.

2.4.3. Solid phase micro-extraction–gas chromatography–mass spectrometry (SPME–GC–MS) analysis

The furan in urine samples was analyzed using a published method with minor modifications (Becalski and Seaman, 2005; Forsyth et al., 2004; Goldmann et al., 2005; Perez Locas and Yaylayan, 2004). The fiber in the SPME experiment was 75 μm carboxen-poly(dimethylsiloxane) (CAR-PDMS: Supelco, Bellefonte, PA, USA). The fiber was exposed at 60 °C for 20 min under constant agitation (200 rpm) in a 20-ml sample vial. Before use, the fiber was conditioned in the injection port of the gas chromatograph at 250 °C for 1 h. The injection was carried out by desorbing the fiber for 5 min at 250 °C. The fiber was baked for 5 min at 250 °C before the next extraction to blow out any residual analyte. An Agilent 6890 gas chromatograph (Agilent Technology, Palo Alto, CA, USA) equipped with an MSD5975 mass spectrometer (Agilent Technology) was used for the GC–MS analysis. The chromatographic separation was carried out in an HP-PLOT Q column (0.32 mm I.D × 15 m length × 20 μm film; J & W Scientific, Folsom. CA, USA). Helium was used as the carrier gas at a flow rate of 44 cm/s; the gas chromatograph was operated in splitless mode with the injector held at a temperature of 250 °C. The amount of sample injected was 1 μl. The following GC oven temperature program was applied: 50 °C for 5 min, 25 °C/min to 230 °C, 230 °C for 2 min. The mass spectrometer was operated in selective-ion monitoring mode (SIM) by recording the current of the following ions: m/z 68 and 39 for furan and m/z 72 and 42 for d4-furan. The corresponding ion ratios for furan and d4-furan were determined for each

measurement. In SIM mode, full scan electron ionization (EI) data were acquired simultaneously to determine appropriate masses for SIM.

2.5. Statistical analysis

The data were analyzed using SAS software, version 9.1 (SAS Institute, Cary, USA). Descriptive statistics were calculated using the PROC UNIVARIATE procedure. The measured variables were age, height, body weight, systolic and diastolic blood pressure, urinary pH, hemoglobin levels, plasma glucose, total, HDL, and LDL cholesterol, triglycerides, and urinary furan levels. The Duncan test was used to compare the differences among means by sex and age. Pearson correlation analysis examined the correlation among urinary furan concentrations, liver enzyme levels, and other clinical characteristics. Both univariate and multiple linear regression analyses were performed with the PROC GLM procedure using γ-GT, AST, and ALT as the dependent variables. Age, sex, body weight, and diastolic blood pressure were included as covariates in a multiple model predicting γ-GT. Since diastolic blood pressure was more strongly correlated with γ-GT than systolic blood pressure was, it was included as a covariate in the multiple models. Linear regression analysis was performed using individual data that had detectable urinary furan levels. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Subject characteristics and liver enzyme levels

Blood and urine samples were collected from 100 healthy subjects who were not being treated for any disease. There were approximately ten subjects in each group classified according to sex and age. Table 1 shows the characteristics of the subjects. The males tended to be heavier than the females. The body mass index (BMI) was the highest in males in their 70s and lowest in females in their 30s. Total cholesterol levels did not differ significantly among all groups, although, interestingly, females in their 30s had the highest HDL levels, but the lowest LDL levels.

Table 1
Characteristics of the study subjects

Characteristic	Age group					
	30s	40s	50s	60s	70s	Average
Subjects	22	23	22	21	12	
Males	11	11	10	11	6	9.8
Females	11	12	12	10	6	10.2
Body weight, kg						
Males	64.1 ± 5.6^{abc}	69.5 ± 9.9^{a}	68.0 ± 5.9^{ab}	67.7 ± 5.7^{ab}	68.5 ± 8.6^{ab}	67.5 ± 7.2
Females	54.1 ± 5.1^{e}	57.2 ± 6.3^{de}	56.5 ± 3.2^{de}	61.6 ± 8.8^{bcd}	60.3 ± 10.3^{cde}	57.6 ± 6.9
Body mass index, kg/m²						
Males	22.7 ± 2.5^{bc}	24.1 ± 3.0^{ab}	24.2 ± 1.7^{ab}	24.5 ± 2.1^{ab}	25.2 ± 2.4^{ab}	24.0 ± 2.4
Females	21.6 ± 1.8^{c}	23.2 ± 2.2^{bc}	23.7 ± 1.8^{abc}	25.1 ± 3.0^{ab}	26.0 ± 4.2^{a}	23.7 ± 2.8
Total cholesterol, mg/dl						
Males	193.8 ± 31.2^{a}	204.1 ± 28.1^{a}	209.0 ± 44.5^{a}	202.5 ± 24.0^{a}	198.8 ± 24.8^{a}	201.8 ± 30.8
Females	184.7 ± 21.3^{a}	184.3 ± 26.4^{a}	202.7 ± 33.1^{a}	212.9 ± 51.7^{a}	210.5 ± 25.0^{a}	197.4 ± 34.4
HDL cholesterol, mg/dl						
Males	41.4 ± 16.3^{ab}	43.1 ± 13.3^{ab}	46.7 ± 9.4^{ab}	38.8 ± 9.7^{ab}	36.7 ± 10.4^{b}	41.7 ± 12.2
Females	48.9 ± 10.5^{a}	40.5 ± 10.8^{ab}	43.8 ± 7.2^{ab}	44.9 ± 10.3^{ab}	41.8 ± 12.8^{ab}	44.1 ± 10.2
LDL cholesterol, mg/dl						
Males	124.9 ± 28.9^{ab}	134.6 ± 31.2^{ab}	136.4 ± 45.9^{ab}	142.1 ± 23.1^{ab}	141.7 ± 31.2^{ab}	135.6 ± 32.0
Females	116.3 ± 22.5^{b}	122.0 ± 26.2^{ab}	144.2 ± 28.7^{ab}	143.2 ± 54.3^{ab}	152.5 ± 23.7^{a}	133.7 ± 34.7
Triglyceride, mg/dl						
Males	135.7 ± 91.0^{a}	132.3 ± 52.2^{ab}	129.7 ± 54.5^{ab}	107.7 ± 49.2^{ab}	101.9 ± 50.3^{ab}	123.0 ± 60.8
Females	97.5 ± 55.5^{ab}	108.5 ± 2.3^{ab}	73.5 ± 26.4^{b}	124.1 ± 77.6^{ab}	81.1 ± 18.2^{ab}	97.7 ± 53.2
Glucose, mg/dl						
Males	86.3 ± 5.4^{d}	97.7 ± 10.5^{ab}	95.6 ± 7.2^{abc}	93.7 ± 7.7^{bcd}	198.8 ± 24.8^{a}	94.3 ± 9.5
Females	92.1 ± 7.0^{bcd}	90.0 ± 5.85^{bcd}	88.7 ± 5.0^{cd}	94.0 ± 10.0^{bcd}	92.3 ± 7.4^{bcd}	91.2 ± 7.1
Aspartame aminotransferase, U/L						
Males	22.8 ± 9.5^{ab}	24.4 ± 8.3^{ab}	23.2 ± 7.6^{ab}	22.5 ± 5.7^{ab}	22.0 ± 5.7^{ab}	23.1 ± 7.4
Females	19.6 ± 4.4^{ab}	17.4 ± 3.2^{b}	22.2 ± 3.4^{ab}	23.0 ± 5.6^{ab}	25.0 ± 6.5^{a}	21.0 ± 5.0
Alanine aminotransferase, U/L						
Males	27.1 ± 15.2^{a}	26.8 ± 19.2^{a}	24.7 ± 10.9^{a}	20.7 ± 7.4^{a}	21.8 ± 4.4^{a}	24.5 ± 13.0
Females	17.9 ± 8.5^{a}	21.8 ± 19.7^{a}	17.8 ± 4.7^{a}	19.5 ± 9.0^{a}	19.0 ± 7.2^{a}	19.2 ± 11.3
γ-Glutamyltranspeptidase, U/L						
Males	49.5 ± 42.6^{ab}	32.6 ± 8.3^{ab}	85.7 ± 187.8^{a}	31.7 ± 27.1^{ab}	35.3 ± 10.0^{ab}	47.4 ± 87.2
Females	17.2 ± 7.5^{b}	21.2 ± 10.5^{ab}	16.8 ± 8.1^{b}	22.1 ± 8.6^{ab}	23.5 ± 15.1^{ab}	19.7 ± 9.7

Each value in the table is the mean ± standard deviation. Means within each characteristic with different letters are significantly ($p < 0.05$) different.

Moreover, the HDL levels were lowest in males in their 70s and LDL levels were highest in females in their 70s. Triglyceride levels were highest in males in their 30s and lowest in females in their 50s, although the differences with the other groups were not significant. At 198.8 mg/dl, glucose levels in males in their 70s were much higher than in the other groups. AST levels were similar and did not differ significantly across groups. The γ-GT levels were lowest in females in their 50s (16.8 mg/dl) and highest in males in their 50s (85.7 U/L) because one individual had a very high γ-GT of 620 U/L. The overall average for males was higher than for females for each parameter, except for HDL levels, and the differences in body weight, ALT, γ-GT, and triglyceride levels were significant ($p < 0.05$).

3.2. Urinary furan levels

Urinary furan was detected in 56 individuals (31 males, 25 females, Table 2) and was not detected in 44 individuals (18 males, 26 females, Table 2). The furan concentration in urine according to sex and age was shown in Fig. 1. The highest furan level was 3.14 ppb, and 15 urine samples exceeded the limit of quantification (LOQ), which was 1.00 ppb in this study. In urine, the mean furan levels were higher in males than in females and a similar trend was observed for the plasma furan.

As described in method section, urine samples were handled with extra caution to minimize the loss of furan. Samples were collected on ice, immediately put on dry ice, and stored at −80 °C till analysis. The experimenters wore cryo-globes cooled down on ice and try to minimize the time exposed on room temperature. To validate the procedure, we performed recovery test by adding 32 ng of internal standard (d4-furan) in the random samples. Recovery rate of urine sample was 101.6 ± 1.85% and the limit of quantification (LOQ) was 1.0 ppb. Urinary furan was detectable in 56 subjects (31 males, 25 females) and was undetectable in 44 subjects. This is probably mainly due to low consumption of dietary furan. As described in the method section, these individuals ate regular Korean diet that does not contain high level of dietary furan. Additionally, reported furan metabolism rate is higher than the metabolism rate in rodents, which may accelerate the metabolic degradation of the furan in humans. In healthy individuals, dietary furan might be quickly metabolized thus present at extremely low concentration, e.g. >1 ppb. We

Table 2
Correlation coefficients (r) between the clinical variables and liver enzymes

	Age	Male gender	Body weight	SBP	DBP	Glucose	AST	ALT	γ-GT	Urinary furan
AST	0.01	−0.01	0.21	0.22	0.20	0.14	–	–	–	0.17
ALT	−0.19	0.24*	0.37***	0.11	0.03	0.14	0.78‡	–	–	−0.01
γ-GT	0.004	0.18	0.07	0.32**	0.47†	0.27**	0.48†	0.27**	–	0.56‡

SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Significant at $0.05 < p < 0.1$.
** Significant at $p < 0.05$.
*** Significant at $p < 0.01$.
† Significant at $p < 0.001$.
‡ Significant at $p < 0.0001$.

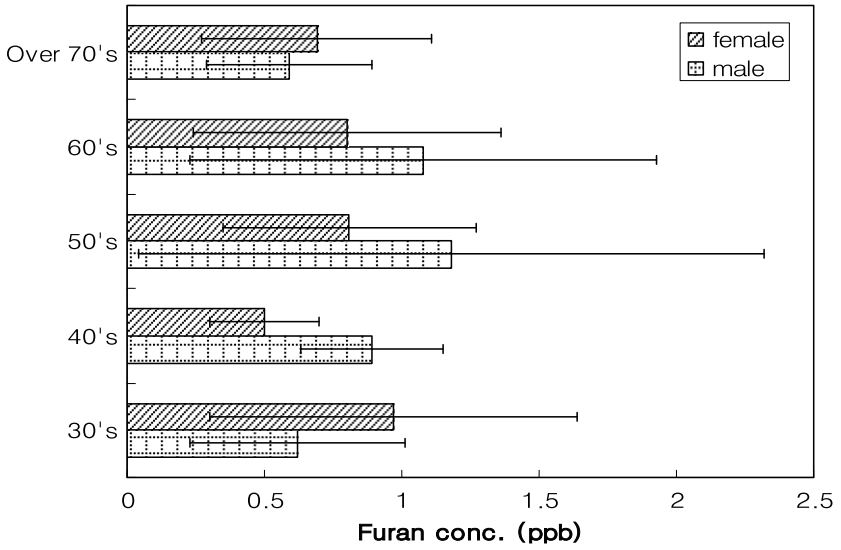

Fig. 1. Furan concentration in urine as the sex and age. The graph shows average urinary furan levels in each age group. Error bar indicates standard deviation.

believe these may be major reasons why urinary furan was not detectable in many individuals. We additionally measured plasma furan levels, however, were unable to quantify detectable amount of furan in human plasma samples (data not shown).

3.3. Correlation between liver enzymes and urinary furan levels

The ALT, AST, and γ-GT are conventional biomarkers for assessing hepatotoxicity. Since furan biotransformation occurs in the liver (Kedderis and Held, 1996) and may cause hepatotoxicity, we estimated the correlations among the plasma liver enzymes, urinary furan levels, and other clinical characteristics. As expected, the AST, ALT, and γ-GT were significantly positively correlated (Table 2). Interestingly, the urinary furan levels were strongly correlated with γ-GT levels, but not with the AST and ALT levels. The correlation coefficient between γ-GT and the urinary furan levels was 0.56 ($p < 0.001$).

3.4. Linear regression analysis

To further test the association between liver enzymes and urinary furan levels, we performed a linear regression analysis. In the univariate analysis, liver enzymes were weak, but significantly, associated with the plasma triglyceride levels: AST ($\beta = 0.03$, $p = 0.02$), ALT ($\beta = 0.06$, $p = 0.02$), and γ-GT ($\beta = 0.4$, $p = 0.02$) (Table 3). Urinary furan was not associated with AST or ALT, but was strongly associated with γ-GT levels (estimate = 78.42, $p < 0.0001$). The multiple model for predicting γ-GT levels included age, sex, body weight, triglyceride levels, and diastolic blood pressure as covariates. The association became weaker, but was still highly significant (estimate = 5.68, $p = 0.0001$) (Table 4).

Table 4
Multiple regression model for predicting plasma γ-GT levels

Variable	γ-GT	
	Parameter estimate	*p*
Age (y)	−0.46	0.55
Male gender	14.1	0.49
Body weight (kg)	−0.69	0.63
Triglyceride (mg/dl)	0.25	0.11
DBP (mmHg)	3.46	0.003
Urinary furan (ppb)	5.68	0.0001

DBP, diastolic blood pressure.

4. Discussion

Furan is a potentially carcinogenic substance produced during heat treatment in a number of foods. To our knowledge, furan is not formed directly *in vivo*; thus, the urinary furan concentration should be a surrogate marker for dietary furan intake. In this study, we examined whether urinary furan levels could be potentially associated with liver toxicity. To investigate this, we recruited 100 subjects matched for age (10-year intervals) and sex. All subjects were disease free and nearly all had normal clinical characteristics, and the values were fairly similar among the groups classified according to sex and age. All of volunteers consumed regular Korean diet and did not intake extreme amount of food items with high levels of furan. Furan was detected in 56 urine samples, with higher levels in males than in females.

Previous studies have reported the effects of furan on plasma lipid levels in animal models. In rats, furan

Table 3
Univariate linear regressions to predict liver enzyme levels

Dependent variable	AST		ALT		γ-GT	
Variables	Parameter estimate	*p*-Value	Parameter estimate	*p*-Value	Parameter estimate	*p*-Value
Age (y)	0.003	NS	−0.17	NS	0.03	NS
Male gender	−0.10	NS	5.46	NS	28.8	NS
Height (cm)	−0.03	NS	0.36	NS	1.48	NS
BW (kg)	0.16	NS	0.54	0.006	0.73	NS
SBP (mmHg)	0.12	NS	0.11	NS	2.41	0.02
DBP (mmHg)	0.14	NS	0.05	NS	4.55	0.0003
Urinary pH	−0.61	NS	0.04	NS	−5.52	NS
Hemoglobin	−0.04	NS	1.36	NS	6.46	NS
Glucose (mg/dl)	0.10	NS	0.19	NS	2.63	NS
Total cholesterol (mg/dl)	−0.03	NS	−0.05	NS	−0.04	NS
AST (U/L)	–	–	1.46	<.0001	6.52	0.0002
ALT (U/L)	0.413	<.0001	–	–	1.93	0.04
γ-GT (U/L)	0.04	0.0002	0.04	0.04	–	–
LDL cholesterol (mg/dl)	−0.006	NS	0.0004	NS	−0.06	NS
HDL cholesterol (mg/dl)	0.07	NS	0.05	NS	−0.15	NS
Triglyceride (mg/dl)	0.03	0.02	0.06	0.02	0.40	0.03
Urinary furan (ppb)	1.82	NS	−0.0007	NS	78.42	<0.0001

BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoproteins; HDL, high-density lipoproteins; NS, $p > 0.05$.

exposure increased the total cholesterol and triglyceride levels (Hamadeh et al., 2004), and furan treatment led to a significant 2- to 3-fold increase in bile acids in blood compared to a control group (Fransson-Steen et al., 1997). However, in our study, urinary furan levels in individuals with undetectable levels was not correlated with the plasma lipid levels including total and LDL cholesterol and triglyceride levels, thus the associations between furan and plasma lipid levels in humans observed in our experiment were weaker than the findings in rodents. This may be due to relatively low levels of urinary furan in the human subjects. Low level of urinary furan may be resulted from the elevated metabolism of furan in humans compared to rats, as reported by several groups. This could enhance the degradation of furan during digestion and metabolism, which could result in low urinary levels of furan. There is evidence that furan catabolism in humans is much faster than in rodents. The hepatic concentration of *cis*-2-butene-1,4-dial, a furan degradation intermediate, is approximately 3 and 10 times lower in humans than in rats and mice, respectively (Kedderis and Held, 1996). The initial rates of furan oxidation in the rat, mouse, and human liver are approximately 13, 24, and 37 times greater than the respective rates of blood flow delivery of furan to the liver after 4-h exposure to 300 ppm (Burka et al., 1991). In addition, experiments in rats with ^{14}C-furan showed that furan is absorbed rapidly, metabolized extensively, and eliminated after ingestion; the highest concentration of the absorbed dose is retained in the liver (Burka et al., 1991). Moreover, a number of urinary metabolites of furan have been observed, but not identified. These data suggest that the low levels of urinary furan may be due to the rapid elimination of furan during metabolism. In addition, dietary furan could be vaporized during digestion. This is an important issue and could be examined with the administration of a known amount of furan *in vivo*, and could be readily tested on animals but probably not be doable on humans. Although we were unable to estimate total loss of furan either by vaporization during digestion or by degradation during metabolism, we found that dietary furan could be measurable in urine and its level was associated with γ-GT, a marker for liver toxicity. Another potential explanation for the low levels of urinary furan is the dietary pattern of study subjects. According to our questionnaire data, none of the subjects consumed abnormally high levels of furan containing food items. They rarely consumed smokes salmon; powdered milk (<1 serving/month); apple juice, spaghetti (<2 servings/wk); potato and peas (<3 servings/wk); coffee (<2 cup/day). We believe this was one of the reasons why urinary furan was not detectable in many individuals and low levels were found among study subjects.

On the other hand, an animal study also investigated the correlation between gene expression and liver pathology on exposure to furan, using microarray tools to assess the clinical chemistry and pathology parameters (Hamadeh et al., 2004). They found that genes involved in cell proliferation and inflammation pathways were elevated in rat liver by exposure to furan. They have also shown that the endpoint of their gene expression profile is in agreement with clinical findings and pathology changes caused by proliferation and inflammation in rat livers. In this study, we have shown that the level of γ-GT, a marker for liver damage, was strongly correlated with the urinary furan concentration ($r = 0.56$, $p < 0.0001$). Several data suggest that γ-GT could be an indicator for inflammation such as in adjuvant arthritis (Bauerova et al., 2006), endothelial inflammatory response to stroke (Yu et al., 2007). The liver damage due to cell proliferation and inflammation may induce plasma level of γ-GT since the enzyme, usually resides within the liver cells, is spilled into the blood stream. Thus, it is reasonable to mention that furan-derived inflammation causes liver damage thus increases γ-GT levels, which was observed in our study.

In conclusion, we measured urinary furan in 100 healthy individuals consumed normal diet (49 men, 51 women) and was detected in 56 subjects (31 males, 25 females) and ranged up to 3.14 ppb. In individuals with detectable urinary furan, the level of γ-glutamyltranspeptidase (γ-GT), a marker for liver damage, was strongly correlated with the urinary furan concentration ($r = 0.56$, $p < 0.0001$). To our knowledge, this is the first study to measure detectable levels of furan in human urine. However, the levels and effects of furan on humans are not known. Therefore, the metabolic rate and potential toxicity of furan remain to be investigated.

Conflict of interest statement

None.

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