表 1. 2011年の関西における大気中SCCPsの日平均濃度（ng m^{-3}）の測定値とモデルによる計算値。計算値は日本、韓国の排出量について最適化した値をモデルに与えた。瀋陽市と撫順市は大気モデルでは同一グリッドに相当する。

測定点	測定日	日平均濃度 (ng m^{-3})	計算値 (ng m^{-3})
京都市左京区 (Yoshida)	2011/01/24	2.81	3.67
	2011/01/25	4.05	2.44
	2011/01/26	1.80	1.85
	2011/01/27	1.64	2.12
	2011/01/28	1.90	5.96
	2011/01/29	2.58	1.60
	2011/01/30	4.89	1.82
	幾何平均値	2.60	2.50
京都市伏見区 (Ujigawa)	2011/02/10	0.57	1.90
	2011/02/11	1.93	2.22
	2011/02/12	1.73	1.19
	2011/02/13	7.47	2.82
	2011/02/14	13.7	1.99
	2011/02/15	3.26	6.72
	2011/02/16	2.99	8.92
	幾何平均値	2.94	2.89
尼崎市 (Amagasaki)	2011/02/25	3.35	5.04
	2011/02/26	2.99	11.1
	2011/02/27	9.14	10.2
	2011/02/28	8.37	3.73
	2011/03/01	4.85	5.05
	2011/03/02	2.15	2.35
	2011/03/03	2.90	2.55
	幾何平均値	4.20	4.86
柏原市 (Kashiwara)	2011/03/11	2.51	6.41
	2011/03/12	9.83	11.6
	2011/03/13	2.79	11.0
	2011/03/14	1.91	2.61
	2011/03/15	0.963	3.54
	幾何平均値	2.63	5.92
釜山市 (Busan)	2008/12/14	3.78	3.31
	2008/12/15	7.25	8.42
	2008/12/16	6.11	6.89
	幾何平均値	5.51	5.77
北京市 (Beijing)	2008/10/18	242	261
	2008/10/19	166	183
	2008/10/19	348	247

	2008/10/20	190	313
	幾何平均値	227	247
瀋陽市	2012/01/04	15.7	8.46
(Shenyang)	2012/01/05	17.7	8.97
	2012/01/06	19.2	7.83
	幾何平均値	17.5	8.41
撫順市	2012/01/08	16.8	23.9
(Fushun)	2012/01/09	22.6	15.7
	2012/01/10	7.18	6.49
	幾何平均値	14.0	13.5
上海市	2012/06/29	4617	1069
(Shanghai)	2012/06/30	4650	2474
	2012/07/01	2338	2199
	2012/07/02	5470	2045
	2012/07/03	5498	2056
	幾何平均値	4323	1895

表２．京都市左京区（Sakyo）における大気中SCCPsの平均濃度（ng m^{-3}）の測定値と計算値。測定は期間中の１回の連続測定による。計算値は日本、韓国の排出量について最適化した値をモデルに与えた。

期間	測定値	計算値
2011年1月24－31日	2.81	2.78
2011年5月9－16日	23.6	27.1
2011年6月6－13日	39.8	88.2
2011年7月21－27日	50.0	37.4
2011年7月27日－8月3日	23.3	72.5
2011年9月8－15日	51.5	46.4
2011年12月18－25日	6.34	4.73
2012年1月16－23日	9.86	3.73
2012年2月27日－3月5日	11.4	4.22
2012年4月16－23日	26.5	10.4
2012年5月10－11日	6.02	9.64
2012年7月9－16日	108	71.7
2012年7月16－17日	197	49.6
2012年7月18－20日	120	47.1
2012年7月24－26日	135	103

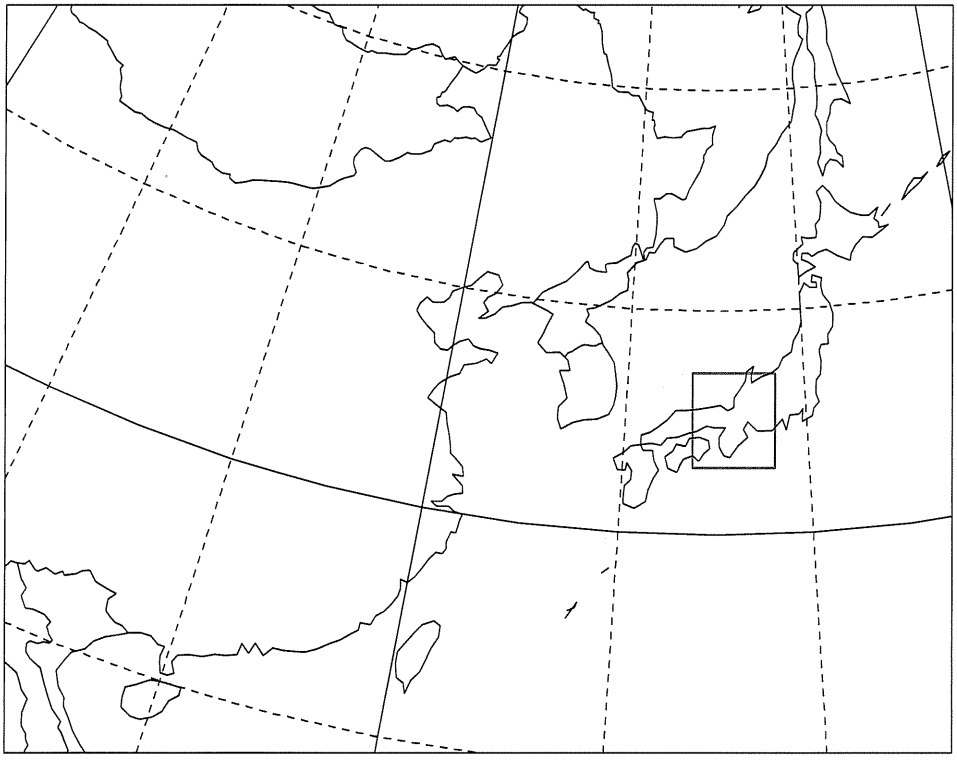

図１．計算領域。東西 4500km、南北 3600km、水平解像度 90km の領域１（黒枠）と、東西南北 450km、水平解像度 9km の領域２（赤枠）を結合した。

図２．ハイボリュームエアサンプラー設置の様子。2011 年 2 月 10 日京都大学防災研究所宇治川オープンラボラトリー構内（Fushimi）。

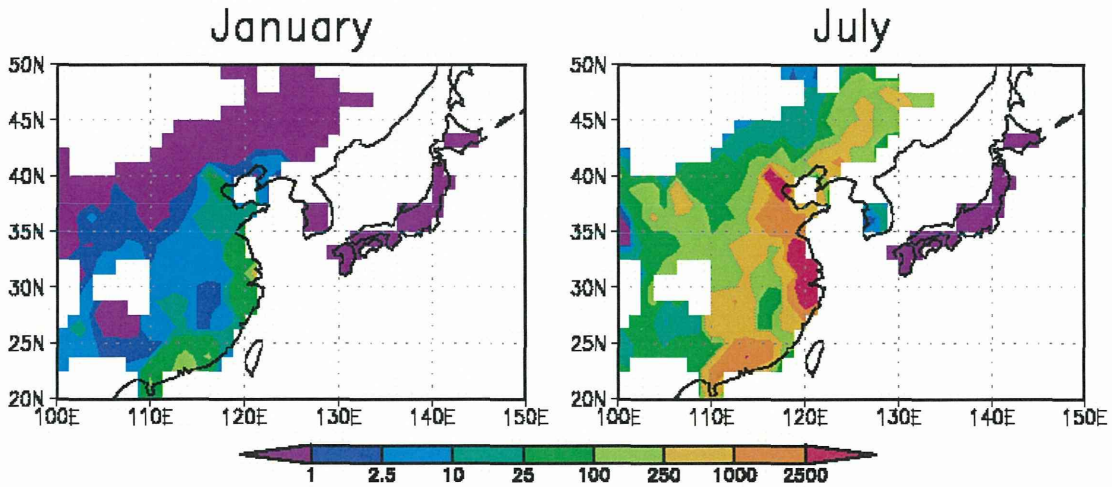

図３．１月と７月における日本、韓国、中国からの大気への SCCPs 推定排出分布（μg m^{-2} mon^{-1}）。国ごとの排出量はそれぞれ 0.08 t yr^{-1}、5.2 t yr^{-1}、12000 t

yr^{-1}。

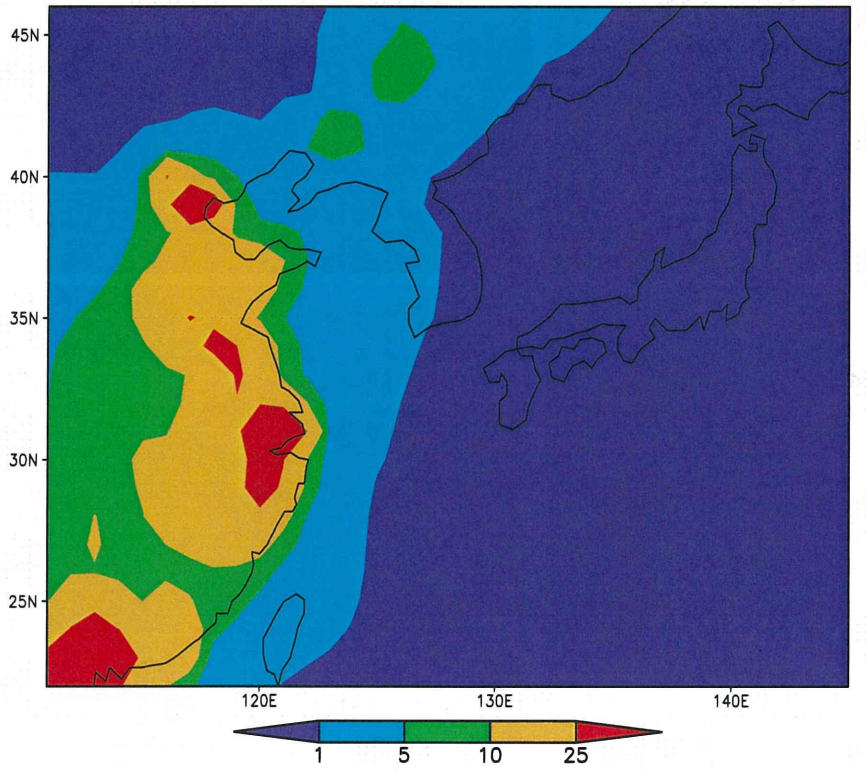

図４．従来の知見に従って推定した排出量（図３）をモデルに与えて計算した2012年１月における地表付近の大気中SCCPsの月平均濃度分布（ng m^{-3}）。日本全体が１ng m^{-3}に満たない領域にある。

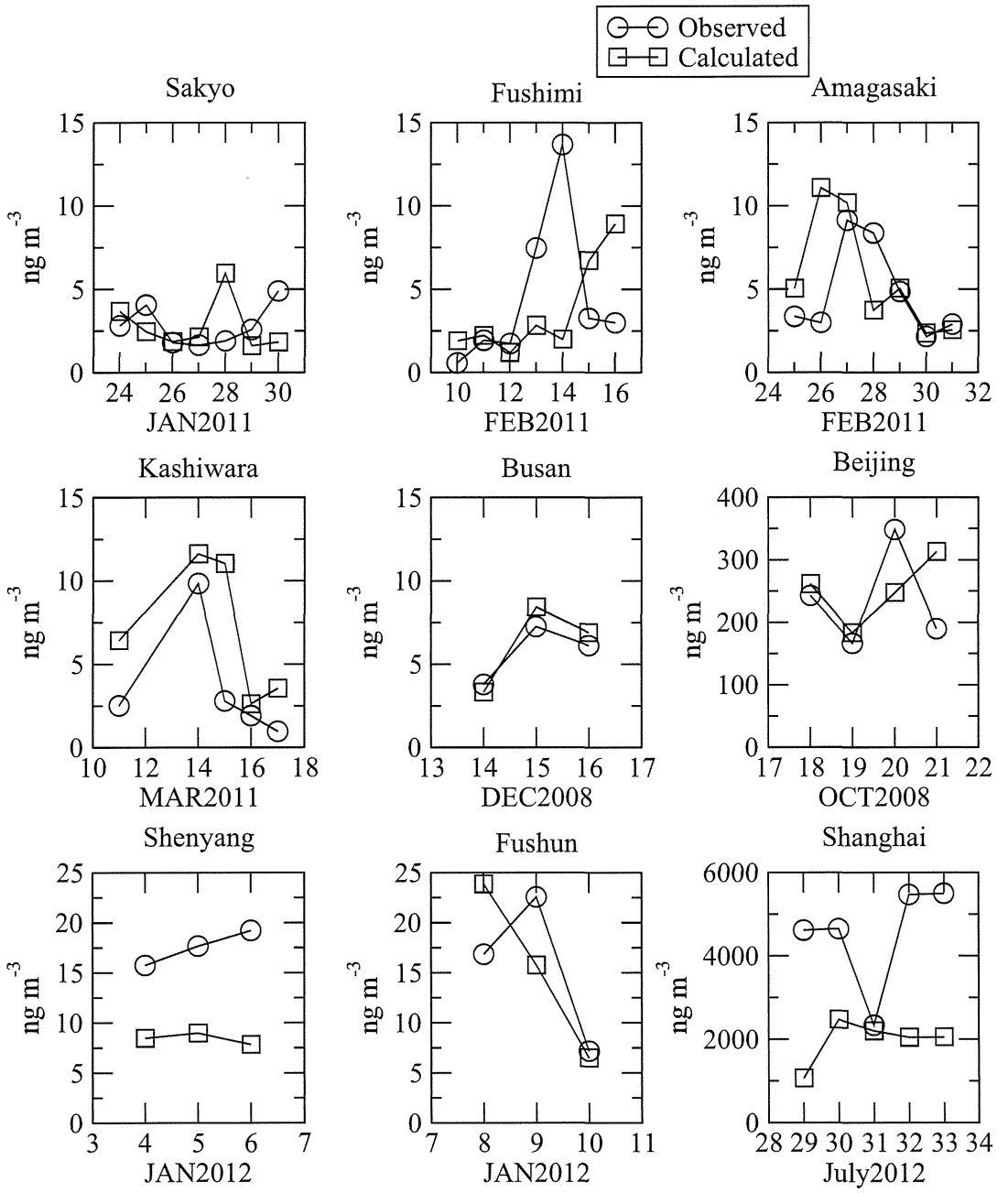

図５．大気中 SCCPs 濃度の日平均値（ng m^{-3}）の計算値（□）と実測値（○）との比較。

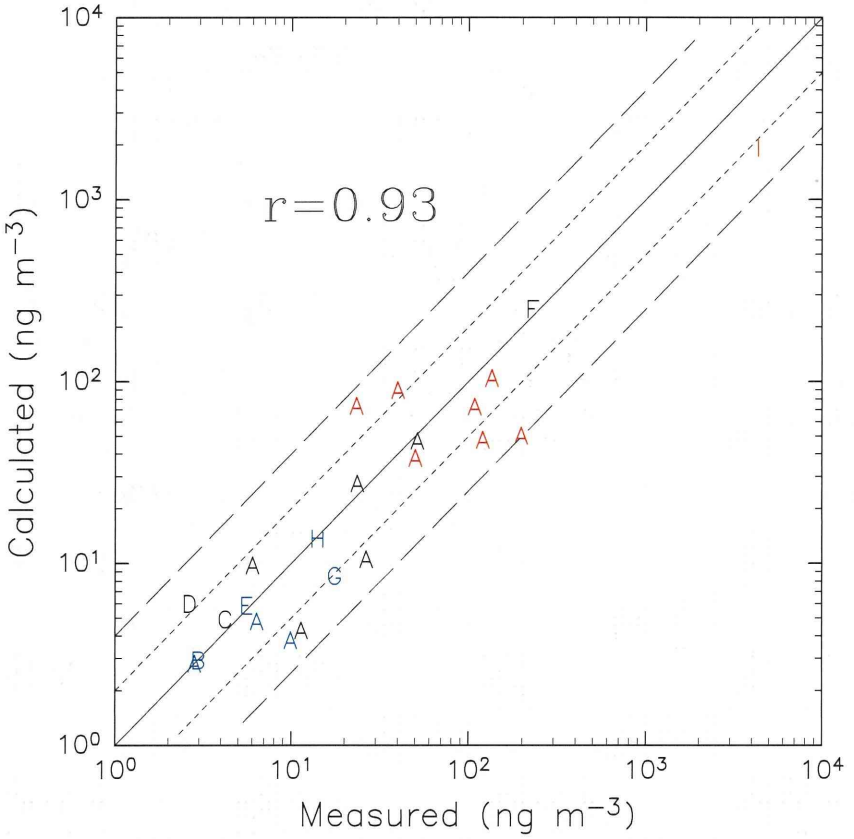

図６．地表面大気中 SCCPs 濃度（ng m^{-3}）の計算値と実測値との比較。表１，２に示した連続測定の期間中の平均値を用いた。実線と破線はそれぞれ factor 2 および 4 の誤差を表す。A: Sakyo、B: Fushimi、C: Amagasaki、D: Kashiwara、E: Busan、F: Beijing、 G: Shenyang、 H: Fushun、 I: Shanghai。赤は夏季、青は冬季の測定であることを示す。r は相関係数（$p = 1.37 \times 10^{-10}$）。

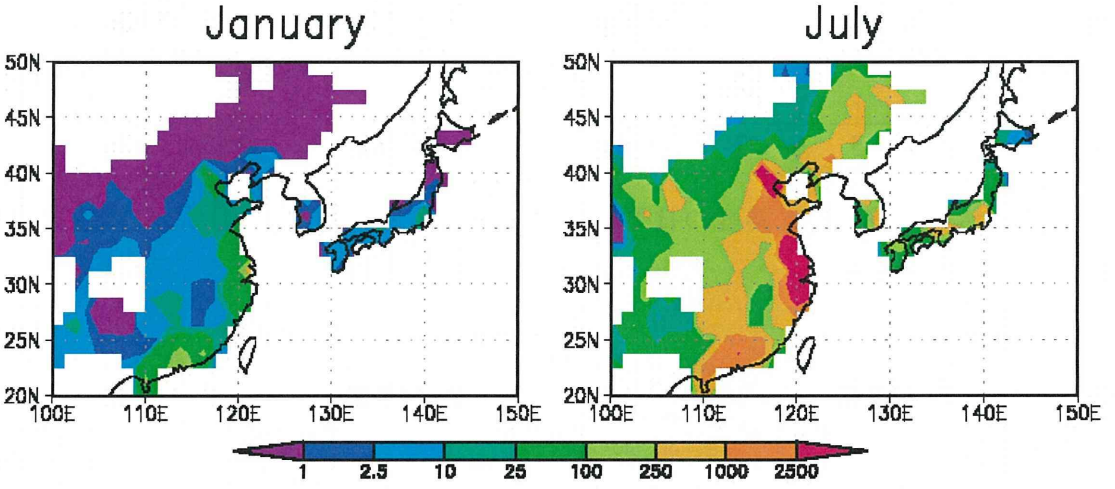

図７．モデルにより最適化したSCCPs排出量（μg m^{-2} mon^{-1}）。日本、韓国からの年間排出量はそれぞれ320 t yr^{-1}、100 t yr^{-1}となった。

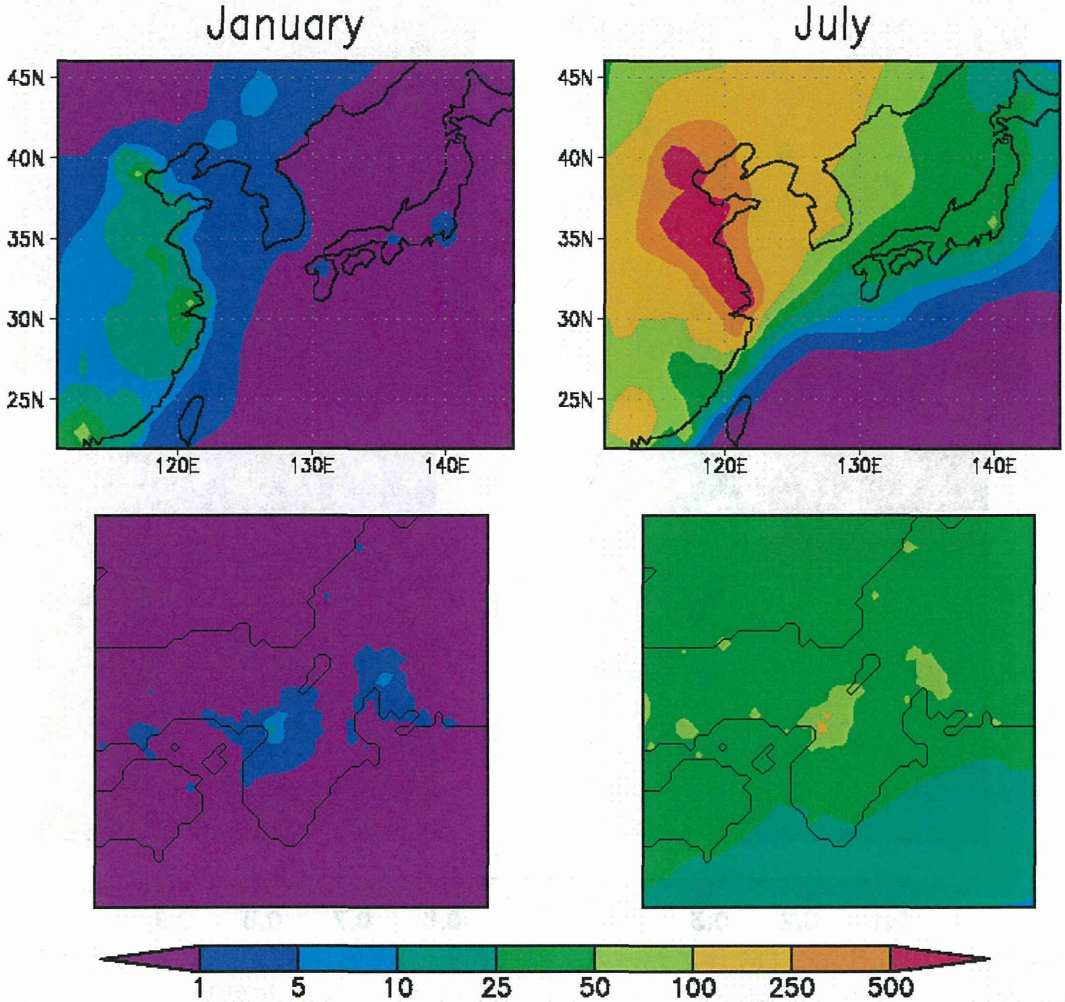

図８．2012年1月および7月における地表面付近の大気中SCCPs濃度の月平均値（ng m^{-3}）。上段は領域１、下段は領域２による計算結果。

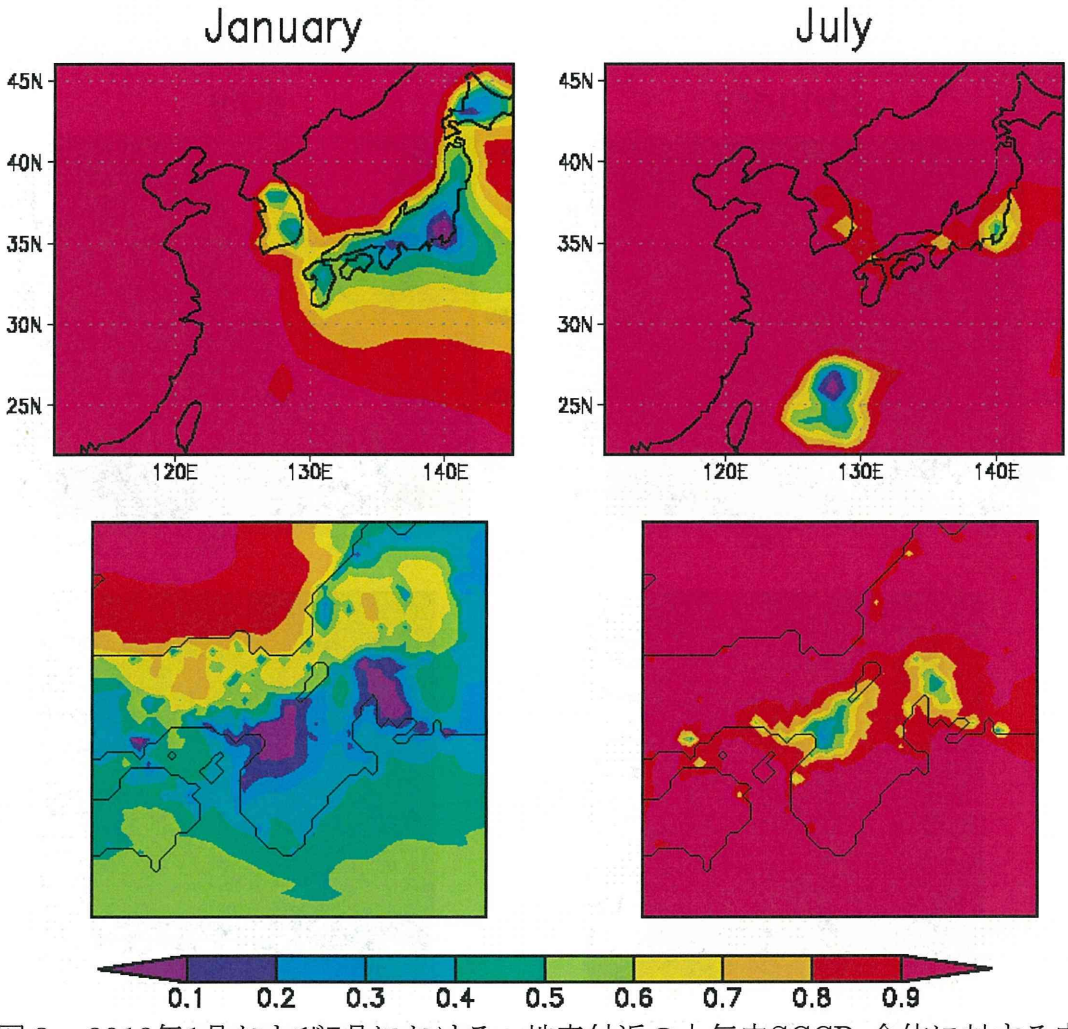

図９．2012年1月および7月における、地表付近の大気中SCCPs全体に対する中国起源成分の寄与。上段は領域１、下段は領域２による計算結果。

III. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Akio Koizumi, Kouji Harada, Yukiko Fujii.	Comparing pesticides in human breast milk from China, Korea and Japan.	Sherma Zibadi, Ronald Ross Watson and Victor R. Preedy	Handbook of dietary and nutritional aspects of human breast milk: Prevention, treatment and toxicity.	Wageningen Academic Publishers	オランダ	2013	In press

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Liu W, Tanabe M, Harada KH, Koizumi A.	Levels of urinary isoflavones and lignan polyphenols in Japanese women.	Environ Health Prev Med	In press	In press	2013
Liu W, Takahashi S, Sakuramachi Y, Harada KH, Koizumi A.	Polyfluorinated telomers in indoor air of Japanese houses.	Chemosphere	90	1672-1677	2013
Harada KH, Fujii Y, Adachi A, Tsukidate A, Asai F, Koizumi A.	Dietary Intake of Radiocesium in Adult Residents in Fukushima Prefecture and Neighboring Regions after the Fukushima Nuclear Power Plant Accident: 24-h Food-Duplicate Survey in December 2011.	Environ Sci Technol	47	2520-2526	2013
Endo T, Hayasaka M, Hisamichi Y, Kimura O, Haraguchi K.	Carbon and nitrogen stable isotope ratios and mercury concentration in the scalp hair of residents from Taiji, a whaling town.	Mar Pollut Bull	69	116-121	2013
Endo T, Hisamichi Y, Kimura O, Ogasawara H, Ohta C, Koga N, Kato Y, Haraguchi K.	Levels of Mercury in Muscle and Liver of Star-Spotted Dogfish (Mustelus manazo) from the Northern Region of Japan: A Comparison with Spiny Dogfish (Squalus acanthias).	Arch Environ Contam Toxicol.	64	467-474	2013

Matsubara F, Haraguchi K, Harada K, Koizumi A.	Screening for antibodies to human T-cell leukemia virus type I in Japanese breast milk.	Biol Pharm Bull	35	773-776	2012
Koizumi A, Harada KH, Niisoe T, Adachi A, Fujii Y, Hitomi T, Kobayashi H, Wada Y, Watanabe T, Ishikawa H.	Preliminary assessment of ecological exposure of adult residents in Fukushima Prefecture to radioactive cesium through ingestion and inhalation.	Environ Health Prev Med	17	292-298	2012
Fujii Y, Harada KH, Koizumi A.	Analysis of perfluoroalkyl carboxylic acids in composite dietary samples by gas chromatography/mass spectrometry with electron capture negative ionization.	Environ Sci Technol	46	11235-11242	2012
Fujii Y, Ito Y, Harada KH, Hitomi T, Koizumi A, Haraguchi K.	Comparative survey of levels of chlorinated cyclodiene pesticides in breast milk from some cities of China, Korea and Japan.	Chemosphere	89	452-457	2012
Fujii Y, Ito Y, Harada KH, Hitomi T, Koizumi A, Haraguchi K.	Regional variation and possible sources of brominated contaminants in breast milk from Japan.	Environ Pollut	162	269-274	2012
Fujii Y, Yan J, Harada KH, Hitomi T, Yang H, Wang P, Koizumi A.	Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia.	Chemosphere	86	315-321	2012
Kato Y, Tamaki S, Haraguchi K, Ikushiro S, Sekimoto M, Ohta C, Endo T, Koga N, Yamada S, Degawa M.	Comparative study on 2,2′,4,5,5′-pentachlorobiphenyl-mediated decrease in serum thyroxine level between C57BL/6 and its transthyretin-deficient mice.	Toxicol Appl Pharmacol	263	323-329	2012
Endo T, Hotta Y, Hisamichi Y, Kimura O, Sato R, Haraguchi K, Funahashi N, Baker CS.	Stable isotope ratios and mercury levels in red meat products from baleen whales sold in Japanese markets.	Ecotoxicol Environ Saf.	79	35-41	2012
Kato Y, Okada S, Atobe K, Endo T, Haraguchi K.	Selective determination of mono- and dihydroxylated analogs of polybrominated diphenyl ethers in marine sponges by liquid-chromatography tandem mass spectrometry.	Anal Bioanal Chem.	404	197-206	2012
Endo T, Minoshima Y, Hisamichi Y, Kimura O, Hayasaka M, Ogasawara H, Haraguchi K.	Levels of mercury and organohalogen compounds in the muscle and liver of kidako moray eels (Gymnothorax kidako) caught off the southern region of Japan.	Biol Pharm Bull.	35	1745-1751	2012

IV. 研究成果の刊行物・別刷

Environ Health Prev Med
DOI 10.1007/s12199-013-0338-6

REGULAR ARTICLE

Levels of urinary isoflavones and lignan polyphenols in Japanese women

Wanyang Liu · Miyako Tanabe · Kouji H. Harada · Akio Koizumi

Received: 14 December 2012 / Accepted: 29 March 2013

Abstract
Objectives High consumption of soybean products has been associated with a reduced risk of hormone-sensitive tumors. Soybean products contain phytoestrogens, such as daidzein, and sesame seeds contain secoisolariciresinol. These compounds are further metabolized to equol, enterodiol, and enterolactone by intestinal bacteria. However, individual differences in the metabolizing potential remain unclear. The aim of this study was to evaluate the urinary daidzein, equol, enterodiol, and enterolactone concentrations in women from several different regions of Japan according to age group.
Methods Five hundred urine samples collected from Japanese women living in Sapporo, Sendai, Kyoto, Kochi, and Naha were analyzed for daidzein, equol, enterodiol, and enterolactone concentration by gas chromatography–mass spectrometry.
Results The urinary isoflavone and lignan polyphenol levels did not differ significantly among the sampling sites, except for daidzein, which was highest in urine collected at Naha. The prevalence of equol producers was 39 % in the total study cohort. In equol producers, a positive correlation was observed between the urinary daidzein and equol levels ($r = 0.399$, $p < 0.001$). However, there was no significant difference between daidzein concentrations in equol producers and non-producers. Moreover, the levels of enterodiol and enterolactone were higher in equol producers than in equol non-producers. In the multivariate logistic analyses, two factors, Sendai dwelling and current smoking, were found to be significant [equol producers to non-producers: odds ratio 2.15 (95 % confidence interval: 1.17–4.02) and odds ratio 0.32 (0.15–0.63), respectively].
Conclusions Our data suggest that geographic factors and smoking status should be considered during the evaluation of equol in urine samples and that the same pathway may be responsible for the metabolism of both isoflavones and lignan polyphenols.

Keywords Phytoestrogen · Isoflavone · Lignan polyphenol · Equol producer · Japanese

Introduction

The risks of breast cancer and prostate cancer in Japan tend to be lower than those in Europe and the USA [1]. Differences in food habits are thought to be one of the reasons for this difference [2], which has led researchers to focus on the high consumption of soybean products by Japanese. Soybean products contain phytoestrogens with isoflavone structures, such as daidzein [3], while sesame seeds also contain lignan polyphenols, such as secoisolariciresinol. These polyphenolic compounds bind to estrogen receptors alpha and beta, with a preference for the latter [4]. Several epidemiological studies have suggested that high phytoestrogen levels are associated with a reduced risk of hormone-sensitive diseases [5–10].

These compounds are further metabolized to equol, enterodiol, and enterolactone by intestinal bacteria [11]. These metabolites, especially equol, show more potent

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binding affinities to estrogen receptors [12] than the respective substrate polyphenolic compound. However, they do show individual differences in metabolizing potential [13–15], and factors such as age, ethnicity, dietary fiber intake, and fat intake have been reported as candidate determinants for these differences. In addition, given that dietary and life habits are candidate determinants, there may be intergenerational and interzonal differences.

The aim of this study was to evaluate the concentrations of daidzein, equol, enterodiol, and enterolactone in urine samples from five regions in Japan according to broad age groups.

Materials and methods

Experimental design and study population

A total of 13,910 participants were originally recruited through medical check-ups of participants aged 20–70 years living in 11 prefectures in Japan between 2000 and 2001 [16]. Age, number of births, smoking habit, and menstrual function were recorded using a self-reported questionnaire. Urine samples were stored at −30 °C until analysis at the Kyoto University Human Specimen Bank [17, 18].

To evaluate geographical differences in Japan, we compared 500 samples collected from Hokkaido (Sapporo), Miyagi (Sendai), Kyoto (Kyoto), Kochi (Kochi), and Okinawa (Naha) between November 2000 and December 2001. At each study site, urine samples were collected from 25 adult Japanese females ranging in age across four age groups (30–39, 40–49, 50–59, and 60–69 years). The characteristics of the participants are summarized in Table 1. There were no significant differences in participant characteristics among the five study sites.

Written informed consent was obtained from all subjects prior to participation in the study. The research protocol for the study was reviewed and approved by the Ethics Committee of Kyoto University Graduate School of Medicine on 14 November 2003 (E25). All experiments were carried out in compliance with the Helsinki Declaration.

Reagents

Daidzein, equol, enterodiol, and enterolactone were obtained from Fujicco Co. Ltd. (Kobe, Japan), Enzo Life Sciences Inc. (Farmingdale, NY), ChromaDex Inc. (Irvine, CA), and Cayman Chemical Company (Ann Arbor, MI), respectively. D_6-daidzein was purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Methanol, ethyl acetate, and acetonitrile (pesticide analysis grade) were obtained from Kanto Chemicals (Tokyo, Japan). *Helix pomatia*-derived glucuronidase/sulfatase was purchased from Sigma-Aldrich Inc. (St. Louis, MO). Methyl t-butyl ether (pesticide analysis grade) and ascorbic acid were purchased from Wako Pure Chemicals (Osaka, Japan). *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1 % trimethylchlorosilane was obtained from Thermo Fisher Scientific Inc. (Waltham, MA).

Determination of phytoestrogens in urine

Daidzein, equol, enterodiol, and enterolactone were analyzed. The urine samples were subjected to a clean-up procedure using a solid-phase extraction. Briefly, 0.5 mL of a urine sample, 50 μL of 0.1 M ascorbic acid, and an internal standard (20 ng D_6-daidzein) were placed in a 1.5-mL polypropylene tube and 20 μL of glucuronidase/sulfatase solution (2,500 U) was added. The samples were shaken on a vertical shaker overnight at 37 °C, and then each solution was passed through a Sep-Pak Plus C_{18} solid-phase cartridge (particle size 55–105 μm; sorbent weight 360 mg; Waters Corp., Milford, MA) previously conditioned with 4 mL of methanol and 4 mL of 5 % methanol in water. Subsequent loading of the sample was followed by washing the sorbent with 4 mL of 5 % methanol in water. The analytes were eluted into a glass tube using

Table 1 Characteristics of the study population

Characteristics	Total	Hokkaido	Miyagi	Kyoto	Kochi	Okinawa	*p* value[a]
Age (mean ± SD)	49.2 ± 10.1	49.4 ± 10.7	50.1 ± 10.7	48.9 ± 9.4	49.3 ± 10.1	48.5 ± 9.9	0.8
Number of delivery (mean ± SD)	1.9 ± 1	1.7 ± 0.9	2.0 ± 0.9	1.8 ± 0.9	1.9 ± 0.9	2.0 ± 1.4	0.3
Post menopause (%)	37	34	33	46	36	37	0.6
Smoking habit (%)							
Non-smoker	86	83	83	91	80	90	0.2
Current smoker	11	13	15	8	15	7	
Ex-smoker	3	4	2	1	5	3	

SD Standard deviation

[a] Differences among residential areas were tested by analysis of variance or the χ^2 test

3 mL of 1:1 (v/v) acetonitrile and ethyl acetate. The solution was evaporated to 1 mL using dry N_2 and extracted with 2 mL of methyl t-butyl ether. The methyl t-butyl ether layer was dried up using dry N_2, after which 100 μL of methyl t-butyl ether and 50 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1 % trimethylchlorosilane were added. The solution was transferred to an autosampler vial and heated for 1 h at 60 °C. The extracts were analyzed by gas chromatography-mass spectrometry (model 6890GC/5973MSD; Agilent Technologies Japan Ltd., Tokyo, Japan) in the electron impact ionization mode using single ion monitoring. The trimethylsilyl derivatives were separated on a DB-5MS column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm) with a helium carrier gas. Splitless injections (1 μL) were performed with the injector set at 280 °C, and the split was opened after 1.5 min. The oven temperature was initially 200 °C, then ramped to 300 °C at 30 °C/min, and held for 12 min. The monitored ions are listed in Electronic Supplementary Material Table 1. The instrumental detection limits (IDLs) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of 3, and ranged from 2 (enterodiol) to 100 pg (daidzein) (Table 2). Since blank samples (0.5 mL of distilled water) contained no detectable concentrations, the method detection limits (MDLs) were considered to be equal to the IDLs, corresponding to 0.6 ng/mL for enterodiol and 28 ng/mL for daidzein (Table 2).

Quality assurance

Quantification was performed using an internal standard method with the external standards dissolved in 100 μL of methyl t-butyl ether and 50 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1 % trimethylchlorosilane. D_6-labeled daidzein was used as the internal standard for all analytes. All samples were quantified using a seven-point calibration curve, with a relative standard deviation (RSD) of the relative response factors of <15 % for all compounds. The recoveries were evaluated by ten replicate fortifications (fortified by 10× the original concentration of urine) of a sample from equol non-producers (Table 2). The procedural blank levels were evaluated in duplicate for 11 samples each using 0.5 mL of distilled water.

Statistical analysis

All statistical analyses were carried out using JMP software (ver. 4; SAS Institute, Cary, NC). Values of $p < 0.05$ were considered to indicate statistical significance. Concentrations of less than the detection limit were all approximated to half of the detection limit for statistical analyses. The urinary levels of phytoestrogens were corrected by the urinary creatinine (Cr) concentration. As the levels in the samples displayed right-skewed patterns, and the geometric means (GMs) were comparable to the medians, statistical analyses were conducted after log-transformation of the concentrations. Differences between mean values were tested by Tukey–Kramer's honestly significant difference test after analysis of variance or Student's *t* test. To reveal the relationships between equol-producing function and subjects' characteristics, we performed multivariate logistic analyses. Presence of equol-producing function was defined by the detection of equol in urine samples above the MDL.

Results

Urinary levels of phytoestrogens in study cohort of Japanese women

The descriptive statistics for the phytoestrogens levels are presented in Table 3. All samples contained detectable amounts of daidzein and enterolactone. Equol and enterodiol were detected in 39 and 77 % of samples, respectively. The GMs were as follows: daidzein, 1610 μg/g-Cr; equol, 78.4 μg/g-Cr; enterolactone, 36.7 μg/g-Cr; enterodiol,

Table 2 Recovery, detection limits, and quality assurance for isoflavones and lignan polyphenols in human urine samples

Compound	Quantification (confirmation value)	Recovery (%) and (reproducibility, RSD %) ($n = 10$)	Instrument detection limit[a] (pg)	Method detection limit[b] (ng/mL)
Equol	386 (371)	103.2 (9.4)	60	20
Daidzein	398 (383)	98.9 (7.3)	100	28
Enterodiol	410 (500)	94.7 (9.2)	2	0.6
Enterolactone	442 (263)	97.3 (10.0)	10	3
D_6-daidzein	404 (389)	–	–	–

RSD Relative standard deviation

[a] 1-μl injection

[b] 0.5-mL urine sample

Table 3 Geometric means, medians, and 95th percentiles of urinary phytoestrogen concentrations in our study cohort of Japanese women

Sample sites	Isoflavones (soybean)[a]		Lignan polyphenols (sesame seeds)[a]	
	Equol (μg/g-Cr)	Daidzein (μg/g-Cr)	Enterolactone (μg/g-Cr)	Enterodiol (μg/g-Cr)
Total	78.4 (6.4)	1,610 (4.6)	36.7 (6.0)	23.8 (7.1)
	–	1,946	42.3	28.8
	1,385	6,761	279	228
Hokkaido	56.8 (6.4)	1,509 (5.2)[a,b]	34.7 (6.2)	19.2 (7.1)
	–	1,758	45.9	27.4
	1,325	7,120	248	166
Miyagi	110.6 (6.8)	1,454 (4.7)[a]	28.7 (6.0)	20.6 (7.0)
	–	1,785	35.4	24.3
	1,827	7,120	229	179
Kyoto	75.2 (6.8)	1,334 (4.7)[a]	48.6 (6.1)	36.8 (7.2)
	–	1,541	50.1	61.3
	1,285	6,380	350	310
Kochi	66.5 (6.1)	1,419 (5.5)[a]	41.6 (6.0)	25.0 (7.2)
	–	1,634	50.1	30.3
	1,382	9,063	295	240
Okinawa	93.2 (5.9)	2,616 (2.6)[b]	32.7 (5.8)	20.3 (6.8)
	–	2,762	38.3	24.2
	812	5,025	203	200

GM Geometric mean, *GSD* geometric standard deviation

Comparisons were made among the residential areas. The geometric means within the same column followed by the same lower-case letter are significantly different ($p < 0.05$). The geometric means within the same column followed by the same lower-case letter of not followed by a lower-case letter do not differ significantly ($p > 0.05$)

[a] Urinary phytoestrogen concentrations for the total study cohort and for each study region are given as: GM (GSD) (top row), median (middle row), and 95th percentile (bottom row)

23.8 μg/g-Cr. The 95th percentiles were as follows: daidzein, 6761 μg/g-Cr; equol, 1385 μg/g-Cr; enterolactone, 279 μg/g-Cr; enterodiol, 228 μg/g-Cr. The urinary daidzein level was highest in Okinawa, followed by Hokkaido and the other sites ($p < 0.05$). There were no significant differences between the GMs for equol, enterodiol, and enterolactone ($p > 0.05$).

Urinary phytoestrogen concentrations were also compared between the equol producers and non-producers (Table 4). In both groups, no significant differences in the GMs of the phytoestrogens were observed among the five sampling sites ($p > 0.05$). The urinary levels of daidzein were comparable between the equol producers and non-producers ($p > 0.05$). In contrast, the GMs of enterodiol and enterolactone were higher in the equol producers than in the non-producers ($p < 0.001$). These trends were consistent over all five sampling sites.

The correlation coefficients among the phytoestrogens in the 500 samples are listed in Table 5. Significant correlations were observed between enterolactone and enterodiol in both the equol non-producers and producers ($\rho = 0.592$ and $\rho = 0.622$, respectively). In the equol producers, the equol concentration was significantly associated with not only the daidzein concentration ($\rho = 0.399$) but also the enterolactone and enterodiol concentrations ($\rho = 0.162$ and $\rho = 0.149$, respectively).

Relationships between equol-metabolizing function and participants' characteristics

The demographic status of equol producers and non-producers is summarized in Table 6. There were no significant differences in age, number of births, and ratio of postmenopause ($p > 0.05$). The smoker ratio was 6 and 15 % in the equol producers and non-producers, respectively, which was a significant difference ($p = 0.009$). The samples from Miyagi showed a marginally higher ratio of equol producers than those from the other four sites ($p = 0.16$). In multivariate logistic analyses, two factors, Miyagi residence and current smoking, were significantly associated with equol-producing function [equol producers to non-producers: odds ratio 2.15, (95 % confidence interval 1.17–4.02) and odds ratio 0.32 (0.15–0.63), respectively].

Table 4 Urinary phytoestrogen concentrations in equol producers and non-producers

Equol producers and non-producers	Isoflavones (soybean)[a]		Lignan polyphenols (sesame seeds)[a]	
	Equol (μg/g-Cr)	Daidzein (μg/g-Cr)	Enterolactone (μg/g-Cr)	Enterodiol (μg/g-Cr)
Total				
E(+)	564 (4.0)	1,531 (4.6)	56.7 (4.4)***	62.4 (4.4)***
(n = 195)	565	1,825	63.1	74.8
E(−)	–	1,662 (4.5)	27.8 (6.9)	12.8 (7.3)
(n = 305)	–	2,010	30.6	10.1
Hokkaido				
E(+)	681 (3.7)	1,738 (4.8)	57.3 (4.4)	48.3 (3.7)***
(n = 30)	916	1,779	42.7	58.6
E(−)	–	1,420 (5.4)	27.9 (6.8)	13.0 (7.9)
(n = 70)	–	1,758	46.3	11.54
Miyagi				
E(+)	606 (3.9)	1,191 (4.8)	35.7 (4.8)	54.4 (4.1)***
(n = 47)	636	1,324	41.8	70.8
E(−)	–	1,736 (4.5)	23.7 (7.2)	8.6 (7.1)
(n = 53)	–	2,292	27.7	5.8
Kyoto				
E(+)	614 (4.3)	1,164 (4.1)	70.9 (4.0)	101.7 (3.4)***
(n = 38)	518	1,265	68.1	88.8
E(−)	–	1,448 (5.0)	38.7 (7.5)	19.9 (8.1)
(n = 62)	–	1,976	38.7	17.7
Kochi				
E(+)	404 (4.7)	1,361 (6.0)	78.1 (3.7)**	62.5 (4.8)***
(n = 40)	382	1,647	78.6	61.1
E(−)	–	1,459 (5.4)	27.4 (6.9)	13.7 (7.3)
(n = 60)	–	1,536	22.4	9.2
Okinawa				
E(+)	579 (3.5)	2,745 (3.1)	56.6 (4.6)**	54.4 (5.6)***
(n = 40)	490	3,520	70.3	64.6
E(−)	–	2,533 (2.3)	22.5 (6.1)	10.4 (5.9)
(n = 60)	–	2,570	24.5	6.0

** $p < 0.01$, *** $p < 0.001$, vs. equol non-producers by Student's t test after log-transformation

$E(+)$ Equol producers, $E(-)$ equol non-producers, Cr creatinine

[a] Urinary phytoestrogen concentrations for equol producers and non-producers in the total study cohort and for each study region are given as: GM (GSD) (top row) and median (bottom row)

Discussion

We have evaluated the urinary levels of phytoestrogens in a sample of women residing in five different areas of Japan. Daidzein was found to be the predominant component of the phytoestrogens present in the urine of the Japanese women sampled and lignan polyphenols were minor components.

Overall, 39 % of the urine samples contained detectable levels of equol. Equol-producing function was also associated with smoking status. Previous studies in Japan reported equol detection rates of 20 % in female subjects [15] and 24 % in male subjects [19]. In our study, the equol detection rates ranged from 30 to 47 %. These variations might be associated with differences in dietary and life habits. Indeed, we found an association between smoking and low equol-producing function. A previous study indicated that the proportion of equol producers is low in young males compared with older males [19]. In our study, no such trend was observed in the study women. This trend is likely to reflect the smoking rate in each age group in Japan [20]. Although the proportion of daidzein to equol in the urine was around 30 % in equol producers, there was no significant difference in the urinary levels of daidzein

Table 5 Correlations among phytoestrogens

Combination	ρ	p value
Equol non-producers		
Enterodiol–Daidzein	−0.057	0.309
Enterolactone–Daidzein	−0.003	0.959
Enterolactone–Enterodiol	0.592	<0.001
Equol producers		
Daidzein–Equol	0.399	<0.001
Enterodiol–Equol	0.149	0.034
Enterodiol–Daidzein	−0.059	0.403
Enterolactone–Equol	0.162	0.021
Enterolactone–Daidzein	−0.123	0.079
Enterolactone–Enterodiol	0.622	<0.001

ρ Spearman's rank correlation coefficient

Table 6 Relationships between equol-producing status and demographic characteristics

Demographic characteristics	Equol producer	Equol non-producer	Odds ratio (95 % confidence interval)[a]
Age[b]	49.7 ± 9.7	48.9 ± 10.4	0.40 (0.07–2.04)
Number of births[b]	1.9 ± 1.0	1.9 ± 1.1	0.94 (0.37–2.43)
Post-menopause (%)	38	36	1.61 (0.87–3.01)
Smoking habit (%)			
Non-smoker	91	82	–[c]
Current smoker	6	15	0.32 (0.15–0.63)*
Ex-smoker	3	3	0.93 (0.30–2.70)
Log_{10} urinary daidzein (μg/g-Cr)[b]	3.2 ± 0.7	3.2 ± 0.7	0.75 (0.25–2.30)
Sampling sites (n)			
Hokkaido	30	70	–[c]
Miyagi	47	53	2.15 (1.17–4.02)*
Kyoto	38	62	1.22 (0.67–2.23)
Kochi	40	60	1.36 (0.74–2.50)
Okinawa	40	60	1.28 (0.69–2.38)

* Significantly difference at $p < 0.05$

[a] Odds ratios were calculated by multivariate logistic analyses

[b] Continuous values are presented as the mean ± SD

[c] Set to the reference level

between equol producers and non-producers. This phenomenon could result from differences in the dietary intake of isoflavones between equol producers and non-producers or in the pharmacokinetics between equol and daidzein. Levels of another soybean isoflavone, genistein, were comparable between equol producers and non-producers (1,192 and 1,070 μg/g-Cr, respectively). Genistein has a similar biological half-life to daidzein while the formation of 4-hydroxy-equol is considered to be rare. Therefore, dietary intake of isoflavones was unlikely to differ between two groups. The half-life of daidzein is relatively shorter than that of equol, and the conversion of daidzein into equol is time-dependent and slow [21]. Therefore, daidzein could be excreted rapidly in urine before its conversion into equol. Another possibility is that more daidzein is likely to be converted into *O*-desmethyl-angolensin (O-DMA) in non-equol producers than in equol producers [22]. This hypothesis needs to be investigated in the future.

The presence or absence of equol-producing function was clearly dichotomized despite the high daidzein levels. Intestinal microflora play important roles in the metabolism of nutrients, and the composition of the intestinal flora shows individual differences [23]. The effects of smoking on the intestinal microflora remain unknown, but smoking has been reported to influence colonic mucus production and mucosal immune systems [24]. These differences in the intestinal environment might discriminate the equol-metabolizing bacteria. As shown in Table 5, the reported association between lignan polyphenols and equol suggests that they are metabolized by the same pathway [25]. It has been suggested that some of the bacterium strains, for example, *Eggerthella sp*, were associated with both the metabolism of daidzein to equol and that of lignan polyphenols to enterodiol and enterolactone [26, 27]. However, the specific intestinal bacteria and pathway responsible for metabolism of these two groups of phytoestrogens need to be identified in the future. Importantly, this phenotype provides an insight for the identification of equol-metabolizing bacteria.

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Conflict of interest None.

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Polyfluorinated telomers in indoor air of Japanese houses

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HIGHLIGHTS

- 6:2 FTOH, 8:2 FTOH, 10:2 FTOH and 8:2 FTOAc were detected in most samples.
- 8:2 FTOH is the predominant component among fluorotelomers in indoor air.
- 8:2 FTOH level is associated with sampled housing location and sampling season.
- There are fluorotelomer sources in indoor environments in the Keihan area, Japan.

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ABSTRACT

The fluorotelomer alcohols (FTOHs) have been detected in various environmental compartments, including indoor and outdoor air, in North America and Europe. In our previous studies, FTOHs were detected at a relative higher concentration in outdoor air in the Keihan (Kyoto–Osaka, one of the major industrial zones) area, Japan compared to reported data. The exposure level of FTOHs in indoor air in the Keihan area remains unclear. In the present study, indoor air FTOH concentrations were investigated using a passive air sampler containing activated carbon felts. The indoor air sampling was conducted in 49 households of the Keihan area, during winter and summer 2008. Most samples contained 6:2 FTOH, 8:2 FTOH, 10:2 FTOH and 8:2 FTOAc. The median concentration of 8:2 FTOH (5.84 ng m^{-3}) was highest among fluorotelomers, followed by those of 10:2 FTOH (1.12 ng m^{-3}), 6:2 FTOH (0.29 ng m^{-3}), and others. Significant correlations among fluorotelomers were observed in collected samples. The association between housing conditions and 8:2 FTOH concentrations showed that samples collected from bed rooms have higher 8:2 FTOH concentrations than those collected from other locations. In addition, samples collected in winter showed lower levels of 8:2 FTOH than those collected in summer. These findings suggest that 8:2 FTOH is the predominant component among fluorotelomers in indoor air, and that there are emission sources of fluorotelomers in indoor environments of the Keihan area. Further investigations into the origins of fluorotelomers are needed to evaluate indoor contamination with fluorotelomers.

1. Introduction

Fluorotelomer alcohols (FTOHs) belong to a class of perfluoroalkyl and polyfluoroalkyl substances (PFASs), that find use in a diverse range of commercial and industrial applications including coatings, polymers, and paints, and so on (Kissa, 2001). 6:2, 8:2, and 10:2 FTOH are now widespread in indoor and outdoor air in the North America and Europe as a result of their widespread use (Stock et al., 2004; Barber et al., 2007; Jahnke et al., 2007; Shoeib et al., 2011). Several lines of experimental evidence have demonstrated the toxic effects of FTOHs on the laboratory animals (Kudo et al., 2005; Ishibashi et al., 2007, 2008; Oda et al., 2007; Phillips et al., 2007; Liu et al., 2010). Therefore, further consideration of the potential biological effects and the risks of FTOHs on human health are warranted. Although there is lack of

Abbreviations: PFASs, perfluoroalkyl and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; FTOHs, fluorotelomer alcohols; PAS, passive air sampler; ACFs, activated carbon fiber felts; 6:2 FTOH, 1H,1H,2H,2H-perfluorooctanol; 8:2 FTOH, 1H,1H,2H,2H-perfluorodecanol; 10:2 FTOH, 1H,1H,2H,2H-perfluoro-1-dodecanol; 8:2 FTOAc, 1H,1H,2H,2H-perfluorodecyl acrylate; 8:2 FTOMac, 1H,1H,2H,2H-heptadecafluorodecyl methacrylate; 8:1 FA, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-1-nonanol; IDL, instrument detection limits; MDL, method detection limits; HSD test, Tukey–Kramer's honestly significant difference test; ANOVA, analysis of variance; GM, geometric mean; GSD, geometric standard deviation; CV, coefficient of variation.

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