Research Article

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Associations of Acrylamide Intake with Circulating Levels of Sex Hormones and Prolactin in Premenopausal Japanese Women

Chisato Nagata[1], Kie Konishi[1], Takashi Tamura[1], Keiko Wada[1], Michiko Tsuji[1,2], Makoto Hayashi[3], Noriyuki Takeda[4], and Keigo Yasuda[3]

Abstract

Background: It has been hypothesized that alteration of hormone systems is involved in the carcinogenesis of acrylamide. The aim of the present study was to examine the cross-sectional associations between dietary acrylamide intake and sex hormone levels in premenopausal Japanese women.

Methods: Study subjects were 393 women who had regular menstrual cycles less than 40 days long. Acrylamide intake was assessed with a food-frequency questionnaire and was based on acrylamide concentration reported from analyses of Japanese foods. We measured the plasma concentrations of estradiol, testosterone, dehydroepiandrosterone sulfate, sex hormone–binding globulin, follicle-stimulating hormone, luteinizing hormone, and prolactin.

Results: After controlling for age, the phase of the menstrual cycle, and other covariates, acrylamide intake was statistically significantly inversely associated with total and free estradiol levels and statistically significantly positively associated with follicle-stimulating hormone level. Total and free estradiol levels were 18.2% and 19.3% lower, respectively, in women in the highest quartile of acrylamide intake than in those in the lowest quartile of intake. Follicle-stimulating hormone levels were 23.5% higher in women in the highest quartile of acrylamide intake than in those in the lowest quartile of intake.

Conclusion: The data suggest that acrylamide intake may alter estradiol and follicle-stimulating hormone levels.

Impact: High estradiol levels have been associated with an increased risk of breast cancer. Although the results need confirmation, they highlight the need to investigate the relationships among dietary acrylamide, sex hormones, and breast cancer risk.

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Introduction

Acrylamide is formed when foods high in carbohydrates and low in proteins are cooked at a high temperature and is found in numerous common foods, such as potato chips, French fries, bread, cereals, and coffee (1). Acrylamide's presence in foods has caused public health concern because acrylamide is known to have carcinogenic properties in experimental animals (2). Rodent studies have shown that administration of acrylamide induces tumors in the predominantly hormonally sensitive sites such as the thyroid and mammary glands (3). In human studies, increased risk of hormone-related cancers, such as ovarian cancer, endometrial cancer, and estrogen receptor–positive breast cancer, was observed (4, 5). On the basis of these data, it has been hypothesized that acrylamide may interfere with hormone systems in such a way that it causes carcinogenesis (3). In fact, several studies have found that acrylamide affects the levels of estradiol, progesterone, testosterone, and prolactin in rats (6–9). Multiple lines of evidence support a central role of sex hormones in the etiology of breast, endometrial, and ovarian cancers (10). Therefore, it is worth examining the associations between acrylamide intake and circulating levels of sex hormones in humans. However, to our knowledge, only one study has examined these associations, and it observed no overall associations among the U.S. women in the Nurses' Health Studies (11). Recently, the food content data for acrylamide have been gathered in Japan. The present study assessed the associations between acrylamide intake and circulating sex hormone levels in premenopausal Japanese women.

Materials and Methods

This study was part of one designed to assess the relationships among lifestyle, environmental factors, and women's health, as described previously (12). Study subjects were participants in a medical health check-up program provided by a general hospital in Gifu, Japan, between October 2003 and March 2006, including 1,545 premenopausal and postmenopausal women (the response proportion: 74.5%). When the response proportion was calculated only for new visitors to the program during the study period, it was 83.2% (1,103 of 1,325 individuals; ref. 12). The study was approved by the ethical board of the Gifu University Graduate School of Medicine. Written informed consent was obtained from all subjects.

During the health check-up, information about demographic characteristics, smoking status, medical and reproductive

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[1]Department of Epidemiology and Preventive Medicine, Gifu University Graduate School of Medicine, Gifu, Japan. [2]Department of Food and Nutrition, Japan Women's University, Tokyo, Japan. [3]Department of Internal Medicine, Matsunami General Hospital, Gifu, Japan. [4]Department of Endocrinology and Metabolism, Murakami Memorial Hospital, Asahi University, Gifu, Japan.

Corresponding Author: Chisato Nagata, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan. Phone: 81-58-230-6411; Fax: 81-58-230-6413; E-mail: chisato@gifu-u.ac.jp

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histories, diet, and exercise was collected with a self-administered questionnaire. Fasting blood samples were drawn from participants at around 8:00 a.m. of the same day and plasma was stored at −80°C until the assay.

Data on the frequency of consumption of a large variety of foods and dishes (169 items) and usual portion size over the course of one year were obtained from a validated food-frequency questionnaire and then transformed into intake estimates of a series of nutrients according to the Japanese Standard Tables of Food Composition, 5th revised and enlarged edition, published by the Science and Technology Agency of Japan. Fatty acid composition was evaluated using data published by Sasaki and colleagues (13). To calculate acrylamide intake, we generally used values of acrylamide concentration reported from analyses of Japanese foods. The Japanese ministries of Agriculture, Forestry and Fisheries and of Health, Labour and Welfare published acrylamide values for more than 120 food items (14–16). In addition, all other available data from published articles on acrylamide concentrations in Japanese foods were included (17–29), and the average of all reported values was allotted to individual foods and dishes considering cooking methods and food preparation techniques. For fried vegetables, data for 15 individual vegetables and their products were obtained. For example, for the item "beef mixed with vegetables, such as Japanese beef-potato stew, sukiyaki, stew, etc.," the vegetables included as ingredients except perilla, such as onion, welsh onion, cabbage, carrot, green pepper, bean sprout, and eggplant, were regarded to be fried. For the acrylamide concentration for potato chips, the average value reported from the Ministry of Agriculture, Forestry and Fisheries was allotted because it was based on more than 500 samples (14). The Ministry of Agriculture, Forestry and Fisheries repeated the acrylamide measurements for bread, cookies, rice cracker, tea, and coffee and published data in two separate reports (14, 15). For these food items except for French bread, data published in the first report were used because updated data in the second report may reflect the efforts of food industry to reduce acrylamide levels (data for French bread were not available in the first report). When acrylamide values were not available for Japanese food samples, we referred to data from the FDA (30). We confirmed that acrylamide values for most of these foods were below the detection level, and we used the FDA values for 5 food items (meat sauce, rye bread, pancakes, Danish pastries, and apple pie) which were above the detection level. When acrylamide levels were lower than the quantification limit, a value half of the quantification limit was assigned because they may still contain some acrylamide. However, dairy products and cereals, potatoes, meat, fish, and vegetables when boiled or raw were assigned values of zero, following previous studies (31, 32). As the acrylamide concentration of steamed white rice was reported to be less than 0.5 ng/g, this was also regarded to be a value of zero. Of the 169 food items or dishes on questionnaire, 111 corresponded to acrylamide-containing foods or dishes. Detailed description of the food-frequency questionnaire, its reliability, and validity for calculating nutrient intakes has been published previously (33). Although the validity of the values for acrylamide intake could not be directly tested, we examined correlations between the questionnaire and 12 daily diet records kept over a 1-year period for the major food sources of acrylamide, like other studies (31, 32); the Spearman correlation coefficients were 0.49 for potatoes, 0.57 for coffee, 0.62 for meats and meat products, and 0.49 for vegetables.

Study subjects were restricted to women who were neither pregnant nor breast feeding and had regular menstrual cycles less than 40 days. A total of 501 women who were not pregnant or breast feeding reported that they had regular menstrual cycles of less than 40 days. Women were excluded if her date of blood donation differed by more than 40 days from the onset of the last menses ($n = 20$), if they had cancer, diabetes mellitus, chronic hepatitis, or thyroid disease ($n = 22$), if they were using oral contraceptives, hormone therapy, or steroid ($n = 16$), or if their blood samples were not obtained ($n = 7$). Because 43 women did not respond to the dietary questionnaire, remaining 393 women ages 20 to 54 years comprised the study population. These women had been previously examined for relationships among body size, reproductive factors, dietary factors, and hormone levels (34, 35).

Plasma hormones were measured by direct immunoassays. Plasma estradiol and testosterone were measured using electro chemiluminescent immunoassay with kits purchased from Roche Diagnostic Japan. Plasma dehydroepiandrosterone sulfate (DHEAS) was measured by chemiluminescent enzyme immunoassay using kits purchased from Beckman Coulter. Plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin were measured using chemiluminescent immunoassay with kits purchased from Abbot Japan Co. Ltd. Sex hormone–binding globulin (SHBG) was measured using immunoradiometric assay with kits purchased from Diagnostic Products Corporation. The interassay coefficients of variation (CV) based on 10 randomly chosen samples for each hormone were $\leq$3.5% for estradiol, $\leq$3.6% for testosterone, $\leq$10.6% for DHEAS, $\leq$7.9% for SHBG, $\leq$5.7% for LH, $\leq$5.3% for FSH, and $\leq$3.6% for prolactin. All measurements were conducted at SRL, Inc. Free estradiol and testosterone were calculated using the measured estradiol, testosterone, albumin, and SHBG concentrations (36).

For statistical analysis, plasma hormone and SHBG concentrations were logarithmically transformed. The acrylamide intake was also logarithmically transformed and adjusted for total energy using the methods proposed by Willett (37). Subjects were divided into four equal groups according to quartile of energy-adjusted acrylamide intake. The geometric means of hormones and SHBG for each category were provided using analysis of covariate models. A linear trend was assessed using the median values of categories. We estimated the date of the start of woman's next menses after blood donation from the date of her last menses and her reported usual cycle length. The day of the menstrual cycle at blood donation was determined by "backward dating" counted backwards from the estimated date of her next menses. The phase of the menstrual cycle at blood donation, categorized into the early-follicular (days ≤ -21 of the cycle), late-follicular (days −20 to −17), periovulatory (days −16 to −12), early-luteal (days −11 to −9), midluteal (days −8 to −4), or late-luteal (days −3 to 0) phases, was used for adjustment. In addition, age, body mass index (BMI), parity, age at first birth, breast feeding, duration of breast feeding, years of smoking, and saturated fat intake were included in the models as covariates because these factors were associated with at least one of the hormones measured in our previous studies (34, 35). All the statistical analyses were performed using SAS programs (Version 9.2; SAS Institute Inc.).

Table 1. Characteristics of 393 premenopausal women[a]

Age, years	39.8 (5.4)
Height, cm	158.7 (5.3)
Weight, kg	52.7 (7.7)
BMI, kg/m^2	20.9 (2.7)
Parous	314 (79.9)
Age at first birth[b], years	26.5 (3.0)
Duration of breast feeding[b], months	19.9 (14.3)
Current smokers	21 (5.3)
Ex-smokers	17 (4.3)
Diet	
Acrylamide, µg/d	24.1 (11.1)
Total energy, kcal/d	2,132 (673)
Saturated fat, g/d	19.6 (9.2)
Alcohol, g/d	7.7 (17.0)
Coffee, mL/d	247 (171)
Blood levels, median (10th to 90th percentile)	
Total estradiol, pg/mL	109.0 (38–292)
Free estradiol, pg/mL	2.23 (0.75–5.55)
Testosterone, ng/dL	27.0 (13.0–46.0)
Free testosterone, ng/dL	0.38 (0.17–0.83)
DHEAS, µg/dL	128.0 (69.0–209.0)
SHBG, nmol/L	66.2 (32.1–124.0)
FSH, U/L	4.82 (2.25–10.6)
LH, U/L	3.85 (1.76–11.1)
Prolactin, ng/mL	14.1 (8.5–27.0)

[a]Values are mean (SD), *n* (%), or median (10th to 90th percentile).
[b]Among parous women.

Results

Characteristics of study subjects are shown in Table 1. The estimated mean value for acrylamide intake was 24.1 µg/d (SD, 11.1; range, 7.5–127.3). The main dietary sources of acrylamide were potatoes including potato chips (17.0% of total exposure) followed by coffee (16.6%), meat products (12.4%), and vegetables and their products (10.3%).

Higher acrylamide intake was significantly associated with lower levels of total and free estradiol. The geometric means of total and free estradiol in women in the highest quartile of acrylamide intake were 18.2% and 19.3% lower, respectively, than those in the lowest quartile of intake after controlling for covariates (Table 2). Acrylamide intake was significantly positively associated with FSH levels. The geometric mean of FSH levels in women in the highest quartile of acrylamide intake was 23.5% higher than those in the lowest quartile of intake. There were no statistically significant associations between acrylamide intake and other hormone levels. To see if the observed associations may be due to some other component of foods containing high acrylamide, additional adjustments for major sources of dietary acrylamide intake were done. Additional adjustment for potato chips intake somewhat attenuated these associations, but did not affect the results greatly; the geometric means of total and free estradiol levels were 15.8% and 17.0% lower, respectively, in women in the highest quartile of intake than in those in the lowest (*P* values for trend were 0.09 and 0.06, respectively). The geometric means of FSH levels were 24.0% higher in women in the highest quartile of intake than in those in the lowest (*P* for trend was 0.02). Adjustment for coffee intake somewhat strengthened the associations; the geometric means of total and free estradiol levels were 24.4% and 25.9% lower, respectively, in women in the highest quartile of intake than in those in the lowest (*P* values for trend were 0.01 and 0.005, respectively). The corresponding value for FSH was 25.5% higher (*P* for trend was 0.02). Exclusion of 8 women with higher FSH levels (>30 U/L) somewhat attenuated the associations, but the statistical significance remained for free estradiol and FSH; the geometric means of total and free estradiol levels were 13.7% and 14.0% lower, respectively, in women in the highest quartile of intake than in those in the lowest (*P* values for trend were 0.08 and 0.048, respectively). The corresponding value for FSH was 15.3% higher (P for trend $= 0.04$). In 4 women, total estradiol levels at the phases except for periovulatory phase were higher than the absolute value of the 75th percentile plus three times the interquartile range of the entire subjects (>512 pg/mL). Exclusion of these women did not alter the results greatly; the geometric means of total and free estradiol levels were 14.4% and 15.6% lower, respectively, in women in the highest quartile of intake than in those in the lowest (*P* values for trend were 0.07 and 0.048, respectively). The corresponding value for FSH was 23.0% higher (P for trend $= 0.02$).

Stratified analysis according to BMI revealed that the inverse associations of acrylamide intake with total and free estradiol levels were only statistically significant in women with higher BMIs (>20.5 kg/m^2; Table 3). The geometric means of total and free estradiol levels were 28.2% and 29.7% lower, respectively, in women in the highest quartile of intake than in those in the lowest. The results were not altered substantially when subjects were restricted to never smokers.

As a previous study reported statistically significant positive associations of acrylamide intake with luteal total and free estradiol levels among normal-weight premenopausal women (BMI < 25 kg/m^2; ref. 11), we repeated analyses, restricting study subjects to normal-weight women in the luteal phase ($n = 168$). The geometric means of total and free estradiol levels were 5.1% and 12.0% lower, respectively, in women in the highest quartile of intake than in those in the lowest (*P* values for trend were 0.61 and 0.37, respectively).

Table 2. Adjusted geometric means of sex hormones and SHBG by quartile of acrylamide intake in premenopausal women

	Median (µg/d)	Range (µg/d)	N	Total E2 (pg/mL)	Free E2 (pg/mL)	Total T (ng/dL)	Free T (ng/dL)	DHEAS (µg/dL)	SHBG (nmol/L)	FSH (U/L)	LH (U/L)	PRL (ng/mL)
Q1	16.3	6.0–18.5	98	116.7	2.33	25.0	0.37	122.1	61.6	4.40	4.13	14.8
Q2	20.6	18.6–22.3	98	111.2	2.27	25.4	0.39	114.9	57.7	4.92	4.04	15.5
Q3	24.5	22.4–26.4	99	99.5	1.97	24.3	0.36	117.8	60.4	4.96	4.05	14.3
Q4	30.1	26.5–56.3	98	95.5	1.88	27.8	0.41	135.7	64.1	5.42	4.38	13.8
P trend				0.04	0.02	0.24	0.54	0.08	0.61	0.02	0.61	0.19

NOTE: Data are adjusted for age, BMI, parity, age at first birth, duration of breast feeding, years of smoking, saturated fat intake, and the phase of the menstrual cycle using analysis of covariance models.
Abbreviations: E2, estradiol; PRL, prolactin; T, testosterone.

Table 3. Adjusted geometric means of sex hormones and SHBG by quartile of acrylamide intake in premenopausal women stratified by low and high BMI[a]

	Total E2 (pg/mL)		Free E2 (pg/mL)		Total T (ng/dL)		Free T (ng/dL)		DHEAS (μg/dL)		SHBG (nmol/L)		FSH (U/L)		LH (U/L)		PRL (ng/mL)	
	Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
All premenopausal women																		
Q1	120.3	114.4	2.22	2.46	24.3	26.1	0.33	0.43	118.0	127.7	75.5	50.0	4.69	4.22	4.27	4.04	15.0	14.6
Q2	120.7	100.6	2.29	2.21	22.9	27.5	0.32	0.47	111.5	117.4	69.4	46.9	4.77	5.17	3.67	4.61	14.6	16.8
Q3	109.0	92.4	2.00	1.95	25.7	23.9	0.34	0.38	124.0	113.7	75.7	50.0	4.89	4.87	4.08	3.99	14.2	14.3
Q4	109.8	82.1	2.02	1.73	27.9	27.6	0.37	0.44	133.6	138.0	74.0	55.4	5.70	5.10	4.79	3.91	13.2	14.7
P trend	0.43	0.04	0.37	0.02	0.12	0.90	0.28	0.79	0.06	0.53	0.97	0.47	0.12	0.17	0.39	0.64	0.16	0.71
Never-smoking premenopausal women																		
Q1	120.0	109.1	2.23	2.38	23.6	25.7	0.32	0.43	117.2	128.4	75.0	49.4	4.89	4.29	4.42	3.98	15.0	14.5
Q2	123.0	105.8	2.30	2.37	22.8	27.5	0.31	0.48	110.8	113.8	71.9	45.3	4.93	5.29	3.86	4.84	14.7	17.6
Q3	109.3	88.7	1.99	1.89	26.2	23.0	0.34	0.37	122.6	105.3	76.5	49.9	4.76	4.96	3.78	4.00	13.4	14.3
Q4	111.1	81.7	2.07	1.71	27.2	26.9	0.37	0.43	129.8	137.3	71.8	55.8	5.86	5.19	4.79	3.82	13.0	14.5
P trend	0.50	0.06	0.47	0.03	0.12	0.97	0.24	0.56	0.13	0.75	0.82	0.38	0.21	0.20	0.67	0.61	0.10	0.63

NOTE: Data are adjusted for age, BMI, parity, age at first birth, duration of breast feeding, years of smoking, saturated fat intake, and the phase of the menstrual cycle using analysis of covariance models.

Abbreviations: E2, estradiol; PRL, prolactin; T, testosterone.

[a]The cut point for BMI is 20.5 kg/m^2.

Discussion

We observed a statistically significant inverse association between acrylamide intake and total and free estradiol among premenopausal Japanese women. Decreased estradiol after administration of acrylamide was reported from a study of female rats (7). However, the results from another human study that evaluated the association between acrylamide intake and sex hormones differed from our results; overall, there were no associations among premenopausal women, but among normal-weight premenopausal women, acrylamide intake was positively associated with luteal total and free estradiol level with statistical significance (11). In the present study, the restriction of subjects to normal-weight women in the luteal phase did not reveal a positive association.

It is known that high estrogen levels are associated with an increased risk of premenopausal breast cancer (38). If acrylamide could cause the development of breast cancer by causing increased estrogen levels, our findings would be unexpected. Among 9 previous epidemiologic studies on dietary acrylamide and the risk of premenopausal or postmenopausal breast cancer (reviewed by Lipworth and colleagues; ref. 39), only one study reported a statistically significant association (5); the study included postmenopausal women only and the positive association was seen between acrylamide-hemoglobin adduct levels and estrogen receptor–positive breast cancer. Among 4 studies of premenopausal women (4, 40–42), the relative risk of premenopausal breast cancer for the highest versus lowest quantile ranged from 0.92 (41) to 1.47 (42). The relative risk of 1.47 [95% confidence interval (CI), 0.96–2.27] was attenuated to 1.17 (95% CI, 0.69–2.00) when subjects were restricted to never smokers (42). These results suggest a possibility that acrylamide is not breast carcinogen. From our findings among premenopausal women, it is also possible that the carcinogenicity of acrylamide may be negated by its estrogen-lowering effect.

Some epidemiologic studies, but not all, reported a positive association between dietary acrylamide and the risk of endometrial and ovarian cancers (39). Although data about the relationship of estrogen levels to these cancers are limited (10), the observed inverse association of dietary acrylamide with estradiol levels is also unexpected. The observed increase in FSH levels associated with high acrylamide intake may be compensatory, resulting from reduced negative feedback by low estrogen levels. Exposure to FSH has been hypothesized to increase the risk of ovarian cancer (43). However, so far two previous studies among premenopausal women have found either null or inverse association (10). For breast cancer and endometrial cancer, to our knowledge, there has been no study reporting positive or inverse association with FSH levels.

The main sources of acrylamide vary between populations. Potato chips, French fries, coffee, pastry and cookies, and bread have been reported to be main sources of acrylamide in the Western populations (44). Vegetables, other than potatoes, cooked in high temperature also contain acrylamide but have not been considered well in the estimation of acrylamide intake in the previous studies probably because of the low consumption among the Western populations. Fried vegetables are common menu in Japan. We attempted to estimate numerous acrylamide-contributing foods including fried vegetables, more than used in previous studies (41). However, similar to other studies using food-frequency questionnaires, acrylamide intake could be difficult to assess, due to the large variations among different brands and cooking methods, such as length of cooking and temperature. As we did not measure hemoglobin adducts of acrylamide, we could not evaluate whether hemoglobin adduct level was correlated with the estimated acrylamide intake. However, it has been argued that hemoglobin adducts may not be a good standard because hemoglobin adducts may represent relatively short-term exposure and may be influenced by seasonality, smoking, recent exposure, etc. (3).

Other limitations of the present study include the use of samples obtained at a single point in time without restriction to the day of the cycle, which can lead to large random measurement errors for hormone levels and consequently to attenuated estimates of associations. However, some large studies have succeeded in detecting associations between potential breast cancer risk factors and sex hormone levels using untimed blood samples (45–47). We also confirmed some of these associations in our dataset (34, 35). We cannot rule out the possibility that unmeasured or unknown confounding factors accounted for the associations observed in our study. We made multiple comparisons, which could have resulted in some associations occurring by chance.

In conclusion, we observed an inverse association between acrylamide intake and total and free estradiol levels and a positive association between acrylamide intake and FSH levels in premenopausal Japanese women. This suggests that acrylamide intake could influence estrogen profile which requires confirmation by additional studies. Because higher estrogen levels have been implicated in an increased risk of premenopausal breast cancer, if confirmed, the relevance of our findings to the breast cancer risk should also be explored. Analytic epidemiologic studies including measurements of both acrylamide intake and estrogen levels would be helpful.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C. Nagata, M. Hayashi, K. Yasuda
Development of methodology: C. Nagata, M. Hayashi, K. Yasuda
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Nagata, M. Hayashi, N. Takeda, K. Yasuda
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Nagata, K. Konishi, T. Tamura, K. Wada, M. Tsuji
Writing, review, and/or revision of the manuscript: C. Nagata, K. Konishi, M. Tsuji, N. Takeda
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Nagata, N. Takeda
Study supervision: C. Nagata

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References

1. Tareke E, Rydberg P, Karlsson P, Eriksson S, Tornqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 2002;50:4998–5006.
2. International Agency for Research on Cancer (IARC). Monographs on the evaluation of carcinogen risk to humans: some industrial chemicals. Lyon: IARC; 1994.
3. Hogervorst JG, Baars B-J, Schouten LJ, Konings EJM, Goldbohm RA, van den Brandt PA. The carcinogenicity of dietary acryamide intake: a comparative discussion of epidemiological and experimental animal research. Crit Rev Toxicol 2010;40:485–512.
4. Wilson KM, Mucci LA, Rosner BA, Willett WC. A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. Cancer Epidemiol Biomarkers Prev 2010;19:2503–15.
5. Olsen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjönneland A. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, cancer and Health study. Int J Cancer 2008;122:2094–100.
6. Uphouse LL, Neneroff CB, Mason G, Prange A, Bondy SC. Interactions between "handling" and acrylamide on endocrine responses in rats. Neurotoxicology 1982;3:121–5.
7. Mannaa F, Abdel-Wahhab MA, Ahmed HH, Park MH. Protective role of Panax ginseng extract standardized with ginsenoside Rg3 against acrylamide-induced neurotoxicity in rats. J Appl Toxicol 2006;26:198–206.
8. Camacho L, Latendress JR, Muskhelishvili L, Patton R, Bowyer JF, Thomas M, et al. Effects of acrylamide exposure on serum hormones, gene expression, cell proliferation, and histopathology in male reproductive tissues of Fischer 344 rats. Toxicol Lett 2012;211:135–43.
9. Wei Q, Li J, Li X, Zhang L, Shi F. Reproductive toxicity in acrylamide-treated female mice. Reprod Toxicol 2014;46:121–8.
10. Eliassen AH, Hankinson SE. Endogenous hormone levels and risk of breast, endometrial and ovarian cancers: prospective studies. Adv Exp Med Biol 2008;630:148–65.
11. Hogervorst JG, Fortner R, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE, et al. Association between dietary acrylamide intake and plasma sex hormone levels. Cancer Epidemiol Biomarkers Prev 2013; 22:2024–36.
12. Nagata C, Nakamura K, Oba S, Hayashi M, Takeda N, Yasuda K. Association of intakes of fat, dietary fiber, soy isoflavone, and alcohol with uterine fibroids in Japanese women. Br J Nutr 2009;101:1427–31.
13. Sasaki S, Kobayashi M, Tsugane S. Development of substituted fatty acid composition table for the use in nutritional epidemiologic studies for Japanese populations: its methodological backgrounds and the evaluation. J Epidemiol 1999:9:190–207.
14. Ministry of Agriculture, Forestry and Fisheries, Japan. [cited 2014 Aug. 2] (in Japanese). Available from: http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/survei/pdf/chem_15-22.pdf.
15. Ministry of Agriculture, Forestry and Fisheries, Japan. [cited 2014 Aug. 2] (in Japanese). Available from: http://www.maff.go.jp/j/syouan/seisaku/risk_analysis/survei/pdf/chem_23-24.pdf2012.
16. Ministry of of Health, Labour and Welfare, Japan. [cited 2014 Aug. 2] (in Japanese). Available from: http://www.mhlw.go.jp/topics/2002/11/tp1101-1a.html.
17. Ono H, Chuda Y, Ohnishi-Kameyama M, Yada H, Ishizuka M, Kobayashi H, et al. Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. Food Addit Contam 2003;20:215–20.
18. Totani N, Yawata M, Takeda M, Moriya M. Acrylamide content of commercial frying oil. J Oleo Sci 2007;56:103–6.
19. Mukai N, Kiso K. Analysis of acrylamide in alcohol beverages. Research Results, National Research Institute of Brewing 2004;176:121–25 (in Japanese).
20. Ishihara K, Nara K, Yonezawa M, Hoshino A, Arima M, Koga H. Acrylamide content in vegetables home-cooked by heating. J Cookery Sci Jpn 2009;42:32–7. (in Japanese).
21. Tsutsumiuchi K, Kambe M, Okajima N, Takamura M, Okada M, Miwa J, et al. Acrylamide in foods. Annual Report of Research Institute for Biological Function 2007;7:7–12 (in Japanese).
22. Matsuoka A, Taniguchi M, Koba K. The acrylamide content of carbohydrate foodstuffs processed in high temperature. Kassuisyu, Kassui Women's College 2009;52:63–8 (in Japanese).
23. Yokoyama R, Kobayashi C, Sakane Y. Investigation of acrylamide contents in processed foods. B Shizuoka Inst Environ Hyg 2011;54:55–9 (in Japanese).
24. Yokoyama R, Kuboyama M, Owada K. Investigation of acrylamide contents in processed foods.(2nd report). B Shizuoka Inst Environ Hyg 2012;55: 55–60 (in Japanese).
25. Takatsuki S, Nemoto S, Sasaki K, Maitani T. Production of acrylamide in agricultural products by cooking. Shokuhin Eiseigaku Zasshi 2004;45: 44–8 (in Japanese).
26. Yoshida M. National Agriculture and Food Research Organization. [cited 2014 Aug. 2] (in Japanese). Available from: http://www.naro.affrc.go.jp/nfri/seikatenji/files/2009_P10.pdf.
27. Yoshida M, Miyoshi K, Horibata K, Mizukami Y, Takenaka M, Yasui A. Estimation of acrylamide intake from cooked rice in Japan. Nippon Shokuhin Kagaku Kogaku Kaishi 2011;58:525–30 (in Japanese).
28. Aoyagi M, Kubo A, Niiyama K, Kanetoshi A, Oda A. Determination of acrylamide in bread. Rep Hokkaido Inst Pub Health 2004;54:55–8 (in Japanese).

29. Matsuoka A, Koba K. The formation and control of acrylamide in plant-based foods during high thermal cooking: in frying tempe, potato and sweet potato. Kassuisyu, Kassui Women's College 2008;51:97–106 (in Japanese).
30. U.S. Food and Drug Administration. The 2006 exposure assessment for acrylamide. [cited 2014 Aug. 2]. Available from: www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm2006782.htm
31. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm A, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2304–13.
32. Larsson SC, Åkesson A, Wolk A. Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. Cancer Epidemiol Biomarkers Prev 2009;18:994–7.
33. Shimizu H, Ohwaki A, Kurisu Y, Takatsuka N, Ido M, Kawakami N, et al. Validity and reproducibility of a quantitative food frequency questionnaire for a cohort study in Japan. Jpn J Clin Oncol 1999;29:38–44.
34. Nagata C, Wada K, Nakamura K, Hayashi M, Takeda N, Yasuda K. Associations of body size and reproductive factors with circulating levels of sex hormones and prolactin in premenopausal Japanese women. Cancer Causes Control 2011;22:581–8.
35. Tsuji M, Tamai Y, Wada K, Nakamura K, Hayashi M, Takeda N, et al. Associations of intakes of fat, dietary fiber, soy isoflavones, and alcohol with levels of sex hormones and prolactin in premenopausal Japanese women. Cancer Causes Control 2012;23:683–9.
36. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17$\beta$to human plasma proteins at body temperature. J Steroid Biochem 1983;16: 801–10.
37. Willett W. Implication of total energy intake for epidemiological analyses: In:Willett W, editor. Nutritional Epidemiology. Oxford: Oxford University Press; 1990; p. 245–71.
38. Endogenous Hormones and Breast Cancer Collaborative Group, Key TJ, Appleby PN, Reeves GK, Travis RC, Alberg AJ, et al.Sex hormones and risk of breast cancer in premenopausal women: a collaborative reanalysis of individual participant data from seven prospective studies. Lancet Oncol 2013;14:1009–19.
39. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. Eur J Cancer Prev 2012;21:375–86.
40. Mucci LA, Sandin S, Bälter K, adami HO, Nagnusson C, Weiderpass E. Acrylamide intake and breast cancer risk in Swedish women. JAMA 2005;293:1326–7.
41. Wilson KM, Mucci LA, Cho E, Hunter DJ, Chen WY, Willett WC. Dietary acrylamide intake and risk of premenopausal breast cancer. Am J Epidemiol 2009;169:951–61.
42. Burley VJ, Greenwood DC, Hepworth SJ, Fraser LK, de Kok TM, van Breda SG, et al. Dietary acrylamide intake and risk of breast cancer in the UK women's cohort. Br J Cancer 2010;103:1749–54.
43. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II Inferences regarding pathogenesis. J Natl Cancer Inst 1983;71:712–21.
44. WHO. Evaluation of certain food contaminants. Sixty-fourth report of the joint FAO/WHO expert committee on food additives. Geneva: WHO; 2006.
45. Verkasalo PK, Thomas HV, Appleby PN, Davey GK, Key TJ. Circulating levels of sex hormones and their relations to risk factors for breast cancer: a cross-sectional study in 1092 pre- and postmenopausal women (United Kingdom). Cancer Causes Control 2011;12:47–59.
46. Bezemer ID, Rinaldi S, Dossus L, van Gils CH, Peeters PHM, van Noord PAH, et al. C-peptide, IGF-I, sex-steroid hormones and adiposity: a cross-sectional study in healthy women within the European Prospective Investigation into Cancer and Nutrition (EPIC). Cancer Causes Control 2005;16:561–72.
47. Rinaldi S, Peeters PH, Bezemer ID, Dossus L, Biessy C, Sacerdote C, et al. Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: the European Prospective Investigation into Cancer and Nutrition. Cancer Causes Control 2006;17: 1033–43.