Validity of Estimated Acrylamide Intake by the Dietary Record Method and Food Frequency Questionnaire in Comparison with a Duplicate Method: A Pilot Study

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Summary Acrylamide, classified as a probable carcinogen to humans, forms during high-temperature cooking. Dietary exposure among the Japanese is unknown. To evaluate the validity of estimated acrylamide intake using a dietary record (DR) and the food frequency questionnaire (FFQ) in comparison with the duplicate diet method (DM) in a Japanese population. Design: A validation study was performed with 14 participants (age, 32–50 y; 11 women) from 11 households. Food samples were simultaneously collected for the DM and DR on the same day over 2 consecutive days. The FFQ was administered after collecting samples for the DM and DR. For the DM, dietary acrylamide was calculated from chemical analyses of each food. For the DR and FFQ, acrylamide intake for each food was calculated using the database of acrylamide contents of foods. Correlation coefficients were calculated using the Spearman rank method. Average acrylamide intake values calculated using the DM, DR, and FFQ were 0.106, 0.233, and 0.128 mg/kg body weight/d, respectively; these values showed a marginally positive correlation between the DM and DR ($r=0.52$), but a low correlation between the DM and FFQ ($r=-0.011$). For the DR, non-alcoholic drinks had the highest contribution, followed by confectionery and vegetables. For the DM, the contribution of confectionery was the highest, followed by vegetables and non-alcoholic drinks. In conclusion, the validity of acrylamide intake estimation using the DR was reasonably high when compared to the analytical value of the simultaneous DM. However, further improvement is required for estimating acrylamide intake using the FFQ.

Key Words acrylamide, dietary record, duplicate method, food frequency questionnaire, validity

Acrylamide is a water-soluble compound mainly produced during food processing and the cooking process by a reaction between asparagine and reducing sugars at high temperatures (1–3). According to several reports based on animal experiments, acrylamide increases the risk of developing breast, lung, or ovarian cancer (4–6). According to the classification of carcinogenicity, the International Agency for Research on Cancer classifies it as group 2A, “probably carcinogenic to humans” (7). However, observational epidemiological research has not shown consistent results regarding the association between dietary acrylamide intake and cancer (8, 9). This inconsistency may be caused by the attenuation of the effect of risk due to an error in dietary measurement.

A database of acrylamide-containing foods is necessary to determine dietary exposure assessment methods, such as the dietary record (DR), 24-h recall, and food frequency questionnaire (FFQ). On the other hand, a database of acrylamide-containing European foods has been developed in the Netherlands to evaluate the exposure to acrylamide intake (10). This acrylamide database was developed using chemical analysis values derived from all acrylamide-containing Dutch foods (i.e., potato crisps, coffee, and cookies). In Japan, the acrylamide contents of specific foods have been published mainly from the reports of the Food Safety Commission of Japan (https://www.fsc.go.jp/fsciis/technicalResearch/download/retrievalId=kai20111222sf&fileld=520). Using this database, the dietary intake of acrylamide and acrylamide-contributing foods among the Japanese population was estimated using the Monte Carlo simulation using data from the National Health and Nutrition Survey (https://www.fsc.go.jp/fsciis/technicalResearch/
Validity of Various Methods Used for Estimating Acrylamide Intake from Foods

In this previous study, the contribution of cooked vegetables to acrylamide production was greater in the Japanese population than that in the Western population. These findings indicated that the contribution of home-cooked vegetables to acrylamide production was high among the Japanese population, and that the sources of dietary acrylamide differ due to differences in dietary habits. Based on these findings, we developed a comprehensive database of acrylamide contents in food for Japanese (11). In this database, 282 of 1,878 food items from the Standard Tables of Food Composition in Japan, Fifth Revised and Enlarged Edition (5th FCT) were designated as acrylamide-containing foods. This database also included information on how acrylamide is affected by various cooking methods and household food preparations (i.e., rice, bread, potatoes, batter, and vegetables). In addition, we used this database to estimate the dietary intake of acrylamide using the DR and FFQ. In this study, we aimed to validate this method of estimation using our database by comparing the estimated acrylamide intake to that in the simultaneous duplicate diet method (DM).

**EXPERIMENTAL**

**Study design and participants.** The study was designed as a cross-sectional dietary assessment. We intended to recruit 10 volunteers aged between 20 and 74 y living in the Kanagawa prefecture, Japan, through posters and flyers between June 2014 and August 2014. We explained the details of the study to the participants in person (or via telephone or email), and ultimately, obtained informed consent and individual information from 14 volunteers (11 women and 3 men) from 11 households.

**Data collection.** Data were collected in September 2014. Food samples for the DM and weighed DR were simultaneously collected over 2 consecutive days (Sunday and Monday) at the participants’ homes. Before the start of data collection, the procedures were explained to the participants in person or via telephone using the protocol manuals. We supplied plastic bags, containers, cooler bags, and ice packs for the DM, as well as specially designed recording sheets, a digital scale, and measuring cups and spoons for the DR to each participant.

Participants saved the duplicates of all foods and drinks (including mixed dishes) they consumed for the 2 consecutive days. Each food and drink sample was separately placed in a plastic bag or container and stored in the refrigerator at their respective homes. Simultaneously, participants weighed all the foods and drinks during the meal preparation using a digital scale and measuring spoons and cups, and recorded the items consumed on a specially designed sheet for the DR.

Following data collection for 2 d, participants from each household brought food samples and recording sheets to the research office, and interviews were conducted by trained dieticians to assess the DR. Food samples were placed in the freezer on the day of the interview and stored at −30°C until analysis. As an incentive for participation, we paid a small remuneration to the participants.

In addition, we administered the FFQ in April 2015 because our FFQ was designed to assess habitual dietary intake for the previous year. The FFQ was originally developed and validated to be used in the Japan Public Health Center-based Prospective Study (12–14). The FFQ consists of 138 food and beverage items and 9 frequency categories, ranging from ‘almost never’ to ‘7 or more times per day’ (or ‘9 glasses per day’ for beverages), and it contains questions about the usual consumption of foods during the previous year. Standard portion sizes were specified for each food item as follows: small (50% smaller), medium (same as the standard), and large (50% larger). The cooking method was considered for 7 vegetables, 2 potatoes, rice, and bread. The proportions of each cooking method were extrapolated from the proportions calculated from the DR. In addition, the frequency of eating fried foods with batter was included as a question in our FFQ.

**Exposure assessment of acrylamide by DR and FFQ.** The development of the database of acrylamide intake from DR data has been described elsewhere. In brief, we selected the analytical values of acrylamide in Japanese foods from 10 reports (15–18, http://www.fsc.go.jp/chousa/kenkyu/kenkyu_happyo.data/29_kenkyu_happyo_1507.pdf, https://www.fsc.go.jp/fscis/technicalResearch/show/cho99920151507, http://www.maff.go.jp/j/syoun/sekiso/risk_analysis/priority/pdf/150807_rp_aa.pdf, http://www.mhlw.go.jp/topics/2002/11/t11011-1a.html, http://www.who.int/foodsafety/publications/acryamide-food/en/). After consolidation of the database, 282 of 1,878 food items from 5th FCT were designated as acrylamide-containing foods. In addition to the food items from the 5th FCT, some cooking methods at home such as stir-frying, baking, and deep-frying were also considered when calculating acrylamide intake from cooked foods (i.e., potatoes, onions, toasted bread, deep-fried batter, and stir-fried rice).

The DR was coded by trained dieticians according to the Standardized Tables of Food Composition, 5th FCT. In addition, cooking methods (raw, boiled, deep-fried, deep-fried with batter, baked, stir-fried, steamed, stir-fried lightly as preparation, and unclear) were coded. Dietary acrylamide was calculated by multiplying the concentration and amount consumed for each food and summing these values daily. Furthermore, energy intake was calculated to determine the over- or underestimation compared to their estimated energy requirement using the 5th FCT.

To assess the long-term intake in epidemiological studies, we also developed a method to estimate acrylamide intake using the FFQ (11). We used the same FFQ that was used in the Japan Public Health Center-based (JPHC) Study, a prospective study of a large Japanese cohort. For calculations using the FFQ, we used the same database of acrylamide-containing foods used in the DR. Acrylamide intake from fried batter was estimated by multiplying the frequency of eating fried foods...
with batter and the amount of acrylamide intake from fried batter per day calculated from the DR. Acrylamide intake from each food was calculated by multiplying the concentration of acrylamide for each food by the consumption frequency and portion size. The total daily acrylamide intake from the FFQ was calculated by summing the acrylamide intake for each food.

**Analysis of food samples.** Quantitation of acrylamide in food samples were performed by modifying the method used by Takatsu et al. (19). Frozen food samples were analyzed at a commercial laboratory (Kankyou Kagaku Kenkyujo K.K., Aichi, Japan). Approximately 10 g of each sample was added to 100 mL of water and 200 μL of internal standard solution (100 μg/mL 13C3-acrylamide solution). The mixture was homogenized for 1 min and centrifuged at 5,000 rpm for 5 min. To defat, 10 mL of the supernatants was strongly shaken with an equivalent amount of dichloromethane and centrifuged at 3,000 rpm for 5 min. A 3-mL aliquot of the supernatant was purified thorough cartridge columns of Bond Elut C18 (500 mg/3 mL), Bond Elut PSA (500 mg), and Bond Elut ACCUCAT (600 mg/3 mL) (all from Agilent Technologies Japan, Ltd., Tokyo, Japan). The purified sample was then applied to LC–MS/MS on an ODS column (InertSustain C18, 2.1×250 mm; GL Sciences Inc., Tokyo, Japan) with 0.1% acetic acid/methanol (5/95) (flow rate, 0.15 mL/min; column temperature, 40°C). The quantitation limit was 0.005 mg/kg.

**Statistical analysis.** The mean, standard deviation, median, minimum, and maximum acrylamide intake from the DR, FFQ, and DM were calculated. Spearman rank correlation coefficients between the DR and DM were calculated. The 18 food groups (grains, potatoes and starches, vegetables, fruits, mushrooms, algae, fishes and shellfishes, meats, eggs, dairy products, fats and oils, confectioneries, beverages, seasonings and spices, and prepared and processed foods) that contributed to acrylamide intake were identified and their percentage contribution were calculated.

**Ethics statements.** This study was approved by the Institutional Review Board (IRB) of Sagami Women’s University (no. 1404) on May 29, 2014. Additional IRB approval was provided by Osaka University to analyze the acrylamide content of food samples (no. 15131) on September 28, 2015, and by Azabu University for data transfer (no. 1321) on September 9, 2017. Written informed consent from the participants was obtained at the study orientation.

**RESULTS**

**Study participants and data collection by DM and DR**

The participants’ characteristics are presented in Table 1. Almost all participants were aged 30–50 y, and 80% were women. The self-reported body mass indexes were similar to the reference value for the Japanese population (http://www.mhlw.go.jp/file/04-Hou douhappyou-10904750-Kenkouzoushkoku/Gantaisakuke nkouzoushinka/0000106403.pdf). All participants simultaneously completed the 2-d DM and DR. Therefore, the range of energy intake calculated by the DR was 1,509–2,414 kcal, and there was no remarkable underestimation compared to their estimated energy requirement (data not shown) (http://www.mhlw.go.jp/file/05-Shingikai-10901000-Kenkouzoushinka/Sou muka/0000114399.pdf). Items in the duplicate collection almost perfectly (99.5%) matched those in the DR. Therefore, the type of food and amount consumed almost completely agreed for the DR and DM.

**Validity of acrylamide intakes of DR and FFQ when compared with DM**

Table 2 shows average values for acrylamide intake calculated from the DM, DR, and FFQ. The DM is a reference method in this study and reveals short-term exposure from food. The DR was also used as a method to estimate short-term exposure and was carried out at the same time as the DM in this study. In contrast, the FFQ is a method for estimating habitual dietary intake to assess the long-term exposure. Average values for acrylamide intake calculated from the DM, DR, and FFQ were 0.106, 0.233, and 0.128 μg/kg body weight/d, respectively; the DR showed higher values than the DM and the FFQ had a similar value to the DM (Table 2).

On the other hand, the coefficient for correlation calculated by the Spearman rank method between the DM and DR had a marginally significant positive value of 0.52 (p=0.057) (Fig. 1) and there was no correlation between the DM and FFQ (r=-0.011, p=0.970) (Fig. 2). This suggested that participants with high levels of acrylamide intake according to the DM showed low values in the FFQ.
Contribution of each food group to total acrylamide intake from the DM and DR

Figure 3 demonstrates the contribution of 18 food groups to the total acrylamide intake in our study population. According to the DR, non-alcoholic drinks (i.e., green tea and coffee) had the highest contribution (48%), followed by confectionery (i.e., snacks) and vegetables. According to the DM, the contribution of confectionery to acrylamide production was highest (31%), followed by vegetables and non-alcoholic drinks. In addition, several foods (i.e., curry rice and stewed dishes) which could not be classified into a particular food group, accounted for 36% in the DM. In the DM analysis, potato chips (1.2 μg/kg), grilled eggplants (0.18 μg/kg) and snacks (0.14 μg/kg) contained higher values of acrylamide.

**DISCUSSION**

The estimated acrylamide intake according to the DR was marginally positively, but insignificantly ($r=0.52$, $p=0.057$), correlated with the DM (Fig. 1). However, in the dietary assessment studies in epidemiology, the median of the correlation coefficient of nutrient intake calculated from the two dietary survey methods ranged between 0.31 and 0.56 (20) because some errors are uncontrollable among free living people. Taking this into account, the correlation coefficient between the DM and DR in our study was reasonably high. On the other hands, the estimated intake according to the FFQ did not correlate with the DM (Fig. 2). These results suggest the potential usefulness of the acrylamide database to estimate dietary intake among the Japanese population. To the best of our knowledge, this study is the first to report the estimates of acrylamide exposure individually using three dietary surveys (DM, DR, and FFQ) among the Japanese population, and to investigate the validity of the estimated intake.

In this pilot study, we also intended to examine the feasibility of conducting the DR and DM simultaneously for 2 consecutive days, which was very burdensome to the participants (21). Volunteers who responded to the flyers were all women, and 3 agreed to help their husbands participate. We observed that their energy intake did not indicate remarkable underestimation compared with their estimated energy requirement (http://www.mhlw.go.jp/file/05-Shingikai-10901000-Kenkoukyoku-Soumuka/0000114399.pdf).
Yamamoto J et al.

This should be considered for the underestimated per-
perform a separate analysis for each food by the DM. We have included various food groups, including vegetables mixed dishes and beverage group. Mixed dishes could DM and DR were somewhat inconsistent in terms of the study, percent contribution by food groups between the DM (Fig. 3). In the DR data analysis, non-alcoholic drinks had the highest contribution (48%), followed by confectionery and vegetables (Fig. 3). In Western countries, reports indicate that wheat products (i.e., bread and breakfast cereals) and processed potato products (i.e., crisps and fried potatoes) account for a large proportion of acrylamide intake (10). Conversely, in Japan, composite cooked dishes (i.e., stir-fried vegetables) account for a large proportion of the sources of acrylamide intake from food (https://www.fsc.go.jp/osirase/acrylamide1.data/acrylamide_hyokasyo1.pdf). In our study, percent contribution by food groups between the DM and DR were somewhat inconsistent in terms of the mixed dishes and beverage group. Mixed dishes could have included various food groups, including vegetables and potatoes, and it was not possible or reasonable to perform a separate analysis for each food by the DM. This should be considered for the underestimated percent contribution in the vegetable group and potatoes and starch group in the DM. For beverages, overestimation of the acrylamide intake from green tea could be a possible reason for this. The amount of acrylamide in green tea in our database was estimated based on the report of Mizukami et al. that the level of acrylamide in green tea leaves ranged from 27 to 110 ng/g and that in green tea ranged from 0.7 to 2.1 ng/mL (15). Among Japanese, it is a popular custom to brew tea in a teapot and drink a few cups from the pot. Because acrylamide is a water-soluble substance (22), the acrylamide content of green tea after the second steeping may become weaker. Therefore, it is considered that the green tea after the second steeping has a lower content of acrylamide. However, this issue was not addressed by Mizukami et al. (15) nor considered in our database, and we could not determine how many cups of tea the participants drank from one pot in the DR. This should be considered for the overestimation of the beverage group in the DM.

A low correlation was found between the FFQ and DM, with a potentially markedly lower acrylamide intake for one participant who responded to the FFQ. It can be assumed that the listed foods in the FFQ had a considerable effect on the estimated acrylamide intake using the FFQ. We used the same FFQ as the JPHC study that consists of 138 food and beverage items (23). There were considerable differences in the estimated acrylamide intake between the DM or DR and FFQ among participants who consumed baked sweet potato or potato chips. These foods have a high level of acrylamide (http://www.mhlw.go.jp/topics/2002/11/tp1101-1a.html). However, our FFQ did not cover eating habits of baked sweet potato and potato chips, and therefore, we could not estimate the consumption of these foods and the acrylamide intake in the FFQ. It may be necessary to assess acrylamide intake using an FFQ that includes foods with high acrylamide content, such as potato chips and baked sweet potato. Overall, two participants (14%) consumed potato chips and one participant (7%) consumed sweet potatoes. Therefore, the influence should be minimum.

Furthermore, it is assumed that the period of the survey has a considerable effect. The FFQ is a dietary survey method used to assess dietary intake over a long-term period (23), whereas the DM, which was used as a reference in this study, is a 2-d survey. Therefore, it is possible that there was low correlation between the 2 surveys because the DM does not account for long-term acrylamide exposure (http://www.mhlw.go.jp/file/05-Shingikai-10901000-Kenkoukyoku-Soumuka/0000114399.pdf). Kotemori et al. reported that the intake of dietary acrylamide for 28-d surveys of 215 and 350 participants, in which energy-adjusted correlation coefficients between the DR and FFQ ranged from 0.34 to 0.48 (11). Therefore, from the results of this study, it could not be inferred that the validity of acrylamide intake estimated by the FFQ is low. Further studies are required to assess the day-to-day variation in acrylamide intake.

The DM is a standard method for estimating the intake of food containing ingredients without a common database. However, the DM cannot be applied to large numbers of participants because of the considerable expense involved and the burden on participants (http://www.mhlw.go.jp/file/05-Shingikai-10901000-Kenkoukyoku-Soumuka/0000114399.pdf). Therefore, different survey methods are required to estimate acrylamide intake with larger populations. In this study, the estimated acrylamide intake by the DR calculated using our database was marginally positively correlated with the DM. Therefore, the present study suggested that it is possible to estimate dietary acrylamide intake from food using the DR in a Japanese population. However, it may be overestimating the amount of acrylamide intake calculated from the DR; the influence by beverages may be especially strong. Further studies are required to update our database.

This study has a few limitations. First, the small sample size contributed to a lack of statistical power for the correlation. We conducted this study as a pilot to test the feasibility of a 2-d simultaneous execution of the DR and DM to estimate acrylamide intake, as well as to investigate the validity of estimating acrylamide intake from foods by different diet research methods. Based on this research, a further large-population study of more than 100 participants is already in progress. Second,
volunteer-based recruitment may have resulted in difficulty in generalizing the results. In addition, collecting data from the same family members may have not been the best method because they have a similar dietary intake. This is an unavoidable problem associated with dietary assessment methods with a heavy burden; however, it does not affect the results too severely if the correlation between 2 methods is non-differential. In addition, when we analyzed the data without including the same family members, the mean intake and coefficient correlations hardly changed (data not shown). Because there were some differences in dietary contents between family members, we included all participants in this study in our analysis. In a further study, we changed the study design based on the limitation of this feasibility study, such as the sample size, recruitment, and number of days for data collection. Third, variability of the acrylamide values in the foods may have resulted in the low reliability of the DM. The amount of acrylamide formation can be easily affected by asparagine and reducing sugar levels in the ingredients and by cooking temperatures (1–3. https://www.fsc.go.jp/osirase/acrylamide1data/acrylamide_hyokasyo1.pdf). Although this is generally an unavoidable limitation for the DM, the pertinent effects can be possibly reduced by using a larger sample size. Lastly, the DM may not have the ability to capture long-term exposure to acrylamide. The number of days necessary to estimate habitual intake, which was calculated using day-to-day variation, was 3 d among Japanese (24). However, these variations in dietary acrylamide intake have not been reported; further studies are required to verify the day-to-day variation in acrylamide intake from food among Japanese. Additionally, to be able to evaluate the validity of the FFQ, long-term exposure markers, such as biomarkers, may be more suitable. Acrylamide biomarkers (i.e., acrylamide hemoglobin adducts and glycidamide), which may be more useful for reflecting a long-term diet in the reference method, should be evaluated (25, 26).

CONCLUSION

The validity of estimating acrylamide intake by means of using a dietary record survey was reasonably high when compared to the analytical value of the simultaneous duplicated diet. However, further improvement is required for estimating acrylamide intake using the FFQ.

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