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The effect of 5-year vitamin C supplementation on serum pepsinogen level and *Helicobacter pylori* infection

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We conducted a population-based, double-blind, randomized controlled trial to examine the effect of vitamin C supplementation on serum pepsinogen (PG) level, *Helicobacter pylori* (*H. pylori*) infection, and cytotoxin-associated gene A (*Cag A*) status. Subjects aged 40 to 69 years living in one village in Akita prefecture, a high-risk area for gastric cancer in Japan, were recruited through annual health check-up programs. Among 635 subjects diagnosed as having chronic gastritis on the basis of serum PG levels, after excluding ineligible cases, 439 subjects were assigned to one of four groups using a 2x2 factorial design (0 or 15 mg/day β-carotene and 50 or 500 mg/day vitamin C). However, based on the results from two β-carotene trials in the United States, we discontinued β-carotene (vitamin C supplementation was continued). Finally, 120 subjects in the low-dose group (vitamin C 50 mg), and 124 subjects in the high-dose group (vitamin C 500 mg) completed the 5-year supplementation. The difference in the change of PGI/II ratio between baseline and after 5-year follow up was statistically significant between the intervention groups among those who completed the supplementation: −0.25 for the low-dose group and −0.13 for the high-dose group (P=0.046). To conclude, vitamin C supplementation may protect against progression of gastric mucosal atrophy. (Cancer Sci 2003; 94: 378–382)

The association between low intake of vegetables and fruits and epithelial, non-hormone-dependent cancers is one of the most consistent findings in epidemiologic studies.[1)] Among many compounds contained in vegetables and fruits, much attention has been focused on antioxidants, including vitamin C, as they are especially abundant in these foods.[1)] Ascorbic acid is known to exert a preventative effect against gastric carcinogenesis through its ability to inactivate oxygen free-radicals, as well as to inhibit nitrosoamine formation.[2,3)] Based on the above-mentioned findings and biological plausibility, vitamin C seems to have potential as a chemopreventive agent in interventional trials.

A possible relation between *Helicobacter pylori* (*H. pylori*) infection and ascorbic acid is also under investigation, as some authors have suggested that high-dose vitamin C may inhibit *H. pylori* infection.[4,5)]

Previous studies have shown that serum pepsinogen (PG) levels reflect the morphological and functional status of the gastric mucosa and may thus be useful for the screening of gastric atrophy, which is a precancerous lesion of gastric cancer.[6)] Herein we describe a population-based, double-blind, randomized controlled trial to examine the effects of vitamin C supplementation on serum PG level as a marker of atrophic gastritis and *H. pylori* infection.

Materials and Methods

Our methods have been described in detail[7)] and are briefly summarized below.

Participants. Subjects aged 40 to 69 years living in four municipalities (three towns and one village) of Yokote Public Health Center District in Akita prefecture, one of the regions with the highest mortality from gastric cancer in Japan, were recruited through annual health check-up programs for circulatory diseases conducted by each municipality under the National Health and Welfare Services Law for the Aged. Eligibility required diagnosis with chronic atrophic gastritis (defined as PGI<70 ng/ml and PGI/PGII ratio<3.0), no past history of gastric cancer, gastric surgery, liver cancer, cirrhosis, or other cancers within the last 5 years, no abnormal liver function (aspartate aminotransferase>100 IU/liter, alanine aminotransferase>100 IU/liter, or alkaline phosphatase>800 IU/liter), no use of diet supplements containing β-carotene or vitamin C, and no expectation of moving outside the study area within 1 year. Written informed consent was obtained from each participant and the Ethics Committee of the National Cancer Center approved the protocol.

Study design and procedures. At first we conducted a "run-in phase," offering full doses of β-carotene (15 mg/day) and vitamin C (500 mg/day) to all participants for 4 weeks to identify and exclude at an early stage the subjects who either did not comply or showed side effects. Remaining participants were then randomized to one of four groups for 5-year supplementation using a 2×2 factorial design (0 or 15 mg/day β-carotene and 50 or 500 mg/day vitamin C).

Participants were asked to visit community centers every 3 months, where public health nurses checked clinical symptoms and compliance (by counting numbers of unconsumed capsules), and provided further capsules. Health condition (including lipid profile, liver function tests) was also monitored every year at the annual health check-up for circulatory diseases. Blood samples were drawn and stored three times (at baseline, and after the first, and the fifth year) in order to measure serum level of ascorbic acid and *H. pylori* antibody and twice (at baseline and the fifth year) for serum levels of PGs, and cytotoxin-associated gene A (*Cag A*) antibody. The participants were also asked to complete a semiquantitative food frequency questionnaire at baseline and the fifth year inquiring about smoking habits, alcohol consumption, and medical history, as well as dietary habits.

However, in response to a National Cancer Institute press re-

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port released on January 18, 1996, indicating that two β-carotene trials had shown no benefit and potential harm,[8] we were obliged to amend the study protocol. First, β-carotene was stopped, but prescription of vitamin C was continued for 5 years. Second, the study area was restricted to the village where participants had already been recruited, and no new participants were recruited from the three other municipalities. Finally, the primary endpoint of the trial was changed from the 10-year cumulative incidence of gastric cancer to the 5-year change in serum levels of PGs and other biomarkers. We explained in detail the results of the two US studies and the amendment of the study protocol, and collected the discontinued capsules from each participant. Signed consent was obtained again from individuals willing to remain in the study, and new capsules containing vitamin C only (50 mg/day or 500 mg/day) were provided. The vitamin C dosage of 50 mg was set based on current Recommended Dietary Allowance (RDA).[9] According to a recent report,[10] safe doses of vitamin C are less than 1000 mg daily, while bioavailability declines and the absorbed amount is largely excreted at single doses of 500 mg and higher. Thus, we set 500 mg as the dosage for the high-dose group.

Laboratory analysis. Fasting blood samples collected at baseline and after 5 years were analyzed for serum ascorbic acid levels, PGI, PGII, *H.pylori* antibody, and Cag A status. The subjects were asked not to eat or drink anything except water after 9 PM on the day before blood sampling. The serum was sampled be-

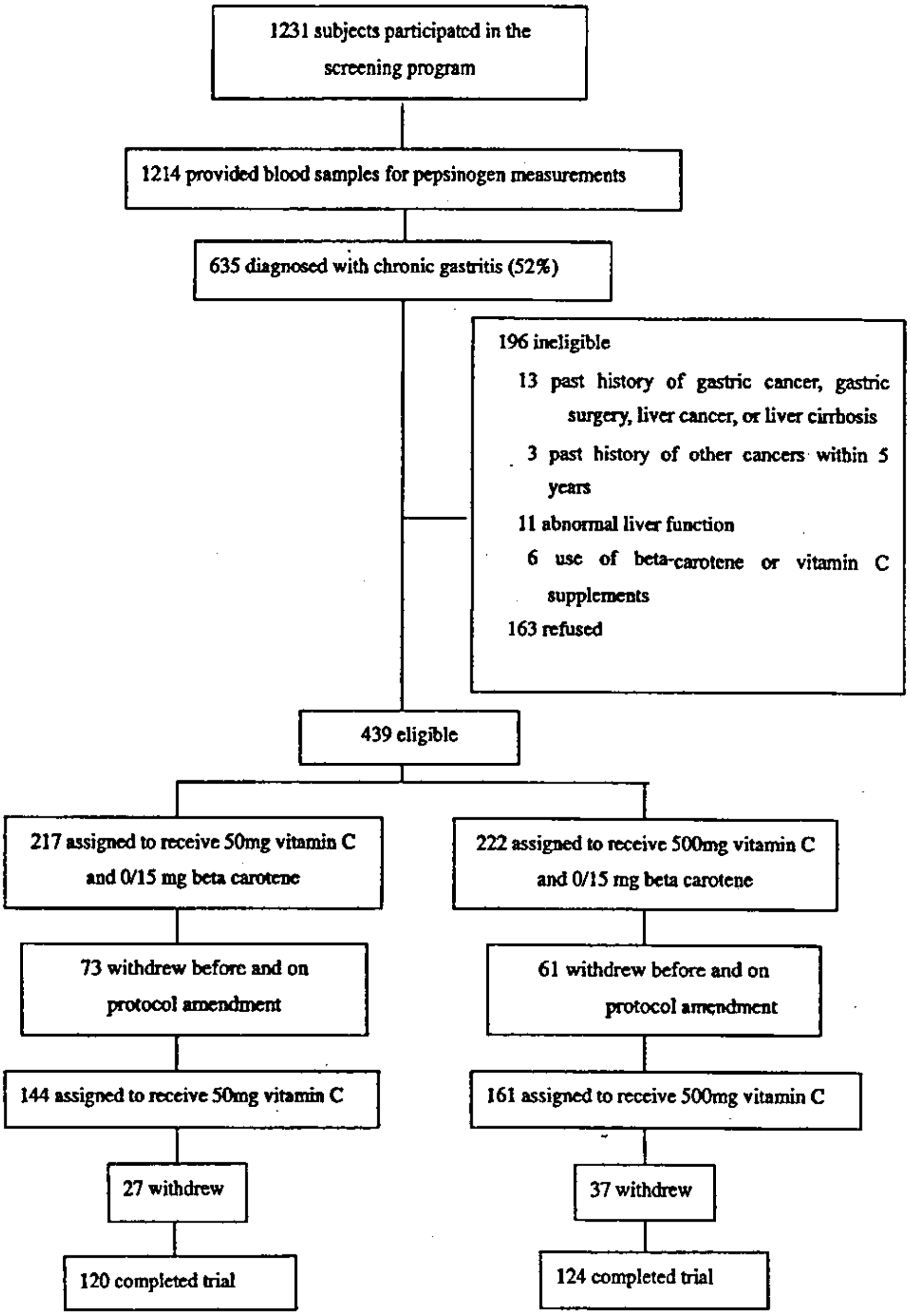

Fig. 1. Study flow.

tween 7 and 10 AM. All samples were stored at −70 to −85°C and were analyzed simultaneously after the completion of the 5-year supplementation. All assays were conducted by persons who were blinded as to the intervention assignment and the questionnaire data.

Serum for ascorbic acid measurement was stabilized by addition of *meta*-phosphoric acid. The level of serum ascorbic acid was analyzed fluorimetrically (iodine oxidation and condensation with 1,2-phenylenediamine). Serum levels of PGI and PGII were measured by radioimmunoassay (RIA) in a commercial laboratory (DINABOT, SRL Co., Ltd., Tokyo). Assays of Cag A was performed by enzyme-linked immunosorbent assay (ELISA), with horseradish peroxidase as the enzyme tracer (Cag A immunoglobulin G (IgG) EIA, Sceti Co., Ltd., Tokyo). The anti-Cag A IgG antibody concentration in standards and samples was measured in a spectrophotometer at 450 nm. IgG antibodies to *H. pylori* were measured with a direct ELISA kit (E Plate 'Eiken' *H. pylori* Antibody, Eiken Kagaku Co., Ltd., Tokyo). Levels of IgG were categorized as seropositive and seronegative for *H. pylori* according to the selected cut off value (492 nm).

Statistical analysis. Baseline characteristics by randomized group were assessed using one-way analysis of variance (ANOVA) for continuous variables and Fisher's exact test for discrete variables. The *P* value was calculated for the difference between the low-dose group and high-dose group. The paired *t* test or one-sample Z test for rates was used for analysis of within-group change of several biomarkers or seropositive rates. One-way ANOVA or one-sample Z test for rates were conducted to investigate the difference of change in serum biomarkers between groups. Multivariate ANOVA and multiple logistic analysis was conducted to control the effect of sex, age and baseline level of *H.pylori* titer. As the distribution of *H. pylori* titer was skewed to the right side, natural logarithms of *H. pylori* titer were always used in the statistical analysis. Reported *P* values were two-sided, and all statistical analyses were done by using the Statistical Analysis System (SAS) version 6.12 (SAS Institute, Inc., Cary, NC).

Results

A total of 1231 subjects, aged 40 to 69 years, entered the annual health check-up program for circulatory diseases which was conducted from June through July, 1995 (Fig. 1). Among them, 1214 subjects provided blood samples for PG measurements and 635 (52%) were diagnosed as having chronic gastritis on the basis of PG level. One hundred and ninety-six men were considered unfit for further examination because they did not meet the inclusion criteria: 13 had a past history of gastric cancer, gastric surgery, liver cancer, or liver cirrhosis; 3 had a past history of other cancers within 5 years; 11 had abnormal liver function; 6 reported use of β-carotene or vitamin C supplements; 163 refused to participate. Finally, 439 subjects (73%) participated in the study. After amendment of the protocol, 305 men (144 men assigned for taking 50 mg of vitamin C and 161 men assigned for taking 500 mg of vitamin C) remained in the study (intention-to-treat cohort). During the follow up, 24 subjects of the 50 mg assigned group and 37 subjects of the 500 mg assigned group withdrew from the study. Thus, 120 subjects for the 50 mg group and 124 subjects for the 500 mg group completed the supplementation (completed group). Subjects for analysis were restricted to those who had supplied blood both at baseline and after 5 years of follow up. This left 117 subjects for each intervention group. Results of completed group analysis are presented in the tables. The same analyses were repeated for the intention-to-treat group, and essentially similar results were obtained.

Baseline characteristics were compared between the low-dose group (vitamin C supplemented with 50 mg), the high-dose group (vitamin C supplemented with 500 mg) and the drop-out group (drop-out before modification of the protocol) (Table 1). No statistically significant difference was found, including the baseline level of serum ascorbic acid, between the intervention groups. Baseline levels of titer of *H. pylori* and Cag A and PG levels were also compared between the intervention groups. No difference was seen, except in the log-transformed *H. pylori* titer; 4.05 for low-dose group and 4.33 for high-dose group ($P=0.005$).

Table 2 shows the changes between baseline and after 5 years of follow up of serum ascorbic acid, *H. pylori* infection, Cag A, and PG levels within the vitamin C supplemented groups and the difference in the changes between groups (completed group analysis). Serum ascorbic acid significantly increased in both groups and the change was significantly higher in the high-dose group ($P=0.0001$). For *H. pylori* and Cag A status, more subjects became seronegative in the high-dose group, though the difference in percentage was significant only for Cag A. As for PG status, about 10% of subjects were not determined as having atrophic gastritis serologically in both groups. While statistically significantly enhanced values of PGII and a decreased

Table 1. Baseline characteristics by randomized group

	Intervention group			
	Low dose (vitamin C 50 mg) n=117	High dose (vitamin C 500 mg) n=117	P for difference[1]	Drop out[2] n=134
Age	59.3 (0.6)	57.4 (0.7)	0.05	57.8 (0.7)
Male (%)	33.3	35.0	0.89	35.8
Current smoker (%)	10.3	13.7	0.55	13.4
Alcohol drinker, 1+/week (%)	36.8	40.2	0.69	24.6
Body mass index (kg/m^2)	23.4 (0.3)	23.3 (0.3)	0.72	24.0 (0.3)
Dietary intakes				
Vitamin C (mg/day)	150.9 (9.7)	153.4 (9.6)	0.86	133.8 (8.5)
Fruit (g/day)	205.7 (20.3)	211.2 (20.5)	0.85	157.9 (12.5)
Green or yellow vegetables (g/day)	56.3 (5.3)	56.4 (5.4)	0.98	50.9 (4.2)
Other vegetables (g/day)	140.6 (13.8)	151.6 (13.9)	0.58	128.6 (12.1)
Serum ascorbic acid (mg/dl)	1.38 (0.03)	1.35 (0.03)	0.49	1.4 (0.03)

Values are means (SE) unless otherwise specified.
1) Difference between low-dose group and high-dose group. Based on one-way analysis of variance (ANOVA), or Fisher's exact test.
2) Drop out before design modification.

Table 2. Change in serum ascorbic acid, *Helicobacter pylori (H. pylori)*, Cag A, and pepsinogen (PG) level between baseline (I) and after 5-year follow up (II) and difference of the changes (II–I) between randomized groups —completed group analysis

	Within-group change						Between group difference		
	Low dose (vitamin C 50 mg) (n=117)			High dose (vitamin C 500 mg) (n=117)			Low dose	High dose	P for
	1995 (i)	2000 (ii)	P[1]	1995 (i)	2000 (ii)	P[1]	ii–i	ii–i	difference[2]
Ascorbic acid	1.38	1.48	0.002	1.35	1.73	0.0001	0.10	0.37	0.0001
(mg/dl)	(1.32–1.44)	(1.42–1.54)		(1.29–1.41)	(1.65–1.81)		(0.02–0.18)	(0.29–0.45)	
H. pylori status,[3] no. (%)	113 (96.6%)	111 (94.9%)	0.40	116 (99.1%)	113 (96.6%)	0.14	−1.7%	−2.6%	0.47
ln *H. pylori* titer	4.05	3.97	0.04	4.33	4.24	0.04	−0.07	−0.09	0.75
(U/ml)	(3.91–4.19)	(3.81–4.13)		(4.32–4.34)	(4.08–4.40)		(−0.17–0.03)	(−0.19–0.01)	
Cag A status,[3] no. (%)	98 (83.8%)	100 (85.5%)	0.61	106 (90.6%)	105 (89.7%)	0.75	1.7%	−0.9%	<0.001
Cag A titer	101.20	89.72	0.02	118.81	108.70	0.05	−11.48	−10.11	0.85
(RU/ml)	(87.24–115.16)	(76.34–103.1)		(104.49–133.13)	(93.97–123.40)		(−21.34—1.62)	(−19.97—0.25)	
PG status,[3] no. (%)	117 (100%)	105 (89.7%)	<0.001	117 (100%)	106 (90.6%)	<0.001	−10.3%	−9.4%	0.74
PGI (ng/ml)	37.91	39.09	0.24	39.58	41.22	0.15	1.18	1.65	0.76
	(34.78–41.04)	(37.14–42.95)		(36.57–42.59)	(37.60–44.84)		(−0.78–3.14)	(−0.45–3.75)	
PGII (ng/ml)	19.28	22.78	0.0001	20.93	24.10	0.0001	3.50	3.17	0.70
	(17.97–20.59)	(20.92–24.64)		(19.56–22.30)	(22.28–25.92)		(2.33–4.67)	(2.00–4.34)	
PGI/II ratio	1.97	1.71	0.0001	1.89	1.76	0.01	−0.25	−0.13	0.05
	(1.85–2.09)	(1.65–1.83)		(1.79–1.99)	(1.64–1.88)		(−0.33−−0.17)	(−0.21−−0.05)	

Values are means (95% CI) unless otherwise specified.
1) Based on paired t test or one-sample Z test for rates.
2) Based on one-way analysis of variance (ANOVA) or one-sample Z test for rates.
3) Number and percentage of subjects positive for *H. pylori* and Cag A, and serologically determined atrophic gastritis for PG (PGI<70 ng/ml and PGI/PGII ratio<3.0).

ratio of PGI/II between baseline and after 5 years of follow up were observed for both groups, the change between the two groups was only significant for PGI/II ratio; −0.25 in the low-dose group, and −0.13 in the high-dose group (P for difference=0.046). Although the strength of the relation was slightly diminished, the overall result did not differ essentially when the same analysis was conducted on the intention-to-treat basis (P=0.06).

Adjusted mean concentrations of change in serum ascorbic acid, titer of *H. pylori* and Cag A, and PGs between baseline and after 5-year follow up were compared between the intervention groups. After adjustment for sex and age, and also baseline level of *H. pylori* titer, the difference in change of serum ascorbic acid and PGI/II ratio between baseline and 5-year follow up between intervention groups still remained statistically significant (P=0.02) (completed group analysis). When the same analysis was repeated on the intention-to-treat basis, the difference in PGI/II ratio between the intervention groups remained statistically significant (P=0.03).

The numbers of subjects with unchanged (seropositive to seropositive, seronegative to seronegative) or worsened (seronegative to seropositive) status of PG were 94 (90.4%) in the low-dose group and 96 (89.7%) in the high-dose group. When the low-dose group was considered as the reference group, the adjusted odds ratio (OR) of unchanged or worsened PG status for the high-dose group was 0.98 (95% confidence interval (95% CI) 0.4–2.5).

Discussion

About 10% of subjects converted to negative PG status in both groups, which may be explained to some extent by what is called 'regression to the mean,' including physiological fluctuation. Even in the absence of treatment, all follow up studies on chronic gastritis show apparent regression in a minority of subjects. In a large cohort follow up study in Colombia, 'regression' rates for the change from atrophy to normal (or superficial gastritis) and from intestinal metaplasia to atrophy were 7.5 and 4.4/100 person years, respectively.[11] The increase of PGII and decrease of PGI/II ratio in both groups contradict our hypothesis that vitamin C may be protective against the development of atrophy. When the change was compared between the two groups, the value was significant only for PGI/II ratio, while it failed to reach the level of statistical significance for PGII. This may be partially explained by the fact that PGI is relatively stable during aging, while PGII is known to increase with age.[12] In contrast, the difference of change in PGI/II ratio between the two groups remained statistically significant even after sex and age were controlled. Several interpretations may exist for the difference in the change of PGI/II ratio between the groups. The change, larger for PGI and smaller for PGII in the high-dose group compared to the low-dose group, became more distinct by taking the ratio of these variables (PGI as numerator and PGII as denominator), but it is not clear whether the difference in ratio, which is known to be related to corpus atrophy, reflects the characteristics of the subjects in this study, or is merely a chance finding. The PGI/II ratio was reported to show an association with severe corpus atrophy,[13] as well as hypochlorhydria.[14] This would be consistent with the high percentage of *H. pylori* infection, which is known to be related to distal gastric cancer, in the subjects in this study.[15] Also, gastric mucosal inflammation may have been reversed to some extent by the role of vitamin C in scavenging free radicals and protecting against lipid peroxidation.[3] Nevertheless, the possibility of a chance finding cannot be ruled out in view of the small number of subjects in both groups. Previous studies have failed to show any association between plasma vitamin C levels and PG levels.[16,17] It seems that while gastric juice vitamin C levels are associated closely with gastric pathology, the effect of serum vitamin C levels on PG levels is much smaller.

H. pylori infection is known to be related to gastric cancer.[15] On the other hand, both epidemiological and laboratory studies suggest that high intake of dietary vitamin C reduces the risk of gastric cancer.[1-3] Moreover, a significantly lower level of ascorbic acid in gastric juice has been reported in *H. pylori*-infected patients, and it has also been found that the value rises after eradication of *H. pylori*.[18,19] We also measured serum IgG antibodies against Cag A. Cag A-positive *H. pylori* causes more extensive inflammation of the gastric mucosa,[20-22] which is more likely to progress to atrophic gastritis,[23] or gastric malignancy.[24] On this basis, one of the aims of this study was to evaluate the possible anti-*H. pylori* effect of vitamin C, as well as the change in Cag A status. However, the number of subjects whose status changed from seropositive to seronegative for *H. pylori* and Cag A was too small to allow detection of any association. The observed mild changes in *H. pylori* and Cag A status may be interpreted as contributing to the change in PGI/II ratio, rather than as reflecting any eradication effect due to vitamin C supplementation.

When the intention-to-treat cohort was set before amendment of the protocol, that is 217 subjects for the low-dose group and 222 subjects for the high-dose group, the conclusion was essentially the same, although the magnitude of the difference became smaller: the change of PGI/II ratio between baseline and after 5-year follow up was −0.19 for the low-dose group and −0.13 for the high-dose group (P=0.26). As nearly half of the subjects dropped out by the end of follow up from these groups, we considered it more appropriate to conduct the intention-to-treat analysis among subjects who remained after the protocol amendment.

The lack of a placebo arm may be critical to evaluate the supplemental effect of vitamin C. However, the mean dietary intakes of vitamin C were 150.9 mg, and 153.4 mg for the low-dose group and the high-dose group, respectively, as shown in Table 1. Thus, supplementation of 50 mg of vitamin C for the low-dose group may be interpreted as allowing this group to play a similar role to a placebo group. Further, the change of serum ascorbic acid level between baseline and after 5 years was statistically significantly different between the intervention groups (Table 2).

Although the difference in change of serum ascorbic acid was significantly different between the intervention groups, the observed changes in *H. pylori* infection status, Cag A status, and PG levels were rather moderate. It has been shown that in the presence of *H. pylori*-associated gastritis, the secretion of ascorbic acid from serum to stomach is severely impaired.[17] In this study, most of the subjects thus may not have had a high enough ascorbic acid level in the stomach to influence the seroprevalence of *H. pylori* or Cag A, or the level of PGs. According to Correa, ascorbic acid may mainly play a role at the phase from atrophic to metaplastic mucosa.[25] Thus, our findings do not conflict with the hypothesis that vitamin C is protective against gastric cancer.

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Validation of a food-frequency questionnaire for cohort studies in rural Japan

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Abstract

Objectives: To examine the validity and reproducibility of a self-administered food-frequency questionnaire (FFQ) used for two cohort studies in Japan.
Design: Cross-sectional study.
Setting: Two rural towns in the Miyagi Prefecture, in north-eastern Japan.
Subjects: Fifty-five men and 58 women.
Results: A 40-item FFQ was administered twice, 1 year apart. In the mean time, four 3-day diet records (DRs) were collected in four seasons within the year. We calculated daily consumption of total energy and 15 nutrients, 40 food items and nine food groups from the FFQs and the DRs. We computed Spearman correlation coefficients between the FFQs and the DRs. With adjustment for age, total energy and deattenuation for measurement error with the DRs, the correlation coefficients for nutrient intakes ranged from 0.25 to 0.58 in men and from 0.30 to 0.69 in women, with median of 0.43 and 0.43, respectively. Median (range) of the correlation coefficients was 0.35 (−0.30 to 0.72) in men and 0.34 (−0.06 to 0.75) in women for food items and 0.60 (−0.10 to 0.76) and 0.51 (0.28–0.70) for food groups, respectively. Median (range) of the correlation coefficients for the two FFQs administered 1 year apart was 0.49 (0.31–0.71) in men and 0.50 (0.40–0.64) in women for nutrients, 0.43 (0.14–0.76) and 0.45 (0.06–0.74) respectively for food items, and 0.50 (0.30–0.70) and 0.57 (0.39–0.66) respectively for food groups. Relatively higher agreement percentages for intakes of nutrients and food groups with high validity were obtained together with lower complete disagreement percentages.
Conclusions: The FFQ has a high reproducibility and a reasonably good validity, and is useful in assessing the usual intakes of nutrients, foods and food groups among a rural Japanese population.

Keywords
Validity
Reproducibility
Food frequency questionnaire

Food-frequency questionnaires (FFQs) have been used in epidemiological studies to investigate the association between diet and chronic diseases[1]. The validity and reproducibility of FFQs in terms of consumption of nutrients has been assessed in variety of studies in Western countries[1] and Japan[2–11].

Epidemiological interest may focus on examining the associations between health and individual foods[12,13] or food groups[14–18]. There are several foods that are eaten frequently in the very localised area of Japan and are hypothesised to have health-protective effects, such as green tea[19–22] and soy products[23–26]. However, relatively few studies[11,27–29] have examined the validity and reproducibility of FFQs in terms of the consumption of foods or food groups, especially in non-Western countries.

We employed a self-administered 40-item FFQ for two population-based prospective cohort studies in Miyagi Prefecture, in north-eastern Japan, that were started in 1990[30] and in 1994[31]. In the present work, we examined the validity and reproducibility of this questionnaire for total energy and 15 nutrients, 40 food items and nine food groups, using intakes measured by 12 days of diet records as the reference standard.

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Subjects and methods

Study design and subjects

The subjects were a sub-sample of participants in one or two of the cohort studies. Fifty-nine men and 60 women were selected on a voluntary basis. Written informed consent was obtained from all subjects. We surveyed the participants with the questionnaire twice, 1 year apart, in November 1996 (FFQ1) and in November 1997 (FFQ2). In the mean time, we collected a total of 12 days of diet records, on three consecutive days (3-day DRs) four times in the year, in November 1996 and February, May and August 1997.

Food-frequency questionnaire

This FFQ was originally developed for the national collaborative cohort study of cancer in Japan[32]. The questionnaire included 40 food items and supplementary questions about the use of vitamin supplements and milk and sugar in coffee and tea. It was not originally intended to calculate the consumption of nutrients from responses on food frequencies. The questionnaire did not specify the time frame for reporting the consumption of food items and did not query specify portion size information. The questionnaire asked about the average frequency of consumption of each food. Regarding the foods consumed differently between seasons, it asked about the frequency in the season in which they are consumed most frequently within a year. Five frequency categories were used for the majority of food items (almost never, 1–2 days per month, 1–2 days per week, 3–4 days per week, almost every day). For rice and bean paste soup, the number of bowls consumed per day was asked. The frequency of alcohol consumption was asked with five frequency categories (almost never, less than once per week, 1–2 days per week, 3–4 days per week, almost every day) and the usual amount was asked with six categories. For four non-alcohol beverages, we used five categories (almost never, sometimes, 1–2 cups per day, 3–4 cups per day, more than 5 cups per day).

Diet records

The DRs were collected so as to cover both weekdays and weekends and also four different seasons of the year. We instructed the participants to record all foods and beverages consumed in a standardised booklet. We asked them to provide detailed descriptions of each food (open-ended) including the weights prepared and proportions consumed. Research dietitians checked their records in a standardised way after completion by the participants.

Statistical analysis

We excluded six subjects who failed to complete the full 12 days of diet records or the two FFQs, and used the remaining 113 subjects (55 men and 58 women) for subsequent analysis.

We calculated the daily consumption of total energy and 15 nutrient variables from the DRs using the *Standard Tables of Food Composition* published by the Science and Technology Agency of Japan[33]. Regarding alcohol, we limited the calculation to alcohol consumed as beverages and excluded alcohol used for cooking. For calculation from the FFQs, we developed a food composition table that corresponded to the items listed in the questionnaire. We determined a portion size for each food item based on the median values observed in the DRs, separately for men and women. Finally, we computed daily nutrient intakes by multiplying the consumption frequency of each food by the nutrient content of the assigned portion size and summing these values for all the foods. We did not consider nutrient intakes from vitamin supplements because the prevalence of daily users was low (20 subjects, 17.7%).

For calculating the daily consumption of the individual foods from DRs, we summed the amount of all the food codes in the DRs corresponding to the 40 items in the FFQ. We then examined the daily consumption of 40 items from the FFQ by converting the selected frequency category for each food to a daily intake. Daily intakes were calculated by multiplying an average number of daily servings by assigned portion sizes. We calculated the daily consumption of nine food groups by combining the individual food items. The nine food groups are the following: (1) total meats (consisting of four items); (2) dairy products (three items); (3) pulses (three items); (4) total fruits (three items); (5) total fruits and vegetables (nine items); (6) total fruits and vegetables excluding pickles (eight items); (7) total vegetables (six items); (8) total vegetables excluding pickles (five items); and (9) yellow & green vegetables (three items). We constructed these food group categories based on the availability of food items in the questionnaire and interest in their potential associations with health outcomes; thus the grouping was not necessarily comprehensive to cover the whole variety of food items.

To assess the validity of the FFQ, we first compared the mean daily intakes between the DRs and FFQ2. We then calculated Spearman correlation coefficients (95% confidence intervals) between the DRs and FFQ2. In addition to crude correlation coefficients, we computed coefficients with adjustment for age and total energy intake by the residual method[34] and with correction for measurement error (within-person variation) in the 12-day DRs[35]. We also calculated Spearman correlation coefficients between the two FFQs (FFQ1 and FFQ2) to assess the 1-year reproducibility.

Second, we divided the daily intakes from DRs into thirds and compared them with thirds calculated from the FFQ, expressing the results as agreement, adjacent agreement and complete disagreement percentages. We did not calculate the percentages for food items because the intakes from the FFQ were expressed as categorical variables and could not be divided into thirds.

Results

The male subjects were aged 45–77 years (mean ± standard deviation (SD), 62.1 ± 8.5 years) and the female subjects 47–76 years (mean ± SD, 61.0 ± 8.5 years). Their major occupations were farmers, self-employed and housewives. The percentage of current smokers in men was 49.1% (27 subjects). No women currently smoked.

We observed significant seasonal differences in the consumption of carotene, ascorbic acid, fruits and vegetables. The daily consumption of carotene and ascorbic acid was high in November (3971 μg for carotene and 166 mg for ascorbic acid in men; 3808 μg for carotene and 186 mg for ascorbic acid in women) and February (3418 μg and 128 mg; 3714 μg and 141 mg, respectively) and low in May (3150 μg and 100 mg; 2995 μg and 121 mg, respectively) and August (2827 μg and 100 mg; 3046 μg and 116 mg, respectively). The daily consumption of fruits was high in November (128.2 g in men and 143.0 g in women) and February (118.5 g and 150.2 g, respectively) and low in May (86.9 g and 113.7 g, respectively) and August (62.4 g and 108.1 g, respectively). Consumption of vegetables was high in November (157.0 g in men and 163.0 g in women) and February (154.9 g and 172.9 g, respectively) and low in May (131.8 g and 142.9 g, respectively) and August (121.2 g and 133.7 g, respectively). For other nutrients and food groups, we did not observe significant seasonal differences.

Table 1 presents the mean daily nutrient intakes in the DRs and FFQs, Spearman correlation coefficients between the DRs and the FFQs, and Spearman correlation coefficients between the two FFQs for both men and women. Compared with the DRs, the questionnaire underestimated the absolute amount of consumption for most of the nutrients except for retinol for both men and women. Adjusted and deattenuated Spearman correlation coefficients between the DRs and the FFQs ranged from 0.25 for protein to 0.58 for ascorbic acid in men and from 0.30 for retinol to 0.69 for phosphorus in women, with the median of 0.43 and 0.43, respectively. Median (range) of Spearman correlation coefficients between the two FFQs administered at 1-year interval was 0.43 (0.31 for niacin to 0.71 for energy) in men and 0.43 (0.40 for protein and carbohydrates to 0.64 for riboflavin and ascorbic acid) in women.

Tables 2 and 3 present the mean daily consumption of 40 food items in the DRs and the FFQs, Spearman correlation coefficients between the DRs and the FFQs, and Spearman correlation coefficients between the two FFQs for men and women, respectively. Adjusted and deattenuated Spearman correlation coefficients for food intakes ranged from −0.30 for dried fish to 0.72 for milk in men and from −0.06 for fresh juice to 0.75 for pork in women, with a median of 0.35 and 0.34, respectively. Median (range) of Spearman correlation coefficients administered at 1-year interval was 0.43 (0.14 for chicken to 0.76 for alcoholic beverages) in men and 0.45 (0.06 for deep-fried dishes to 0.74 for milk) in women.

Table 4 presents the mean daily consumption of nine food groups in the DRs and FFQs, Spearman correlation coefficients between the DRs and the FFQs, and Spearman correlation coefficients between the two FFQs for both men and women. Adjusted and deattenuated Spearman correlation coefficients for food groups intakes ranged from −0.10 for total meats to 0.76 for total fruits in men and from 0.28 for pulses to 0.70 for total fruits in women, with a median of 0.60 and 0.51, respectively. Median (range) of the Spearman correlation coefficients administered at 1-year interval was 0.50 (0.30 for pulses to 0.70 for dairy products) in men and 0.57 (0.39 for yellow & green vegetables to 0.66 for total meats) in women.

Tables 5 and 6 present agreement, adjacent agreement and complete disagreement percentages in the nutrient and food group intakes between the DRs and FFQ (crude and energy-adjusted). Median (range) of agreement percentages for energy-adjusted nutrient intakes was 43% (from 56% for ascorbic acid to 36% for sodium and riboflavin) in men and 47% (from 55% for phosphorus to 33% for retinol) in women. Median (range) of agreement percentages for energy-adjusted food group intakes was 53% (from 65% for dairy products to 33% for total meats) in men and 50% (from 59% for total fruits to 41% for total meats) in women. Median (range) of complete disagreement percentages for energy-adjusted nutrient intakes was 13% (from 15% for fat and thiamine to 4% for phosphorus) in men and 12% (from 17% for retinol to 3% for calcium and phosphorus) in women. Median (range) of complete disagreement percentages with energy-adjusted food group intakes was 9% (from 25% for total meats to 4% for total fruits and vegetables) in men and 10% (from 16% for total vegetables excluding pickles to 5% for dairy products) in women.

Discussion

In this study, we assessed the validity and reproducibility of a 40-item FFQ used for two large-scale cohort studies among a rural Japanese population. The food lists in our questionnaire were not originally intended to calculate the consumption of nutrients. They were not selected for the questionnaire according to the cumulative contribution to absolute total nutrient intakes[36] or the ability of the food items to discriminate between individual variations in nutrient intake[37]. Nevertheless, the questionnaire has a high reproducibility and a reasonably good validity for many nutrients in terms of correlation coefficients.

Median values of coefficients were almost the same between men and women in the nutrients, foods and food groups, but there were differences between sexes for several nutrients, foods and food groups. For example, regarding the validity for nutrients, there was a relatively large sex difference in correlations for energy (0.55 in men

Table 1 Validity and reproducibility of the FFQ for the consumption of nutrients

	Nutrient	Mean daily intake (SD)		Spearman correlation coefficients between DRs and FFQ2		Spearman correlation coefficients between FFQ1 and FFQ2	
		DRs	FFQ	Crude	Age- and energy-adjusted and deattenuated	Crude	Age- and energy-adjusted
Men	Energy (kcal)	2386 (435)	2009 (469)	0.58 (0.38–0.78)	0.55 (0.35–0.76)*	0.72 (0.56–0.88)	0.71 (0.55–0.87)†
	Protein (g)	94.7 (16)	64.8 (14.7)	0.28 (0.03–0.53)	0.25 (−0.03 to 0.52)	0.62 (0.46–0.78)	0.39 (0.12–0.66)
	Fat (g)	56.3 (12.1)	35.5 (9.3)	0.25 (−0.02 to 0.52)	0.37 (0.06–0.68)	0.51 (0.31–0.71)	0.47 (0.22–0.72)
	Carbohydrate (g)	329.8 (66.1)	310.5 (77)	0.59 (0.39–0.79)	0.57 (0.35–0.79)	0.77 (0.65–0.89)	0.61 (0.41–0.81)
	Calcium (mg)	682 (185)	485 (145)	0.35 (0.08–0.62)	0.57 (0.32–0.82)	0.62 (0.44–0.80)	0.60 (0.40–0.80)
	Phosphorus (mg)	1311 (232)	911 (202)	0.32 (0.08–0.56)	0.52 (0.29–0.75)	0.68 (0.54–0.82)	0.54 (0.30–0.78)
	Iron (mg)	13.1 (2.2)	8.5 (2.1)	0.26 (−0.01 to 0.53)	0.35 (0.07–0.63)	0.51 (0.29–0.73)	0.44 (0.19–0.69)
	Sodium (mg)	6328 (1220)	2605 (694)	0.39 (0.15–0.63)	0.37 (0.11–0.62)	0.65 (0.47–0.83)	0.48 (0.24–0.72)
	Potassium (mg)	3195 (607)	2052 (545)	0.30 (0.05–0.55)	0.45 (0.17–0.72)	0.56 (0.36–0.76)	0.55 (0.35–0.75)
	Retinol (μg)	355 (298)	414 (540)	0.44 (0.22–0.66)	0.38 (0.07–0.69)	0.55 (0.37–0.73)	0.34 (0.10–0.58)
	Carotene (μg)	3367 (1375)	1465 (611)	0.37 (0.13–0.61)	0.56 (0.31–0.80)	0.49 (0.25–0.73)	0.51 (0.29–0.73)
	Thiamin (mg)	1.15 (0.20)	0.80 (0.19)	0.20 (−0.07 to 0.47)	0.33 (0.03–0.62)	0.57 (0.37–0.77)	0.52 (0.28–0.76)
	Riboflavin (mg)	1.64 (0.32)	1.23 (0.33)	0.23 (−0.02 to 0.48)	0.43 (0.18–0.69)	0.51 (0.27–0.75)	0.48 (0.24–0.72)
	Niacin (mg)	19.7 (4.6)	12.0 (3.6)	0.24 (0.00–0.48)	0.33 (0.06–0.60)	0.43 (0.19–0.67)	0.31 (0.04–0.58)
	Ascorbic acid (mg)	123 (38)	96 (34)	0.48 (0.26–0.70)	0.58 (0.34–0.83)	0.40 (0.15–0.65)	0.49 (0.27–0.71)
Women	Energy (kcal)	1857 (257)	1359 (225)	0.30 (0.06–0.54)	0.36 (0.11–0.62)*	0.58 (0.40–0.76)	0.54 (0.34–0.74)†
	Protein (g)	79.6 (12.8)	54.6 (12.0)	0.33 (0.09–0.57)	0.49 (0.26–0.73)	0.71 (0.53–0.89)	0.40 (0.16–0.64)
	Fat (g)	52.0 (10.5)	33.4 (8.5)	0.39 (0.17–0.61)	0.50 (0.25–0.74)	0.65 (0.47–0.83)	0.42 (0.20–0.64)
	Carbohydrate (g)	267.5 (41.4)	208.3 (34.2)	0.34 (0.12–0.56)	0.43 (0.21–0.66)	0.51 (0.31–0.71)	0.40 (0.16–0.64)
	Calcium (mg)	671 (174)	518 (132)	0.62 (0.46–0.78)	0.67 (0.50–0.84)	0.61 (0.41–0.81)	0.50 (0.28–0.72)
	Phosphorus (mg)	1139 (191)	817 (181)	0.45 (0.23–0.67)	0.69 (0.55–0.84)	0.70 (0.54–0.86)	0.50 (0.30–0.70)
	Iron (mg)	12.0 (2.3)	8.0 (2.0)	0.40 (0.16–0.64)	0.47 (0.24–0.71)	0.73 (0.55–0.91)	0.50 (0.26–0.74)
	Sodium (mg)	5650 (1014)	2482 (637)	0.32 (0.08–0.56)	0.33 (0.06–0.59)	0.77 (0.67–0.87)	0.55 (0.33–0.77)
	Potassium (mg)	3076 (572)	2149 (508)	0.44 (0.24–0.64)	0.45 (0.22–0.68)	0.68 (0.50–0.86)	0.49 (0.27–0.71)
	Retinol (μg)	309 (271)	335 (272)	0.30 (0.06–0.54)	0.30 (−0.05 to 0.64)	0.66 (0.46–0.86)	0.58 (0.40–0.76)
	Carotene (μg)	3399 (1313)	2089 (735)	0.37 (0.15–0.59)	0.45 (0.21–0.69)	0.55 (0.35–0.75)	0.45 (0.23–0.67)
	Thiamin (mg)	1.01 (0.17)	0.75 (0.18)	0.30 (0.06–0.54)	0.31 (0.02–0.61)	0.64 (0.46–0.82)	0.46 (0.24–0.68)
	Riboflavin (mg)	1.55 (0.30)	1.21 (0.31)	0.47 (0.27–0.67)	0.54 (0.33–0.76)	0.74 (0.60–0.88)	0.64 (0.46–0.82)
	Niacin (mg)	16.1 (3.6)	10.6 (2.9)	0.23 (−0.02 to 0.48)	0.47 (0.19–0.74)	0.68 (0.48–0.88)	0.41 (0.17–0.65)
	Ascorbic acid (mg)	141 (41)	117 (34)	0.39 (0.17–0.61)	0.43 (0.16–0.69)	0.69 (0.51–0.87)	0.64 (0.48–0.80)

FFQ – food-frequency questionnaire; SD – standard deviation; DR – diet record.
*Age-adjusted and deattenuated, using the ratio of the within-person to the between-person variance components from the data of the 12 days of diet records.
† Age-adjusted.

Table 2 Validity and reproducibility of the FFQ for the consumption of individual foods: men

	Mean daily intake in g (SD)		Spearman correlation coefficients between DRs and FFQ2		Spearman correlation coefficients between FFQ1 and FFQ2	
Food	DRs	FFQ	Crude	Age- and energy-adjusted and deattenuated	Crude	Age- and energy-adjusted
Rice	563.4 (184.7)	726.0 (236.0)	0.56 (0.34–0.78)	0.16 (−0.06 to 0.39)	0.80 (0.70–0.90)	0.44 (0.34–0.54)
Miso soup	16.2 (9.0)	24.7 (9.8)	0.49 (0.24–0.74)	0.42 (0.13–0.71)	0.75 (0.61–0.89)	0.70 (0.56–0.84)
Beef	0.8 (1.9)	3.8 (4.7)	0.40 (0.20–0.60)	0.36 (0.16–0.56)*	0.70 (0.52–0.88)	0.69 (0.51–0.87)
Pork (excluding ham, sausage)	16.4 (9.3)	10.4 (6.4)	0.05 (−0.24 to 0.34)	0.20 (−0.63 to 1.00)	0.50 (0.30–0.70)	0.43 (0.23–0.63)
Ham, sausage	6.2 (6.1)	3.1 (3.3)	0.29 (0.05–0.53)	0.37 (0.05–0.70)	0.57 (0.39–0.75)	0.59 (0.41 – 0.77)
Chicken	12.9 (14.3)	8.5 (6.5)	0.07 (−0.18 to 0.32)	0.07 (−0.37 to 0.50)	0.28 (0.03–0.53)	0.14 (−0.11 to 0.39)
Liver	0.7 (2.1)	2.0 (4.0)	0.27 (0.00–0.54)	−0.08 (−0.53 to 0.37)	0.45 (0.23–0.67)	0.27 (0.05–0.49)
Egg	44.8 (20.0)	41.4 (17.4)	0.47 (0.25–0.69)	0.53 (0.28–0.78)	0.57 (0.35–0.79)	0.56 (0.34–0.78)
Milk	110.9 (111.6)	121.8 (88.2)	0.71 (0.57–0.85)	0.72 (0.57–0.86)	0.77 (0.65–0.89)	0.72 (0.60–0.84)
Yoghurt	12.3 (26.5)	18.1 (26.6)	0.57 (0.35–0.79)	0.56 (0.33–0.79)	0.60 (0.40–0.80)	0.56 (0.36–0.76)
Cheeses	1.6 (3.6)	1.4 (3.2)	0.37 (0.12–0.62)	0.36 (0.06–0.66)	0.43 (0.19–0.67)	0.40 (0.16–0.64)
Butter	0.6 (0.9)	0.5 (0.9)	0.25 (0.00–0.25)	0.20 (−0.23 to 0.63)	0.58 (0.36–0.80)	0.50 (0.28–0.72)
Margarine	0.2 (0.9)	0.2 (0.6)	0.25 (−0.02 to 0.52)	0.28 (−0.04 to 0.60)	0.57 (0.35–0.79)	0.68 (0.46–0.90)
Deep-fried dishes, tempura	32.6 (17.3)	27.0 (20.0)	0.31 (0.06–0.56)	0.33 (0.08–0.58)*	0.39 (0.14–0.64)	0.33 (0.08–0.58)
Fried vegetables	28.3 (18.6)	32.8 (25.8)	0.30 (0.03–0.57)	0.70 (0.15–1.00)	0.39 (0.15–0.63)	0.41 (0.17–0.65)
Raw fish, fish broiled with soy, roast fish	78.4 (36.1)	54.2 (30.4)	0.23 (−0.01 to 0.47)	0.39 (0.10–0.67)	0.38 (0.16–0.60)	0.31 (0.09–0.53)
Boiled fish paste	8.9 (7.6)	5.8 (4.4)	0.32 (0.07–0.57)	0.49 (0.11–0.88)	0.19 (−0.06 to 0.44)	0.19 (−0.06 to 0.44)
Dried fish	7.0 (7.2)	2.2 (2.1)	−0.25 (−0.49 to −0.01)	−0.30 (−0.68 to 0.07)	0.50 (0.28–0.72)	0.39 (0.17–0.61)
Green vegetables	17.7 (16.9)	13.6 (7.3)	0.28 (0.03–0.53)	0.35 (0.03–0.66)	0.42 (0.17–0.67)	0.41 (0.16–0.66)
Carrot, pumpkin	22.0 (16.0)	9.6 (6.0)	0.23 (−0.02 to 0.48)	0.24 (−0.05 to 0.54)	0.38 (0.13–0.63)	0.38 (0.13–0.63)
Tomato	21.8 (19.7)	19.3 (16.5)	0.41 (0.17–0.65)	0.63 (0.28–0.99)	0.53 (0.29–0.77)	0.50 (0.26–0.74)
Cabbage, lettuce	29.2 (18.9)	23.0 (14.4)	0.31 (0.07–0.55)	0.40 (0.10–0.70)	0.34 (0.09–0.59)	0.32 (0.07–0.57)
Chinese cabbage	18.0 (15.5)	27.5 (14.0)	0.14 (−0.13 to 0.41)	0.09 (−0.32 to 0.50)	0.13 (−0.12 to 0.38)	0.15 (−0.10 to 0.40)
Wild plant	1.1 (2.1)	2.5 (3.0)	0.10 (−0.17 to 0.37)	0.26 (−0.16 to 0.69)	0.27 (0.02–0.52)	0.27 (0.02–0.52)
Mushrooms (shiitake, enokitake)	7.6 (5.9)	5.8 (4.8)	0.22 (−0.05 to 0.49)	0.32 (−0.08 to 0.72)	0.27 (0.02–0.52)	0.30 (0.05–0.55)
Potatoes	47.9 (22.8)	23.0 (15.5)	0.39 (0.19–0.59)	0.53 (0.26–0.80)	0.47 (0.25–0.69)	0.42 (0.20–0.64)
Seaweeds	12.2 (8.6)	4.8 (2.7)	0.24 (−0.01 to 0.49)	0.44 (0.02–0.85)	0.34 (0.09–0.59)	0.35 (0.10–0.60)
Pickles (radish, Chinese cabbage)	35.1 (20.8)	29.6 (14.0)	0.53 (0.33–0.73)	0.65 (0.42–0.87)	0.64 (0.42–0.86)	0.60 (0.38–0.82)
Food boiled with soy	0.7 (1.3)	1.5 (1.8)	0.09 (−0.16 to 0.34)	0.19 (−0.22 to 0.61)	0.37 (0.15–0.59)	0.39 (0.17–0.61)
Boiled beans	2.1 (4.6)	6.4 (10.3)	0.36 (0.16–0.56)	0.26 (−0.04 to 0.56)	0.50 (0.19–0.81)	0.55 (0.24–0.86)
Soybean (tofu, fermented soybeans)	88.3 (31.5)	61.6 (24.8)	−0.03 (−0.32 to 0.26)	0.02 (−0.33 to 0.38)	0.19 (−0.08 to 0.46)	0.25 (−0.02 to 0.52)
Orange	23.1 (22.2)	46.8 (28.8)	0.51 (0.31–0.71)	0.56 (0.29–0.82)	0.51 (0.27–0.75)	0.61 (0.37–0.85)
Other fruits	72.1 (53.0)	46.5 (27.7)	0.52 (0.30–0.74)	0.70 (0.43–0.98)	0.40 (0.16–0.64)	0.42 (0.18–0.66)
Fresh juice	2.4 (9.0)	26.0 (48.0)	0.15 (−0.03 to 0.33)	0.07 (0.16–0.56)*	0.46 (0.22–0.70)	0.44 (0.20–0.68)
Confectioneries	21.2 (15.6)	17.0 (14.5)	0.43 (0.19–0.67)	0.58 (0.28–0.88)	0.51 (0.31–0.71)	0.61 (0.41–0.81)
Green tea	382.4 (271.3)	510.0 (330.0)	0.73 (0.59–0.87)	0.71 (0.56–0.85)	0.62 (0.42–0.82)	0.63 (0.43–0.83)
Black tea	2.0 (7.0)	42.0 (57.0)	0.25 (0.00–0.50)	0.25 (−0.13 to 0.63)	0.55 (0.33–0.77)	0.50 (0.28–0.72)
Coffee	8.7 (36.5)	174.0 (193.5)	0.15 (−0.12 to 0.42)	0.07 (−0.21 to 0.36)	0.77 (0.63–0.91)	0.75 (0.61–0.89)
Chinese tea	18.3 (41.3)	58.5 (91.5)	0.35 (0.11–0.59)	0.20 (−0.08 to 0.48)	0.47 (0.23–0.71)	0.15 (−0.09 to 0.39)
Alcoholic beverages	23.5 (25.5)	23.4 (24.3)	0.77 (0.61–0.93)	0.70 (0.54–0.86)	0.83 (0.73–0.93)	0.76 (0.66–0.86)

FFQ – food-frequency questionnaire; SD – standard deviation; DR – diet record.
*Without deattenuation because of large within- to between-person variance in the DRs.

Table 3 Validity and reproducibility of the FFQ for the consumption of individual foods: women

Food	Mean daily intake in g (SD)		Spearman correlation coefficients between DRs and FFQ2		Spearman correlation coefficients between FFQ1 and FFQ2	
	DRs	FFQ	Crude	Age- and energy-adjusted and deattenuated	Crude	Age- and energy-adjusted
Rice	356.4 (90.0)	399.0 (93.8)	0.55 (0.35–0.75)	0.65 (0.44–0.86)	0.39 (0.19–0.59)	0.43 (0.23–0.63)
Miso soup	13.3 (8.4)	18.7 (5.9)	0.17 (−0.10 to 0.42)	0.21 (−0.07 to 0.50)	0.66 (0.41–0.91)	0.54 (0.29–0.79)
Beef	0.8 (1.5)	2.7 (3.5)	0.31 (0.0–0.55)	0.13 (−0.11 to 0.37)*	0.63 (0.39–0.87)	0.65 (0.41–0.89)
Pork (excluding ham, sausage)	10.8 (8.6)	8.7 (6.3)	0.36 (0.11–0.61)	0.75 (0.38–1.00)	0.56 (0.31–0.81)	0.50 (0.25–0.75)
Ham, sausage	4.9 (5.4)	3.2 (3.2)	0.38 (0.13–0.63)	0.43 (0.11–0.76)	0.58 (0.33–0.83)	0.49 (0.24–0.74)
Chicken	9.8 (15.9)	9.3 (7.8)	0.12 (−0.20 to 0.39)	0.22 (−0.16 to 0.60)	0.60 (0.33–0.87)	0.49 (0.22–0.76)
Liver	0.8 (2.0)	1.2 (1.8)	0.52 (0.36–0.68)	0.21 (0.05–0.37)*	0.61 (0.45–0.77)	0.50 (0.34–0.66)
Egg	36.6 (18.1)	37.0 (17.0)	0.61 (0.43–0.79)	0.58 (0.38–0.78)	0.68 (0.50–0.86)	0.53 (0.35–0.71)
Milk	135.3 (87.5)	149.1 (77.7)	0.71 (0.57–0.85)	0.65 (0.50–0.79)	0.74 (0.60–0.88)	0.74 (0.60–0.88)
Yoghurt	21.9 (28.6)	33.3 (28.8)	0.62 (0.46–0.78)	0.60 (0.43–0.77)	0.41 (0.25–0.57)	0.45 (0.29–0.61)
Cheeses	1.3 (2.4)	1.2 (1.5)	0.32 (0.08–0.56)	0.36 (0.09–0.64)	0.47 (0.23–0.71)	0.35 (0.11–0.59)
Butter	0.8 (1.2)	0.5 (0.6)	0.10 (−0.20 to 0.37)	0.11 (−0.27 to 0.49)	0.49 (0.22–0.76)	0.56 (0.29–0.83)
Margarine	0.2 (0.6)	0.4 (0.5)	0.30 (0.03–0.57)	0.41 (0.02–0.80)	0.50 (0.23–0.77)	0.54 (0.27–0.81)
Deep-fried dishes, tempura	29.7 (20.4)	23.2 (15.4)	0.21 (0.00–0.46)	0.09 (−0.16–0.34)*	0.36 (0.11–0.61)	0.06 (−0.19 to 0.31)
Fried vegetables	32.1 (23.0)	51.3 (30.0)	0.13 (−0.10 to 0.38)	0.26 (−0.25 to 0.77)	0.46 (0.21–0.71)	0.38 (0.13–0.63)
Raw fish, fish broiled with soy, roast fish	58.6 (25.0)	48.0 (24.0)	0.30 (0.06–0.54)	0.60 (0.26–0.94)	0.50 (0.26–0.74)	0.33 (0.09–0.57)
Boiled fish paste	8.0 (6.8)	7.7 (6.1)	0.25 (0.01–0.49)	0.41 (0.08–0.73)	0.57 (0.33–0.81)	0.44 (0.20–0.68)
Dried fish	6.0 (6.7)	2.3 (2.1)	0.17 (−0.10 to 0.44)	0.35 (−0.09 to 0.79)	0.34 (0.07–0.61)	0.28 (0.01–0.55)
Green vegetables	15.9 (11.4)	14.2 (6.2)	0.18 (−0.10 to 0.43)	0.37 (−0.02 to 0.76)	0.48 (0.23–0.73)	0.40 (0.15–0.65)
Carrot, pumpkin	24.8 (17.7)	17.0 (8.3)	0.31 (0.07–0.55)	0.35 (0.08–0.62)	0.50 (0.26–0.74)	0.44 (0.20–0.68)
Tomato	34.2 (31.4)	33.6 (24.5)	0.29 (0.02–0.56)	0.33 (−0.02 to 0.67)	0.45 (0.18–0.72)	0.41 (0.14–0.68)
Cabbage, lettuce	27.9 (15.9)	25.2 (13.2)	0.17 (−0.10 to 0.42)	0.15 (−0.23 to 0.52)	0.49 (0.24–0.74)	0.40 (0.15–0.65)
Chinese cabbage	16.2 (12.0)	30.9 (14.6)	0.24 (0.00–0.49)	0.17 (−0.08 to 0.42)*	0.53 (0.28–0.78)	0.49 (0.24–0.74)
Wild plant	0.8 (2.0)	2.4 (2.4)	0.13 (−0.10 to 0.37)	0.15 (−0.24 to 0.54)	0.25 (0.01–0.49)	0.22 (−0.02 to 0.46)
Mushrooms (shiitake, enokitake)	6.7 (5.4)	3.4 (2.3)	0.27 (0.03–0.51)	0.55 (0.17–0.93)	0.46 (0.22–0.70)	0.33 (0.09–0.57)
Potatoes	44.3 (19.2)	31.5 (15.5)	0.24 (0.00–0.49)	0.08 (−0.27 to 0.43)	0.51 (0.26–0.76)	0.32 (0.07–0.57)
Seaweeds	12.1 (7.6)	6.4 (3.0)	0.09 (−0.20 to 0.36)	0.00 (−0.40 to 0.40)	0.32 (0.05–0.59)	0.13 (−0.14 to 0.40)
Pickles (radish, Chinese cabbage)	34.5 (24.3)	32.0 (16.7)	0.67 (0.51–0.83)	0.67 (0.50–0.84)	0.75 (0.59–0.91)	0.57 (0.41–0.73)
Food boiled with soy	0.4 (0.8)	1.7 (2.4)	0.11 (−0.10 to 0.36)	0.25 (0.00–0.50)*	0.48 (0.23–0.73)	0.44 (0.19–0.63)
Boiled beans	3.2 (6.1)	7.2 (8.8)	0.20 (0.00–0.44)	0.47 (0.06–0.88)	0.60 (0.36–0.84)	0.41 (0.17–0.65)
Soybean (tofu, fermented soybeans)	72.2 (28.3)	56.0 (19.3)	0.17 (−0.10 to 0.42)	0.23 (−0.08 to 0.54)	0.58 (0.33–0.83)	0.60 (0.35–0.85)
Orange	29.8 (24.0)	72.3 (31.3)	0.35 (0.10–0.60)	0.51 (0.18–0.85)	0.51 (0.26–0.76)	0.53 (0.28–0.78)
Other fruits	95.9 (50.6)	68.0 (33.0)	0.46 (0.24–0.68)	0.61 (0.35–0.87)	0.53 (0.31–0.75)	0.59 (0.37–0.81)
Fresh juice	2.4 (8.0)	22.0 (32.0)	0.01 (−0.20 to 0.26)	−0.06 (−0.43 to 0.31)	0.61 (0.36–0.86)	0.47 (0.22–0.72)
Confectioneries	39.2 (25.5)	20.0 (16.2)	0.34 (0.12–0.56)	0.27 (0.02–0.52)	0.53 (0.31–0.75)	0.41 (0.19–0.63)
Green tea	535.2 (280.9)	504.0 (240.0)	0.53 (0.33–0.73)	0.53 (0.33–0.74)	0.66 (0.46–0.86)	0.64 (0.44–0.84)
Black tea	4.6 (8.5)	40.5 (57.0)	0.31 (0.06–0.56)	0.32 (0.07–0.57)*	0.24 (−0.01 to 0.49)	0.22 (−0.03 to 0.47)
Coffee	9.6 (31.6)	120.0 (142.5)	0.41 (0.19–0.63)	0.27 (0.04–0.50)	0.66 (0.44–0.88)	0.64 (0.42–0.86)
Chinese tea	13.9 (32.1)	36.0 (60.0)	0.00 (−0.30 to 0.25)	0.03 (−0.39 to 0.46)	0.32 (0.07–0.57)	0.38 (0.13–0.63)
Alcoholic beverages	0.7 (1.5)	0.6 (1.4)	0.71 (0.53–0.89)	0.60 (0.4–0.80)	0.75 (0.57–0.93)	0.66 (0.48–0.84)

FFQ – food-frequency questionnaire; SD – standard deviation; DR – diet record.
*Without deattenuation because of large within- to between-person variance in the DRs.

Table 4 Validity and reproducibility of the FFQ for the consumption of food groups

	Food group	Mean daily intake in g (SD)		Spearman correlation coefficients between DRs and FFQ2		Spearman correlation coefficients between FFQ1 and FFQ2	
		DRs	FFQ	Crude	Age- and energy-adjusted and deattenuated	Crude	Age- and energy-adjusted
Men	Total meats	36.3 (16.9)	25.8 (15.0)	−0.06 (−0.33 to 0.21)	−0.10 (−0.51 to 0.31)*	0.53 (0.31–0.75)	0.45 (0.23–0.67)
	Dairy products	124.8 (114.1)	142.7 (100.2)	0.67 (0.49–0.85)	0.71 (0.52–0.89)	0.69 (0.53–0.85)	0.70 (0.54–0.86)
	Pulses	106.5 (31.3)	92.5 (29.8)	0.05 (−0.24 to 0.34)	0.11 (−0.25 to 0.46)	0.27 (0.00–0.54)	0.30 (0.03–0.57)
	Total fruits	97.6 (60.1)	119.6 (74.6)	0.62 (0.46–0.78)	0.76 (0.51–1.00)	0.44 (0.20–0.68)	0.50 (0.26–0.74)
	Total fruits and vegetables	241.4 (92.5)	242.2 (109.0)	0.61 (0.41–0.81)	0.75 (0.50–1.00)	0.52 (0.30–0.74)	0.54 (0.32–0.76)
	Total fruits and vegetables excluding pickles	206.3 (90.2)	212.4 (104.4)	0.61 (0.41–0.81)	0.74 (0.50–1.00)	0.49 (0.25–0.73)	0.50 (0.26–0.74)
	Total vegetables	143.8 (51.5)	122.7 (49.1)	0.50 (0.26–0.74)	0.60 (0.33–0.87)	0.49 (0.25–0.73)	0.43 (0.19–0.67)
	Total vegetables excluding pickles	108.8 (48.3)	92.9 (43.0)	0.40 (0.16–0.64)	0.49 (0.20–0.78)	0.41 (0.16–0.66)	0.37 (0.12–0.62)
	Yellow & green vegetables	61.5 (35.3)	42.5 (23.3)	0.46 (0.22–0.70)	0.54 (0.24–0.84)	0.55 (0.33–0.77)	0.53 (0.31–0.75)
Women	Total meats	26.3 (18.7)	24.0 (15.6)	0.36 (0.12–0.60)	0.51 (0.39–1.00)	0.64 (0.44–0.84)	0.66 (0.46–0.86)
	Dairy products	158.5 (94.6)	183.4 (84.1)	0.63 (0.45–0.81)	0.60 (0.45–0.82)	0.58 (0.36–0.80)	0.61 (0.39–0.83)
	Pulses	88.6 (30.9)	82.5 (23.7)	0.21 (0.00–0.46)	0.28 (0.03–0.61)	0.60 (0.40–0.80)	0.57 (0.37–0.77)
	Total fruits	128.2 (60.3)	162.9 (65.3)	0.56 (0.34–0.78)	0.70 (0.62–1.00)	0.56 (0.34–0.78)	0.58 (0.36–0.80)
	Total fruits and vegetables	281.6 (100.3)	316.2 (106.4)	0.51 (0.29–0.73)	0.57 (0.39–0.87)	0.59 (0.39–0.79)	0.59 (0.39–0.79)
	Total fruits and vegetables excluding pickles	247.1 (92.6)	284.1 (99.0)	0.44 (0.20–0.68)	0.51 (0.31–0.84)	0.57 (0.35–0.79)	0.54 (0.32–0.76)
	Total vegetables	153.4 (65.2)	153.3 (60.5)	0.47 (0.23–0.71)	0.45 (0.22–0.79)	0.66 (0.52–0.80)	0.53 (0.33–0.73)
	Total vegetables excluding pickles	119.0 (55.4)	121.2 (52.3)	0.36 (0.12–0.60)	0.35 (0.11–0.70)	0.60 (0.44–0.76)	0.46 (0.22–0.70)
	Yellow & green vegetables	74.9 (44.8)	65.1 (32.3)	0.45 (0.21–0.69)	0.44 (0.21–0.79)	0.49 (0.27–0.71)	0.39 (0.15–0.63)

FFQ – food-frequency questionnaire; SD – standard deviation; DR – diet record.
The nine food groups consist of the following: (1) total meats (four food items – beef, pork, ham and sausage, chicken); (2) dairy products (three food items – milk, yoghurt and cheeses); (3) pulses (three food items – boiled beans, soybean dishes and miso soup); (4) total fruits (three food items – orange, other fruits and fresh juice); (5) total fruits and vegetables (nine food items – orange, other fruits, fresh juice, green vegetables, carrot and pumpkin, tomato, cabbage and lettuce, Chinese cabbage and pickles): (6) total fruits and vegetables excluding pickles (eight food items – orange, other fruits, fresh juice, green vegetables, carrot and pumpkin, tomato, cabbage and lettuce, and Chinese cabbage); (7) total vegetables (six food items – green vegetables, carrot and pumpkin, tomato, cabbage and lettuce, Chinese cabbage and pickles); (8) total vegetables excluding pickles (five food items – green vegetables, carrot and pumpkin, tomato, cabbage and lettuce, and Chinese cabbage); and (9) yellow & green vegetables (three food items – green vegetables, carrot and pumpkin, and tomato).
*Deattenuation by using log-transformed intra- and inter-individual variance components.

Table 5 Percentages of agreement, adjacent agreement and complete disagreement according to tertile classification of daily nutrient intakes based on the diet records and food-frequency questionnaire

		Crude			Energy-adjusted		
	Nutrient	Agreement (%)	Adjacent agreement (%)	Complete disagreement (%)	Agreement (%)	Adjacent agreement (%)	Complete disagreement (%)
Men	Energy	56	36	7	–	–	–
	Protein	35	47	18	44	44	13
	Fat	47	36	16	42	44	15
	Carbohydrate	55	40	5	51	40	9
	Calcium	55	33	13	47	42	11
	Phosphorus	40	47	13	51	45	4
	Iron	36	47	16	40	47	13
	Sodium	42	47	11	36	53	11
	Potassium	45	44	11	44	45	11
	Retinol	42	51	7	49	38	13
	Carotene	38	51	11	42	45	13
	Thiamin	36	44	20	38	47	15
	Riboflavin	36	45	18	36	51	13
	Niacin	35	51	15	42	45	13
	Ascorbic acid	53	36	11	56	36	7
Women	Energy	40	45	16	–	–	–
	Protein	43	45	12	47	40	14
	Fat	45	41	14	50	41	9
	Carbohydrate	47	45	9	52	36	12
	Calcium	55	38	7	47	50	3
	Phosphorus	43	48	9	55	41	3
	Iron	47	45	9	48	41	10
	Sodium	41	45	14	43	43	14
	Potassium	48	41	10	40	52	9
	Retinol	36	52	12	33	50	17
	Carotene	36	48	16	43	45	12
	Thiamin	36	50	14	48	36	16
	Riboflavin	48	41	10	50	38	12
	Niacin	45	40	16	53	31	16
	Ascorbic acid	38	48	14	45	41	14

Table 6 Percentages of agreement, adjacent agreement and complete disagreement according to tertile classification of daily intakes of food groups based on the diet records and food-frequency questionnaire

		Crude			Energy-adjusted		
	Food group	Agreement (%)	Adjacent agreement (%)	Complete disagreement (%)	Agreement (%)	Adjacent agreement (%)	Complete disagreement (%)
Men	Total meats	38	33	29	33	42	25
	Dairy products	65	29	5	65	29	5
	Pulses	35	45	20	38	38	24
	Total fruits	53	44	4	56	35	9
	Total fruits and vegetables	64	31	5	60	36	4
	Total fruits and vegetables excluding pickles	58	35	7	58	35	7
	Total vegetables	60	29	11	53	38	9
	Total vegetables excluding pickles	49	36	15	51	38	11
	Yellow & green vegetables	45	45	9	47	40	13
Women	Total meats	47	38	16	41	45	14
	Dairy products	60	34	5	57	38	5
	Pulses	34	48	17	52	38	10
	Total fruits	55	33	12	59	33	9
	Total fruits and vegetables	48	45	7	50	40	10
	Total fruits and vegetables excluding pickles	53	34	12	48	34	17
	Total vegetables	59	28	14	48	40	12
	Total vegetables excluding pickles	52	33	16	48	36	16
	Yellow & green vegetables	48	41	10	52	38	10

and 0.36 in women) and protein (0.25 and 0.49, respectively).

The absolute mean levels of consumption for energy and most nutrients were lower in our questionnaire than in the diet records. This is probably due to the relatively small number of food items included in our questionnaire. A questionnaire with a larger number of food items may better estimate the absolute amount of food and nutrient consumption. In contrast, the absolute consumption levels for individual food items were not necessarily lower in our questionnaire than in the diet records, which would indicate that the validity of our questionnaire in assessing the absolute amount of intake varies by food item. However, the problem of our questionnaire estimates of absolute intake should be of less concern when they are applied to the main cohort studies, since we would use energy-adjusted values rather than absolute values, and the primary objective of analyses would be to rank individuals within the cohorts according to the relative levels of consumption.

Table 7 summarises studies conducted in Japan examining the validity and reproducibility of FFQs in assessing the consumption of multiple nutrients. Among these studies, median correlation coefficients between the DRs and the FFQs ranged from 0.36 to 0.61, and median reproducibility between the FFQs ranged from 0.32 to 0.72. Our questionnaire showed validity and reproducibility comparable with those reported in other Japanese studies.

The validity and reproducibility of individual food items[27,28] and dietary patterns[38] in the FFQ were examined for the US population. For the Japanese population, Wakai *et al.*[29] examined the validity and reproducibility of 20 food group intakes assessed by a 97-item FFQ. Median (range) of correlation coefficients with the DRs was 0.56 (0.16 for noodles to 0.83 for milk and dairy products). Median (range) of correlation coefficients for 1-year reproducibility was 0.54 (0.34 for eggs to 0.78 for breads). Tokudome *et al.*[11] also examined the validity of 15 food group intakes assessed by a 102-item FFQ. Median (range) of correlation coefficients with the DRs was 0.52 (0.17 for beverages to 0.83 for rice). Our results are similar to the findings in these studies.

Regarding the nutrients and food groups with high correlation coefficients, agreement and complete disagreement percentages according to tertile classification of daily intakes based on DRs and FFQ were generally high and low, respectively, in our study. The agreement and complete disagreement percentages of ascorbic acid in men (Spearman correlation coefficient, 0.58) were 56% and 7% (energy-adjusted), respectively. In contrast, the agreement and complete disagreement percentages of retinol in women (Spearman correlation coefficient, 0.30) were 33% and 17% (energy-adjusted), respectively. Because we cannot assume that the two variables (daily intake from DR and daily intake from FFQ) are normally distributed and their relationship is linear, we calculated

Table 7 Summary of validation studies for FFQs among the Japanese population

Author	Number of food items	Number of frequency categories	Subjects	Days of DRs	Number of nutrients	Validity: Correlation coefficients between DRs and FFQ†	Reproducibility: Correlation coefficients between FFQs†	Reproducibility: Interval between FFQs
Nakamura *et al.*, 1994[2]	21	Open-ended	19 women	7	13	0.56 (0.27–0.90)	–	1 week
Date *et al.*, 1996[3]	122	Open-ended	67 men and women	56–63	14	0.48 (0.21–0.74)	0.71 (0.28–0.78)	1 week
Katagiri *et al.*, 1998[4]	24	6	72 men and women	7	11	0.37 (0.15–0.57)	0.67 (0.59–0.90)	1 week
Sasaki *et al.*, 1998[5]	110	*	47 women	3	18	0.48 (0.19–0.75)	–	–
Egami *et al.*, 1999[6]	97	9	88 men and women	16	19	0.61 (0.42–0.83)	0.67 (0.48–0.82)	1 year
Shimizu *et al.*, 1999[7]	169	8	117 men and women	3	15	0.42 (0.10–0.56) men 0.37 (0.10–0.66) women	0.62 (0.46–0.78) men 0.57 (0.13–0.67) women	1 year
Yamaoka *et al.*, 2000[8]	65	7	71 men	7	18	0.36 (−0.10 to 0.65)	0.72 (0.59–0.81)	10 months
Tsubono *et al.*, 2001[9]	141	9	113 men and women	12	16	0.43 (0.24–0.85)	0.68 (0.47–0.91)	1 year
Tsubono *et al.*, 2001[10]	44	4	211 men and women	28	15	0.37 (0.15–0.57) men 0.39 (0.12–0.51) women	0.32 (0.06–0.58) men 0.51 (0.36–0.61) women	5 years
Tokudome *et al.*, 2001[11]	102	8	79 women	28	31	0.45 (0.23–0.71)	–	–
Present study	40	5	113 men and women	12	16	0.49 (0.27–0.65)	0.57 (0.44–0.69)	1 year

FFQ – food-frequency questionnaire; DR – diet record.
*Combination of the two questions: (1) per day, week or month, and (2) once, twice, three times or more than or equal to four times.
† Values are given as mean or median (range).

agreement and complete disagreement percentages to ascertain the usefulness of the FFQ for dividing individuals according to their level of consumption.

For interpreting the results presented in Tables 5 and 6, we must consider what would be expected by chance alone. For example, the probability of agreement and complete disagreement expected by chance alone in tertile classification is 33% and 22%, respectively. Thus, we might have overestimated agreement percentages or underestimated complete disagreement percentages.

Some traditional Japanese foods, such as green tea and soybean products (tofu, miso, etc.), have recently drawn attention as being potentially health-protective. Although many studies have been conducted in Japan to examine the associations between consumption of green tea[19–22], soy products[23–26] and various health outcomes, most studies did not document the validity and reproducibility of questionnaires used to measure the usual consumption of these food items. In this study, the correlation between our questionnaire and the DRs was high for green tea (adjusted and deattenuated Spearman correlation coefficient of 0.71 in men and 0.53 in women), moderate for miso soup (0.42 and 0.21, respectively) and low for soybean products (0.02 and 0.23, respectively). These results suggest that the validity of FFQs for Japanese populations may vary by the food item examined.

A possible reason for the low correlation for soybean products may be the lack of variation in the questionnaire responses owing to a limited number of frequency categories, since the majority of men (65.5%) and women (75.9%) chose the highest category (almost daily). A questionnaire with a larger number of frequency categories at the high end may have improved validity for the assessment of soybean intake. Another reason may be the lack of between-person variation in our subjects in the actual consumption of soybeans. The ratio of between- to within-person variation in soybean intake, estimated from the diet record data, was 5.92 in men and 5.52 in women. These ratios are relatively higher compared with other popular foods such as rice (0.69 and 1.18, respectively), miso soup (3.50 and 3.14, respectively) and green tea (0.99 and 1.20, respectively), indicating the relatively limited between-person variation for intake of soy foods.

We have reported that consumption of green tea, as measured by another FFQ with high validity, is not associated with decreased risk of gastric cancer in a different cohort study conducted in the same area[13]. Using the two cohort studies in which the present FFQ is used, we are currently examining the associations between green tea, soybean products and various cancers. We are also beginning to examine the relationship between level of intake of food groups such as fruit and vegetables and cancer risk at several sites, because we can show that our FFQ is useful to divide individuals according to their relative level of intake.

Information regarding validity is important and indispensable in interpreting study results[1]. For example, when a calculated relative risk suggests 'no association' between diet and diseases, it may come from too small correlations between the DR and FFQ to detect differences between dietary exposures. Dietary exposures are often expressed in quintiles. Our results suggest that gross misclassification exists in several nutrients or food groups calculated by our FFQ. The attenuation of relative risks towards the null hypothesis can be caused by this non-differential misclassification[39].

In summary, we examined the validity and reproducibility of a 40-item FFQ used for two prospective cohort studies in rural Japan. Our results indicate comparable validity and reproducibility with regard to the consumption of nutrients, foods and food groups. This brief FFQ is useful to examine the association between diet and health in the Japanese population.

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VEGETABLES, FRUIT AND RISK OF GASTRIC CANCER IN JAPAN: A 10-YEAR FOLLOW-UP OF THE JPHC STUDY COHORT I

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The association between vegetables and fruit consumption and gastric cancer risk was investigated in a population-based prospective study in 4 public health center areas in Japan. Dietary and other exposure data were obtained in 1990 from a cohort of 19,304 men and 20,689 women with a self-administered questionnaire. After 10 years of follow-up, a total of 404 cases of gastric cancer were documented among them. After adjustment for age, gender, areas and other potential confounding factors and after exclusion of the cases diagnosed in first and second follow-up years, the relative risk associated with intake 1 or more days per week compared to less than 1 day per week was 0.64 (95% CI 0.45–0.92) for yellow vegetable, 0.48 (95% CI 0.25–0.89) for white vegetable and 0.70 (95% CI 0.49–1.00) for fruit. Relative risks associated with quintile of total vegetable consumption were 1.00, 0.86, 0.75, 0.90 and 0.75 (*p* for trend = 0.17). In the differentiated type of gastric cancer, the association became clearer: 1.00, 0.96, 0.78, 0.88 and 0.53 (*p* for trend = 0.03). This prospective study suggests that vegetable and fruit intake, even in low amounts, is associated with a lower risk of gastric cancer. Although no striking differences in the association were seen between cardia and noncardia cancer, an inverse association was higher in differentiated rather than in undifferentiated types of gastric cancer.

Key words: *gastric cancer: histologic classification; vegetables; fruit; prospective studies*

Although gastric cancer mortality has been declining during the last few decades, it remains the second most common cancer worldwide.[1] High consumption of vegetables and fruit has been hypothesized to have protective effects against gastric cancer. Although in many previous case-control studies a high consumption of vegetables and fruit has been associated with a reduced risk of gastric cancer,[2–4] the evidence from 11 prospective cohort studies has not been consistent.[5–15]

Trends of gastric cancer incidence reportedly differ depending on the subsites and histologic types. The incidence of cardia cancer has recently increased, while the rate of distal cancer has been stable for intestinal type or decreasing for diffuse type.[16–19] On the other hand, recent studies have indicated that infection by *Helicobacter pylori (H. pylori)*, an established risk factor for gastric cancer, is associated with an increased risk for noncardia cancer[20] but not for cardia cancer.[21] These findings indicate that discrepancies in incidence trends between the two subsites (noncardia and cardia) and 2 histologic types (intestinal and diffuse) may be linked with not only *H. pylori* infection but also dietary habits or nutrient factors including the consumption of vegetable and fruits. However, only a few case-control studies of fruits, vegetables and gastric cancer have analyzed the data according to subsites and histology,[22–25] and no prospective study has been conducted.

To further examine the association between the consumption of vegetables and fruit and the risk of gastric cancer, we conducted a population-based prospective study in four public health center areas as part of the Japan Public Health Center-based prospective study on cancer and cardiovascular disease (JPHC Study). Furthermore, we examined the association between the risk of different gastric cancer subsites and histologic types and vegetables and fruit consumption.

MATERIAL AND METHODS

Study cohort

JPHC Study Cohort I is a prospective cohort study that began in January 1990. The study design has been described previously.[26] Briefly, the cohort included 27,063 men and 27,435 women, aged 40–59 years at baseline who registered their addresses in 4 public health center (PHC) areas: Ninohe PHC area of Iwate Prefecture, Yokote PHC area of Akita, Saku PHC area of Nagano and Ishikawa PHC area of Okinawa. These 4 PHC areas were selected to represent the extent of variation in the mortality rate of gastric cancer based on our previous ecologic study.[27,28] After the initiation of the study, 65 men and 38 women were found to be ineligible and were thus excluded (28 persons of non-Japanese nationality, 73 with delayed reports of out-migration before the start of the follow-up and 2 with mistakenly recorded birthdays), leaving 26,998 men and 27,397 women eligible for the study.

Exposure data

A self-administered questionnaire was distributed to all registered residents in 1990 and was collected from 20,658 men (76%) and 22,482 (82%) women.

It included a food frequency questionnaire (FFQ), which asked about the average consumption of 44 food items during the previous month. The questionnaire included 4 items on vegetables ("green leafy vegetables such as spinach", "yellow vegetables such as carrot, pumpkin", "white vegetables such as chinese cabbage,

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radish, tomato, cucumber" and "pickled vegetables"), 1 item on total fruit and 2 on juices (vegetable and fruit). The consumption frequency of vegetables and fruit was asked using 4 categories: less than 1 day per week, 1–2 days per week, 3–4 days per week and almost daily. For the 2 juices, we used 6 frequency categories (less than 1 day per week, 1–2 days per week, 3–4 days per week, almost daily and 1–2 cups per day, almost daily and 3–4 cups per day and almost daily and 5 or more cups per day).

For calculating the amount of vegetables and fruit intakes, we determined the portion size and the content of each food item based on the observed median values on 14–28-day diet record data.[29] This diet record was conducted to assess the validity of the questionnaire. The Spearman correlation coefficients between the diet record and the questionnaire in men (n = 94) and women (n = 107), respectively, were 0.26 and 0.36 for the amount of total vegetable intake (g/day), which is defined as the sum of intake of green, yellow and white vegetables, 0.52 and 0.41 for the amount of fruit intake (g/day), 0.39 and 0.40 for frequency of vegetable intake (times/day) and 0.59 and 0.44 for frequency of fruit intake (times/day). For the amount of vegetables and fruit intake (g/day), we adjusted for total energy intake by the residual method.[30]

Participants also provided information on their frequency consumption of highly salted fish roe and gut (sodium chloride content: 7–12%), use of vitamin supplements, smoking status (never, past smoker and current smoker), alcohol consumption (g/day), educational level (up to primary school, high school and college or higher) history of peptic ulcer (yes or no) and family history of gastric cancer (yes or no).

Of 43,140 subjects who responded to the questionnaire, 1,086 men and 1,195 women who reported extreme total energy intake (upper 2.5% or lower 2.5%) and 268 men and 598 women who reported a past history of cancer were excluded, leaving 19,304 eligible men and 20,689 eligible women for the analysis.

Follow-up

We followed the subjects from January 1, 1990, through December 31, 1999. Incident cases of cancer occurring in the cohort have been identified through continuous surveillance of hospital records, population-based cancer registries and death certificates. In Ninohe and Ishikawa PHC areas, prefecture-wide cancer registries were available. Of the 2,610 cancer cases diagnosed in 1990–1999, 245 (9.4%) were first identified by death certificate as a supplementary information source for the cancer registry (Death Certificate Notification, DCN). Among them, 55 cases not confirmed by other medical records accounted for 2.1% (Death Certificate Only, DCO) of all entries, reflecting a reasonably high quality of cancer registration in this cohort. Each case was confirmed by a histologic diagnosis, based on biopsy or surgery. The histologic classification was based on the available pathology records, and no collection and reclassification of original specimens were done. As of November 2000, a total of 404 cases of gastric cancer, 294 in 19,304 men and 110 in 20,689 women, were documented with a histologically confirmed diagnosis in 1990–1999.

Cardia cancer has been defined as a tumor located in the esophagogastric junction or upper third of the stomach (International Classification of Disease for Oncology [ICD-O] code C160–161).[31] Until quite recently in Japan, the upper third of the stomach has been called the "cardia" based on the guidelines for gastric cancer classification.[32] Because of the difficulty in distinguishing the actual "cardia" from the upper third of the stomach, we combined them into one site for analysis in our study. A tumor located on the lower side of the stomach was classified as distal gastric cancer (ICD-O code C162-167). Those subsites that could not be classified for its diffuse lesion (ICD-O code C168) or those with no information (ICD-O code C169) were categorized as unclassified. The following histologic subdivisions were made in our study: the differentiated types (corresponding to intestinal types of Lauren's classification) were composed of papillary adenocarcinoma, tubular adenocarcinoma (well-differentiated type) and tubular adenocarcinoma (moderately differentiated type); the undifferentiated types (corresponding to diffuse types of Lauren's classification) included poorly differentiated adenocarcinoma, mucinous adenocarcinoma and signet-ring cell carcinoma. Adenosquamous carcinoma, squamous cell carcinoma, carcinoid tumor, undifferentiated carcinoma and miscellaneous others belonged to unclassified types. In accordance with this classification, 47 cardia cancer, 289 noncardia cancer and 64 unclassified subsite cancer patients were identified. The noncardia cancers included 111 undifferentiated types (corresponding to diffuse types of Lauren's classification), 164 differentiated (corresponding to intestinal types of Lauren's classification) and 14 unclassified types.

Statistical analysis

All analyses were conducted for men and women combined because of the relatively small number of gastric cancer cases among women. We computed the gastric cancer incidence rate for a frequency category of vegetables and fruit and a quintile category of vegetable consumption by dividing the number of gastric cancer cases by person-years of follow-up. We calculated person-years of follow-up for each subject from January 1, 1990 until the dates of diagnosis of gastric cancer, death or moving from a PHC area or the end of follow-up (December 31, 1999), whichever occurred first. We used the Cox proportional-hazards regression model to estimate the rate ratios (RRs) using the SAS PHREG procedure.[33] RRs were adjusted for the following variables: age (5-year age groups), gender, PHC areas, educational level (up to primary school, high school, college or higher), smoking status (never, former, current), BMI (less than 22, ≥22 and <25, ≥25), alcohol consumption (none, < 250 g/week, ≥250 g/week), use of supplements for vitamin A, C or E, total energy intake (in quintiles), highly salted food (sum of salted fish roe and salted fish gut intake category), history of peptic ulcer and family history of gastric cancer (yes or no). We repeated all the analyses after excluding the 66 patients with gastric cancer diagnosed in the first 2 years of follow-up (41 men and 25 women). The p-values for the test of linear trend were 2-sided.

RESULTS

The distribution of the baseline characteristics according to total vegetable consumption is shown in Table I. Persons with high vegetable consumption were older and had higher gastric cancer rates in their family history. Vegetable consumption was not clearly associated with a history of peptic ulcer or the educational level. Participants with high vegetable consumption also reported a higher prevalence of healthy behavior as indicated by lower rates of current smoking and lower alcohol consumption. However, participants with high vegetable consumption also reported a higher consumption of highly salted food. No appreciable difference was observed in body mass index, rates of any vitamin supplement use and total energy intake. Vegetable consumption was positively correlated with vegetable juice or fruit juice consumption in men but not in women.

Table II presents RRs of gastric cancer by frequency of vegetable and fruit consumption. Compared to those who consumed less than 1 day per week of vegetables (except pickled vegetables) and fruit, subjects who consumed them more frequently tended to have a lower risk of gastric cancer. However, the risk did not decline in a stepwise manner as the consumption frequencies increased from 1–2 days per week through almost daily except for yellow vegetables. When the upper 3 categories were combined and compared to the lowest category (less than 1 day per week), fruit consumption was associated with a significantly lower risk, while yellow and white vegetables were associated with a nonsignificantly lower risk. Pickled vegetables were not associated with either a higher or lower risk of gastric cancer. After exclusion of the gastric cancer cases diagnosed in the first and second follow-up