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Isoflavone consumption and subsequent risk of hepatocellular carcinoma in a population-based prospective cohort of Japanese men and women

Norie Kurahashi[1]*, Manami Inoue[1], Motoki Iwasaki[1], Yasuhito Tanaka[2], Masashi Mizokami[2] and Shoichiro Tsugane[1], for the JPHC Study Group

[1]*Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan*
[2]*Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Mizuho-ku, Nagoya, Japan*

The incidence of hepatocellular carcinoma (HCC) is much higher in men than in women. Several experiment and epidemiological studies have suggested that estrogen might play an inhibitory role in the development of HCC. Because isoflavones have a similar structure as 17β-estradiol and appear to have an anti-estrogenic effect in women and estrogenic effect in men, we hypothesized that the effect of isoflavones on HCC differs by sex. We investigated the association between isoflavones (genistein and daidzein) and soy products and HCC in Japan in a population-based prospective study in 19,998 Japanese (7,215 men and 12,783 women) aged 40–69 years. During 11.8 years of follow-up, 101 subjects (69 men and 32 women) were newly diagnosed with HCC. Case patients were grouped according to consumption of isoflavones and soy products and stratified by hepatitis virus infection. Hazard ratios (HRs) and 95% confidence intervals (CIs) for HCC were calculated by Cox proportional-hazards modeling. In women, genistein and daidzein were dose-dependently associated with an increased risk of HCC, with multivariable HRs for the highest versus lowest tertile of 3.19 (95%CI = 1.13–9.00, p_{trend} = 0.03) and 3.90 (95% CI = 1.30–11.69, p_{trend} = 0.01), respectively. No association between isoflavones and HCC was observed in men. These results persisted when analysis was restricted to subjects positive for either or both hepatitis C and B virus. In conclusion, isoflavone consumption may be associated with an increased risk of HCC in women. Women with hepatitis virus infection may be advised to abstain from isoflavone consumption. Further studies are warranted to confirm these findings.
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Key words: isoflavone; hepatocellular carcinoma (HCC); hepatitis C virus (HCV); hepatitis B virus (HBV); JPHC study

Hepatocellular carcinoma (HCC) is an important disease worldwide. In Japan, HCC ranks as the third- and fourth-leading cause of death from cancer among men and women, respectively.[1] The most important risk factors for the development of HCC in humans are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).[2] However, despite a similar prevalence of chronic HCV or HBV infection in men and women,[3,4] the incidence of HCC is higher in men, with the International Agency for Research on Cancer (IARC) reporting a 2-fold or greater difference.[5] Although this may partly result from differences in exposure to environmental risk factors such as alcohol consumption and cigarette smoking, several human and nonhuman studies point to a possible role of hormonal factors. In laboratory experiments, ovariectomy in mice increased susceptibility to chemically induced hepatocarcinogenesis,[6,7] whereas administration of estrogens inhibited the development of HCC in male mice.[7] Additionally, Naugler *et al.*[8] showed that estrogen-mediated inhibition of interleukin-6 (IL-6) reduced HCC risk in female mice. In an epidemiological study, Yu *et al.* reported that natural menopause at a younger age and ovariectomy during premenopause were associated with an increased risk of HCC.[9] These various observations suggest that sex hormones, especially estrogen, may confer a protective effect against the development of HCC.

Isoflavones are structurally similar to 17β-estradiol and have the ability to bind to estrogen receptors (ERs),[10] suggesting that they may influence the development of HCC. However, to date this possibility has received relatively scant interest.[11–16] Isoflavones act as estrogen agonists and also as antagonists competing for estradiol at the receptor complex.[17] Because physiological levels of estradiol differ substantially between men and women,

Abbreviations: CI, confidence interval; ER, estrogen receptor; HBsAg, hepatitis B virus antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IL, interleukin.

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Members of the JPHC Study Group: S. Tsugane (principal investigator), M. Inoue, T. Sobue and T. Hanaoka, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan; J. Ogata, S. Baba, T. Mannami and A. Okayama, National Cardiovascular Center, Suita, Japan; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto and T. Ikuta, Iwate Prefectural Ninohe Public Health Center, Ninohe, Japan; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi and N. Nagai, Akita Prefectural Yokote Public Health Center, Yokote, Japan; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe and Y. Miyagawa, Nagano Prefectural Saku Public Health Center, Saku, Japan; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa, Japan; K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito and F. Shoji, Katsushika Public Health Center, Tokyo, Japan; A. Murata, K. Minato, K. Motegi and T. Fujieda, Ibaraki Prefectural Mito Public Health Center, Mito, Japan; K. Matsui, T. Abe, M. Katagiri and M. Suzuki, Niigata Prefectural Kashiwazaki and Nagaoka Public Health Center, Kashiwazaki and Nagaoka, Japan; M. Doi, A. Terao, Y. Ishikawa and T. Tagami, Kochi Prefectural Chuo-higashi Public Health Center, Tosayamada, Japan; H. Sueta, H. Doi, M. Urata, N. Okamoto and F. Ide, Nagasaki Prefectural Kamigoto Public Health Center, Arikawa, Japan; H. Sakiyama, N. Onga, H. Takaesu, and M. Uehara, Okinawa Prefectural Miyako Public Health Center, Hirara, Japan; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Suita, Japan; S. Matsushima and S. Natsukawa, Saku General Hospital, Usuda, Japan; M. Akabane, Tokyo University of Agriculture, Tokyo, Japan; M. Konishi, K. Okada, and I. Saito, Ehime University, Toon, Japan; H. Iso, Osaka University, Suita, Japan; Y. Honda and K. Yamagishi, Tsukuba University, Tsukuba, Japan; H. Sugimura, Hamamatsu University, Hamamatsu, Japan; Y. Tsubono, Tohoku University, Sendai, Japan; M. Kabuto, National Institute for Environmental Studies, Tsukuba, Japan; S. Tominaga, Aichi Cancer Center Research Institute, Nagoya, Japan; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan; S. Sato, Osaka Medical Center for Health Science and Promotion, Osaka, Japan; N. Yasuda, Kochi University, Nankoku, Japan; S. Kono, Kyushu University, Fukuoka, Japan; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita, Japan; Y. Takashima, Kyorin University, Mitaka, Japan; E. Maruyama, Kobe University, Kobe, Japan; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo, Japan; T. Kadowaki, Tokyo University, Tokyo, Japan; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo, Japan; and H. Shimizu, Sakihae Institute, Gifu, Japan.

***Correspondence to:** Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji Chuo-ku Tokyo 104-0045, Japan. Fax: +81-3-3547-8578.
E-mail: nkurahas@ncc.go.jp

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we speculated that the effects of isoflavones on HCC might differ by sex. Long-term isoflavone consumption in typical daily life appears to have an anti-estrogenic effect in women and an estrogenic effect in men, although several studies have reported that short-term dietary soy showed a weak estrogenic response in the breast in women.[18–20] Indeed, in epidemiological studies, isoflavones have been inversely associated with breast cancer in women[10,21,22] and prostate cancer in men.[10,23,24] Additionally, the inverse association between isoflavones and breast cancer was more pronounced in women with high blood levels of estradiol.[25] On the basis of these, the effects of isoflavones may be dependent on endogenous levels of estradiol, and we hypothesized that their effects on HCC may differ by sex. However, previous epidemiological findings for isoflavones or soy food intake and HCC are inconsistent, and most studies did not analyze by sex,[11,13–16] nor consider HCV or HBV infection status.[11,12,14,15]

Here, we investigated the presence of an association between isoflavone consumption and HCC in Japanese men and women in a large-scale population-based cohort study in Japan, with due consideration for HCV and HBV infection status.

Material and methods

Study population

The Japan Public Health Center-based Prospective Study (JPHC study) Cohort II was initiated in 1993–1994. This cohort consisted of 6 PHC areas (Ibaraki, Niigata, Kochi, Nagasaki, Okinawa and Osaka) across Japan. The study design has been described in detail previously.[26] The study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan. The study population was defined as all residents aged 40–69 years at the start of the respective baseline survey. In the present analysis, we excluded some subjects from the Osaka area for whom different definitions were used. Initially, we defined a population-based cohort of 68,974 subjects (33,888 men and 35,086 women) after the exclusion of ineligible subjects ($n = 103$).

Baseline survey

At baseline, participants completed a self-administered questionnaire that assessed information on personal medical history, smoking and drinking habits, diet and other lifestyle factors. Completed questionnaires were received from 26,850 men (response rate, 79%) and 29,785 women (response rate, 85%). Subjects with a self-reported history of cancer at baseline were excluded from analysis ($n = 1,219$).

Blood collection

Subjects were asked to voluntarily provided 10 mL of blood during health checkups in 1993–1995. Samples were divided into plasma and buffy layers, and preserved at −80°C until analysis. Among respondents to the baseline questionnaire, a total of 20,406 subjects (36%) (7,442 men and 12,964 women) donated blood.

Food frequency questionnaire

The questionnaire asked about the usual consumption of 52 foods, including beverages, during the previous year. We then calculated the consumption of isoflavones (genistein and daidzein) and soy food. Soy food referred to the consumption of *tofu*, *miso* (soybean paste) and *natto* (fermented soybeans), for which the major ingredient is soybean. Standard portion sizes were specified for each food item in 3 amounts: small (50% smaller), medium (same as the standard) and large (50% larger). The frequency of soy food intake was divided into 5 categories: almost never, sometimes, 1 or 2 times per week, 3 or 4 times per week and almost daily. The total consumption of soy food (g/day) was calculated from these responses, whereas isoflavone consumption was calculated using values in a specially developed food composition table for isoflavones in Japanese foods.[27,28] Energy was calculated using the fifth revised edition of the Standard Tables of Food Composition in Japan.[29]

Validity was assessed in subsamples using 14- or 28-day dietary records. Spearman's correlation coefficients between energy-adjusted intake of soy food from the questionnaire and from dietary records for men and women were 0.47 and 0.44, respectively, whereas those for energy-adjusted intake of genistein and daidzein were 0.56 and 0.55 for men, and 0.51 and 0.49 for women, respectively (unpublished data).

Among the 20,406 subjects who responded to the questionnaire and provided a blood sample, 408 who reported extreme total energy intake (upper 1.0% or lower 1.0%) were excluded, leaving 19,998 subjects (7,215 men and 12,783 women) for analysis.

Follow-up and identification of HCC

Subjects were followed from the baseline survey until December 31, 2005. Changes in residence status, including survival, were identified annually through the residential registry in their public health center area. Among study subjects, 1,070 (5.4%) moved out of their study area and 49 (0.2%) were lost to follow-up during the study period.

Incidence data on HCC were identified by active patient notification from major local hospitals in the study area and data linkage with population-based cancer registries, with permission from the local governments responsible for the registries. Death certificates were used as a supplementary information source. In our cancer registry system, 5.9% of cases were based on death certificate only. Cases were coded using the International Classification of Diseases for Oncology, Third Edition (ICD-O-3; C22.0).[30] We identified 101 (69 for men, 32 for women) newly diagnosed cases of HCC during the study period among subjects who had returned the baseline questionnaire and provided blood samples.

Laboratory assays

Plasma samples were screened for anti-HCV using a third-generation immunoassay (Lumipulse II Ortho HCV, Ortho-Clinical Diagnostics K.K., Tokyo, Japan)[31] and for hepatitis B virus antigen (HBsAg) by reversed passive hemagglutination with a commercial kit (Institute of Immunology, Tokyo, Japan). The virus-positive group consisted of subjects positive for either or both anti-HCV and HBsAg.

Statistical analysis

Person-years of follow-up were calculated for each subject from the date of completion of the baseline questionnaire to the date of HCC diagnosis, date of emigration from the study area, or date of death, whichever occurred first; or if none of these occurred, follow-up was through to the end of the study period (December 31, 2005). Subjects who were lost to follow-up were censored at the last confirmed date of presence in the study area. Hazard ratios (HRs) of HCC were calculated by tertiles of isoflavones and soy food consumption, with the lowest consumption category as the reference. HRs and 95% confidence intervals (CIs) were calculated by the Cox proportional hazards model, adjusting for age at baseline survey (5-year age categories) and study area (6 PHC areas) according to the SAS PHREG procedure (Version 9.1; SAS Institute, Cary, NC). For further adjustment, additional possible confounders were incorporated into the model: smoking status (never, former, current); alcohol intake (non- and ex-drinkers, less than weekly, weekly or more [<150 g/week, 150-300 g/week or ≥300 g/week]); intake of coffee (almost never, 1–4 days/week, 1–4cups/day or 5 or more cups/day) and vegetables (continuous); and HCV or HBV infection status (positive or negative). In females, further adjustment was made for menopausal status (yes or no). These variables are either known or suspected risk factors for cancer or were previously associated with the risk of HCC.[32] When covariates were entered into the statistical model, isoflavones, soy food and vegetable intakes were adjusted for total energy intake using the residual method.[33] Vegetable intakes were

TABLE I – SUBJECT CHARACTERISTICS AT BASELINE ACCORDING TO GENISTEIN CONSUMPTION

	Men			Women		
	Genistein consumption			Genistein consumption		
	Low	Middle	High	Low	Middle	High
Age, years ± SD	56.6 ± 8.6	57.5 ± 8.2	59.0 ± 7.6	55.9 ± 8.5	56.2 ± 8.3	57.3 ± 7.9
Current smoker (%)	47.0	41.5	36.9	5.4	3.9	3.0
Regular drinker (yes, %)	64.6	63.6	59.3	12.1	9.0	7.2
Postmenopausal (%)	–	–	–	69.7	70.6	76.9
Coffee, daily (%)	46.6	36.3	31.6	42.4	34.7	27.3
Vegetables (g/day)	49.4 ± 67.1	59.3 ± 65.9	71.9 ± 68.9	55.2 ± 68.2	63.5 ± 68.5	69.6 ± 64.6
Soy food (g/day)	21.7 ± 10.2	49.5 ± 14.4	81.9 ± 18.8	23.1 ± 11.1	49.7 ± 15.5	76.1 ± 17.3
Genistein (mg/day)	6.0 ± 2.7	13.8 ± 3.5	24.2 ± 6.3	6.4 ± 2.9	13.9 ± 3.8	23.2 ± 6.3
Daidzein (mg/day)	3.6 ± 1.6	8.2 ± 2.1	14.5 ± 3.8	3.8 ± 1.7	8.4 ± 2.3	13.9 ± 3.8
Infection status						
HCV(−)/HBV(−)	89.43	90.89	90.61	92.65	93.24	93.22
HCV(−)/HBV(+)	3.29	2.83	2.99	2.00	1.92	2.06
HCV(+)/HBV(−)	7.20	6.24	6.28	5.19	4.76	4.62
HCV(+)/HBV(+)	0.08	0.04	0.12	0.16	0.07	0.09

Values are mean unless otherwise indicated. SD = Standard deviations, HCV = hepatitis C virus, HBV = hepatitis B virus.

TABLE II – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR HEPATOCELLULAR CARCINOMA (HCC) ACCORDING TO ISOFLAVONE AND SOY FOOD CONSUMPTION AMONG JAPANESE MEN AND WOMEN

		No. of cases	Person-years of follow-up	HR (95%CI)[1]	HR (95%CI)[2]
Men (N = 7,215)					
Genistein (mg/day)					
Low	<12.0	26	27,304	1	1
Middle	12.0–19.9	16	27,793	0.66 (0.35–1.23)	0.78 (0.41–1.49)
High	≥20.0	27	27,863	1.07 (0.61–1.89)	1.13 (0.60–2.11)
p_{trend}				0.93	0.80
Daidzein (mg/day)					
Low	<8.0	26	27,310	1	1
Middle	8.0–12.7	17	27,781	0.69 (0.37–1.29)	0.81 (0.43–1.54)
High	≥12.8	26	27,869	1.03 (0.58–1.82)	1.09 (0.58–2.05)
p_{trend}				0.98	0.87
Soy food (g/day)					
Low	<37.6	26	27,318	1	1
Middle	37.6–64.9	16	27,738	0.65 (0.34–1.21)	0.77 (0.40–1.47)
High	≥65.0	27	27,904	1.05 (0.60–1.84)	1.10 (0.59–2.03)
p_{trend}				0.97	0.84
Women (*N* = 12,783)					
Genistein (mg/day)					
Low	<12.2	6	50,398	1	1
Middle	12.2–19.5	12	51,424	2.36 (0.88–6.32)	2.36 (0.85–6.51)
High	≥19.6	14	51,029	2.86 (1.07–7.64)	3.19 (1.13–9.00)
p_{trend}				0.03	0.03
Daidzein (mg/day)					
Low	<8.1	5	50,402	1	1
Middle	8.1–12.5	13	51,408	3.08 (1.09–8.70)	3.10 (1.07–8.99)
High	≥12.6	14	51,041	3.46 (1.21–9.83)	3.90 (1.30–11.69)
p_{trend}				0.02	0.01
Soy food (g/day)					
Low	<38.2	8	50,403	1	1
Middle	38.2–62.7	11	51,056	1.51 (0.60–3.78)	1.68 (0.65–4.35)
High	≥62.8	13	51,393	1.74 (0.71–4.28)	1.74 (0.67–4.52)
p_{trend}				0.22	0.25

[1]Adjusted for age and area.–[2]Adjusted for age, area, HCV, HBsAg, smoking status, alcohol consumption, and intake of coffee and vegetables. Further adjusted for menopausal status in women.

calculated from 6 items in the questionnaire. Testing of the proportional hazards assumption by Schoenfeld and scaled Schoenfeld residuals found no violation of proportionality. We additionally analyzed the association between isoflavone intake and HCC in subjects who were either or both anti-HCV- or HBsAg-positive.

Trends were assessed by assignment of the median value in each category. All *p*-values were 2-sided, and statistical significance was determined at the $p < 0.05$ level.

Results

During 235,811 person-years of follow-up (average follow-up, 11.8 years) for 19,998 subjects (7,215 men and 12,783 women), a total of 101 cases (69 for men, 32 for women) of HCC were newly diagnosed and included in the analyses.

Subject characteristics at baseline according to tertile of energy-adjusted isoflavone consumption are shown in Table I, with the results for genistein used as a surrogate for isoflavones owing to the high correlation among results for genistein and daidzein. Subjects with high genistein consumption were older, smoked and drank less, consumed less coffee, and consumed more vegetables, notwithstanding sex. The proportion of postmenopausal women increased as genistein intake increased. As expected, soy food and daidzein increased as genistein intake increased. The proportion of subjects positive for anti-HCV,

TABLE III – HAZARD RATIO (HR) AND 95% CONFIDENCE INTERVAL (CI) FOR HEPATOCELLULAR CARCINOMA (HCC) ACCORDING TO ISOFLAVONE AND SOY PRODUCT CONSUMPTION AMONG JAPANESE MEN AND WOMEN WHO WERE ANTI-HCV- OR HBSAG-POSITIVE

	No. of cases	Person-years of follow-up	HR (95%CI)[1]	HR (95%CI)[2]
Men (N = 699)				
Genistein				
Low	22	2,481	1	1
Middle	12	2,493	0.58 (0.29–1.20)	0.59 (0.28–1.24)
High	23	2,451	1.06 (0.56–2.00)	1.05 (0.52–2.12)
p_{trend}			0.99	0.96
Daidzein				
Low	22	2,481	1	1
Middle	13	2,483	0.63 (0.31–1.26)	0.63 (0.31–1.31)
High	22	2,460	1.00 (0.52–1.89)	0.97 (0.48–1.98)
p_{trend}			0.88	0.84
Soy food				
Low	22	2,483	1	1
Middle	12	2,482	0.61 (0.30–1.26)	0.61 (0.29–1.28)
High	23	2,459	1.10 (0.58–2.06)	1.10 (0.55–2.20)
p_{trend}			0.86	0.87
Women (N = 890)				
Genistein				
Low	4	3,426	1	1
Middle	11	3,504	3.28 (1.02–10.48)	3.11 (0.92–10.51)
High	10	3,507	3.07 (0.94–10.09)	3.30 (0.92–11.82)
p_{trend}			0.06	0.06
Daidzein				
Low	4	3,427	1	1
Middle	11	3,498	3.27 (1.02–10.47)	3.12 (0.92–10.56)
High	10	3,512	3.08 (0.94–10.13)	3.32 (0.93–11.88)
p_{trend}			0.06	0.06
Soy food				
Low	8	3,393	1	1
Middle	8	3,518	1.11 (0.41–3.05)	0.97 (0.34–2.77)
High	9	3,526	1.18 (0.44–3.12)	1.02 (0.36–2.94)
p_{trend}			0.74	0.98

[1]Adjusted for age and area.–[2]Adjusted for age, area, smoking status, alcohol consumption, and intake of coffee and vegetables. Further adjusted for menopausal status in women.

HBsAg or both among tertiles of genistein consumption were similar.

Table II shows minimally adjusted and multivariable HRs and 95% CIs for HCC by tertile of genistein, daidzein and soy food consumption in men and women. Consumption of genistein, daidzein and soy food showed no association with HCC in men, with respective multivariable HRs for the highest versus lowest tertile of 1.13 (95% CI = 0.60–2.11), 1.09 (95% CI = 0.58–2.05) and 1.10 (95% CI = 0.59–2.03). In women, in contrast, genistein and daidzein were dose-dependently associated with an increased risk of HCC, with multivariable HRs for the highest versus lowest tertile of 3.19 for genistein (95% CI = 1.13–9.00, p_{trend} = 0.03) and 3.90 for daidzein (95% CI = 1.30–11.69, p_{trend} = 0.01). Similarly, soy food consumption also tended to be associated with an increased risk of HCC in women, but without statistical significance (highest versus lowest: multivariable HR = 1.74, 95% CI = 0.67–4.25). Miso soup, natto and tofu consumption also showed no association with HCC in men (data not shown). In women, natto and tofu consumption was positively associated with HCC. Multivariable HRs for the highest *versus* lowest tertile of natto and tofu consumption was 3.71 (95% CI = 1.42–9.71) and 1.67 (95% CI = 0.65–4.28), respectively (data not shown).

These results remained essentially unchanged when analysis was restricted to subjects who were either or both anti-HCV- or HBsAg-positive (Table III); the positive association between genistein and daidzein and HCC in these women remained, albeit with attenuation of the test for linear trend (p_{trend} = 0.06 and 0.06 for genistein and daidzein, respectively). In contrast, soy food was not associated with HCC in women who were either or both HCV- and HBV-positive. No association between isoflavones and soy food and HCC was observed in men. Further, no association with individual soy foods was seen in men (data not shown). In women, the positive association between natto consumption and HCC remained, whereas tofu and miso soup consumption were not associated with HCC risk (data not shown).

Because the effects of isoflavones on HCC might differ between premenopausal and postmenopausal women due to difference in their estrogen levels, we also analyzed the association between soy foods and isoflavones and HCC in postmenopausal women. Results were similar to those for total women in Table II (data not shown). Hazard ratios among premenopausal women could not be calculated, because only one case occurred among them.

Discussion

We found a dose-dependent increase in the risk of HCC with consumption of isoflavones in Japanese women, even after consideration of infection status of hepatitis virus. In contrast, no association between isoflavones and HCC was seen in men. To our knowledge, this is the first study to report a positive association between the consumption of isoflavones and HCC in women.

Previous epidemiological findings for isoflavone and soy food intake and HCC are inconsistent.[11–16] Two prospective[11,12] and one case–control study[13] reported an inverse association between frequency of miso soup intake and HCC mortality. Lei *et al.*[14] reported that genistein consumption was lower at first diagnosis in patients with HCC than in those with cirrhosis. In several case-control studies, in contrast, no association with HCC was seen for frequency of tofu[15] and pulses intake.[16] However, most of these studies did not control for the potentially important confounding effects of infection with either or both HCV and HBV.[11,12,14,15] Additionally, most of these previous studies did not analyze by sex,[11,13–16] notwithstanding that the effects of isoflavones on HCC may differ between men and women.[10]

Although the relation between estrogen and HCC remains obscure,[2,34] previous epidemiological studies have reported the

preventive effects of estrogen against HCC or the progression of liver fibrosis. Yu *et al*. reported that the use of hormone replacement therapy was associated with a lower risk of HCC, and that younger age at menopause and ovariectomy during premenopause were risk factors for HCC.[9] Tanaka *et al*. reported that elevated serum testosterone, together with decreased serum estrogens, may promote the development of HCC in patients with cirrhosis.[35] Additionally, menopause seems to play a role in accelerating the progression of fibrosis.[36] In animal experiments, the degree of fibrosis was increased in males and females with hypoestrogenemia compared with females with normal levels of estrogen.[37] Moreover, variant ERs were more frequently expressed in male HCC patients than female subjects, even in an early stage of chronic liver disease,[38,39] while expression of both ERβ and wild ERα was lower in patients with HCC than in those with chronic liver disease.[40] Taken together, these findings may indicate that a loss of estrogen responsiveness might lead to HCC, and suggest that estrogen and ER status may play a role in hepatic defense.

Several mechanisms may explain the association we found between isoflavone consumption and increased risk of HCC in women. First, because isoflavones compete for estradiol at the receptor complex, they may have an anti-estrogenic effect in women.[17] Indeed, a number of epidemiological studies reporting an association between isoflavone intake and decreased breast cancer risk have suggested that this finding is ascribable to the possibility of anti-estrogenic effects of isoflavone.[10,21,22] Second, serum estradiol concentration shows a significant inverse correlation with soy product intake in women.[41,42] These findings suggest that isoflavones inhibit the preventive effects of estrogen against HCC in women. Moreover, the anti-estrogenic effects of isoflavone in women might impede the preventive effects of estrogen-mediated inhibition of IL-6 on HCC, given that estrogen's inhibitory effect on IL-6 secretion reduced HCC risk in female mice,[8] and that an isoflavone-rich diet increased IL-6 levels in women.[43]

We attempted to specify which kinds of soy products contributed to the increased risk of HCC in women. Our results showed a stronger association for natto (fermented soybean) than other soyfoods. Given that natto is the greatest contributor to isoflavones intake in Japan,[44] this result is plausible. Additionally, the isoflavone aglycones in fermented foods may have greater bioavailability than their glucosides, because genistein and daidzein are absorbed as isoflavone aglycones following hydrolysis of the glycoside by beta-glucosidases present in not only human gut bacteria but also in foods.[45] These findings indicate the need for further study of risk and bioavailability using plasma data.

In this study, most women (97%) who developed HCC during the follow-up period were postmenopausal at baseline. Isoflavone may have not competed with estrogen in postmenopausal women due to their low estrogen levels. However, even when analysis was restricted to postmenopausal women, a positive association between isoflavones and HCC risk remained. This lack of change between premenopausal and postmenopausal women has also been reported for breast cancer.[21,22,46,47] Given the extended period required for carcinogenesis to occur, an anti-estrogenic effect of isoflavones in premenopausal women might remain in postmenopausal women. In contrast, given that isoflavones may have an estrogenic effect in men,[10,21,22] they might be expected to decrease the risk of HCC in men. Here, however, we saw no association between isoflavone consumption and HCC in men. The predominance of androgens in men may obfuscate any estrogenic impact of isoflavones, because testosterone level is positively associated with the risk of HCC in men.[35,48]

The strength of the present study is its prospective design and negligible proportion of loss to follow-up (0.2%). Information on isoflavones and soy food consumption was collected before the subsequent diagnosis of HCC, thereby diminishing the probability of the recall bias that is inherent to case–control studies. Another strength was that virus infection status was determined at baseline for the entire population, allowing us to clarify the association between isoflavones and HCC in a high-risk population.

Several limitations of the study also warrant mention. First, because we estimated consumption from self-reports and at a single point (at baseline), and that validity for isoflavones was moderate, some measurement error in the assessment of isoflavones and soyfoods consumption is inevitable. If present, however, this was probably nondifferential and would have lead to the underestimation of results. Second, it would have been preferable if we had been able to confirm the association between plasma isoflavone level and HCC. Further studies using plasma samples are needed. However, Spearman's correlation coefficients for daidzein and genistein between intakes from the questionnaire and from serum concentrations in a validation study using subsamples in the JPHC Study were 0.31 and 0.33, respectively.[44] Additionally, we previously reported similar results regarding the effects of isoflavone between studies using plasma isoflavone levels and using a FFQ in both breast[21,22] and prostate cancer.[23,24] On the basis of these findings, we expect that results using plasma samples would be similar to our present results. Third, we had no information on the clinical severity of hepatitis or on the treatment of subjects with hepatitis virus infection before and during the study period. If infected subjects had received treatment, the occurrence of HCC may have been decreased. However, this might have lead to the underestimation of HCC occurrence, which would also bias the results toward the null. Finally, any generalization of our results should be done with caution.[49] Our subjects were restricted to those who provided a blood sample and participated in the baseline health checkup survey (28% for men, 45% for women), and subjects already under care for hepatitis infection may have been less willing to provide blood samples.

In conclusion, we found that isoflavones have a relevant role in HCC risk in women. In particular, the unfavorable effect of isoflavones was independent of other major risk factors, namely HBV and HCV infection. It might be therefore necessary for women with hepatitis virus infection to abstain from isoflavone. Because our cases numbers were relatively small, confirmation of these findings in further studies is required.

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