Table 2. RRs and 95% CIs for total prostate cancer according to quartile of energy-adjusted intake of dairy products

	Intake by quartile (median)				P_{trend}
	Lowest (12.8 g/d)	Second (66.1 g/d)	Third (165.2 g/d)	Highest (339.8 g/d)	
Total dairy products					
No. cases	60	71	83	115	
Person-years of follow-up	80,805	81,640	82,137	79,066	
Age-area adjusted RR (95% CI)	1.00	1.26 (0.90-1.78)	1.28 (0.92-1.79)	1.73 (1.26-2.37)	<0.01
Multivariate RR (95% CI)	1.00	1.34 (0.92-1.95)	1.29 (0.90-1.86)	1.63 (1.14-2.32)	0.01
	Lowest (2.3 g/d)	Second (32.4 g/d)	Third (125.8 g/d)	Highest (290.5 g/d)	
Milk					
No. cases	62	69	92	106	
Person-years of follow-up	79,972	81,201	82,827	79,648	
Age-area adjusted RR (95% CI)	1.00	1.26 (0.89-1.78)	1.39 (1.00-1.92)	1.54 (1.12-2.11)	< 0.01
Multivariate RR (95% CI)	1.00	1.41 (0.97-2.05)	1.49 (1.04-2.14)	1.53 (1.07-2.19)	0.01
	Lowest (1.9 g/d)	Second (2.2 g/d)	Third (3.0 g/d)	Highest (6.2 g/d)	
Cheese					
No. cases	74	98	85	73	
Person-years of follow-up	83,006	77,857	81,744	81,040	
Age-area adjusted RR (95% CI)	1.00	1.30 (0.96-1.77)	1.40 (1.02-1.91)	1.29 (0.93-1.79)	0.26
Multivariate RR (95% CI)	1.00	1.40 (1.00-1.96)	1.49 (1.06-2.09)	1.32 (0.93-1.89)	0.30
	Lowest (1.9 g/d)	Second (2.1 g/d)	Third (5.2 g/d)	Highest (31.5 g/d)	
Yogurt					
No. cases	73	66	85	105	
Person-years of follow-up	84,974	80,313	79,925	78,436	
Age-area adjusted RR (95% CI)	1.00	0.90 (0.64-1.25)	1.13 (0.82-1.55)	1.55 (1.14-2.09)	<0.01
Multivariate RR (95% CI)	1.00	0.93 (0.65-1.33)	1.15 (0.81-1.62)	1.52 (1.10-2.12)	<0.01

NOTE: Multivariate RRs were adjusted for age, area, smoking status, drinking frequency, marital status, and intake of green tea and genistein.

versus lowest quartile of calcium of 1.24 (95% CI, 0.85-1.81; P_{trend} = 0.16) and 1.37 (95% CI, 0.97-1.95; P_{trend} = 0.11), respectively. Intake of some specific saturated fatty acids dose-dependently increased the risk of total prostate cancer. Multivariable RRs for the highest versus lowest quartiles of myristic acid and palmitic acid were 1.62 (95% CI, 1.15-2.29; P_{trend} < 0.01) and 1.53 (95% CI, 1.07-2.20; P_{trend} = 0.04), respectively. Multivariate RRs of stearic acid were attenuated when we controlled for potential confounding factors. However, this fatty acid did tend to increase the risk of total prostate cancer, albeit without statistically significance (highest versus lowest: multivariate RR, 1.35; 95% CI, 0.94-1.94; P_{trend} = 0.14).

We also analyzed the energy-adjusted intake of dairy products, calcium, and saturated fatty acid in relation to prostate cancer according to local staging (Table 4). Analysis of energy-adjusted intake of total dairy products and saturated fatty acid showed stronger associations for localized than for total prostate cancer. The multivariate RR for total dairy products was 1.69 (95% CI, 1.10-2.59; P_{trend} = 0.02) in the highest compared with the lowest category. Saturated fatty acid intake tended to increase the risk of localized prostate cancer, but without statistical significance (highest versus lowest: multivariate RR, 1.51; 95% CI; 0.98-2.31; P_{trend} = 0.08). No association was observed between calcium intake and localized prostate cancer (highest versus lowest: multivariate RR, 1.25; 95% CI, 0.80-1.97; P_{trend} = 0.21). Similarly, increased RR estimates for total dairy products were also seen for advanced prostate cancer but did not show statistical significance because of the limited number of advanced cases. Multivariate RR for the highest versus lowest category was 1.41 (95% CI, 0.73-2.73) for total dairy products. Intake of saturated fatty acid showed no association with advanced prostate cancer (highest versus lowest; multivariate RR, 1.21; 95% CI, 0.62-2.35). Results for calcium intake in advanced cancer were not substantially different to those in localized cancer (highest versus lowest: multivariate RR, 1.14; 95% CI, 0.54-2.41). To weaken the influence of prostate cancer detected by prostate-specific antigen (PSA) screening, we also analyzed the association between prostate cancer and the three items after excluding screening-detected tumors, notwithstanding that screening information was not available for 15% of cases.

Results for both localized and advanced prostate cancer were similar to those in Table 4 when screening-detected prostate cancer was included, although statistical significance was lost due to the small numbers. Multivariable RRs for the highest versus lowest quartile were 1.81 (95% CI, 0.88-3.71) for dairy products, 1.47 (95% CI, 0.71-3.05) for saturated fatty acid, and 1.15 (95% CI, 0.56-2.35) for calcium in localized prostate cancer and 1.13 (95% CI,

0.50-2.54) for dairy products, 1.12 (95% CI, 0.45-2.83) for saturated fatty acid, and 1.15 (95% CI, 0.42-3.15) for calcium in advanced prostate cancer (data not shown).

Discussion

We found a dose-dependent increase in the risk of prostate cancer with intake of dairy products in Japanese men. For specific saturated fatty acids, myristic acid and palmitic acid increased the risk of prostate cancer in a dose-dependent manner. To our knowledge, this is the first prospective study to investigate the association between the intake of dairy products, saturated fatty acid, and calcium and prostate cancer in an Asian population.

An association between dairy products and prostate cancer has been reported in many previous papers. Recently, two meta-analyses showed an 11% and 68% increase in the risk of prostate cancer in the highest category of dairy products (11) and milk consumption (12), respectively. Our present findings support the results of these meta-analyses. The mechanism of this increased risk has been proposed to owe to the calcium and fat content of dairy products. One mechanism is the effect of calcium in suppressing circulating levels of the active form of vitamin D (1,25-hydroxyvitamin D), or increasing those of insulin-like growth factor-I, which have been shown to be related to the risk of prostate cancer (32-34). A recent meta-analysis of prospective studies estimated that men with a higher intake of calcium had an increased risk of prostate cancer compared with those with a lower intake (RR, 1.39; 95% CI, 1.09-1.77; ref. 11). Another mechanism is that increased fat intake might lead to increased testosterone levels (35), and this might lead to increased cell division, activation of proto-oncogenes, and inactivation of tumor suppressor genes (36) and that high testosterone levels may therefore influence prostate cancer risk (37). Findings from previous epidemiologic studies examining the intake of saturated fatty acid in relation to prostate

Table 3. RRs and 95% CIs for prostate cancer according to quartile of energy-adjusted calcium and saturated fatty acid

	Intake by quartile (median)				P_{trend}
	Lowest (282.8 mg/d)	Second (403.6 mg/d)	Third (521.9 mg/d)	Highest (725.1 mg/d)	
Calcium					
No. cases	56	68	98	107	
Person-years of follow-up	80,438	81,652	82,005	79,554	
Age-area adjusted RR (95% CI)	1.00	1.09 (0.76-1.55)	1.40 (1.00-1.95)	1.43 (1.03-1.97)	0.01
Multivariate RR (95% CI)	1.00	1.03 (0.70-1.51)	1.32 (0.92-1.90)	1.24 (0.85-1.81)	0.16
	Lowest (9.7 g/d)	Second (13.8 g/d)	Third (17.3 g/d)	Highest (22.9 g/d)	
Saturated fatty acid					
No. cases	70	77	75	107	
Person-years of follow-up	82,409	81,945	80,180	79,114	
Age-area adjusted RR (95% CI)	1.00	1.13 (0.82-1.56)	1.10 (0.80-1.53)	1.53 (1.12-2.08)	0.01
Multivariate RR (95% CI)	1.00	1.09 (0.77-1.55)	0.99 (0.69-1.42)	1.37 (0.97-1.95)	0.11
	Lowest (0.6 g/d)	Second (1.0 g/d)	Third (1.4 g/d)	Highest (2.0 g/d)	
Myristic acid (14:0)					
No. cases	68	68	81	112	
Person-years of follow-up	82,223	81,990	81,325	78,110	
Age-area adjusted RR (95% CI)	1.00	1.08 (0.77-1.51)	1.27 (0.92-1.75)	1.82 (1.34-2.47)	< 0.01
Multivariate RR (95% CI)	1.00	1.10 (0.77-1.58)	1.24 (0.87-1.75)	1.62 (1.15-2.29)	< 0.01
	Lowest (6.0 g/d)	Second (8.1 g/d)	Third (10.0 g/d)	Highest (12.9 g/d)	
Palmitic acid (16:0)					
No. cases	63	82	76	108	
Person-years of follow-up	82,455	81,801	79,954	79,438	
Age-area adjusted RR (95% CI)	1.00	1.32 (0.95-1.84)	1.22 (0.87-1.70)	1.65 (1.20-2.28)	<0.01
Multivariate RR (95% CI)	1.00	1.27 (0.89-1.82)	1.09 (0.75-1.58)	1.53 (1.07-2.20)	0.04
	Lowest (2.1 g/d)	Second (3.1 g/d)	Third (4.0 g/d)	Highest (5.5 g/d)	
Stearic acid (18:0)					
No. cases	65	84	82	98	
Person-years of follow-up	82,497	81,801	79,858	79,491	
Age-area adjusted RR (95% CI)	1.00	1.31 (0.94-1.81)	1.31 (0.94-1.82)	1.46 (1.05-2.02)	0.03
Multivariate RR (95% CI)	1.00	1.23 (0.87-1.74)	1.15 (0.80-1.65)	1.35 (0.94-1.94)	0.14

NOTE: Multivariate RRs were adjusted for age, area, smoking status, drinking frequency, marital status, and intake of green tea and genistein.

Table 4. RRs and 95% CIs for prostate cancer according to quartile of energy-adjusted intake of dairy products, calcium, and saturated fatty acid by local staging

	Intake in quartile				P_{trend}
	Lowest	Second	Third	Highest	
Localized prostate cancer					
Total dairy products					
No. cases	40	46	60	81	
Multivariate RR (95% CI)	1.00	1.32 (0.84-2.09)	1.37 (0.88-2.12)	1.69 (1.10-2.59)	0.02
Calcium					
No. cases	39	47	70	71	
Multivariate RR (95% CI)	1.00	1.03 (0.65-1.62)	1.36 (0.88-2.10)	1.25 (0.80-1.97)	0.21
Saturated fatty acid					
No. cases	45	55	55	72	
Multivariate RR (95% CI)	1.00	1.21 (0.79-1.84)	1.15 (0.75-1.78)	1.51 (0.98-2.31)	0.08
Advanced prostate cancer					
Total dairy products					
No. cases	19	22	22	27	
Multivariate RR (95% CI)	1.00	1.30 (0.66-2.56)	1.17 (0.59-2.32)	1.41 (0.73-2.73)	0.37
Calcium					
No. cases	15	20	26	29	
Multivariate RR (95% CI)	1.00	1.13 (0.55-2.34)	1.27 (0.62-2.61)	1.14 (0.54-2.41)	0.72
Saturated fatty acid					
No. cases	22	20	20	28	
Multivariate RR (95% CI)	1.00	0.95 (0.49-1.83)	0.84 (0.42-1.68)	1.21 (0.62-2.35)	0.74

NOTE: Multivariate RRs were adjusted for age, area, smoking status, drinking frequency, marital status, and intake of green tea and genistein.

cancer are inconsistent, although dietary fat may be related to prostate cancer risk (13). Only one prospective and four case-control studies have reported that the intake of saturated fatty acid increased the risk of prostate cancer (20, 38-41).

In Japanese men, dairy products are the main source of not only calcium but also saturated fatty acid, with data from a validation study in this cohort showing a mean intake and cumulative percent of 120.2 mg/d and 19.3% for calcium and 2.6 g/d and 16.0% for saturated fatty acid, respectively (19). In a previous study, a stronger association between saturated fat intake and prostate cancer risk was reported among Japanese Americans and Chinese Americans than among Blacks and Whites (20). These findings may explain our result that saturated fatty acid seemed to increase the risk of localized prostate cancer. In contrast, although calcium intake is reported to have increased the risk of prostate cancer in many epidemiologic studies, our lack of an association between calcium and prostate cancer may be due to the low intake of calcium in this study: the positive associations in several previous studies were limited to men with high intakes of >1,000 mg/d (14-18), whereas average intake here was only 500 mg/d, and only 3.5% consumed >1,000 mg/d. These results suggest that saturated fatty acid may play a relatively more important role than calcium in the incidence of prostate cancer in Japanese, to some extent at least. Owing to the relatively high correlation between dairy products, calcium, and saturated fatty acid shown in this study, however, we were unable to clarify what component of dairy is relevant to prostate cancer. Spearman's correlation coefficient between the energy-adjusted intake of dairy products and saturated fatty acid was 0.51, that of dairy products and calcium was 0.79, and that of calcium and saturated fatty acid was 0.48. These high correlations made it difficult to separate their effect on prostate cancer.

With regard to specific saturated fatty acids, our results showed that myristic acid and palmitic acid were associated with a dose-dependent increase in risk even after controlling for potential confounding factors. The main saturated fatty acids in dairy products are palmitic acid, myristic acid, and stearic acid, with palmitic acid levels more than twice those of the others (42). Our study accordingly showed a higher intake of palmitic acid than myristic acid, and we expected that palmitic acid would have the strongest relation with prostate cancer but instead found the greatest risk with myristic acid. Our results are supported by a nested case-control study, which found that high serum myristic acid levels were associated with a 2-fold increase in risk over low levels (41). In contrast, a second prospective study showed no association between dietary palmitic and stearic acid and prostate cancer risk, albeit without describing risk for myristic acid (43). In this study, Spearman's correlation coefficients between the energy-adjusted intake of dairy products and myristic acid, palmitic acid, and stearic acid were 0.76, 0.41, and 0.35, respectively. Thus, myristic acid may well reflect the intake of dairy products. Regarding specific saturated fatty acids, epidemiologic and laboratory data are sparse, and further studies on the effects of specific saturated fatty acids are required.

It has also been suggested that a high consumption of dairy products increases the risk of prostate cancer, especially advanced disease (11). However, our results for dairy products in advanced cancer were not substantially different from those in localized cancer, although RRs for them in advanced prostate cancer were smaller than those in localized cancer. The difference with some previous studies may be due to the difference in the proportion of PSA-detected cancers. PSA-based detection is less common in Japanese (38% in this study) than in western populations (e.g., ~80% in the United States; ref. 44). Giovannucci et al. (44) reported that the

association between several risk factors and prostate cancer in the pre-PSA era differed from these associations in the PSA era, that is, because these cancers may have been largely diluted by the high prevalence of PSA-detected cancers.

Given that our results were not changed after the exclusion of screening-detected cancers, however, PSA screening in this study may have had less effect on the association between dairy products, calcium, and saturated fatty acid and prostate cancer than in other studies. Overall, moreover, the total prostate cancers in this study may have been more aggressive than those in western populations due to the lower PSA screening. On this basis, the results of total prostate cancers in this study may be similar to those for advanced cancer in western populations. An additional reason might be the small number of advanced cases; if so, a larger sample size may have detected positive effects of dairy products on advanced prostate cancer with greater precision.

The major strengths of our study were its prospective design that diminished the probability of recall bias, which is inherent to case-control studies. Because the study subjects were selected from the general population, the response rate was high (~80%), and the proportion of loss to follow-up was negligible (0.3%), the findings can be generalized to middle-aged and elderly Japanese men. Further, dietary information was ascertained using a validated FFQ.

Several limitations also warrant mention. First, misclassification of exposure due to changes in dairy product consumption during the study period might have occurred because information on consumption was obtained at one point only. If present, however, such misclassification would probably be nondifferential and may underestimate the true RR. Second, we could not distinguish between regular and low-fat milk intake, because the FFQ unfortunately did not enquire about the kind of milk. In Japan, regular milk accounts for nearly 90% of total milk consumption (45). Although our results showed that intake of milk and that of a specific saturated fatty acid increased the risk of prostate cancer, we could not clarify the association between low-fat milk and prostate cancer in Japanese men, notwithstanding several previous studies that it did indeed increase risk (46, 47). Finally, we did not consider the effect of calcium from supplements on prostate cancer. However, the proportion of calcium supplement users in this study was markedly low (0.2% of total subjects), and our results were not substantially changed after the exclusion of subjects who used calcium supplements (data not shown).

In summary, we found that the intake of dairy products was associated with an increased risk of prostate cancer. Given other findings that increased intake of dairy products may be protective for bone health, hypertension and colorectal cancer (48), further research is required to clarify the potential benefits and risks of a high intake of dairy foods.

Appendix A

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

Acknowledgments

We thank all the staff members in each study area and in the central offices for cooperation and technical assistance and the Iwate, Aomori, Ibaraki, Niigata, Osaka, Kochi, Nagasaki, and Okinawa Cancer Registries for provision of incidence data.

References

1. Parkin DM, Whelan SL, Ferlay J, Thomas DB. Cancer incidence in five continents. Vol. 8. Lyon: IARC; 2002.

2. Yatani R, Kusano I, Shiraishi T, Hayashi T, Stemmermann GN. Latent prostatic carcinoma: pathological and epidemiological aspects. Jpn J Clin Oncol 1989;19:319–26.
3. Tsugane S, de Souza JM, Costa ML, Jr., et al. Cancer incidence rates among Japanese immigrants in the city of Sao Paulo, Brazil, 1969-78. Cancer Causes Control 1990;1:189–93.
4. Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer 1991;63:963–6.
5. Tominaga S, Kuroishi T. An ecological study on diet/nutrition and cancer in Japan. Int J Cancer 1997;Suppl 10:2–6.
6. Center for Cancer Control and Information Services. Japan: National Cancer Center; 2007.
7. The National Health and Nutrition Survey in Japan, Ministry of Health, Labour and Welfare of Japan; 2004.
8. Mishina T, Watanabe H, Araki H, Nakao M. Epidemiological study of prostatic cancer by matched-pair analysis. Prostate 1985;6:423–36.
9. Allen NE, Sauvaget C, Roddam AW, et al. A prospective study of diet and prostate cancer in Japanese men. Cancer Causes Control 2004;15:911–20.
10. Sonoda T, Nagata Y, Mori M, et al. A case-control study of diet and prostate cancer in Japan: possible protective effect of traditional Japanese diet. Cancer Sci 2004;95:238–42.
11. Gao X, LaValley MP, Tucker KL. Prospective studies of dairy product and calcium intakes and prostate cancer risk: a meta-analysis. J Natl Cancer Inst 2005;97:1768–77.
12. Qin LQ, Xu JY, Wang PY, Kaneko T, Hoshi K, Sato A. Milk consumption is a risk factor for prostate cancer: meta-analysis of case-control studies. Nutr Cancer 2004;48:22–7.
13. Kolonel LN, Nomura AM, Cooney RV. Dietary fat and prostate cancer: current status. J Natl Cancer Inst 1999;91:414–28.
14. Giovannucci E, Rimm EB, Wolk A, et al. Calcium and fructose intake in relation to risk of prostate cancer. Cancer Res 1998;58: 442–7.
15. Chan JM, Giovannucci E, Andersson SO, Yuen J, Adami HO, Wolk A. Dairy products, calcium, phosphorous, vitamin D, and risk of prostate cancer (Sweden). Cancer Causes Control 1998;9:559–66.
16. Rodriguez C, McCullough ML, Mondul AM, et al. Calcium, dairy products, and risk of prostate cancer in a prospective cohort of United States men. Cancer Epidemiol Biomarkers Prev 2003;12: 597–603.
17. Giovannucci E, Liu Y, Stampfer MJ, Willett WC. A prospective study of calcium intake and incident and fatal prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:203–10.
18. Mitrou PN, Albanes D, Weinstein SJ, et al. A prospective study of dietary calcium, dairy products and prostate cancer risk (Finland). Int J Cancer 2007;120:2466–73.
19. Sasaki S, Takahashi T, Iitoi Y, et al. Food and nutrient intakes assessed with dietary records for the validation study of a self-administered food frequency questionnaire in JPHC Study Cohort I. J Epidemiol 2003;13:S23–50.
20. Whittemore AS, Kolonel LN, Wu AH, et al. Prostate cancer in relation to diet, physical activity, and body size in Blacks, Whites, and Asians in the United States and Canada. J Natl Cancer Inst 1995; 87:652–61.
21. Tsugane S, Sobue T. Baseline survey of JPHC study-design and participation rate. Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol 2001;11: S24–9.
22. Science and Technology Agency, editors. Standard tables of food composition in Japan. The fifth revised edition [in Japanese]. Tokyo: Printing Bureau, Ministry of Finance; 2000.
23. Science and Technology Agency, editors. Fatty acids, cholesterol, vitamin E composition table of Japanese foods [in Japanese]. Tokyo: Ishiyaku Shuppan Publishers; 1990.
24. Tsugane S, Kobayashi M, Sasaki S; JPHC. Validity of the self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I: comparison with dietary records for main nutrients. J Epidemiol 2003;13:S51–6.
25. Sasaki S, Kobayashi M, Tsugane S; JPHC. Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I: comparison with dietary records for food groups. J Epidemiol 2003;13:S57–63.
26. Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Tsugane S; JPHC. Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess fatty acid intake: comparison with dietary records and serum phospholipid level. J Epidemiol 2003;13:S64–81.
27. Sasaki S, Ishihara J, Tsugane S; JPHC. Reproducibility of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess food and nutrient intake. J Epidemiol 2003;13:S115–24.
28. Ishihara J, Sobue T, Yamamoto S, et al. Validity and reproducibility of a self-administered food frequency questionnaire in the JPHC Study Cohort II: study design, participant profile and results in comparison with Cohort I. J Epidemiol 2003;13:S134–47.
29. WHO. International classification of diseases for oncology. 3rd ed. Geneva (Switzerland): WHO; 2000.
30. Kurahashi N, Iwasaki M, Sasazuki S, Otani T, Inoue M, Tsugane S. Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. Cancer Epidemiol Biomarkers Prev 2007;16: 538–45.
31. Kurahashi N, Sasazuki S, Iwasaki M, Inoue M, Tsugane S. Green tea consumption and prostate cancer risk in Japanese men: a prospective study. Am J Epidemiol 2008;167:71–7.
32. Giovannucci E. Dietary influences of 1,25(OH)2 vitamin D in relation to prostate cancer: a hypothesis. Cancer Causes Control 1998;9:567–82.
33. Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. Cancer Epidemiol Biomarkers Prev 2002;11:852–61.
34. Gunnell D, Oliver SE, Peters TJ, et al. Are diet-prostate cancer associations mediated by the IGF axis? A cross-sectional analysis of diet, IGF-I and IGFBP-3 in healthy middle-aged men. Br J Cancer 2003;88:1682–6.
35. Dorgan JF, Judd JT, Longcope C, et al. Effects of dietary fat and fiber on plasma and urine androgens and estrogens in men: a controlled feeding study. Am J Clin Nutr 1996;64:850–5.
36. Ross RK, Henderson BE. Do diet and androgens alter prostate cancer risk via a common etiologic pathway? J Natl Cancer Inst 1994;86: 252–4.
37. Garn PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. J Natl Cancer Inst 1996;88:1118–26.
38. Heshmat MY, Kaul L, Kovi J, et al. Nutrition and prostate cancer: a case-control study. Prostate 1985;6:7–17.
39. West DW, Slattery ML, Robison LM, French TK, Mahoney AW. Adult dietary intake and prostate cancer risk in Utah: a case-control study with special emphasis on aggressive tumors. Cancer Causes Control 1991;2:85–94.
40. Bairati I, Meyer F, Fradet Y, Moore L. Dietary fat and advanced prostate cancer. J Urol 1998;159:1271–5.
41. Mannisto S, Pietinen P, Virtanen MJ, et al. Fatty acids and risk of prostate cancer in a nested case-control study in male smokers. Cancer Epidemiol Biomarkers Prev 2003;12:1422–8.
42. Science and Technology Agency, editors. Standard tables of food composition in Japan. The fifth revised and enlarged edition, fatty acids section [in Japanese]. Tokyo: Printing Bureau, Ministry of Finance; 2005.
43. Schuurman AG, van den Brandt PA, Dorant E, Brants HA, Goldbohm RA. Association of energy and fat intake with prostate carcinoma risk: results from The Netherlands Cohort Study. Cancer 1999;86:1019–27.
44. Giovannucci E, Liu Y, Platz EA, Stampfer MJ, Willett WC. Risk factors for prostate cancer incidence and progression in the Health Professionals Follow-up Study. Int J Cancer 2007;121:1571–8.
45. Statistics of Agriculture, Forestry and Fisheries. Statistic on milk and dairy products. Ministry of Agriculture, Forestry and Fisheries; 2007.
46. Tseng M, Breslow RA, Graubard BI, Ziegler RG. Dairy, calcium, and vitamin D intakes and prostate cancer risk in the National Health and Nutrition Examination Epidemiologic Follow-up Study cohort. Am J Clin Nutr 2005;81:1147–54.
47. Torniainen S, Hedelin M, Autio V, et al. Lactase persistence, dietary intake of milk, and the risk for prostate cancer in Sweden and Finland. Cancer Epidemiol Biomarkers Prev 2007;16:956–61.
48. Alvarez-Leon EE, Roman-Vinas B, Serra-Majem L. Dairy products and health: a review of the epidemiological evidence. Br J Nutr 2006; 96:S94–9.

VOLUME 26 · NUMBER 36 · DECEMBER 20 2008

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Plasma Isoflavones and Subsequent Risk of Prostate Cancer in a Nested Case-Control Study: The Japan Public Health Center

Norie Kurahashi, Motoki Iwasaki, Manami Inoue, Shizuka Sasazuki, and Shoichiro Tsugane

From the Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan.

Submitted February 20, 2008; accepted July 22, 2008; published online ahead of print at www.jco.org on November 17, 2008.

Supported by Grants-in-Aid for Cancer Research (Grant No. 19shi-2), for the 3rd Term Comprehensive 10-Year Strategy for Cancer Control (Grant No. H18-sanjigan-ippan-001), and for Research on Risk of Chemical Substances (Grant No. H17-kagaku-ippan-014) from the Ministry of Health, Labour and Welfare of Japan, and Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology (Grant No. 17015049).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Norie Kurahashi, MD, PhD, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji Chuo-ku Tokyo 104-0045 Japan; e-mail: nkurahas@gan2.res.ncc.go.jp.

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

© 2008 by American Society of Clinical Oncology

0732-183X/08/2636-5923/$20.00

DOI: 10.1200/JCO.2008.16.8807

ABSTRACT

Purpose
The incidence of prostate cancer is much lower in Japanese than Western populations. Given the preventive effects of isoflavones on carcinogenesis in the prostate in many nonhuman studies and the high consumption of isoflavones in Japanese, this low incidence may be partly due to the effects of soy.

Patients and Methods
We conducted a nested case-control study within the Japan Public Health Center–based Prospective Study. A total of 14,203 men aged 40 to 69 years who had returned the baseline questionnaire and provided blood samples were observed from 1990 to 2005. During a mean of 12.8 years of follow-up, 201 newly diagnosed prostate cancers were identified. Two matched controls for each case were selected from the cohort. Conditional logistic regression model was used to estimate the odds ratios (ORs) and 95% CIs for prostate cancer in relation to plasma levels of isoflavone.

Results
Plasma genistein level tended to be inversely associated with the risk of total prostate cancer. Although plasma daidzein showed no association, the highest tertile for plasma equol, a metabolite of daidzein, was significantly associated with a decreased risk of total prostate cancer (OR = 0.60; 95% CI, 0.36 to 0.99; P_{trend} = .04). These inverse associations were strengthened after analysis was confined to localized cases, with ORs in the highest group of plasma genistein and equol compared with the lowest of 0.54 (95% CI, 0.29 to 1.01; P_{trend} = .03) and 0.43 (95% CI, 0.22 to 0.82; P_{trend} = .02), respectively. Plasma isoflavone levels were not statistically significantly associated with the risk of advanced prostate cancer.

Conclusion
Isoflavones may prevent the development of prostate cancer.

J Clin Oncol 26:5923-5929. © 2008 by American Society of Clinical Oncology

INTRODUCTION

Although the incidence of prostate cancer has increased in Japan, it remains less than one fifth of that in Western countries.[1] Interestingly, however, the incidence of latent or clinically insignificant prostate cancer in autopsy studies among men from Japan and the United States is not substantially different.[2] Moreover, migration data show that incidence increases in men migrating from areas of low incidence to areas of higher incidence.[3,4] These results suggest that the etiology of prostate cancer may involve dietary, lifestyle, and environmental factors.[5-7]

Isoflavones, which include genistein, daidzein, and glycitein, are found in soy and soy products.[8] In some experimental studies, isoflavones have demonstrated protective effects against prostate cancer development as a result of their anticarcinogenic properties and estrogenic activity.[9] Given that Asian populations consume more soy products than Western populations and that mean plasma concentrations of isoflavones are accordingly higher in Japanese than those in men from the United Kingdom[10] and Finland,[11] this low incidence may partly reflect the influence of isoflavones.

Although experimental studies have consistently shown preventive effects of isoflavones on prostate cancer,[9] data from epidemiologic studies have been inconsistent.[5,12-28] We previously reported an association between dietary isoflavones and prostate cancer risk among Japanese men using data from a 5-year follow-up questionnaire in the Japan Public Health Center (JPHC) –based Prospective Cohort Study.[5] In that study, consumption of soy products, genistein, and daidzein was associated with a decreased risk of localized prostate cancer. However, we could not explore the association between equol, which is metabolized from daidzein

Information downloaded from jco.ascopubs.org and provided by Kokuritsu Gan Center on December 18, 2008 from .
Copyright © 2008 by the American Society of Clinical Oncology. All rights reserved.

by intestinal bacteria and known to have stronger estrogenic activity than daidzein,[29,30] and prostate cancer using a food frequency questionnaire (FFQ), because approximately 30% to 50% of adults lack the ability to metabolite daidzein to equol.[8] Additionally, genistein and daidzein are absorbed as isoflavone aglycones after hydrolysis of the glycoside by beta-glucosidases present in not only human gut bacteria but also in foods. On this basis, the isoflavone aglycones in fermented foods, such as miso, natto, and so on, may be more bioavailable than their glucosides.[8] Thus plasma data in Japanese, who consume various soy foods, both fermented and nonfermented, is useful with regard to bioavailability.

We investigated the effect of isoflavones as measured in plasma on subsequent prostate cancer in a nested case-control study within a large prospective cohort study.

PATIENTS AND METHODS

Study Population

The JPHC study was initiated in 1990 for cohort I and in 1993 for cohort II. Cohort I consisted of five Public Health Center (PHC) areas (Iwate, Akita, Nagano, Okinawa, and Tokyo), and cohort II consisted of six PHC areas (Ibaraki, Niigata, Kochi, Nagasaki, Okinawa, and Osaka) across Japan. The study design has been described in detail previously.[31] This 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 to 59 years in cohort I and 40 to 69 years in cohort II at the start of the respective baseline survey. In the present analysis, the Tokyo participants were not included in data analyses because incidence data for them were not available.[31] After initiation of the study, 144 patients were found to be ineligible and were excluded because of non-Japanese nationality (n = 31), late report of emigration occurring before the start of the follow-up period (n = 107), incorrect birth data (n = 1), duplicate registration (n = 2), and self-reported history of prostate cancer (n = 3). Initially, we defined a population-based cohort of 65,657 men.

Questionnaire Survey

A self-administered questionnaire, which included personal and family medical history, smoking and drinking habits, diet, and other lifestyle factors, was distributed to all eligible residents who had registered their address in the respective PHC areas in 1990 to 1994. Completed questionnaires were received from 50,435 men (response rate, 77%). Dietary habits were assessed with an FFQ of 44 food items for cohort I and 52 food items for cohort II.[32]

Blood Collection

Participants voluntarily provided 10 mL of blood during health check-ups in 1990 to 1995. Blood samples were divided into plasma and buffy layers, and preserved at −80°C until analysis. Among respondents to the baseline questionnaire, a total of 14,203 men (28%) donated blood.

Follow-Up

Participants were observed from the baseline survey until December 31, 2005. Changes in residence status, including survival, were identified annually through the residential registry in their PHC area. Among study participants, 749 patients (5.3%) moved out of their study area and 28 patients (0.2%) were lost to follow-up during the study period.

Selection of Cases and Controls

Incidence data on prostate cancer 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. Cases were coded using the International Classification of Diseases for Oncology, Third Edition. We identified 201 prostate cancer cases newly diagnosed after blood collection up to the end of the study period among men who had returned the baseline questionnaire, reported no history of prostate cancer, and provided blood samples. Ninety-seven percent of cases were pathologically confirmed, and 0.5% of cases were based on death certificate only. Among the 201 cases, advanced cases were defined by a diagnosis of extraprostatic or metastatic cancer involving lymph nodes or other organs at first diagnosis of prostate cancer. If this information was not available, they were defined as those with a high Gleason score (8 to 10) or poor differentiation. These criteria were selected to allow the identification of advanced cases with a high likelihood of poor prognosis. The remaining cases were organ-localized. A total of 48 advanced, 144 localized, and nine (4% of total) cases of undetermined stage were identified.

For each case, two controls were selected from among participants with no history of prostate cancer when the case was diagnosed. Controls were matched for each case by age (within 3 years), PHC area, area (city or town and village), date on which blood was obtained (within 60 days), time of day of blood collection (within 3 hours), and duration of fasting at blood collection (within 3 hours).

Laboratory Assays

Concentrations of isoflavones in the plasma samples, namely of genistein, daidzein, glycitein and equol, were measured using triple quadrupole tandem liquid chromatography-mass spectrometry.[33] The isoflavones assayed were genistein, daidzein, glycitein, and equol. Beta-glucuronidase/sulfatase was added to 0.1 mL of plasma. The aglycones of the isoflavones and their metabolites were recovered by diethyl ether extraction. The diethyl ether extract of the sample was dried under nitrogen flow and redissolved in acetonitrile. The ionizing method was electrospray using negative ions, and multiple reaction monitoring was used for mass analysis. To assure quality control (QC), laboratory precision in this measurement was assessed twice using two kinds of sample before and after each assay. Based on 40 replicated measurements of QC samples, interbatch coefficient of variations (CVs) were ≤ 6.08% for genistein, ≤ 4.06% for daidzein, ≤ 5.48% for glycitein, and ≤ 6.15% for equol. QC samples used blood pooled from healthy volunteers, for which mean concentrations were 60.1 and 103.4 ng/mL for genistein, 39.1 and 95.0 ng/mL for daidzein, 3.4 and 48.2 ng/mL for glycitein, and 15.7 and 57.1 ng/mL for equol. Cases and matched controls were assayed in the same batch. Detection limits were less than 1.0 ng/mL for all isoflavones. All samples were analyzed at a single laboratory (SRL, Tokyo, Japan) under blinding to case-control status.

Statistical Analysis

Baseline characteristics between cases and controls were evaluated by the Mantel-Haenszel procedure with matched set strata. Odds ratios (ORs) and 95% CIs for prostate cancer risk were estimated by tertile level of plasma genistein and daidzein using a conditional logistic regression model. Tertile cutoff points were based on the frequency distribution of controls. In analyses for glycitein and equol, the ORs were computed according to three levels: the first comprised study participants with amounts below the detection limits (< 1.0 ng/mL), whereas the middle and high level groups comprised those with detectable levels as equally bisected by the median detected amount. A total of 30.8% and 25.1% of cases and controls were under the detection limit for glycitein and 39.8% and 36.3% were under the detection limit for equol, respectively. In this study, we defined "equol producer" as a participant in whom plasma equol was detected (≥ 1.0 ng/mL).

ORs and 95% CIs were adjusted for the following variables as potential confounders: smoking status (never, former, and current), alcohol intake (almost never, one to three times per month, ≥ 1 times per week), marital status (yes/no), green tea intake (<1 cup/d, 1 to 2 cups/d, 3 to 4 cups/d, ≥ 5 cups/d), and intake of protein, fiber, green or yellow vegetables, and dairy food (continuous). These variables are either known or suspected risk factors for cancer or had previously been associated with the risk of prostate cancer.[6,7] A family history of prostate cancer was not to be evaluated as a potential confounding factor because there was only one participant who reported it. Because the questionnaires for cohorts I and II differed slightly with respect to food items, method of expression, and frequency categories, adjustment was done by calculating separate estimates for cohorts I and II and then analyzing the combined result using a fixed-effects model. The two cohorts were not heterogeneous.

 © 2008 by American Society of Clinical Oncology

Information downloaded from jco.ascopubs.org and provided by Kokuritsu Gan Center on December 18, 2008 from .
Copyright © 2008 by the American Society of Clinical Oncology. All rights reserved.

Table 1. Baseline Characteristics of Case and Matched Control Subjects

Characteristic	Case (n = 201) Mean	SE	Control (n = 402) Mean	SE	P*
Age, years	58.6	6.4†	58.4	6.6†	–
Current smoker					.13
No.	68		163		
%	33.8		40.6		
Alcohol intake ≥ 1-2 times/wk					.42
No.	139		291.0		
%	69.2		72.4		
Marital status, yes					.26
No.	188		364		
%	93.5		90.6		
Family history of prostate cancer, yes					.16
No.	1		0		
%	0.5		0.0		
Green tea, daily					.97
No.	163		323		
%	81.1		80.4		
Total energy intake, kcal/d‡	2,085.0	42.5	2,075.6	30.0	.87
Protein intake, g/d‡	63.5	0.8	61.8	0.6	.06
Total fat intake, g/d‡	32.1	0.7	31.1	0.5	.22
Calcium intake, mg/d‡	504.0	15.9	488.9	11.2	.41
Fiber intake, g/d‡	9.4	0.1	9.1	0.1	.08
Milk/dairy intake, g/d‡	199.9	13.8	194.0	9.8	.14
Meat intake, g/d‡	38.8	1.3	37.4	0.9	.37
Green-yellow vegetable intake, g/d‡	37.2	1.4	34.2	1.0	.08
Fruit intake, g/d‡	87.8	5.1	88.2	3.6	.97
Soy food intake, g/d‡	61.4	1.8	60.53	1.3	.72

Abbreviations: wk, week; d, day.
*P for Mantel-Haenszel test with matched-pair strata.
†Value for age is standard deviation.
‡Adjusted for age and cohort.

Linear trends for OR were tested using the median values of isoflavones. All P values are two-sided, and statistical significance was determined at $P < .05$. Additionally, we estimated the OR of prostate cases stratified by stage as well as that for all cases. All statistical analyses were done with SAS software (version 9.1; SAS Institute Inc, Cary, NC).

RESULTS

Basic characteristics of case and matched controls at baseline are listed in Table 1. Cases tended to smoke less and to consume more protein, fiber, green or yellow vegetables, and dairy products. Further, Table 2 shows that there were no significant differences between cases and controls in median plasma levels of isoflavones.

Table 3 shows ORs and 95% CIs of prostate cancer risk according to isoflavone levels in plasma. When adjusted for potential confounding factors, genistein was inversely associated with the risk of prostate cancer, without statistical significance (OR = 0.66; 95% CI, 0.40 to 1.08, for highest *v* lowest; $P_{trend} = .08$). Further, although daidzein showed no association with prostate cancer, equol, a metabolite of daidzein, was associated with a dose-dependent decrease in risk (OR = 0.60; 95% CI, 0.36 to 0.99, for highest *v* lowest; $P_{trend} = .04$). In contrast, no association with total prostate cancer was seen for glycitein.

When analyzed according to prostate cancer stage, however, these associations were strengthened to statistical significance in localized cases (Table 4): in localized cases, genistein was associated with a dose-dependent decrease in risk of localized prostate cancer (OR = 0.54; 95% CI, 0.29 to 1.01 for highest *v* lowest, $P_{trend} = .03$), whereas the association between equol and localized cases was strengthened to statistical significance (OR = 0.43; 95% CI, 0.22 to 0.82, for highest *v* lowest, $P_{trend} = .02$). Daidzein and glycitein were also inversely associated with the risk of localized cancer, although without statistical significance. In contrast, no statistically significant association was seen between plasma isoflavone and advanced prostate cancer risk. ORs for the highest versus lowest group in these cases were 1.77 for genistein, 1.64 for daidzein, 1.89 for glycitein, and 2.39 for equol.

DISCUSSION

In this nested case-control study conducted as part of the JPHC Study in Japan, we found that high levels of genistein and equol were associated a decreased risk of localized prostate cancer, with dose dependency. Median value in our highest plasma genistein tertile group corresponded to a median dietary intake of 28.1 mg/d for genistein, as estimated by the validation study data. This amount of genistein is equivalent to 50 g of fermented soybeans or 100 g of tofu. Results were similar for daidzein and glycitein, although without statistical significance. To our knowledge, the present study is the first large study to investigate the association between plasma isoflavones and prostate cancer according to stage and to identify preventive effects of plasma isoflavones on localized prostate cancer. To date, only two nested case-control studies of prostate cancer have measured isoflavone levels in blood directly. Low et al[28] showed no protective effect for circulating genistein, daidzein, glycitein, or equol in a United Kingdom population with low isoflavone levels (mean serum level of genistein, 4.4

Table 2. Plasma Level of Isoflavone of Cases and Controls

Isoflavone	Case (n = 201) Median (ng/mL)	Interquartile Range	Control (n = 402) Median (ng/mL)	Interquartile Range	P*
Genistein	89.3	34.6-174.6	86.2	39.3-195.0	.55
Daidzein	37.0	11.4-83.4	35.5	14.0-81.3	.41
Glycitein	2.8	1.0-5.6	2.6	1.0-6.4	.29
Equol	3.7	1.0-16.5	4.7	1.0-23.0	.10

*P for Mantel-Haenszel test with matched-pair strata.

© 2008 by American Society of Clinical Oncology

Information downloaded from jco.ascopubs.org and provided by Kokuritsu Gan Center on December 18, 2008 from .
Copyright © 2008 by the American Society of Clinical Oncology. All rights reserved.

Table 3. ORs and 95% CIs of Prostate Cancer According to Plasma Level of Isoflavone

Plasma Level	Low	Middle	High	*P* for Trend
Genistein, ng/mL	< 57	57-151.7	≥ 151.7	
Cases	73	72	56	
Controls	133	134	135	
OR*	1.00	0.96	0.75	.15
95% CI	Reference	0.63 to 1.47	0.48 to 1.16	
OR†	1.00	0.90	0.66	.08
95% CI	Reference	0.56 to 1.43	0.40 to 1.08	
Daidzein, ng/mL	< 22	22-61.5	≥ 61.5	
Cases	76	59	66	
Controls	132	135	135	
OR*	1.00	0.75	0.83	.58
95% CI	Reference	0.49 to 1.15	0.54 to 1.28	
OR†	1.00	0.84	0.78	.44
95% CI	Reference	0.52 to 1.35	0.49 to 1.25	
Glycitein, ng/mL	< 1.0‡	< 3.9	≥ 3.9	
Cases	61	66	74	
Controls	102	149	151	
OR*	1.00	0.75	0.82	.68
95% CI	Reference	0.49 to 1.14	0.53 to 1.26	
OR†	1.00	0.76	0.78	.51
95% CI	Reference	0.47 to 1.21	0.48 to 1.26	
Equol, ng/mL	< 1.0‡	< 15.0	≥ 15.0	
Cases	79	69	53	
Controls	147	126	129	
OR*	1.00	1.03	0.77	.16
95% CI	Reference	0.68 to 1.54	0.51 to 1.17	
OR†	1.00	1.11	0.60	.04
95% CI	Reference	0.71 to 1.74	0.36 to 0.99	

Abbreviation: OR, odds ratio.
*Calculated for the two cohorts together.
†Calculated from the weighted average of the results from separate conditional logistic regressions fitted to the individual cohorts. Adjusted for smoking status, alcohol intake, marital status, and intake of green tea, protein, fiber, and green or yellow vegetables.
‡Not detected.

ng/mL). In contrast, Ozasa et al[27] suggested that high serum isoflavone (genistein, daidzein, and equol) levels tended to decrease the risk of prostate cancer in Japanese men, with similar levels to those seen in the present study (mean serum level genistein, 99.5 ng/mL). However, they did not stratify by local stage and studied fewer than 100 cases.

In addition to these epidemiologic data, experimental studies in vivo and in vitro have also demonstrated protective effects of isoflavones against prostate cancer development. Among these effects, isoflavones possess weak estrogen activity, inhibit tyrosine protein kinases, angiogenesis, and reduce serum testosterone levels.[12,34,35] They also inhibit 5α-reductase, an enzyme that metabolizes testosterone to dihydrotestosterone.[36] Any or all of these mechanisms may explain the inverse association between isoflavone and localized prostate cancer seen here. Moreover, these results are supported by incidence data, which show a much lower incidence of prostate cancer in Japanese, with a relatively high concentration of plasma isoflavones, than in Western men.[1]

However, when analyzed by stage, our data showed that isoflavones had no preventive effects against advanced prostate cancer. Our results thus were supported by the hypothesis that isoflavones may fail to protect against advanced prostate cancer via the complete or partial loss of estrogen receptor β (ERβ) expression,[37-39] on the basis that the effect of isoflavones on risk seems to involve ERβ in prostate tissue.[9] Additionally, they are also supported by animal studies showing a beneficial role of a soy diet in the early stages of tumor development, but no effect in invasive prostate cancer.[40,41] On these bases, therefore, isoflavones may prevent the early stages of prostate cancer development, and then delay the progression of latent prostate cancer. This speculation is supported by the finding that the incidence of latent prostate cancer is the same in Japanese as in Western men, despite a lower incidence of prostate cancer.[2] Another possibility is that advanced prostate cancer may be different from localized prostate cancer. Consideration should also be given to the possibility of detection bias owing to the reduction in 5α-reductase by isoflavone.[36] A reduction in 5α-reductase will decrease the value of prostate-specific antigen[42] and thus might mask the presence of prostate cancer. Isoflavones might therefore have no effect on the detection of advanced cases because of their substantially high prostate-specific antigen values. Nevertheless, because the number of advanced cases was small, the occurrence of this result by chance cannot be ruled out.

Of note, our present results for plasma isoflavone levels are similar to those we previously obtained in the JPHC Study using an FFQ.[5] It is particularly meaningful that a similar association was shown between the long-term intake of isoflavones versus plasma concentrations obtained at a single time point, notwithstanding their short half lives of 8.4 hours for genistein and 5.8 hours for daidzein.[43] In a validation study using subsamples in the JPHC Study, Spearman's correlation coefficients for daidzein and genistein between intakes from the questionnaire and from serum concentrations were 0.31 and 0.33, respectively.[44] Further, our present results did not substantially change when we excluded participants who provided a nonfasting blood sample, within 6 hours of eating a meal (data not shown). Blood concentrations might be maintained in Japanese by frequent habitual intake of isoflavone-rich foods.

In this study, each isoflavone showed a different effect on prostate cancer. Several experimental studies have reported that genistein may have greater estrogenic activity than daidzein.[8,45] Daidzein is metabolized to equol, which is known to have more estrogenic potency and greater affinity for ERβ than daidzein.[29,30,46] A previous epidemiologic study reported that the highest serum equol level was associated with a statistically significant 60% decrease in total prostate cancer risk among Japanese men.[27] In contrast, our study showed no preventive effect of glycitein on prostate cancer, although several experimental studies have reported that glycitein has weak estrogenic activity[47] and plays a role in the modulation of tyrosine kinase activity.[48] Low et al[28] also reported that serum glycitein is not associated with prostate cancer (OR = 1.08). Nakamura et al[49] estimated daily intake of isoflavones from soy products by Japanese of 13.48 mg/d for genistein, 12.02 mg/d for daidzein, and 2.30 mg/d for glycitein. In our study, plasma glycitein levels were much lower than those of other isoflavones. Glycitein may fail to prevent prostate cancer because of its small amount in food or blood. These previous and present studies thus suggest it is plausible that isoflavones have different effects on prostate cancer.

Several limitations in the interpretation of our findings should be considered. First, plasma isoflavone levels were measured only once. As mentioned above, however, the frequent intake of isoflavone-rich foods by Japanese may keep plasma levels stable. In addition, because our study participants were restricted to those who participated in the baseline health check-up survey, any generalization of our results should be done with caution.[50]

 © 2008 by American Society of Clinical Oncology

Information downloaded from jco.ascopubs.org and provided by Kokuritsu Gan Center on December 18, 2008 from .
Copyright © 2008 by the American Society of Clinical Oncology. All rights reserved.

Table 4. ORs and 95% CIs of Prostate Cancer According to Plasma Level of Isoflavone by Stage

Isoflavone and Stage	Low	Middle	High	*P* for Trend
Localized prostate cancer				
Genistein				
Cases	54	55	35	
Controls	94	95	99	
OR*	1.00	1.00	0.61	.04
95% CI	Reference	0.61 to 1.64	0.36 to 1.04	
OR†	1.00	0.98	0.54	.03
95% CI	Reference	0.56 to 1.72	0.29 to 1.01	
Daidzein				
Cases/controls	59/96	43/97	42/95	
Controls				
OR*	1.00	0.70	0.70	.24
95% CI	Reference	0.43 to 1.16	0.42 to 1.16	
OR†	1.00	0.87	0.63	.12
95% CI	Reference	0.48 to 1.58	0.35 to 1.13	
Glycitein				
Cases/controls	45/75‡	49	50	
Controls		105	108	
OR*	1.00	0.78	0.77	.50
95% CI	Reference	0.48 to 1.28	0.47 to 1.27	
OR†	1.00	0.70	0.78	.68
95% CI	Reference	0.40 to 1.23	0.45 to 1.38	
Equol				
Cases/controls	62/105‡	46	36	
Controls		90	93	
OR*	1.00	0.85	0.66	.11
95% CI	Reference	0.52 to 1.40	0.40 to 1.08	
OR†	1.00	0.75	0.43	.02
95% CI	Reference	0.43 to 1.33	0.22 to 0.82	
Advanced				
Genistein				
Cases/controls	15/34	17	16	
Controls		32	30	
OR*	1.00	1.23	1.25	.67
95% CI	Reference	0.51 to 2.95	0.49 to 3.19	
OR†	1.00	1.25	1.77	.47
95% CI	Reference	0.33 to 4.79	0.42 to 7.41	
Daidzein				
Cases/controls	13/31	16	19	
Controls		31	34	
OR*	1.00	1.28	1.42	.45
95% CI	Reference	0.51 to 3.22	0.55 to 3.68	
OR†	1.00	0.98	1.64	.48
95% CI	Reference	0.25 to 3.88	0.34 to 7.87	
Glycitein				
Cases/controls	12/23‡	15	21	
Controls		36	37	
OR*	1.00	0.84	1.12	.62
95% CI	Reference	0.35 to 2.03	0.44 to 2.87	
OR†	1.00	1.37	1.89	.34
95% CI	Reference	0.24 to 7.75	0.32 to 11.33	
Equol				
Cases/controls	14/39‡	18	16	
Controls		29	28	
OR*	1.00	1.67	1.51	.49
95% CI	Reference	0.72 to 3.85	0.65 to 3.50	
OR†	1.00	2.48	2.39	.50
95% CI	Reference	0.66 to 9.29	0.55 to 10.32	

Abbreviation: OR, odds ratio.
*Calculated for the two cohorts together.
†Calculated from the weighted average of the results from separate conditional logistic regressions fitted to the individual cohorts. Adjusted for smoking status, alcohol intake, marital status, and intake of green tea, protein, fiber, and green or yellow vegetables.
‡Not detected.

© 2008 by American Society of Clinical Oncology

Information downloaded from jco.ascopubs.org and provided by Kokuritsu Gan Center on December 18, 2008 from .
Copyright © 2008 by the American Society of Clinical Oncology. All rights reserved.

In summary, we found that plasma genistein and equol levels were inversely associated with the risk of localized prostate cancer in a nested case-control study in Japan. These findings suggest that these compounds may be protective against the development of prostate cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Norie Kurahashi, Motoki Iwasaki, Manami Inoue, Shizuka Sasazuki, Shoichiro Tsugane
Collection and assembly of data: Norie Kurahashi, Motoki Iwasaki, Manami Inoue, Shizuka Sasazuki, Shoichiro Tsugane
Data analysis and interpretation: Norie Kurahashi, Motoki Iwasaki, Manami Inoue, Shizuka Sasazuki, Shoichiro Tsugane
Manuscript writing: Norie Kurahashi
Final approval of manuscript: Norie Kurahashi, Motoki Iwasaki, Manami Inoue, Shizuka Sasazuki, Shoichiro Tsugane

REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al: Global cancer statistics, 2002. CA Cancer J Clin 55:74-108, 2005
2. Yatani R, Kusano I, Shiraishi T, et al: Latent prostatic carcinoma: Pathological and epidemiological aspects. Jpn J Clin Oncol 19:319-326, 1989
3. Tsugane S, de Souza JM, Costa ML Jr, et al: Cancer incidence rates among Japanese immigrants in the city of Sao Paulo, Brazil, 1969-78. Cancer Causes Control 1:189-193, 1990
4. Shimizu H, Ross RK, Bernstein L, et al: Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer 63:963-966, 1991
5. Kurahashi N, Iwasaki M, Sasazuki S, et al: Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. Cancer Epidemiol Biomarkers Prev 16:538-545, 2007
6. Kurahashi N, Sasazuki S, Iwasaki M, et al: Green tea consumption and prostate cancer risk in Japanese men: A prospective study. Am J Epidemiol 167:71-77, 2008
7. Kurahashi N, Inoue M, Iwasaki M, et al: Dairy product, saturated fatty acid, and calcium intake and prostate cancer in a prospective cohort of Japanese men. Cancer Epidemiol Biomarkers Prev 17:930-937, 2008
8. Kurzer MS, Xu X: Dietary phytoestrogens. Annu Rev Nutr 17:353-381, 1997
9. Magee PJ, Rowland IR: Phyto-oestrogens, their mechanism of action: Current evidence for a role in breast and prostate cancer. Br J Nutr 91:513-531, 2004
10. Morton MS, Arisaka O, Miyake N, et al: Phytoestrogen concentrations in serum from Japanese men and women over forty years of age. J Nutr 132:3168-3171, 2002
11. Adlercreutz H, Markkanen H, Watanabe S: Plasma concentrations of phyto-oestrogens in Japanese men. Lancet 342:1209-1210, 1993
12. Strom SS, Yamamura Y, Duphorne CM, et al: Phytoestrogen intake and prostate cancer: A case-control study using a new database. Nutr Cancer 33:20-25, 1999
13. Kolonel LN, Hankin JH, Whittemore AS, et al: Vegetables, fruits, legumes and prostate cancer: A multiethnic case-control study. Cancer Epidemiol Biomarkers Prev 9:795-804, 2000
14. Lee MM, Gomez SL, Chang JS, et al: Soy and isoflavone consumption in relation to prostate cancer risk in China. Cancer Epidemiol Biomarkers Prev 12:665-668, 2003
15. Sonoda T, Nagata Y, Mori M, et al: A case-control study of diet and prostate cancer in Japan: Possible protective effect of traditional Japanese diet. Cancer Sci 95:238-242, 2004
16. Heald CL, Ritchie MR, Bolton-Smith C, et al: Phyto-oestrogens and risk of prostate cancer in Scottish men. Br J Nutr 98:388-396, 2007
17. Nagata Y, Sonoda T, Mori M, et al: Dietary isoflavones may protect against prostate cancer in Japanese men. J Nutr 137:1974-1979, 2007
18. Oishi K, Okada K, Yoshida O, et al: A case-control study of prostatic cancer with reference to dietary habits. Prostate 12:179-190, 1988
19. Lee MM, Wang RT, Hsing AW, et al: Case-control study of diet and prostate cancer in China. Cancer Causes Control 9:545-552, 1998
20. Jian L, Zhang DH, Lee AH, et al: Do preserved foods increase prostate cancer risk? Br J Cancer 90:1792-1795, 2004
21. Hedelin M, Klint A, Chang ET, et al: Dietary phytoestrogen, serum enterolactone and risk of prostate cancer: The cancer prostate Sweden study (Sweden). Cancer Causes Control 17:169-180, 2006
22. Jacobsen BK, Knutsen SF, Fraser GE: Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States). Cancer Causes Control 9:553-557, 1998
23. Allen NE, Sauvaget C, Roddam AW, et al: A prospective study of diet and prostate cancer in Japanese men. Cancer Causes Control 15:911-920, 2004
24. Nomura AM, Hankin JH, Lee J, et al: Cohort study of tofu intake and prostate cancer: No apparent association. Cancer Epidemiol Biomarkers Prev 13:2277-2279, 2004
25. Akaza H, Miyanaga N, Takashima N, et al: Is daidzein non-metabolizer a high risk for prostate cancer? A case-controlled study of serum soybean isoflavone concentration. Jpn J Clin Oncol 32:296-300, 2002
26. Akaza H, Miyanaga N, Takashima N, et al: Comparisons of percent equol producers between prostate cancer patients and controls: Case-controlled studies of isoflavones in Japanese, Korean and American residents. Jpn J Clin Oncol 34:86-89, 2004
27. Ozasa K, Nakao M, Watanabe Y, et al: Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men. Cancer Sci 95:65-71, 2004
28. Low YL, Taylor JI, Grace PB, et al: Phytoestrogen exposure, polymorphisms in COMT, CYP19, ESR1, and SHBG genes, and their associations with prostate cancer risk. Nutr Cancer 56:31-39, 2006
29. Setchell KD, Brown NM, Lydeking-Olsen E: The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr 132:3577-3584, 2002
30. Setchell KD, Clerici C, Lephart ED, et al: S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. Am J Clin Nutr 81:1072-1079, 2005
31. Watanabe S, Tsugane S, Sobue T, et al: Study design and organization of the JPHC study: Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol 11:S3-S7, 2001
32. Tsubono Y, Kobayashi M, Sasaki S, et al: Validity and reproducibility of a self-administered food frequency questionnaire used in the baseline survey of the JPHC Study Cohort I. J Epidemiol 13:S125-S133, 2003
33. Coward L, Kirk M, Albin N, et al: Analysis of plasma isoflavones by reversed-phase HPLC-multiple reaction ion monitoring-mass spectrometry. Clin Chim Acta 247:121-142, 1996
34. Nagata C, Inaba S, Kawakami N, et al: Inverse association of soy product intake with serum androgen and estrogen concentrations in Japanese men. Nutr Cancer 36:14-18, 2000
35. Holzbeierlein JM, McIntosh J, Thrasher JB: The role of soy phytoestrogens in prostate cancer. Curr Opin Urol 15:17-22, 2005
36. Evans BA, Griffiths K, Morton MS: Inhibition of 5 alpha-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. J Endocrinol 147:295-302, 1995
37. Horvath LG, Henshall SM, Lee CS, et al: Frequent loss of estrogen receptor-beta expression in prostate cancer. Cancer Res 61:5331-5335, 2001
38. Leav I, Lau KM, Adams JY, et al: Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. Am J Pathol 159:79-92, 2001
39. Fixemer T, Remberger K, Bonkhoff H: Differential expression of the estrogen receptor beta (ERbeta) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. Prostate 54:79-87, 2003
40. Landström M, Zhang JX, Hallmans G, et al: Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. Prostate 36:151-161, 1998
41. Bylund A, Zhang JX, Bergh A, et al: Rye bran and soy protein delay growth and increase apoptosis of human LNCaP prostate adenocarcinoma in nude mice. Prostate 42:304-314, 2000
42. Canby-Hagino E, Hernandez J, Brand TC, et al: Looking back at PCPT: Looking forward to new paradigms in prostate cancer screening and prevention. Eur Urol 51:27-33, 2007
43. Watanabe S, Yamaguchi M, Sobue T, et al: Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). J Nutr 128:1710-1715, 1998
44. Yamamoto S, Sobue T, Sasaki S, et al: Validity and reproducibility of a self-administered food-frequency questionnaire to assess isoflavone intake in a japanese population in comparison with dietary

 © 2008 by American Society of Clinical Oncology

Information downloaded from jco.ascopubs.org and provided by Kokuritsu Gan Center on December 18, 2008 from .
Copyright © 2008 by the American Society of Clinical Oncology. All rights reserved.

records and blood and urine isoflavones. J Nutr 131:2741-2747, 2001

45. Branham WS, Dial SL, Moland CL, et al: Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. J Nutr 132:658-664, 2002

46. Morito K, Hirose T, Kinjo J, et al: Interaction of phytoestrogens with estrogen receptors alpha and beta. Biol Pharm Bull 24:351-356, 2001

47. Song TT, Hendrich S, Murphy PA: Estrogenic activity of glycitein, a soy isoflavone. J Agric Food Chem 47:1607-1610, 1999

48. Clubbs EA, Bomser JA: Glycitein activates extracellular signal-regulated kinase via vascular endothelial growth factor receptor signaling in nontumorigenic (RWPE-1) prostate epithelial cells. J Nutr Biochem 18:525-532, 2007

49. Nakamura Y, Tsuji S, Tonogai Y: Determination of the levels of isoflavonoids in soybeans and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake. J AOAC Int 83:635-650, 2000

50. Iwasaki M, Yamamoto S, Otani T, et al: Generalizability of relative risk estimates from a well-defined population to a general population. Eur J Epidemiol 21:253-262, 2006

Acknowledgment

We thank all staff members in each study area and in the central offices for their cooperation and technical assistance. We also thank the Iwate, Aomori, Ibaraki, Niigata, Osaka, Kochi, Nagasaki, and Okinawa Cancer Registries for their provision of incidence data.

© 2008 by American Society of Clinical Oncology

Information downloaded from jco.ascopubs.org and provided by Kokuritsu Gan Center on December 18, 2008 from .
Copyright © 2008 by the American Society of Clinical Oncology. All rights reserved.

Coffee, green tea, and caffeine consumption and subsequent risk of bladder cancer in relation to smoking status: a prospective study in Japan

Norie Kurahashi,[1] Manami Inoue, Motoki Iwasaki, Shizuka Sasazuki and Shoichiro Tsugane, for the Japan Public Health Center (JPHC) Study Group

Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center. 5-1-1 Tsukiji Chuo-ku Tokyo 104-0045 Japan

(Received July 14, 2008/Revised October 06, 2008/Accepted October 14, 2008)

Coffee and caffeine consumption are thought to increase the risk of bladder cancer. However, few studies have stratified this risk by smoking status, which is a potential confounder. Here, we investigated the association between coffee, green tea (another major source of caffeine), and caffeine, and bladder cancer incidence in relation to smoking status. We conducted a population-based prospective study in a cohort of Japanese, comprising a total of 49 566 men and 54 874 women aged 40–69 years who reported their coffee and green tea consumption at baseline. During follow-up from 1990 through 2005, 164 men and 42 women were newly diagnosed with bladder cancer. Cigarette smoking was associated with an increased risk of bladder cancer, with a strong dose–response relationship. Coffee was positively associated with bladder cancer risk in men, without statistical significance. When stratified by smoking status, coffee and caffeine consumption were associated with an increased risk of bladder cancer in never- or former-smoking men, with hazard ratios (95% confidence interval) in the highest categories of coffee (one or more cups per day) and caffeine consumption compared with the lowest of 2.24 (95% CI = 1.21–4.16) and 2.05 (95% CI = 1.15–3.66), respectively. In conclusion, cigarette smoking was confirmed as a risk factor for bladder cancer. Coffee and caffeine may be associated with an increased bladder cancer risk in never or former smokers among Japanese men. (*Cancer Sci* 2008)

The incidence of bladder cancer is increasing slightly worldwide, particularly in industrial countries, including Japan.[1,2] Many epidemiological studies have suggested that bladder cancer is influenced by environmental factors, such as smoking, exposure to industrial chemicals, and several lifestyle factors.[3] While the International Agency for Research on Cancer (IARC) reported that coffee is a possible carcinogenic agent (group 2B) in bladder cancer, it could not exclude the possibility that this was due to bias or confounding.[4] In a recent meta-analysis, Zeegers *et al.* demonstrated a small elevated risk (20%) of bladder cancer for current coffee drinkers,[5] and a pooled analysis of 10 case-control studies in European countries found that heavy coffee consumption showed a positive association with bladder cancer among non-smokers.[6] However, most of these previous studies were conducted in Western countries; and of the five conducted in Asian populations, results have been inconsistent: a prospective study in Japanese-Americans reported that coffee consumption increased the risk of bladder cancer albeit without a dose–response,[7] two case-control[8,9] and a prospective study[10] among Japanese people found that the relative risk of coffee drinking was insignificant; while a case-control study in Japanese-American women showed an inverse association with coffee consumption.[11] These findings indicate that the relationship between coffee consumption and bladder cancer in Asian populations is largely unknown.

Caffeine, which is contained in coffee, has been suggested to contribute to the increased risk of bladder cancer, although its carcinogenicity or otherwise in humans has not been classified (group 3).[4] In Asian populations, green tea is a major source of caffeine, but only three studies have investigated the association between green tea and bladder cancer, and their results are inconsistent.[8-10]

Given that Asian populations have a low incidence of bladder cancer, and that their coffee and green tea consumption habits and amounts differ by geographic area, an understanding of the relationships between coffee, green tea or caffeine consumption and bladder cancer among Asian populations should be useful. Moreover, the distribution of phenotypes of N-acetyltransferase,[12,13] which metabolizes caffeine, differs between Asian and Caucasian populations.[14] The effect of coffee, green tea, or caffeine on bladder cancer in Asian populations may therefore differ from that in other populations.

Here, we analyzed the association between coffee, green tea, or caffeine consumption, and bladder cancer in a population-based prospective study in Japan. In addition, we explored these associations by smoking status, which is a known confounder between them.

Materials and Methods

Study population. The Japan Public Health Center-based Prospective Study (JPHC study) started in 1990 for Cohort I and in 1993 for Cohort II. The study design has been described in detail previously.[15] Cohort I consisted of five Public Health Center (PHC) areas (Iwate, Akita, Nagano, Okinawa, and Tokyo), and Cohort II of six PHC areas (Ibaraki, Niigata, Kochi, Nagasaki, Okinawa, and Osaka) across Japan. When analyzing the present data, we excluded all subjects in Tokyo, whose incidence data were not available. As a whole, this cohort consisted of 133 323 men and women aged 40–69 years at baseline who registered in the study areas. After excluding 239 subjects with non-Japanese nationality ($n = 51$), late report of emigration occurring before the start of the follow-up period ($n = 178$), incorrect birth date ($n = 6$), and duplicate enrollment ($n = 4$), a population-based cohort of 133 084 subjects (65 660 men and 67 424 women) was established. This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Baseline survey. A self-administered questionnaire, which included information on coffee and green tea consumption, smoking history, medical history, and other lifestyle factors, was distributed to all registered residents at baseline. A total of

[1]To whom correspondence should be addressed. E-mail: nkurahas@ncc.go.jp

doi: 10.1111/j.1349-7006.2008.01027.x
© 2008 Japanese Cancer Association

106 326 subjects returned valid responses (response rate 80%). After excluding subjects with self-reported bladder cancer at baseline (11 persons) and incomplete data on coffee and green tea consumption, 104 440 subjects (49 566 men and 54 874 women) were included in analysis.

Information on coffee or green tea consumption was obtained in terms of frequency and amount of intake in six categories: almost none, 1–2 times/week, 3–4 times/week, 1–2 cups/day, 3–4 cups/day, and ≥5 cups/day. Based on the distribution in each category, we divided categories of coffee into the following four categories in men: almost none, 1–4 times/week, 1–2 cups/day, and ≥3 cups/day. The type of coffee consumed (decaffeinated or caffeinated) was not included in the questionnaire, because decaffeinated coffee is rarely consumed in Japan. Similarly, green tea consumption was divided into the following four categories in men: <1 cup/day, 1–2 cups/day, 3–4 cups/day, and ≥5 cups/day. For women, we classified coffee and green tea consumption by three categories because of the small numbers of cases. Caffeine consumption was summed as the total amount contained in coffee, green tea, black tea, and Chinese tea. Caffeine consumption from each was calculated by multiplying the amount per cup by the quantity of caffeine per cup for each beverage. Amount per cup for each beverage was determined as the median value in a validation study among subsamples using 28-day dietary records,[16] and found to be 150, 140, 150, and 120 mL in men, and 120, 130, 150, and 120 mL in women for coffee, green tea, black tea, and Chinese tea, respectively. Quantity of caffeine per cup for each beverage was taken from the fifth revised edition of the Standard Tables of Food Composition in Japan.[17]

The validity of coffee or green tea consumption was assessed among subsamples using 28-day dietary records. Spearman's correlation coefficients between coffee or green tea consumption from the questionnaire and from dietary records in men and women were 0.42 and 0.38 (Cohort I) and 0.59 and 0.51 (Cohort II) for coffee, respectively, and 0.57 and 0.63 (Cohort I) and 0.37 and 0.43 (Cohort II) for green tea, respectively.[16]

Follow-up. We followed subjects from the baseline survey until 31 December 2005. Changes in residence status including survival were identified annually through the residential registry in each area or, for those who had moved out of the study area, through the municipal office of the area to which they had moved. Generally, mortality data for residents included in the residential registry are forwarded to the Ministry of Health, Labour and Welfare and coded for inclusion in the National Vital Statistics. Residency and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are believed to be complete. Among the study subjects, 8897 (8.5%) died, 5892 (5.6%) moved out of the study area, and 413 (0.4%) were lost to follow-up during the study period.

The occurrence of cancer was 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. Cases were coded using the International Classification of Diseases for Oncology, third edition (ICD-O-3). Death certificate information was used as a supplementary information source, with 4.4% of cases of bladder cancer first notified by death certificate (DCN). The proportion of case patients with bladder cancer ascertained by death certificate only (DCO) was 1.9%. The mortality/incidence ratios were 0.19 for bladder cancer and 0.36 for all cancer except skin. These results were considered to be of adequate quality for the present study based on the international standard.[18]

For the present analysis, the earliest date of diagnosis was used in cases with multiple primary cancers at different times. A total of 206 newly diagnosed bladder cancer cases (164 men, 42 women) were identified by 31 December 2005.

Statistical analysis. Person-years of follow-up were calculated for each person from the date of completion of the baseline questionnaire to the date of bladder cancer diagnosis, the date of emigration from the study area, or the date of death, whichever came first; or if none of these occurred, follow-up was through to the end of the study period (31 December 2005). Subjects who were lost to follow-up were censored at the last confirmed date of presence in the study area. The hazard ratios (HRs) of bladder cancer were calculated in four categories for coffee and green tea or in quartiles for the categories of caffeine consumption, with the lowest consumption category as the reference. HRs and 95% confidence intervals (CIs) were calculated by the Cox proportional hazards model according to the SAS PHREG procedure (Version 9.1; SAS Institute, Cary, NC, USA). Covariates used in the model were age at enrollment, study area (10 PHC areas), smoking status (never, former [<10, 10–19, and ≥20 years since cessation of smoking], current [1–19, 20–29, 30–39, 40–49, and ≥50 pack-years, defined by multiplying the years of smoking by the average number of cigarettes divided by 20] in men and never, former, current [<25 and ≥25 pack-years] in women), alcohol consumption (non- or occasional drinkers, 1–150, ≥150 g/week), green tea, and coffee consumption. Moreover, we also calculated the HRs of bladder cancer for combined categories of coffee, green tea, or caffeine consumption and smoking status, and tested statistical interactions using the differences between two likelihood ratios of the models with and without the interaction terms between coffee, green tea, and caffeine consumption and smoking status. Interaction terms were generated by multiplying the ordinal of these consumption categories by ordinal smoking categories (never, former, or current smokers).

P-values for trends for coffee, green tea, and caffeine consumption were calculated by treatment as ordinal variables in the proportional-hazards model. All *P*-values are two-sided, and statistical significance was determined at the $P < 0.05$ level.

Results

During 1314 586 person-years of follow-up (average 12.6 years) of 104 440 subjects (49 566 men and 54 874 women), 164 men and 42 women were newly diagnosed with bladder cancer.

Baseline characteristics of subjects according to coffee and green tea consumption are shown in Table 1. Both men and women with high coffee consumption were younger than those who hardly drank. The proportion of current smokers increased as coffee consumption increased in both men and women. As coffee consumption increased, alcohol intake decreased in men and increased in women. Subjects with higher green tea consumption tended to be older, drink less alcohol, and consume less coffee in both men and women. The proportion of current smokers increased as green tea consumption increased in men, whereas that in women was high in both the lowest and highest categories of green tea consumption. As expected, caffeine consumption increased as coffee and green tea intake increased.

Results for smoking status at baseline in relation to bladder cancer risk are showed in Table 2. Cigarette smoking was associated with an increased risk of bladder cancer, with a strong dose–response relationship in both men and women. Moreover, no increased risk was observed in men who had given up smoking for 10 or more years.

Table 3 shows hazard ratios of bladder cancer in relation to coffee, green tea, and caffeine consumption among men. Most daily caffeine intake was derived from coffee (53%) and green tea (40%) in men. Although coffee drinking was associated with a marginally statistically significant increase in bladder cancer risk (age- and area-adjusted HR for men who drank ≥ 3 cups/day *vs* men who hardly drank was 1.71, 95% CI = 0.99–2.96; $P_{trend} = 0.01$), the results were attenuated after further adjustment

doi: 10.1111/j.1349-7006.2008.01027.x
© 2008 Japanese Cancer Association

Table 1. Baseline characteristics of study subjects according to coffee and green tea intake category

	Coffee intake				Green tea intake			
	Almost none	1–4 times/week	1–2 cups/day	≥3 cups/day	<1 cup/week	1–2 cups/day	3–4 cups/day	≥5 cups/day
Men								
Number of subjects	14 929	14 600	12 966	7071	12 868	11 679	13 092	11 927
Proportion (%)	30.1	29.5	26.1	14.3	26.0	23.6	26.4	24.0
Age (years, mean) ± SD	53.7 ± 7.8	52.1 ± 7.8	50.6 ± 7.9	48.3 ± 7.3	49.7 ± 7.4	50.7 ± 7.9	52.3 ± 8.1	53.9 ± 7.8
Current smokers (%)	43.3	49.7	55.9	71.3	51.4	52.9	51.7	54.1
Regular drinkers (%)	68.7	68.5	68.8	62.3	66.4	71.6	69.1	63.8
Coffee intake (%), daily	–	–	–	–	42.9	47.6	40.4	30.8
Green tea intake (%), daily	75.3	74.9	74.5	68.7	–	–	–	–
Caffeine intake (g, mean) ± SD	0.09 ± 0.06	0.12 ± 0.06	0.21 ± 0.06	0.43 ± 0.09	0.13 ± 0.13	0.16 ± 0.12	0.20 ± 0.12	0.23 ± 0.12
Women								
Number of subjects	17 509	15 968	15 994	5403	13 441	11 492	15 316	14 625
Proportion (%)	31.9	29.1	29.1	9.9	24.5	20.9	27.9	26.7
Age (years, mean) ± SD	55.0 ± 7.7	52.2 ± 7.8	49.8 ± 7.5	47.3 ± 6.7	49.8 ± 7.4	50.8 ± 7.9	52.7 ± 8.2	53.9 ± 7.9
Current smokers (%)	4.4	4.4	7.6	18.0	7.8	6.5	5.4	7.1
Regular drinkers (%)	8.6	10.5	15.8	19.6	12.7	13.6	12.1	11.3
Coffee intake (%), daily	–	–	–	–	43.3	48.8	38.4	27.9
Green tea intake (%), daily	76.8	77.7	75.0	66.2	–	–	–	–
Caffeine intake (g, mean) ± SD	0.09 ± 0.06	0.11 ± 0.06	0.19 ± 0.05	0.35 ± 0.08	0.10 ± 0.10	0.13 ± 0.09	0.17 ± 0.08	0.19 ± 0.09

Table 2. Hazard ratios (HRs) and 95% confidence intervals (CIs) of bladder cancer in relation to smoking

	Number of cases	Person-years of follow up	HR[†] (95% CI)	HR[‡] (95% CI)
Men				
Never smoker	26	147 111	1.0 (reference)	1.0 (reference)
Former smoker	42	143 910	1.32 (0.80–2.16)	1.28 (0.78–2.11)
Current smoker	92	316 526	1.69 (1.09–2.63)	1.46 (0.92–2.31)
P_{trend}			0.01	0.10
Never smoker	26	147 111	1.0 (reference)	1.0 (reference)
Years since cessation of smoking in former smokers				
<10	29	72 905	1.88 (1.10–3.21)	1.82 (1.06–3.13)
10–19	7	48 324	0.71 (0.31–1.63)	0.69 (0.30–1.60)
≥20	6	22 682	0.98 (0.40–2.40)	0.98 (0.40–2.38)
Pack-years in current smokers				
<20	9	55 910	1.14 (0.53–2.44)	0.85 (0.37–1.98)
20–29	16	83 959	1.39 (0.74–2.61)	1.32 (0.70–2.49)
30–39	20	77 819	1.39 (0.77–2.50)	1.23 (0.67–2.26)
40–49	22	45 221	2.24 (1.26–3.99)	1.94 (1.06–3.56)
≥50	24	46 178	2.61 (1.49–4.56)	2.24 (1.24–4.04)
P_{trend}			<0.01	0.04
Women				
Never smoker	26	147 111	1.0 (reference)	1.0 (reference)
Former smoker	0	10 764	–	–
Current smoker	9	43 315	5.45 (2.56–11.61)	6.53 (3.02–14.11)
Pack-years in current smokers				
<25	7	28 118	6.91 (3.00–15.93)	8.30 (3.55–19.43)
≥25	2	12 433	4.06 (0.96–17.17)	5.27 (1.23–22.64)
P_{trend}			<0.01	<0.01

[†]Adjusted for age (continuous) and area (10 public health center areas).
[‡]Adjusted for age (continuous), area (10 public health center areas), alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week), green tea (<1, 1–2, 3–4, ≥5 cups/day), and coffee (almost none, 1–4 times/week, 1–2, ≥3 cups/day).

for smoking status, alcohol drinking, and green tea consumption (multivariate HR = 1.37, 95% CI = 0.75–2.51; $P_{trend} = 0.09$). In contrast, green tea and caffeine consumption were not associated with bladder cancer, and HRs did not change substantially after adjustment for all potential confounding factors.

Table 4 presents the association between coffee, green tea, and caffeine consumption and bladder cancer in women. The proportions of coffee and green tea in caffeine were similar in women (43.3% for coffee and 46% for green tea). Coffee consumption tended to decrease the risk of bladder cancer in women who drank one or more cups of coffee per day (multivariate HR = 0.55, 95% CI = 0.23–1.33). In contrast, green tea dose-dependently increased the risk of bladder cancer in women. Multivariate HR for women who drank 5 or more cups

© 2008 Japanese Cancer Association

Table 3. Hazard ratios (HRs) and 95% confidence intervals (CIs) of bladder cancer in relation to consumption of coffee, green tea, and caffeine in men

Category of intake	Coffee				
	Almost none	1–4 times/week	1–2 cups/day	≥3 cups/day	P_{trend}
Number of cases	50	52	43	19	
Person-years of follow up	185 405	183 367	157 544	83 713	
HR[†] (95% CI)	1.0 (reference)	1.23 (0.83–1.82)	1.60 (1.05–2.43)	1.71 (0.99–2.96)	0.01
HR[‡] (95% CI)	1.0 (reference)	1.26 (0.84–1.88)	1.53 (0.98–2.37)	1.37 (0.75–2.51)	0.09
	Green tea				
	<1 cup/day	1–2 cups/day	3–4 cups/day	≥5 cups/day	P_{trend}
Number of cases	33	39	39	53	
Person-years of follow up	161 557	140 218	158 952	149 301	
HR[†] (95% CI)	1.0 (reference)	1.13 (0.70–1.82)	0.83 (0.51–1.33)	0.99 (0.63–1.56)	0.67
HR[§] (95% CI)	1.0 (reference)	1.18 (0.73–1.91)	0.71 (0.43–1.18)	0.90 (0.56–1.45)	0.31
	Caffeine (median, mg/day)				
	Lowest (0.04)	Second (0.13)	Third (0.18)	Highest (0.32)	P_{trend}
Number of cases	47	34	43	40	
Person-years of follow up	177 874	142 138	132 876	157 140	
HR[†] (95% CI)	1.0 (reference)	0.72 (0.46–1.13)	1.24 (0.82–1.89)	1.26 (0.82–1.94)	0.10
HR[¶] (95% CI)	1.0 (reference)	0.67 (0.42–1.08)	1.23 (0.80–1.89)	1.05 (0.66–1.67)	0.36

[†]Adjusted for age (continuous) and area (10 public health center areas).
[‡]Adjusted for age (continuous), area (10 public health center areas), smoking status (never, former [<10, 10–19, ≥20 years since cessation of smoking], current [<20, 20–29, 30–39, 40–49, ≥50 pack-years]), alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week), and green tea (<1, 1–2, 3–4, ≥5 cups/day).
[§]Adjusted for age (continuous), area (10 public health center areas), smoking status (never, former [<10, 10–19, ≥20 years since cessation of smoking], current [<20, 20–29, 30–39, 40–49, ≥50 pack-years]), alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week), and coffee (almost none, 1–4 times/week, 1–2, ≥3 cups/day).
[¶]Adjusted for age (continuous), area (10 public health center areas), smoking status (never, former [<10, 10–19, ≥20 years since cessation of smoking], current [<20, 20–29, 30–39, 40–49, ≥50 pack-years]), and alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week).

Table 4. Hazard ratios (HRs) and 95% confidence intervals (CIs) of bladder cancer in relation to consumption of coffee, green tea, and caffeine in women

Category of intake	Coffee			
	Almost none	1–4 times/week	≥1 cups/day	P_{trend}
Number of cases	19	15	8	
Person-years of follow up	226 689	207 355	270 514	
HR[†] (95% CI)	1.0 (reference)	1.09 (0.55–2.16)	0.63 (0.26–1.52)	0.38
HR[‡] (95% CI)	1.0 (reference)	1.03 (0.51–2.07)	0.55 (0.23–1.33)	0.23
	Green tea			
	<3 cups/day	3–4 cups/day	≥5 cups/day	P_{trend}
Number of cases	12	9	21	
Person-years of follow up	324 123	193 066	187 369	
HR[†] (95% CI)	1.0 (reference)	1.10 (0.45–2.68)	2.21 (1.05–4.66)	0.03
HR[§] (95% CI)	1.0 (reference)	1.22 (0.49–3.00)	2.29 (1.06–4.92)	0.03
	Caffeine (median, mg/day)			
	Lowest (0.05)	Middle (0.13)	Highest (0.24)	P_{trend}
Number of cases	18	12	12	
Person-years of follow up	260 765	227 409	216 383	
HR[†] (95% CI)	1.0 (reference)	0.84 (0.40–1.76)	1.23 (0.58–2.61)	0.68
HR[¶] (95% CI)	1.0 (reference)	0.88 (0.42–1.86)	1.13 (0.52–2.46)	0.81

[†]Adjusted for age (continuous) and area (10 public health center areas).
[‡]Adjusted for age (continuous), area (10 public health centers), smoking status [never, former, current smokers (<25, ≥25 pack-years)], alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week), and green tea (<1, 1–2, 3–4, ≥5 cups/day).
[§]Adjusted for age (continuous), area (10 public health centers), smoking status [never, former, current smokers (<25, ≥25 pack-years)], alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week), and coffee (almost none, 1–4 times/week, 1–2, ≥3 cups/day).
[¶]Adjusted for age (continuous), area (10 public health centers), smoking status [never, former, current smokers (<25, ≥25 pack-years)], and alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week).

doi: 10.1111/j.1349-7006.2008.01027.x
© 2008 Japanese Cancer Association

Table 5. Hazard ratios (HRs) and 95% confidence intervals (CIs) of bladder cancer in relation to consumption of coffee, green tea, and caffeine in men by smoking status

Category of intake	Coffee				
	Almost none	1–4 times/week	≥1 cups/day	P_{trend}	$P_{interaction}$
Never smoker					
Number of cases	6	9	11		
HR[†] (95% CI)	1.00 (reference)	1.89 (0.67–5.32)	2.48 (0.88–7.05)	0.09	0.25
Former smoker					
Number of cases	13	13	16		
HR[†] (95% CI)	1.00 (reference)	1.25 (0.58–2.71)	2.09 (0.96–4.54)	0.07	
Never/Former smoker					
Number of cases	19	22	27		
HR[†] (95% CI)	1.00 (reference)	1.47 (0.79–2.72)	2.24 (1.21–4.16)	0.01	
Current smoker					
Number of cases	29	30	33		
HR[†] (95% CI)	1.00 (reference)	1.11 (0.65–1.90)	1.13 (0.65–1.97)	0.67	
	Green tea				
Category of intake	<3 cups/day	3–4 cups/day	≥5 cups/day	P_{trend}	$P_{interaction}$
Never smoker					
Number of cases	13	6	7		
HR[‡] (95% CI)	1.00 (reference)	0.81 (0.30–2.19)	0.85 (0.31–2.32)	0.72	0.42
Former smoker					
Number of cases	14	12	16		
HR[‡] (95% CI)	1.00 (reference)	1.17 (0.53–2.56)	1.40 (0.67–2.95)	0.38	
Never/Former smoker					
Number of cases	27	18	23		
HR[‡] (95% CI)	1.00 (reference)	0.98 (0.53–1.79)	1.14 (0.63–2.04)	0.68	
Current smoker					
Number of cases	14	18	30		
HR[‡] (95% CI)	1.00 (reference)	0.44 (0.24–0.80)	0.62 (0.37–1.04)	0.05	
	Caffeine (median, mg/day)				
Category of intake	Lowest (0.06)	Middle (0.15)	Highest (0.28)	P_{trend}	$P_{interaction}$
Never smoker					
Number of cases	12	6	8		
HR[§] (95% CI)	1.00 (reference)	0.95 (0.35–2.58)	1.66 (0.66–4.20)	0.33	0.04
Former smoker					
Number of cases	13	13	16		
HR[§] (95% CI)	1.00 (reference)	1.19 (0.55–2.59)	2.30 (1.08–4.87)	0.03	
Never/Former smoker					
Number of cases	25	29	24		
HR[§] (95% CI)	1.00 (reference)	1.09 (0.59–2.00)	2.05 (1.15–3.66)	0.02	
Current smoker					
Number of cases	38	30	24		
HR[§] (95% CI)	1.00 (reference)	0.87 (0.53–1.44)	0.72 (0.41–1.27)	0.25	

[†]Adjusted for age (continuous), area (10 public health center areas), smoking status (never, former [<10, 10–19, ≥20 years since cessation of smoking], current [<20, 20–29, 30–39, 40–49, ≥50 pack-years]), alcohol drinking (non- or occasional drinkers, 1–150, ≥ 150 g/week), and green tea (<1, 1–2, 3–4, ≥5 cups/day).
[‡]Adjusted for age (continuous), area (10 public health center areas), smoking status (never, former [<10, 10–19, ≥20 years since cessation of smoking], current [<20, 20–29, 30–39, 40–49, ≥50 pack-years]), alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week), and coffee (almost none, 1–4 times/week, 1–2, ≥3 cups/day).
[§]Adjusted for age (continuous), area (10 public health center areas), smoking status (never, former [<10, 10–19, ≥20 years since cessation of smoking], current [<20, 20–29, 30–39, 40–49, ≥50 pack-years]), and alcohol drinking (non- or occasional drinkers, 1–150, ≥150 g/week).

of green tea per day was 2.29 (95% CI = 1.06–4.92; $P_{trend} = 0.03$). We observed no association between caffeine consumption and bladder cancer risk in women.

We also assessed the effect of coffee, green tea, and caffeine consumption according to smoking status (Table 5). To avoid residual confounding by smoking, we first stratified smoking status to never, former, or current. Because the HRs of never and former smokers did not substantially differ, these were then combined. Coffee consumption was positively associated with bladder cancer risk in never or former smokers (for 1–4 times per week, multivariate HR = 1.47; for 1 cups or more per day, HR = 2.24, compared with men who hardly drank). For current smokers, coffee was not substantially associated with bladder cancer risk regardless of the amount of coffee consumption (HR = 1.13, 95% CI = 0.65–1.97 for men who drank 1 or more cups per day compared with men who hardly drank). In contrast, green tea was not statistically significant associated with bladder cancer in either never- or former-smoking men. Among current smokers, green tea appeared to be associated with a decreased risk of bladder cancer. We also found a positive association

© 2008 Japanese Cancer Association

between caffeine intake and bladder cancer in never- or former-smoking men, with a HR (95% CI) in the highest category of caffeine consumption compared with the lowest of 2.05 (95% CI = 1.15–3.66). In addition, we observed that HRs slightly decreased as caffeine consumption increased among current smokers, and detected an interaction between caffeine consumption and smoking status ($P_{interaction} = 0.04$).

Discussion

In this study, coffee consumption was associated with an increased risk of bladder cancer, without statistical significance. In contrast, we found no association between green tea and caffeine consumption and bladder cancer risk in men. In never and former smokers, however, a positive association between coffee and caffeine and bladder cancer was observed. Additionally, significant interaction between caffeine and smoking was found ($P_{interaction} = 0.04$), suggesting that the effects of the caffeine might be modified by smoking status.

Cigarette smoking is an established risk factor for bladder cancer.[19] A meta-analysis of 43 published case-control and cohort studies reported that smoking amount and smoking duration were positively associated and that cessation of cigarette smoking was inversely associated with urinary tract cancer risk (most cases being bladder cancer).[20] Our study confirmed these previous results.

Many previous studies have reported a positive association between coffee drinking and bladder cancer in Western countries,[7,12,21–29] although many others have reported no association.[30–37] However, several studies have shown higher risks in coffee drinkers who were non-smokers,[12,27,31,32,34,36,38,39] suggesting the necessity of separate interpretation by smoking status due to the difference in association between coffee consumption and bladder cancer according to smoking status. Our study showed that HRs of subjects who drank 3 or more cups of coffee were attenuated by adjustment for smoking status. Moreover, when we stratified these results by smoking status, we found a positive association between coffee and bladder cancer only in never or former smokers. Thus, our results suggest that the effect of coffee on bladder cancer risk in Japanese may be similar to that in other populations, notwithstanding that the type, strength, and amount of coffee drinking differ among countries.

In our study, caffeine consumption also showed a positive association with bladder cancer among never or former smokers. Given that a similar association was seen in the Netherlands cohort study,[29] these findings suggest that caffeine contained in coffee might contribute to an increased risk of bladder cancer. Moreover, its ability to modify the apoptotic response and perturb cell checkpoint integrity[40,41] suggest that caffeine might be a causative agent of bladder cancer.[4] It is particularly noteworthy that the adverse effects of coffee and caffeine were observed among never or former smokers, and indeed plausible given a previous report that the clearance of caffeine in smokers is faster than that in non-smokers,[42] and that the urinary caffeine levels of smokers were approximately 70% lower than those of non-smokers.[43] In contrast, our study showed that the hazard ratio of current smokers who were in the highest tertile of caffeine consumption was lower than that of current smokers with lower caffeine consumption. Caffeine stimulates the production of cytochrome P450 (CYP) enzymes in the liver, such as CYP1A2 or NAT2, and these enzymes may increase the metabolic activation of carcinogens like polycyclic aromatic hydrocarbons in cigarette smoking.[44] Thus, the adverse effects of coffee and caffeine may be more clearly expressed among never or former smokers and caffeine may modify the increased bladder cancer risk caused by smoking. However, it should be noted that smoking is a major independent risk of bladder cancer. HRs among current smokers who hardly drank coffee were higher than those among never smokers who hardly drank coffee (HR = 4.17 [95% CI = 1.59–10.96], data not shown). Even though our study appears to show a negative tendency between caffeine and bladder cancer among current smokers, the HRs among current smokers was higher than those among never smokers irrespective of caffeine intake (never smokers in lowest category of caffeine intake: reference, current smokers in lowest category of caffeine intake: HR = 3.23 [95% CI = 1.47–7.08], current smokers in highest category of caffeine intake: HR = 2.13 [95% CI = 0.95–4.79]; data not shown).

Of the few papers which have investigated the association between green tea and bladder cancer, one prospective study showed no relation with bladder cancer,[10] a case-control study showed an elevated risk in heavy drinkers of green tea (5–9 cups/day),[9] while a third showed an inverse association with the consumption of matcha (powdered green tea) in women.[8] In our study, green tea was not associated with bladder cancer risk among men. This result is supported by a recent meta-analysis.[5] We speculate that several polyphenolic components of green tea with antioxidant properties might mask the adverse effects of caffeine on bladder cancer in men. In addition, green tea appeared negatively associated with bladder cancer risk among smoking men. Nevertheless, hazard ratios in current smokers were relatively high independently of green tea drinking, indicating that green tea was unable to cancel all the adverse effects of smoking (HR = 1.72 [95%CI = 0.80–3.70] for current smokers who drank five or more cups of green tea per day *versus* never smokers who drank less than three cups; data not shown). In contrast, a positive association between green tea intake and bladder cancer was observed in women. This sex difference might suggest that the contribution of green tea to total caffeine intake in women is higher than that in men. In any case, the negative tendency in bladder cancer risk with increasing coffee consumption in women is difficult to explain. At present, a biological mechanism that might explain this discrepancy between men and women remains unclear, and may have arisen by chance due to the small sample size in women.

This study was a large prospective study in a general Japanese population. Among its strengths were the high rate of participation (approximately 80%) and negligible proportion of loss to follow-up (0.4%), indicating that selection bias was unlikely. Another strength of the prospective design was that coffee and green tea intake were measured before the disease was diagnosed, thereby avoiding the probability of recall bias that is inherent to case-control studies. On the other hand, several limitations also warrant mention. First, we could not evaluate the validity of caffeine consumption. The amount of caffeine depends on the quantity of coffee beans and tea leaf and the length of time of extraction. Unfortunately, we were unable to obtain such information. Second, we could not evaluate the large number of substances other than caffeine contained in coffee. The risk of bladder cancer might be attributable to ingredients other than caffeine or metabolic substances. Third, the number of bladder cancer cases was small, and that therefore the results in women might have occurred by chance. Fourth, coffee and green tea were sources of total fluid intake, which might be inversely associated with the risk of bladder cancer.[35,45] However, we could not adjust for total fluid intake because we did not obtain such information. Finally, we were unable to determine exposure to chemical substances which might have increased the risk of bladder cancer. For example, coffee and green tea might be sources of potential exposure to drinking water contaminants such as chlorination by-products and nitrates.[28] However, any explanation of the different effects of coffee on bladder cancer in terms of smoking status by contaminants in drinking water would be difficult: if contaminants in coffee and green tea were a risk factor for bladder cancer,

doi: 10.1111/j.1349-7006.2008.01027.x
© 2008 Japanese Cancer Association

coffee consumption would be associated with dose-related increase in risk even in current smokers.

In summary, we confirmed that cigarette smoking is major risk factor of bladder cancer and that coffee and caffeine are associated with an increased risk of bladder cancer in never- or former-smoking men. Considerable research effort is required to clarify the effects of caffeine on carcinogenic processes in the bladder.

Acknowledgments

We wish to thank all staff members in each study area and in the central offices for their cooperation and technical assistance. We also wish to thank the Iwate, Aomori, Ibaraki, Niigata, Osaka, Kochi, Nagasaki, and Okinawa Cancer Registries for their provision of incidence data. This study was supported by Grants-in-Aid for Cancer Research (19shi-2).

Appendix

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

References

1 International Agency for Research on Cancer. *Trends in Cancer Incidence and Mortality*. IARC Press 1993.
2 Marugame T, Kaneko S. Comparison of bladder cancer mortality in five countries: France, Italy, Japan, UK and USA from the WHO Mortality Database (1960–2000). *Jpn J Clin Oncol* 2005; **35**: 357–60.
3 Johansson SL, Cohen SM. Epidemiology and etiology of bladder cancer. *Semin Surg Oncol* 1997; **13**: 291–8.
4 International Agency for Research on Cancer, World Health Organization. Coffee, tea, mate, methylxanthines and methylglyoxal. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. *IARC Monogr Eval Carcinog Risks Hum* 1991; **51**: 1–513.
5 Zeegers MP, Tan FE, Goldbohm RA, van den Brandt PA. Are coffee and tea consumption associated with urinary tract cancer risk? A systematic review and meta-analysis. *Int J Epidemiol* 2001; **30**: 353–62.
6 Sala M, Cordier S, Chang-Claude J *et al*. Coffee consumption and bladder cancer in nonsmokers: a pooled analysis of case-control studies in European countries. *Cancer Causes Control* 2000; **11**: 925–31.
7 Chyou PH, Nomura AM, Stemmermann GN. A prospective study of diet, smoking, and lower urinary tract cancer. *Ann Epidemiol* 1993; **3**: 211–6.
8 Ohno Y, Aoki K, Obata K, Morrison AS. Case-control study of urinary bladder cancer in metropolitan Nagoya. *Natl Cancer Inst Monogr* 1985; **69**: 229–34.
9 Wakai K, Hirose K, Takezaki T *et al*. Foods and beverages in relation to urothelial cancer: case-control study in Japan. *Int J Urol* 2004; **11**: 11–19.
10 Nagano J, Kono S, Preston DL *et al*. Bladder-cancer incidence in relation to vegetable and fruit consumption: a prospective study of atomic-bomb survivors. *Int J Cancer* 2000; **86**: 132–8.
11 Nomura AM, Kolonel LN, Hankin JH, Yoshizawa CN. Dietary factors in cancer of the lower urinary tract. *Int J Cancer* 1991; **48**: 199–205.
12 Donato F, Boffetta P, Fazioli R, Aulenti V, Gelatti U, Porru S. Bladder cancer, tobacco smoking, coffee and alcohol drinking in Brescia, northern Italy. *Eur J Epidemiol* 1997; **13**: 795–800.
13 Hein DW, Doll MA, Fretland AJ *et al*. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 29–42.
14 Hickman D, Sim E. N-acetyltransferase polymorphism. Comparison of phenotype and genotype in humans. *Biochem Pharmacol* 1991; **42**: 1007–14.
15 Tsugane S, Sobue T. Baseline survey of JPHC study – design and participation rate. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. *J Epidemiol* 2001; **11**: S24–9.
16 Tsubono Y, Kobayashi M, Sasaki S, Tsugane S. Validity and reproducibility of a self-administered food frequency questionnaire used in the baseline survey of the JPHC Study Cohort I. *J Epidemiol* 2003; **13**: S125–33.
17 Science and Technology Agency, eds. *Standard Tables of Food Composition in Japan*. The Fifth Revised Edition (in Japanese). Tokyo: Printing Bureau, Ministry of Finance, 2000.
18 Parkin DM, Welan SL, Ferlay J, Teppo L, Thomas DB, eds. *Cancer Incidence in Five Continents*. Lyon: International Agency for Research on Cancer, 2002.
19 Zeegers MP, Kellen E, Buntinx F, van den Brandt PA. The association between smoking, beverage consumption, diet and bladder cancer: a systematic literature review. *World J Urol* 2004; **21**: 392–401.
20 Zeegers MP, Tan FE, Dorant E, van Den Brandt PA. The impact of characteristics of cigarette smoking on urinary tract cancer risk: a meta-analysis of epidemiologic studies. *Cancer* 2000; **89**: 630–9.
21 Claude J, Kunze E, Frentzel-Beyme R, Paczkowski K, Schneider J, Schubert H. Life-style and occupational risk factors in cancer of the lower urinary tract. *Am J Epidemiol* 1986; **124**: 578–89.
22 De Stefani E, Boffetta P, Deneo-Pellegrini H *et al*. Non-alcoholic beverages and risk of bladder cancer in Uruguay. *BMC Cancer* 2007; **7**: 57.
23 Howe GR, Burch JD, Miller AB *et al*. Tobacco use, occupation, coffee, various nutrients, and bladder cancer. *J Natl Cancer Inst* 1980; **64**: 701–13.
24 Mettlin C, Graham S. Dietary risk factors in human bladder cancer. *Am J Epidemiol* 1979; **110**: 255–63.
25 Momas I, Daures JP, Festy B, Bontoux J, Gremy F. Relative importance of risk factors in bladder carcinogenesis: some new results about Mediterranean habits. *Cancer Causes Control* 1994; **5**: 326–32.
26 Pohlabeln H, Jockel KH, Bolm-Audorff U. Non-occupational risk factors for cancer of the lower urinary tract in Germany. *Eur J Epidemiol* 1999; **15**: 411–9.
27 Vena JE, Freudenheim J, Graham S *et al*. Coffee, cigarette smoking, and bladder cancer in western New York. *Ann Epidemiol* 1993; **3**: 586–91.

© 2008 Japanese Cancer Association

28 Villanueva CM, Cantor KP, King WD *et al.* Total and specific fluid consumption as determinants of bladder cancer risk. *Int J Cancer* 2006; **118**: 2040–7.
29 Zeegers MP, Dorant E, Goldbohm RA, van den Brandt PA. Are coffee, tea, and total fluid consumption associated with bladder cancer risk? Results from the Netherlands Cohort Study. *Cancer Causes Control* 2001; **12**: 231–8.
30 Bruemmer B, White E, Vaughan TL, Cheney CL. Fluid intake and the incidence of bladder cancer among middle-aged men and women in a three-county area of western Washington. *Nutr Cancer* 1997; **29**: 163–8.
31 Ciccone G, Vineis P. Coffee drinking and bladder cancer. *Cancer Lett* 1988; **41**: 45–52.
32 D'Avanzo B, La Vecchia C, Franceschi S, Negri E, Talamini R, Buttino I. Coffee consumption and bladder cancer risk. *Eur J Cancer* 1992; **28A**: 1480–4.
33 Demirel F, Cakan M, Yalcinkaya F, Topcuoglu M, Altug U. The association between personal habits and bladder cancer in Turkey. *Int Urol Nephrol* 2008; **40**: 643–7.
34 Escolar Pujolar A, Gonzalez CA, Lopez-Abente G *et al.* Bladder cancer and coffee consumption in smokers and non-smokers in Spain. *Int J Epidemiol* 1993; **22**: 38–44.
35 Michaud DS, Spiegelman D, Clinton SK *et al.* Fluid intake and the risk of bladder cancer in men. *N Engl J Med* 1999; **340**: 1390–7.
36 Risch HA, Burch JD, Miller AB, Hill GB, Steele R, Howe GR. Dietary factors and the incidence of cancer of the urinary bladder. *Am J Epidemiol* 1988; **127**: 1179–91.
37 Stensvold I, Jacobsen BK. Coffee and cancer. a prospective study of 43 000 Norwegian men and women. *Cancer Causes Control* 1994; **5**: 401–8.
38 Clavel J, Cordier S. Coffee consumption and bladder cancer risk. *Int J Cancer* 1991; **47**: 207–12.
39 Marrett LD, Walter SD, Meigs JW. Coffee drinking and bladder cancer in Connecticut. *Am J Epidemiol* 1983; **117**: 113–27.
40 Efferth T, Fabry U, Glatte P, Osieka R. Expression of apoptosis-related oncoproteins and modulation of apoptosis by caffeine in human leukemic cells. *J Cancer Res Clin Oncol* 1995; **121**: 648–56.
41 Taylor WR, Stark GR. Regulation of the G2/M transition by p53. *Oncogene* 2001; **20**: 1803–15.
42 Campbell ME, Spielberg SP, Kalow W. A urinary metabolite ratio that reflects systemic caffeine clearance. *Clin Pharmacol Ther* 1987; **42**: 157–65.
43 Yang MKT, Katoh T, Matsuno K. Effects of lifestyle and genetic polymorphisms on consumption of coffee or black tea and urinary caffeine levels. *Biomarkers* 1998; **3**: 367–77.
44 Porta M, Vioque J, Ayude D *et al.* Coffee drinking: the rationale for treating it as a potential effect modifier of carcinogenic exposures. *Eur J Epidemiol* 2003; **18**: 289–98.
45 Michaud DS, Kogevinas M, Cantor KP *et al.* Total fluid and water consumption and the joint effect of exposure to disinfection by-products on risk of bladder cancer. *Environ Health Perspect* 2007; **115**: 1569–72.

doi: 10.1111/j.1349-7006.2008.01027.x
© 2008 Japanese Cancer Association