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Short Communication

No association between fruit or vegetable consumption and the risk of colorectal cancer in Japan

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In a pooled analysis of two prospective studies with 88 658 Japanese men and women, fruit and vegetable consumptions, were not associated with a lower risk of colorectal cancer (705 cases); multivariate relative risk (95% confidence interval) for the highest *vs* the lowest quartile of intake being 0.92 (0.70–1.19) and 1.00 (0.79–1.27), respectively.
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Although fruit and vegetables have been suggested to confer protection against colorectal cancer, recent prospective studies in Western populations found no or limited associations (Michels *et al*, 2000; Voorrips *et al*, 2000). In Japan, mortality from colorectal cancer increased during 1950–2000, especially in men (age-adjusted rate per 100 000 of 2.9–14.4 for colon and 5.6–9.3 for rectum in men; 3.3–9.5 for colon and 4.2–4.1 for rectum in women) (Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labor, and Welfare of Japan, 2003). Dietary factors may play a part in this increase, but the role of fruit and vegetables remains unclear. We therefore examined the association between fruit and vegetable consumption and the risk of colorectal cancer in the Japan Public Health Center (JPHC) prospective study on cancer and cardiovascular disease.

MATERIALS AND METHODS

The JPHC study has two population-based cohorts, and study designs are described in detail elsewhere (Otani *et al*, 2003). Briefly, Cohort I started in 1990 and included 40 106 subjects (19 345 men and 20 761 women) who were 40–59 years of age, lived in four Public Health Center districts, responded sufficiently to a self-administered questionnaire, and had no history of cancer (73.7% of the eligible subjects). Cohort II started in 1993 and included 48 552 subjects (23 180 men and 25 372 women) who were 40–69 years of age, lived in five Public Health Center districts, responded sufficiently to a self-administered questionnaire, and had no history of cancer (77.9% of the eligible subjects).

Cohort I questionnaire asked about the average consumption during the previous month of 44 food items including two fruit (fruit and fruit juice) and five vegetables (green leafy vegetables, yellow vegetables, white vegetables, pickled vegetables, and vegetable juice). Cohort II questionnaire asked about the average consumption during the previous month of 52 food items including three fruit (apples, oranges, and fruit juice) and six vegetables (green vegetables, carrot, tomatoes, green pickled vegetables, other pickled vegetables, and vegetable juice). The questionnaires had six frequency categories for fruit juice and vegetable juice that ranged from 'rarely' to '5 glasses day^{-1}', and four (Cohort I) or five (Cohort II) categories for other items that ranged from 'never' or 'rarely' to 'almost everyday'. The amount of consumption of total fruit and total vegetables (g day^{-1}) were calculated from these responses. We documented the questionnaire assessment of fruit and vegetable consumption to be reasonably valid (Kobayashi *et al*, 2002).

We followed up vital and residential status of subjects and incidence of cancer until the end of 1999. During 694 074 person-years of follow-up from the two cohorts, 705 cases of histologically confirmed colorectal cancer (456 colon and 249 rectum) were identified. Five percent of the subjects moved out of the study regions and 0.04% were lost to follow-up.

We used Cox's regression to compute from each cohort relative risk (RR) and 95% confidence interval (CI) of colorectal cancer according to quartiles of total fruit or vegetable consumption with adjustment for potential confounders. We pooled these estimates to obtain summary measures using inverse-variance weighting. As we observed no differential findings between the two cohorts, we present the pooled results only. This study has approximately 80% statistical power, with the two-sided α-error level of 5%, in detecting a true RR of 0.75 among the highest *vs* lowest quartiles of total vegetable consumption.

RESULTS

Compared with men in Cohort I in the lowest quartile of total vegetable consumption, men in the highest quartile were more likely to engage in sports and use vitamin supplements, less likely to be current smokers, and consumed higher amount of meats and fish, but lower amount of cereals. The men in the two groups did not differ with respect to age, body mass index, or the prevalence

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Table 1 Pooled multivariate RR and 95% CI of colorectal cancer for total fruit and total vegetable consumption[a]

	Quartiles of total fruit consumption					Quartiles of total vegetable consumption				
	Lowest	**Second**	**Third**	**Highest**	**Trend *P***	**Lowest**	**Second**	**Third**	**Highest**	**Trend *P***
Person-years in Cohort I	94 449	95 035	94 925	95 901		94 394	94 936	95 360	95 620	
Person-years in Cohort II	78 632	78 285	78 545	78 303		78 581	78 766	78 467	77 950	
Men and women										
Colorectum										
No. of cases	114/94	102/81	97/73	64/80		100/85	91/84	95/78	91/81	
RR (95% CI)	1.00	0.89	0.88	0.92 (0.70–1.19)	0.40	1.00	0.98	0.92	1.00 (0.79–1.27)	0.80
Colon										
No. of cases	77/56	70/51	66/48	43/45		67/50	60/53	68/44	61/53	
RR (95% CI)	1.00	0.89	0.93	0.92 (0.66–1.28)	0.61	1.00	0.99	0.96	1.08 (0.80–1.45)	0.73
Rectum										
No. of cases	37/38	32/30	31/25	21/35		33/35	31/31	27/34	30/28	
RR (95% CI)	1.00	0.88	0.78	0.91 (0.59–1.40)	0.47	1.00	0.95	0.84	0.87 (0.58–1.31)	0.37
Men										
Colorectum										
No. of cases	90/80	81/61	61/43	10/28		83/66	62/55	60/45	37/46	
RR (95% CI)	1.00	0.86	0.79	1.06 (0.70–1.61)	0.34	1.00	0.95	0.82	1.18 (0.88–1.59)	0.86
Colon										
No. of cases	59/51	57/36	42/31	8/16		57/40	42/36	41/27	26/31	
RR (95% CI)	1.00	0.83	0.86	1.02 (0.61–1.70)	0.57	1.00	0.96	0.84	1.24 (0.86–1.79)	0.69
Rectum										
No. of cases	31/29	24/25	19/12	2/12		26/26	20/19	19/18	11/15	
RR (95% CI)	1.00	0.91	0.68	1.19 (0.59–2.36)	0.42	1.00	0.91	0.81	1.06 (0.63–1.78)	0.81
Women										
Colorectum										
No. of cases	24/14	21/20	36/30	54/52		17/19	29/29	35/33	54/35	
RR (95% CI)	1.00	1.02	1.15	0.93 (0.61–1.42)	0.77	1.00	1.03	1.08	0.88 (0.57–1.35)	0.48
Colon										
No. of cases	18/5	13/15	24/17	35/29		10/10	18/17	27/17	35/22	
RR (95% CI)	1.00	1.07	1.19	0.87 (0.49–1.52)	0.86	1.00	1.09	1.25	1.01 (0.58–1.76)	0.96
Rectum										
No. of cases	6/9	8/5	12/13	19/23		7/9	11/12	8/16	19/13	
RR (95% CI)	1.00	0.77	0.95	0.84 (0.43–1.65)	0.77	1.00	0.96	0.84	0.71 (0.36–1.38)	0.27

RR = relative risk; CI = confidence interval. [a]RRs have been adjusted for sex, age (5-year groups), Public Health Centre area, body mass index in $kg\,m^{-2}$ (less than 19, 19–22.9, 23–26.9, and 27 or more), frequency of sports (never or 1 day/month or more), smoking (never, past, and current), alcohol consumption (non, occasional, 1–149, 150–299, and 300 g week or more), vitamin supplement use, quartiles of energy, cereals, meats, and fish by each cohort. The lowest quartile serves as reference category. The numbers of colon and rectal cancers are from Cohort I/Cohort II.

of regular drinkers. We observed similar tendencies for women in Cohort I, and for men and women in Cohort II.

We found no significant association between fruit or vegetable intakes and the risk of colorectal cancer (Table 1). Multivariate RRs (95% CI) for the highest *vs* the lowest quartile of intake were 0.92(0.70–1.19) and 1.00(0.79–1.27), respectively, based on 705 cases. We observed no association whether or not colon and rectal cancers were separated, or men and women were separated. Exclusion of colorectal cancer cases diagnosed in the first 3 years of follow-up did not change the findings materially. Stratified analyses by covariates included in multivariate models did not reveal remarkable effect modifications. Analyses based on the octiles of total fruit or vegetable consumption did not show significant associations. No individual fruit or vegetables showed significant relations with risk.

DISCUSSION

This is the first prospective cohort study of fruit and vegetable consumption and incident risk of colorectal cancer in Japan. Our results are consistent with the recent prospective studies in Western populations showing no substantial protective associations (Michels *et al*, 2000; Voorrips *et al*, 2000).

Our food frequency questionnaires had relatively small number of fruit and vegetable items and limited range of frequency categories. Nevertheless, we had observed in Cohort I an inverse association between fruit and vegetable intakes and the risk of gastric cancer (Kobayashi *et al*, 2002). It is therefore unlikely that failure to observe protective association was due to the crude designs of our questionnaires.

While mortality from colorectal cancer in Japan increased during 1950–2000, the average consumption of fruit and vegetables also increased during this period (42–117 and 242–311 g day^{-1}, respectively) (Kenko Eiyo Joho Kenkyukai, 2002). Our results, along with these time trends, suggest that low consumption of fruit and vegetables is not primarily responsible for the increased rate of colorectal cancer in Japan.

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Appendix A

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Body mass index, body height, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan Public Health Center-based Prospective Study

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Abstract

Objective: To investigate the association of body mass index (BMI) or body height with colorectal cancer incidence in a population-based prospective study.
Methods: We identified 986 (626 men and 360 women) newly diagnosed cases of colorectal cancer during the 9.4-year follow-up of a cohort consisting of 102,949 (49,158 male and 53,791 female) middle-aged and elderly Japanese.
Results: Lower BMI groups (lower than 23) were not associated with colorectal cancer compared with the 23–24.9 BMI group. Any categories of 25–26.9, 27–29.9, or 30 or more BMI were associated with an increased risk of colorectal cancer compared with the lower than 25 BMI (RR, 1.2 for 25–26.9, 1.4 for 27–29.9, and 1.5 for 30 or more; p for trend, 0.004) in men. These associations were more evident only in invasive-type cancer analysis. BMI was not associated with the risk of colorectal cancer in women. No significant association with height was obtained for either men or women.
Conclusions: The association of BMI with colorectal cancer was confirmed in a Japanese population as well as Western populations. Only invasive-cancer analysis suggested that BMI was important for tumor growth and proliferation. Approximately 6.7% of colorectal cancer was attributable to a BMI of 25 or higher in middle-aged and elderly Japanese men.

Introduction

Colorectal cancer is one of the most common cancers in both Western and Asian populations, including Japan. Particularly, the Japanese population has shown a rapidly increasing colorectal cancer incidence for several decades [1]. Thus, analytic epidemiology to elucidate risk factors for this cancer is an important and urgent issue in order to provide evidence for its prevention.

Many epidemiologic studies have investigated an association of body mass index (BMI) with colorectal cancer and adenoma [2, 3] mostly in Western populations [4–23] but rarely in Asian populations [24–26]. Most of these studies reported a positive association and a linear trend, especially in men. Obesity causes insulin resistance and leads to a high exposure of insulin-like growth factor I (IGF-I) [27]. These hormones, insulin and IGF-I, relate to colorectal carcinogenesis in animal studies and epidemiologic studies [3, 27, 28]. In the Japanese population, weight, as well as height, has been increasing in recent decades [29]. Simultaneously, body mass index (BMI) has elevated [30]. However, the overweight population (25 or higher BMI; 24% in men and 21% in women in 1991–1995) in Japan is lower than in Western populations [29–32]. Nevertheless, the incidence rate of colorectal cancer in the Japanese population has now reached the highest level in the world [1].

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This phenomenon may be due to an increase in the number of high BMI individuals, and thus the BMI in Japan may more strongly affect colorectal carcinogenesis than in Western populations. Even 25 to 29.9 BMI subjects may be associated with a much higher risk, and this may be causing the rapid increase of colorectal cancer in the Japanese population. In addition, the risk to leaner individuals (lower than 25 BMI) should be carefully examined in Asian populations, because even the lower than 25 BMI subjects have greater disease risks such as type 2 diabetes and cardiovascular diseases than Western individuals [33].

Some studies reported that the effects of BMI differed among site-specific cancers. Distal colon cancer is more strongly associated with BMI than proximal colon cancer in many studies [5, 8, 16, 17, 19], while proximal colon is associated with a clearer risk than distal colon in a few studies [23]. This site-specific evidence, however, is too limited for a conclusive statement and should be confirmed by larger prospective studies.

Some mechanisms of BMI for colorectal cancer include the hypothesis of colonic cell proliferation by insulin and IGF-I [27, 28]. If this hypothesis is true, at least BMI would be associated with a stage of tumor growth and infiltration. In fact, some studies reported a stronger association with large rather than small adenomas [20–23, 26]. However, this evidence is insufficient, and a study on non-invasive-type and invasive-type cancer is needed.

Furthermore, body height has been another body size measure investigated for a possible association with colorectal cancer. The evidence, however, is inconsistent. Most cohort studies [4, 7, 9–11, 25] reported a positive association, but most case-control studies failed to show significant associations [15, 16, 18, 21] despite this variable with a lower recall bias. Japanese mean body height has increased in recent decades [29], and may be associated with an elevated colorectal cancer incidence.

We previously reported a high population-attributable fraction of alcohol consumption and smoking for colorectal cancer incidence in Japanese men [34]. We used here the same population of the Japan Public Health Center-based Prospective Study and investigated an association between BMI or body height and colorectal cancer incidence focusing on differences among tumor sites and the degree of invasion.

Materials and methods

Study population

The Japan Public Health Center-based Prospective Study (JPHC study) Cohort I was defined in 1990 and Cohort II in 1993 [35]. Study subjects were mainly all residents living in several municipalities in each Public Health Center area, aged 40 to 59 for Cohort I and aged 40 to 69 for Cohort II. Additionally, Cohort I included health check-up examinees and Cohort II included health check-up examinees and a random sample aged 40 to 69 from a municipality. The study subjects were identified by the population registry in each municipality. Because cancer incidence data were not available, Cohort I health check-up examinees were excluded in this report. Thus, we defined a cohort of 65,803 men (27,063 in Cohort I; 38,740 in Cohort II) and 67,520 women (27,435 in Cohort I; 40,085 in Cohort II). This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan. The study design is described in detail elsewhere [34–40].

Baseline survey

Study subjects were asked about their personal and familial medical histories, smoking, alcohol consumption, dietary habits, and other lifestyle factors by a self-administered questionnaire [36–38]. Their dietary habits were assessed by a 44-item food frequency questionnaire (FFQ) in Cohort I [41] and a 52-item FFQ in Cohort II. Altogether, 50,456 men (77%) and 55,909 women (83%) returned the questionnaire.

Assessment of exposure

Respondents reported current height (cm) and weight (kg) at baseline. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2). These self-reported height and weight data were validated in our previous report [39]. We categorized BMI as follows: less than 19, 19–20.9, 21–22.9, 23–24.9, 25–26.9, 27–29.9, and 30 or more [39, 40]. Body height was divided into quintiles by sex.

Concerning potential confounding factors, we used age at baseline, alcohol consumption, smoking [34], miso (soybean paste) soup intake, and refraining from salty foods and animal fats in the BMI analysis, because these potential confounding factors were selected by a 10% change-in-estimate strategy in the highest category [42]. In body height analysis, we used age at baseline, alcohol consumption, smoking, and body weight at baseline, selected by the same strategy. Other factors such as medical history, family medical history, medication, health check-up, total energy intake, food intake frequency such as vegetables, meats, fish and rice, physical exercise, occupation, and reproductive health in women were also examined as confounding factors but not included in a final multivariate-adjusted model.

Follow-up

We followed study subjects until 31 December 2001. When subjects died, we used mortality data from the Ministry of Health, Labor and Welfare. Subjects moving to other municipalities were also annually identified through residential registers in PHC areas. Among study subjects, 9.6% moved away, and 0.2% were lost to follow-up during the study period.

Identification of colorectal cancer incidence

Up to 31 December 2001, 1064 incident cases of colorectal cancer were identified (C180–C209 in the International Classification of Diseases for Oncology, Third edition (ICD-O-3); Ref. [43]). For multiple primary cancers of the colon or rectum at different times, the earliest diagnosis was applied. For those occurring simultaneously, the most advanced and most invasive types of tumor were applied. Among these incident cases, 986 were pathologically confirmed as adenocarcinoma (626 in men and 360 in women). Such cases were further classified into two groups according to the depth of tumor invasion, i.e., invasive cancer over a mucosal layer corresponding to code 3 (Malignant, primary site) in "behavior code for neoplasms" (415 colon cases and 259 rectal cases), and non-invasive cancer within a mucosal layer corresponding to code 2 (Carcinoma *in situ*; 219 colon and 63 rectum) in ICD-O-3 (the depth in 19 colon and 11 rectal tumors were unknown). We categorized these colorectal cancer cases into site-specific cases as follows: C180–C189 for colon cancer; C180–C185 for proximal colon cancer; C186 and C187 for distal colon cancer; and C199 and 209 for rectal cancer. The proportion of cases for which information was available only from death certificates (DCO) was 1.7% for colorectal cancer and 4.1% for all cancers during the study period. These figures were considered of satisfactory quality for the present study based on the international standard [1].

Statistical analysis

We excluded ineligible subjects notified during this study period, such as non-Japanese (31 men and 20 women), those who had already moved away at baseline (107 men and 69 women), those outside the age parameters (one man and five women), and any duplication of subjects registered in our cohort (two men and one woman). From baseline questionnaire respondents, we excluded subjects with a self-reported medical history of cancer and with a diagnosis of colorectal cancer before the baseline questionnaire survey (740 men and 1503 women). Finally, we excluded subjects with incomplete body height and weight items (540 men and 597 women), leaving 49,158 men and 53,791 women as study subjects.

We calculated person-years of follow-up from the start in each cohort until the date of diagnosis of colorectal cancer, the date of a subject's death, the date of moving from a PHC area, or 31 December 2001, whichever occurred first. The mean follow-up period was 9.4 years in both cohorts together (11.3 years in Cohort I and 8.1 years in Cohort II).

Relative risks (RR) and 95% confidence intervals (CI) of colorectal cancer incidence for BMI and body height were estimated by the Cox proportional hazards model, according to the SAS PHREG procedure [44]. The estimates were adjusted for the following potentially confounding factors incorporated into the model: age (continuous), alcohol consumption (never, one to three days per month, 1–149 g/week ethanol, 150–299 g/week ethanol, 300 g/week or more ethanol), smoking (never, past, current), miso soup intake (less than 1 cup/day, 1 cup/day, 2 cups/day, 3 or more cups/day), refraining from salty foods (yes or no) and animal fats (yes or no), body weight (quintiles by sex) and PHC area. The linear trend of BMI and body height was assessed by assignment of the median value in each category. p-values for those trends were evaluated using the two-sided test with 0.05 as the significance level.

First, we estimated the RR of all cases of colorectal cancer in each cohort. These RR estimates were integrated by the fixed-effect model, as a weighted mean by the inverse of the variance. Second, after confirming no statistically significant heterogeneity across two cohorts, we combined their datasets and calculated the RRs and the linear trends for BMI. We estimated RRs of site-specific colorectal cancer. In such site-specific analyses, we considered the cancer events in the other sites as censored cases. Similarly, RRs of only invasive cancers were calculated. In such invasive-cancer analyses, non-invasive cancers were defined as censored cases.

The population-attributable fraction (PAF) was estimated by P_e $(RR_a - 1)/RR_a$, where P_e was the prevalence of exposure among incident cases and RR_a was the adjusted RR [45]. The PAFs' 95% CI were estimated by the formula of Greenland [46]. We estimated the PAFs of overweight subjects (25 or higher BMI) to normal weight subjects (lower than 25 BMI).

Results

Moderate body mass index (BMI) categories (21–22.9 and 23–24.9) applied to a large percentage of male and

Table 1. Baseline characteristics by body mass index category in men and women

	Body mass index (kg/m^2)						
	< 19	19–20.9	21–22.9	23–24.9	25–26.9	27–29.9	30+
Men in Cohort I , Number	704	2913	5226	5750	3448	1752	420
Proportion (%)	3.5	14.4	25.8	28.5	17.1	8.7	2.1
BMI (kg/m^2), median	18.3	20.2	22.0	24.0	25.8	27.9	31.2
Age (y), mean	49.9	49.5	49.6	49.3	49.2	49.3	49.5
Alcohol consumption 1 day/week or more (%)	62.3	68.8	69.9	69.9	67.5	62.6	56.5
Current smokers (%)	65.8	64.4	56.8	49.7	47.1	42.8	42.4
Miso soup intake 1 cup/day or more (%)	77.3	80.5	80.9	77.6	74.6	70.9	67.1
Refraining from salty foods (%)	66.1	66.1	72.1	74.6	76.1	76.1	75.9
Refraining from animal fats (%)	52.8	53.5	62.5	68.5	70.4	70.9	71.3
Physical exercise once or more per week (%) >	12.9	14.5	17.2	17.5	19.8	19.1	24.6
Total energy intake per day (kcal), median[a]	2033	2135	2159	2095	2020	1957	1883
Men in Cohort II, Number	1387	4312	7503	7941	4636	2550	616
Proportion (%)	4.9	14.9	26.0	27.5	15.9	8.7	2.0
BMI (kg/m^2), median	18.3	20.2	22.1	23.9	25.9	28.0	31.2
Age (y), mean	55.1	53.6	53.2	52.8	52.3	51.5	51.2
Alcohol consumption 1 day/week or more (%)	59.8	66.7	69.8	69.2	68.0	66.6	62.4
Current smokers (%)	63.7	61.3	56.0	49.0	45.0	44.9	43.2
Miso soup intake 1 cup/day or more(%)	62.7	65.9	65.9	66.2	64.0	63.6	62.1
Refraining from salty foods (%)	63.3	64.5	68.4	70.8	70.4	69.6	69.8
Refraining from animal fats (%)	56.4	61.0	65.1	68.6	69.4	69.5	71.6
Physical exercise once or more per week (%)	16.6	17.8	20.0	22.0	21.3	20.1	19.2
Total energy intake per day (kcal), median[a]	1610	1675	1672	1658	1655	1666	1688
Women in Cohort I, Number	1058	3244	5661	5427	3444	2191	700
Proportion (%)	4.9	14.9	26.1	25.0	15.8	10.1	3.2
BMI (kg/m^2), median	18.3	20.2	22.1	23.9	25.9	28.0	31.5
Age (y), mean	49.0	48.6	49.3	49.7	50.3	50.4	50.4
Alcohol consumption 1 day/week or more (%)	11.8	12.4	11.9	9.9	10.0	8.4	7.3
Current smokers (%)	10.4	7.1	5.2	4.5	4.9	5.9	8.0
Miso soup intake 1 cup/week or more (%)	77.2	75.9	75.9	74.4	74.6	72.4	67.3
Refraining from salty foods (%)	81.3	81.8	85.4	86.8	86.2	86.4	84.7
Refraining from animal fats (%)	64.6	70.8	77.5	81.2	80.8	82.9	81.4
Physical exercise once or more per week (%)	11.6	13.0	14.3	15.6	14.3	13.8	13.8
Total energy intake per day (kcal), median[a]	1370	1364	1372	1368	1361	1346	1318
Women in Cohort II, Number	2145	5572	8492	7432	4509	2907	1009
Proportion (%)	6.7	17.5	26.5	23.2	14.0	9.0	3.1
BMI (kg/m^2), median	18.3	20.2	22.0	23.9	25.9	28.0	31.6
Age (y), mean	52.7	51.4	52.7	53.7	54.8	55.3	54.8
Alcohol consumption 1 day/week or more (%)	17.5	17.8	14.8	12.8	10.6	9.7	7.8
Current smokers (%)	12.2	9.0	7.4	5.4	6.0	6.2	8.7
Miso soup intake 1 cup/day or more (%)	56.7	57.4	59.6	62.0	62.6	62.4	59.0
Refraining from salty foods (%)	81.5	83.5	84.6	85.7	85.1	86.2	85.0
Refraining from animal fats (%)	71.1	73.9	79.5	81.7	81.9	82.5	81.9
Physical exercise once or more per week (%)	17.3	19.9	20.6	20.6	21.4	20.8	17.8
Total energy intake per day (kcal), median[a]	1084	1091	1076	1071	1064	1037	1028

[a] Based on the food frequency questionnaire.

female subjects (approximately 50% in these two categories alone: Table 1). The proportion of men drinking one day per week or more was smaller in both the lowest and the highest category of BMI than in other categories. Female drinkers one day per week or more accounted for a smaller proportion in the higher BMI categories. High BMI men were less likely to have smoking habits. Higher BMI subjects tended to take less miso soup except for Cohort II women and to refrain more from salty foods and animal fats. Taller subjects had greater body weight and more energy intake, and tended to do physical exercise (Table 2).

Table 2. Baseline characteristics by body height category in both sexes

		Body height (cm)				
		Q1	Q2	Q3	Q4	Q5
Men						
	Range	< 160	160–162	163–165	166–169	170+
Cohort I	Number	4212	4029	4829	3400	3743
	Proportion (%)	20.7	19.9	24.0	16.8	18.6
	Height (cm), median	156	160	164	168	172
	Weight (kg), median	57	60	63	65	69
	BMI (kg/m^2), median	23.5	23.4	23.5	23.3	23.2
	Age (y), mean	51.7	50.2	49.4	48.3	47.3
	Alcohol consumption 1 day/week or more (%)	63.3	67.0	69.0	70.0	72.1
	Current smokers (%)	49.7	52.4	52.4	52.8	58.4
	Physical exercise once or more per week (%)	14.1	17.2	18.2	18.9	19.4
	Total energy intake per day (kcal), median[a]	2057	2045	2094	2087	2141
Cohort II	Number	5734	5364	6232	5080	6535
	Proportion (%)	19.3	18.4	21.5	17.8	22.9
	Height (cm), median	156	160	164	168	172
	Weight (kg), median	56	60	63	65	70
	BMI (kg/m^2), median	23.2	23.4	23.3	23.2	23.3
	Age (y), mean	58.1	54.7	53.1	50.9	48.7
	Alcohol consumption 1 day/week or more (%)	63.4	63.9	67.0	70.6	73.2
	Current smokers (%)	44.6	50.1	53.0	54.7	57.6
	Physical exercise once or more per week (%)	18.5	20.2	19.6	20.9	21.6
	Total energy intake per day (kcal), median[a]	1633	1635	1664	1665	1697
Women						
	Range	< 148	148–150	151–153	154–156	157+
Cohort I	Number	4200	5621	4548	3585	3771
	Proportion (%)	19.3	25.8	21.0	16.5	17.3
	Height (cm), median	145	149	152	155	159
	Weight (kg), median	50	52	54	55	58
	BMI (kg/m^2), median	23.8	23.6	23.4	23.2	22.7
	Age (y), mean	51.3	50.1	49.4	49.0	47.7
	Alcohol consumption 1 day/week or more (%)	8.0	9.3	11.3	11.8	13.5
	Current smokers (%)	5.4	4.9	5.3	6.0	7.1
	Physical exercise once or more per week (%)	12.7	12.7	14.9	15.0	16.8
	Total energy intake per day (kcal), median[a]	1338	1351	1374	1370	1389
Cohort II	Number	5696	7316	6317	5745	6992
	Proportion (%)	17.7	22.8	19.7	17.9	21.8
	Height (cm), median	145	149	152	155	159
	Weight (kg), median	49	52	53	55	57
	BMI (kg/m^2), median	23.8	23.1	23.1	22.8	22.3
	Age (y), mean	58.6	55.3	53.0	51.5	49.3
	Alcohol consumption 1 day/week or more (%)	7.1	10.7	13.7	15.5	19.8
	Current smokers (%)	4.8	6.1	7.1	8.1	9.9
	Physical exercise once or more per week (%)	18.4	19.1	20.4	20.9	22.2
	Total energy intake per day (kcal), median[a]	1043	1056	1077	1077	1096

[a] Based on the food frequency questionnaire.

The highest BMI group (30 or more) was associated with a non-significant increased risk of colorectal cancer compared with the 23–24.9 group in men of both cohorts [multivariate relative risk (RR), 1.5; 95% confidence interval (CI), 0.7–3.2 in Cohort I; RR, 1.3; 95% CI, 0.6–3.0 in Cohort II; Table 3]. Because groups of lower than 23 BMI showed almost the same risk as the 23–24.9 group, we combined these categories and repeatedly calculated RRs with a referent group of lower than 25 BMI. As a result, RR for such BMI categories had a non-significant linear trend in Cohort I (p for trend, 0.17) and a significant linear trend in Cohort II (p for trend, 0.005).

In contrast, BMI had no association with colorectal cancer in women in both cohorts (Table 3). RRs of the 30 or higher group were 0.8 (95% CI, 0.3–2.2) in Cohort I and

Table 3. Relative risks (RR) and 95% confidence intervals (CI) of colorectal cancer for body mass index by each cohort in men and women

	Body mass index (kg/m^2)							*p* for trend
	< 19	19–20.9	21–22.9	23–24.9	25–26.9	27–29.9	30+	
Men								
Cohort I (1990–2001)								
Case	13	46	80	81	58	24	10	
Person-year	7536	32122	57958	64133	38206	19395	4562	
Age-adjusted RR[a]	1.3	1.1	1.0	1.0	1.2	1.1	1.9	
95% CI	(0.7–2.3)	(0.7–1.5)	(0.8–1.4)	(reference)	(0.9–1.7)	(0.7–1.7)	(0.97–3.7)	
Multivariate RR1[b]	1.3	1.1	1.0	1.0	1.3	1.1	1.5	
95% CI	(0.7–2.4)	(0.7–1.6)	(0.7–1.4)	(reference)	(0.9–1.8)	(0.7–1.7)	(0.7–3.2)	
Multivariate RR2[b]			1.0		1.2	1.1	1.5	0.17
95% CI			(reference)		(0.9–1.7)	(0.7–1.6)	(0.7–3.0)	
Cohort II (1993–2001)								
Case	10	49	73	86	55	34	7	
Person-year	10689	34309	59693	63285	37189	20644	4861	
Age-adjusted RR[a]	0.6	0.9	0.8	1.0	1.2	1.5	1.3	
95% CI	(0.3–1.1)	(0.7–1.3)	(0.6–1.2)	(reference)	(0.8–1.6)	(0.98–2.2)	(0.6–2.9)	
Multivariate RR1[b]	0.6	1.0	0.8	1.0	1.1	1.6	1.3	
95% CI	(0.3–1.2)	(0.7–1.4)	(0.6–1.1)	(reference)	(0.8–1.6)	(1.1–2.4)	(0.6–3.1)	
Multivariate RR2[b]			1.0		1.2	1.8	1.5	0.005
95% CI			(reference)		(0.9–1.7)	(1.2–2.5)	(0.6–3.3)	
Weighted mean estimates by the inverse of variance between Cohort I and II								
Multivariate RR2[b]			1.0		1.2	1.4	1.5	
95% CI			(reference)		(0.99–1.5)	(1.1–1.9)	(0.86–2.5)	0.003
Women								
Cohort I (1990–2001)								
Case	6	21	49	38	39	18	5	
Person-year	11804	36861	64361	62514	39360	25008	8051	
Age-adjusted RR[a]	0.8	1.0	1.3	1.0	1.6	1.2	1.0	
95% CI	(0.4–2.0)	(0.6–1.7)	(0.8–1.9)	(reference)	(1.02–2.5)	(0.7–2.0)	(0.4–2.6)	
Multivariate RR1[b]	0.8	1.0	1.2	1.0	1.6	1.1	0.8	
95% CI	(0.3–2.0)	(0.6–1.7)	(0.8–1.9)	(reference)	(1.02–2.5)	(0.7–2.0)	(0.3–2.2)	
Multivariate RR2[b]			1.0		1.5	1.1	0.7	0.51
95% CI			(reference)		(1.04–2.1)	(0.7–1.8)	(0.3–2.0)	
Cohort II (1993–2001)								
Case	10	30	53	38	33	15	5	
Person-year	17051	45205	69886	61852	37717	24495	8509	
Age-adjusted RR[a]	1.0	1.3	1.3	1.0	1.3	0.9	0.9	
95% CI	(0.5–2.0)	(0.8–2.0)	(0.9–2.0)	(reference)	(0.8–2.1)	(0.5–1.6)	(0.4–2.3)	
Multivariate RR1[b]	1.0	1.4	1.4	1.0	1.3	1.0	1.0	
95% CI	(0.5–2.3)	(0.9–2.3)	(0.9–2.3)	(reference)	(0.8–2.2)	(0.5–1.9)	(0.4–2.7)	
Multivariate RR2[b]			1.0		1.1	0.8	0.8	0.56
95% CI			(reference)		(0.7–1.6)	(0.5–1.4)	(0.3–2.0)	
Weighted mean estimates by the inverse of variance between Cohort I and II								
Multivariate RR2[b]			1.0		1.3	0.9	0.8	
95% CI			(reference)		(0.98–1.7)	(0.7–1.4)	(0.4–1.5)	0.95

[a] Adjusted for age (continuous) and Public Health Center areas.

[b] Adjusted for age (continuous), Public Health Center areas, smoking (never, past current), alcohol consumption (non-drinkers, 1–3 days/month, 1–149 g/week ethanol, 150–299 g/week, 300 or more g/week), miso soup intake (less than 1 cup/day, 1 cup/day, 2 cups/day, 3 or more cups/day), refraining from salty foods and animal fats.

1.0 (95% CI, 0.4–2.7) in Cohort II, compared with the 23 to 24.9 BMI group. These estimates remained almost unchanged after stratification by age or menopausal status at baseline (data not shown).

Next, RRs of overall and site-specific colorectal cancers were calculated together with both cohorts' data, separated by sex (Table 4). Overall RRs (95% CI) of colorectal cancer in both cohorts were 1.2 (0.98–1.5) for

Table 4. Relative risks (RR) and 95% confidence intervals (CI) of site-specific colorectal cancer for body mass index in both cohorts

	Body mass index (kg/m^2)				*p* for trend
	< 25	25–26.9	27–29.9	30+	
Men					
Person-years	329724	75395	40039	9423	
Colorectal cancer	438	113	58	17	
RR[a]	1.0	1.2	1.4	1.5	0.004
95% CI	(reference)	(0.98–1.5)	(1.04–1.8)	(0.9–2.5)	
Invasive colorectal cancer	285	74	44	15	
RR[a]	1.0	1.2	1.6	1.9	0.001
95% CI	(reference)	(0.9–1.6)	(1.1–2.2)	(1.05–3.4)	
Colon cancer	291	80	41	12	
RR[a]	1.0	1.3	1.5	1.4	0.003
95% CI	(reference)	(1.02–1.7)	(1.08–2.1)	(0.7–2.8)	
Invasive colon cancer	173	47	31	11	
RR[a]	1.0	1.3	1.9	2.2	< 0.001
95% CI	(reference)	(0.9–1.9)	(1.3–2.8)	(1.1–4.4)	
Proximal colon cancer	110	34	17	4	
RR[a]	1.0	1.7	1.8	1.8	0.003
95% CI	(reference)	(1.1–2.5)	(1.1–3.0)	(0.7–5.0)	
Invasive proximal colon cancer	73	17	12	4	
RR[a]	1.0	1.3	1.9	2.7	0.01
95% CI	(reference)	(0.8–2.2)	(1.02–3.6)	(0.99–7.6)	
Distal colon cancer	169	44	23	8	
RR[a]	1.0	1.2	1.4	1.3	0.13
95% CI	(reference)	(0.8–1.6)	(0.9–2.1)	(0.5–3.2)	
Invasive distal colon cancer	96	29	19	7	
RR[a]	1.0	1.3	2.0	1.8	0.006
95% CI	(reference)	(0.9–2.1)	(1.2–3.3)	(0.7–5.0)	
Rectal cancer	147	33	17	5	
RR[a]	1.0	1.0	1.2	1.6	0.40
95% CI	(reference)	(0.7–1.5)	(0.7–1.9)	(0.6–3.9)	
Invasive rectal cancer	112	27	13	4	
RR[a]	1.0	1.1	1.1	1.5	0.39
95% CI	(reference)	(0.7–1.7)	(0.6–2.0)	(0.5–4.1)	
Women					
Person-years	369533	77077	49503	16560	
Colorectal cancer	245	72	33	10	
RR[a]	1.0	1.3	0.9	0.8	0.94
95% CI	(reference)	(0.97–1.7)	(0.6–1.4)	(0.4–1.5)	
Colon cancer	155	48	21	5	
RR[a]	1.0	1.3	0.9	0.5	0.73
95% CI	(reference)	(0.9–1.8)	(0.6–1.4)	(0.2–1.4)	
Proximal colon cancer	79	21	10	2	
RR[a]	1.0	1.1	0.8	0.5	0.47
95% CI	(reference)	(0.7–1.8)	(0.4–1.6)	(0.1–2.1)	
Distal colon cancer	70	26	9	3	
RR[a]	1.0	1.6	0.9	0.6	0.87
95% CI	(reference)	(0.98–2.5)	(0.4–1.8)	(0.1–2.5)	
Rectal cancer	90	24	12	5	
RR[a]	1.0	1.2	1.0	1.3	0.56
95% CI	(reference)	(0.8–2.0)	(0.5–1.8)	(0.5–3.1)	

[a] Adjusted for age (continuous), Public Health Center areas, smoking (never, past current), alcohol consumption (non-drinkers, 1–3 days/month, 1–149 g/week ethanol, 150–299 g/week, 300 or more g/week), miso soup intake (less than 1 cup/day, 1 cup/day, 2 cups/day, 3 or more cups/day), refraining from salty foods and animal fats.

the 25 to 26.9 group, 1.4 (1.04–1.8) for the 27 to 29.9 group, and 1.5 (0.9–2.5) for the 30 or higher group (*p* for trend, 0.004). We also calculated RR for an integrated category of 27 to 29.9 and 30 or higher, because the number of events in the 30 or higher group was very small. This RR was 1.4 (95% CI, 1.1–1.8).

Proximal colon cancer was strongly associated with BMI categories: 1.7 (1.1–2.5) for the 25–26.9 group; 1.8 (1.1–3.0) for the 27–29.9 group; and 1.8 (0.7–5.0) for the 30 or higher group compared with the lower than 25 group (p for trend, 0.003). RR for 27 or higher BMI was similar to the 27–29.9 or 30 or more group (RR, 1.8; 95% CI, 1.1–2.9). In women, however, no association was detected in any site-specific colorectal cancers.

In addition, invasive-type-cancer analyses showed a clearer association with BMI in men (Table 4). RRs of invasive colorectal cancer had a clearer linear trend as follows: RR, 1.2 for 25–26.9, 1.6 for 27–29.9, and 1.9 for 30 or higher [1.6 (1.2–2.2) for 27 or higher; p for trend, 0.001], compared with RRs in non-invasive and invasive analysis (1.2, 1.4, and 1.5 for respective categories in Table 4). RRs trend did not differ between proximal and distal colon cancer in this invasive-type-cancer analysis. RR for 27 or higher was also similar between proximal (RR, 2.1; 95% CI, 1.2–3.6) and distal (RR, 1.9; 95% CI, 1.2–3.1) colon cancer. The results did not change in invasive-type-cancer analyses in women (data not shown).

We estimated the population-attributable fraction (PAF) at 3.3% for the 25–26.9 group, 2.6% for the 27–29.9 group, and 0.9% for the 30 or higher group compared with the lower than 25 BMI group. As a whole, 6.7% (95% CI, 1.6–12) of colorectal cancer and 8.9% (95% CI, 2.5–15) of invasive-type colorectal

Table 5. Relative risks (RR) and 95% confidence intervals (CI) of colorectal cancer for body height by each cohort in men and women

	Body height (cm)					p for trend
	Q1	Q2	Q3	Q4	Q5	
Men						
Range	< 160	160–162	163–165	166–169	170+	
Cohort I (1990–2001)						
Case	67	71	68	61	45	
Person-year	47117	44915	53565	37417	40898	
Multivariate-adjusted RR[a]	1.0	1.2	1.0	1.4	1.0	0.74
95% CI	(reference)	(0.9–1.7)	(0.7–1.4)	(0.95–2.0)	(0.6–1.5)	
Cohort II (1993–2001)						
Case	69	60	75	46	64	
Person-year	46726	43228	49880	40013	50823	
Multivariate-adjusted RR[a]	1.0	1.1	1.2	1.0	1.3	0.37
95% CI	(reference)	(0.7–1.5)	(0.9–1.8)	(0.6–1.5)	(0.8–1.9)	
Weighted mean estimates by the inverse of variance between Cohort I and II						
Case	136	131	143	107	109	
Person-year	93843	88143	103444	77430	91720	
Multivariate-adjusted RR[a]	1.0	1.1	1.1	1.2	1.1	0.39
95% CI	(reference)	(0.9–1.5)	(0.9–1.4)	(0.9–1.6)	(0.8–1.5)	
Women						
Range	< 148	148–150	151–153	154–156	157+	
Cohort I (1990–2001)						
Case	32	45	49	24	26	
Person-year	47978	64167	52043	40957	42814	
Multivariate-adjusted RR[a]	1.0	1.1	1.4	0.9	0.9	0.71
95% CI	(reference)	(0.7–1.7)	(0.9–2.2)	(0.5–1.6)	(0.5–1.6)	
Cohort II (1993–2001)						
Case	37	57	36	25	29	
Person-year	48088	60865	52201	47038	56523	
Multivariate-adjusted RR[a]	1.0	1.5	1.4	1.1	1.3	0.69
95% CI	(reference)	(0.96–2.3)	(0.8–2.2)	(0.6–1.9)	(0.7–2.2)	
Weighted mean estimates by the inverse of variance between Cohort I and II						
Case	69	102	85	49	55	
Person-year	96066	125031	104244	87995	99336	
Multivariate-adjusted RR[a]	1.0	1.3	1.4	1.0	1.1	0.98
95% CI	(reference)	(0.9–1.7)	(0.99–1.9)	(0.7–1.5)	(0.7–1.6)	

[a] Adjusted for age (continuous), Public Health Center areas, smoking (never, past, current), alcohol consumption (non-drinkers, 1–3 days/month, 1–149 g/week ethanol, 150–299 g/week, 300 or more g/week), body weight (quintiles).

cancer was attributable to 25 or higher BMI men compared with lower than 25 BMI men. With regard to subsite cancer, PAF estimates (95% CI) were 14% (3.7–23) for proximal colon cancer, 5.8% (−2.8 to 14) for distal colon cancer, and 2.4% (−6.7 to 11) for rectal cancer.

On the contrary, body height had no association with colorectal cancer in either sex of either cohort (Table 5). Site-specific colorectal cancer analyses also resulted in no association (data not shown). In addition, we calculated RRs for the highest decile category (173 cm or more in men and 159 cm or more in women) and the highest quartile category within the highest quintile category (175 cm or more in men, or 161 cm or more in women). However, these taller subjects were not associated with colorectal cancer either. Furthermore, we calculated stratified RRs by smoking, alcohol consumption, and body weight quintile category. No RR estimates by such analyses showed a significant association (data not shown).

Discussion

Colorectal cancer risk had a linear trend with increased BMI in the 25 or higher BMI men. Obese subjects (30 or higher BMI) showed the highest risk, although it was not statistically significant. These results were consistent with previous studies in both Western populations [2–4, 6–9, 12–15, 17] and Asian populations [25]. Although we hypothesized that even the 25–29.9 BMI subjects may be associated with a much elevated risk and may cause a rapid increase of colorectal cancer in the Japanese population, the RRs in such groups were not so high (1.2 for 25–26.9, 1.4 for 27–29.9). In other words, the relative effect of BMI was similar between Western and Asian populations. In addition, BMI's population-attributable fraction (PAF) was relatively small (6.7%) because of the small percentage of the 25 or higher BMI group. As a result, the PAF was too small to explain the increase in colorectal cancer incidence during recent decades.

The cancer risk similarly increased among proximal colon and distal colon. Proximal colon cancer, however, appeared to have a slightly stronger association with BMI than distal colon cancer. Many previous studies reported that BMI was strongly associated with distal colon cancer rather than proximal colon cancer [5, 8, 16, 17, 19]. A case-control study [47] revealed that high fat and protein intake were more closely associated with sigmoid colon cancer than ascending or transverse colon cancers. Thus, the association of BMI with site-specific colon cancer may differ among dietary lifestyles that lead to high BMI. Further studies may be needed in various populations with various dietary lifestyles, and in animal studies also, to clarify this site-specific association.

In addition, an association of BMI with colorectal cancer was clearer in only invasive-cancer cases rather than in overall colorectal-cancer cases including the non-invasive type. This result suggested that BMI may be associated with the promotion stage of carcinogenesis rather than the initiation stage [21, 26]. This hypothesis is also consistent with its stronger association with large adenoma rather than smaller adenoma [20–23, 26]. In other words, BMI may be related to cell proliferation and tumor growth in line with the insulin-like growth factor hypothesis [20]. High BMI as well as physical inactivity are hypothesized as factors that may lead to insulin resistance and high insulin and insulin-like growth factor concentration in blood, and then cause colorectal epithelial proliferation and carcinogenesis [3, 27]. Some epidemiologic evidence has been accumulated concerning an association between serum insulin or insulin-like growth factors and colorectal cancer by nested case-control studies [28].

In contrast, high BMI female subjects were not associated with colorectal cancer. Moreover, the association between BMI and colorectal cancer in women was inconsistent among previous studies [5, 6, 8, 10–17, 19, 24, 25]. Slattery *et al.* [14] hypothesized that estrogen-positive women (premenopausal women and postmenopausal women with hormone-replacement therapy) differed from estrogen-negative women (postmenopausal women without hormone-replacement therapy). Their result suggested that only estrogen-positive women were associated with an increased risk of colorectal cancer from being overweight or obese. Our further analyses stratified by age or menopausal status, however, did not support this hypothesis (data not shown). At least, women may have a weaker association than men in light of the present and previous studies.

The association of body height with colorectal cancer was inconsistent. Although previous cohort studies [4, 7, 9–11, 25] revealed this association, previous case-control studies failed to show any significant associations [15, 16, 18, 21]. Body height is hypothesized as a surrogate marker of a long large bowel [48] and a large number of colorectal epithelial cells. Such large numbers of cells carry a higher probability of carcinogenesis than smaller numbers of cells [49]. Another hypothesis is that a high caloric intake [49] and a high level of growth hormone in childhood may cause colorectal cell proliferation and carcinogenesis. Le Marchand *et al.* [50] reported that the genotype related to a low concentration of growth hormone in blood was inversely associated with colorectal cancer in a case-control study. However, our result suggested that body height was not associated

with colorectal cancer. Body height may have a threshold for colorectal carcinogenesis or a non-linear risk trend. Some studies [4, 9] reported a significant association of much greater body height, over 175 or 180 cm, with colon cancer in male populations. In these studies, the shortest height category ranged around 167 cm or less [4] or 68 in. (173 cm) or less [9], while in our study, even the highest category was only 170 cm or more in height. Therefore, body height may not be associated with colorectal cancer incidence unless it reaches 180 cm or more, though the proportion of men with a height of 180 cm or more was too small (0.5%) to examine this hypothesis in our study population.

The major strengths of our study include its prospective design, a general population with a high response rate (approximately 80%), and the relatively low proportion of subjects who moved away from the original study areas (9.6%) or were lost to follow-up (0.2%). Information on body height and body weight was collected before any subsequent diagnosis of colorectal cancer, thus avoiding the exposure recall bias inherent in case-control studies. The findings of this study can be generalized to middle-aged and elderly Japanese men and women because the study subjects were selected from the general population, and there was a high response rate. Moreover, the two cohorts starting at different times produced the same results. In addition, any confounding by factors measurable by our questionnaire was examined by the 10% change-in-estimate strategy and was excluded as thoroughly as possible. Although we used categorical variables of alcohol consumption and smoking as covariates to control confounding by these factors, our results did not substantially change when we used continuous variables of weekly ethanol intake and pack-years (number of cigarettes smoked per day divided by 20 and multiplied by years; data not shown in tables).

In conclusion, BMI increased the risk of colorectal cancer in men. Only invasive-cancer analysis suggested that BMI was important for tumor growth and proliferation. Proximal colon cancer appeared to have a slightly stronger association with BMI than distal colon cancer. Body height was not associated with colorectal cancer in men and women. From the risk estimates, 6.7% of colorectal cancer is attributable to a BMI of 25 or higher in middle-aged and elderly Japanese men.

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2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) level in human hair as biomarkers for dietary grilled/stir-fried meat and fish intake

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Abstract

Several case-control studies have reported possible associations between heterocyclic amine (HCA) intake and the risk of cancer. However, the validity of a questionnaire to assess HCA intake has hardly been examined. In particular, no biomarker which could serve as an independent measure of habitual HCA intake has been established. Therefore, the validity of a questionnaire to assess HCA intake by means of a biomarker remains to be investigated. In this study, we examined the availability of hair HCAs as a biochemical indicator of dietary intake of HCAs. Study subjects were 20 volunteers (7 men and 13 women) aged 25–57 years, either residents of Tokyo or the neighboring cities in Japan. We collected individual weighed dietary records (DR) over 28 consecutive days. Approximately 3–5 g of hair was collected twice from all subjects before and after DR at intervals of 1–3 months. The mean (S.D.) 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) level of hair was 1376.0 pg/g hair (928.9) and 16.6 ng/g melanin (12.3). A steady increase in the mean PhIP level in hair from the lowest to the highest tertile of the grilled/stir-fried meat intake was observed ($P=0.009$), but not in the grilled/stir-fried fish intake ($P=0.461$). The PhIP level in hair was highly correlated with the grilled/stir-fried meat intake ($r=0.68$) but not with the grilled/stir-fried fish intake ($r=0.28$). These observations were made of hair with and without melanin adjustment. The present study indicates that the PhIP level in hair can be used as a biological indicator of dietary intake of HCAs.

Keywords: Heterocyclic amines; Biomarker; Dietary record; Human hair

1. Introduction

Although some heterocyclic amines (HCAs) are known to be genotoxic in mammalian cell lines [1,2] and carcinogenic in experimental animals [3–5], the epidemiologic evidence for an etiologic role of HCAs in carcinogenesis is inconsistent [6–10]. One of the prime reasons for this inconsistency is the difficulty of assessing human exposure to HCAs. Because the concentrations of HCAs depend on the cooking method or doneness level of meat or fish, the development of a complete and standardized database of the concentrations of HCAs is difficult, and the estimation of dietary

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HCAs from a questionnaire is likely to be misclassified. Although a database for HCA content has recently been developed for 297 food items [11], these limited food items may present difficulties in estimating the dietary HCAs for different study populations. Thus, measuring HCAs in biological samples offers an attractive alternative approach to estimating human exposure to these substances. Several studies have used human urine levels as an indicator of HCA intake [12,13]. However, urinary metabolites will not provide an accurate assessment of usual HCA intake, because the half-life of HCA metabolite is not enough to accurately assess them in a regular diet [14,15]. Although HCAs in hair samples are reportedly available to determine the HCA contents in regular diet [16], they have been so far compared only with the frequency of meat intake and not quantitative dietary intake.

We have previously reported the ability of an analytical method to detect the 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) level in human hair [17]. It has long been known that PhIP interacts with melanin [18]. Besides, there is a report indicating the role of eumelanin for PhIP uptake into hair, and the PhIP content in the hair based on melanin content tends to negate the effect of melanin content variation on the concentration of PhIP in the hair [14]. Further, we reported a positive correlation between PhIP levels in hair and melanin content in hair in the previous study [17]. Then, in the present study, we examined the availability of PhIP in hair as an indicator of dietary intake of HCAs. The PhIP levels in hair or PhIP levels per melanin content were compared with grilled/stir-fried meat or fish intake from dietary records over 28 days.

2. Materials and methods

2.1. Study population

Study subjects were 20 healthy volunteers (7 men and 13 women) aged 25–57 years, non-smokers, and residents of Tokyo or the neighboring cities in Japan. All subjects gave their informed consent to participate in this study. Mean (S.D.) age was 38.1 (8.6) years and mean (S.D.) body mass index was 21.8 (2.6). We collected individual weighed dietary records (DR) over 28 consecutive days. Approximately 3–5 g of hair was collected twice from all subjects before and after DR at intervals of 1–3 months. Ten (50%) subjects had dyed hair.

2.2. Analysis of hair HCAs

Details of the analysis have been reported elsewhere [17]. Briefly, 3 g of hair was washed in 0.1% SDS (100 ml) by ultrasonication for 5 min, after which the liquid was decanted, and hair was then washed four times with water (100 ml), once with 100% ethanol (50 ml), and dried at room temperature [19]. The dried hair sample was weighed, and 1N NaOH (100 ml) and 10 ng of internal standard (PhIP-d_3) were added. This solution was incubated at 100 °C for 45 min in a capped container (225 ml graduated conical tube with polypropylene cup, Falcon, NJ, USA), and the tube was capped loosely. After centrifugation at 3100 rpm for 10 min, the supernatant was filtrated (5B filter, ADVAN-TEC, Tokyo, Japan), and then neutralized to pH 7–9 with 6N HCl. PhIP in this filtrate was extracted using a Blue-Chitin column absorption method [20]. Briefly, HCAs absorbed on a Blue-Chitin column were eluted with 20 ml of MeOH–28% NH_3 (50:1) (flow rate, 5 ml/min). The eluate was concentrated under vacuum at room temperature using a centrifugal concentrator CC-105 with low temperature trap TV-105 (TOMY, Tokyo, Japan). The residue was then dissolved in 1 ml of 100% MeOH. After centrifugation of 3100 rpm for 10 min, the supernatant was collected, and then concentrated under vacuum. The residue was dissolved in 2 ml of 0.1N HCl. After washing with 2 ml of *n*-hexane, the aqueous layer was adjusted to pH > 10 with 28% NH_3, and then extracted twice with 2 ml of dichloromethane. The organic layer was concentrated under vacuum. The residue was dissolved in 500 μl of 40 μM ammonium acetate: 100% MeOH (1:1, v/v), and then filtrated with 0.45 μM filter (Ultrafree-MC, Millipore, Bedford, MA, USA).

The column-switching liquid chromatography–mass spectrometry (LC–MS) system was LCMS-2010A coupled with a column switching system (Co-sense for BA system, Shimadzu, Kyoto, Japan). A SIL-10APvp automatic injector equipped with a contamination control kit (Shimadzu, Kyoto, Japan) was used. Three hundred microliters of the sample was injected by an auto-sampler, and was loaded onto the extraction column (Shim-pack MAYI-ODS column, 2.0 mm × 10 mm, Shimadzu, Kyoto, Japan) by 10 mM ammonium acetate at a rate of 2.0 ml/min for 4 min. Then, the valve was switched. The analyte was introduced into the analytical column (Mercury MS LUNA 3 μm C18 column, 2.0 mm × 20 mm, Penomenex, Torrance, CA) at a rate of 0.2 ml/min. Then 40 μM ammonium acetate (pH 4.0, A) and methanol (B) were used as an analytical mobile phase. The gradient program was as follows: 13% B (0–5 min)–45% B (5.01 min)–45% B (5.01–13 min)–13% B (13.01 min). The column oven was maintained at 40 °C. The mass spectrometry conditions for electrospray ionization (ESI)-MS were as follows: drying nitrogen gas temperature was set at 250 °C and introduced into the capillary at a flow rate of 4.5 l/min; the capillary was held at a potential of 4.5 kV for the positive ion mode. In selected ion monitoring (SIM) mode, ions at *m/z* 225 were assigned to $[M+H]^+$ of PhIP.

Hair sample (1 mg) and sepia melanin (1 mg) were dissolved in 1 ml of a mixture of Soluene-350 and water (9:1, v/v), followed by heating at 95 °C for 45 min. Optical density was observed at 500 and 650 nm (A_{500} and A_{650}) in Jasco V-550 (Japan Spectroscopic, Tokyo, Japan). A_{500} indicates the quantity of total melanin, and the ratio A_{650}/A_{500} equals the ratio of eumelanin to total melanin in the hair sample. Total melanin

concentrations of hair samples were calculated according to a previous method [21].

2.3. Meat and fish intakes assessed by DR

Weighed DR were collected over 28 consecutive days. The subjects were asked to provide detailed descriptions of meat (beef, pork, chicken, and processed meat) and fish (any fish in fresh, dried, and processed) including the method of preparation (grilled, stir-fried, deep fried, boiled, fresh, and others) and intake of burnt portion. The subjects reported doneness levels (charred, well-done, medium well-done, medium, medium rare, rare) for grilled fish or meat. Grilled or stir-fried fish and meat intake were calculated as a surrogate indicator for HCA intake, because the database of HCA content is not sufficient to estimate HCA intake. We did not include processed fish in total fish, because the burnt portion of grilled or stir-fried processed fish which was made from minced flesh, egg, starch, and other seasonings was not the burnt portion of fish.

2.4. Data analysis

The statistical analyses were performed using SAS (version 9.1; SAS Institute, Inc., Cary, NC). The mean PhIP level in hair which was collected twice was computed and expressed as the crude level (pg/g hair) and level per melanin content (ng/g melanin). To assess whether PhIP in hair can serve as a valid biomarker in epidemiological studies, subjects were categorized into three groups according to tertiles of grilled and stir-fried fish and meat intake from DR. Mean PhIP in hair in different tertile of DR value was computed. Tests for trends of PhIP in hair were conducted using the mantel extension test. Spearman rank correlations were used to assess the degree of association between PhIP in hair and grilled or stir-fried fish and meat intake from DR. Association between PhIP in hair and intake of the burnt portion of fish, and the frequency of the preferable doneness level when the meat was grilled or stir-fried were also examined.

3. Results

The mean (S.D.) PhIP level in hair was 1376.0 pg/g hair (928.9) and 16.6 ng/g melanin (12.3).

Table 1 shows meat and fish intake of grilled/stir-fried meat and fish intake assessed with DR of the study population. Daily meat intake was 86.7 g and daily grilled/stir-fried meat intake was 47.7 g. Grilled/stir-fried beef and pork intake was almost the same or greater than those of grilled/stir-fried chicken and processed meat. Daily fish intake was 48.4 g and daily grilled/stir-fried fish intake was 21.8 g. Grilled/stir-fried and dried fish intake was far less than grilled/stir-fried raw fish intake. Frequency of burnt portion in fish intake was 6.2 (times/month) and well-done meat intake was 7.5 (times/month).

Table 2 shows the mean PhIP level in hair according to the tertiles of the grilled/stir-fried meat or fish intake assessed with DR. A steady increase was observed in the mean PhIP level in hair from the lowest to the highest tertile of the pork ($P=0.035$), chicken ($P=0.020$), total meat ($P=0.009$), and total meat and fish intake ($P=0.004$), but not for grilled/stir-fried fish intake ($P=0.461$). These observations were found with or without hair adjustment with melanin. Table 2 also presents the correlation between the PhIP level in hair and grilled/stir-fried meat and/or fish intake assessed

Table 1
Meat and fish intake assessed with DR[a] of the study population ($n=20$)

	Mean ± S.D.	Percentile		
		25th	50th	75th
Meat and fish intake assessed with DR				
Total meat (g/day)	86.7 ± 40.1	53.6	69.1	113.6
Grilled/stir-fried meat (g/day)				
Total meat	47.7 ± 20.3	33.3	46.0	56.2
Beef	16.9 ± 11.2	10.2	15.2	18.7
Pork	17.7 ± 9.3	11.7	17.9	22.4
Chicken	9.7 ± 8.3	2.9	7.0	14.1
Processed meat	3.4 ± 3.0	0.8	2.5	5.4
Well-done meat (times/month)	7.5 ± 8.9	1.0	5.0	9.0
Total fish (g/day)	48.4 ± 23.2	31.6	40.5	60.3
Grilled/stir-fried fish (g/day)				
Total fish	21.8 ± 12.3	11.2	20.1	28.2
Raw fish	20.1 ± 11.4	11.2	17.5	26.5
Dried fish	1.7 ± 2.7	0.0	0.0	2.7
Burned portion of fish (times/month)	6.2 ± 4.7	2.5	4.5	11.0

[a] Dietary records.

Table 2
PhIP[a] level in hair according to tertiles of grilled/stir-fried meat and fish intake assessed with DR[b] and their correlation ($n = 20$)

	Crude levels (pg/g hair)			Levels per melanin content (ng/g melanin)		
	Mean (95% CI)	Spearman correlation		Mean (95% CI)	Spearman correlation	
		r	P		r	P
Beef		0.34	0.137		0.48	0.031
Lowest	830.7 (53.5, 1607.9)			8.6 (0, 18.4)		
Second	1626.9 (907.4, 2346.4)			16.8 (7.7, 25.9)		
Highest	1592.3 (872.8, 2311.9)			23.1 (14.1, 32.2)		
P for trend[c]	0.152			0.034		
Pork		0.46	0.039		0.39	0.091
Lowest	891.1 (158.0, 1624.2)			10.7 (0, 21.3)		
Second	1200.0 (521.2, 1878.7)			19.0 (9.1, 28.9)		
Highest	1967.5 (1288.8, 2646.3)			19.1 (9.3, 29.0)		
P for trend[c]	0.035			0.229		
Chicken		0.53	0.017		0.52	0.019
Lowest	960.8 (286.6, 1634.9)			11.6 (3.1, 20.0)		
Second	1062.8 (479.0, 1646.6)			11.2 (3.9, 18.5)		
Highest	2208.7 (1534.6, 2882.8)			28.7 (20.2, 37.1)		
P for trend[c]	0.020			0.017		
Processed meat		0.15	0.536		0.13	0.580
Lowest	1694.7 (884.5, 2504.9)			17.7 (7.0, 28.3)		
Second	1053.9 (303.8, 1804.0)			11.6 (1.7, 21.4)		
Highest	1424.8 (674.7, 2174.8)			20.6 (10.7, 30.4)		
P for trend[c]	0.638			0.629		
Total meat		0.68	0.001		0.69	0.001
Lowest	721.7 (44.7, 1398.6)			8.9 (0.2, 17.7)		
Second	1257.9 (631.2, 1884.7)			13.1 (5.0, 21.2)		
Highest	2054.8 (1428.1, 2681.5)			26.6 (18.5, 34.7)		
P for trend[c]	0.009			0.009		
Raw fish		0.20	0.391		0.18	0.448
Lowest	1355.3 (510.1, 2200.4)			13.7 (2.7, 24.6)		
Second	1345.8 (563.3, 2128.2)			15.4 (5.2, 25.5)		
Highest	1423.8 (641.4, 2206.3)			20.2 (10.1, 30.3)		
P for trend[c]	0.891			0.333		
Dried fish		0.09	0.709		−0.03	0.893
Lowest	1428.7 (840.6, 2016.8)			19.2 (11.3, 27.1)		
Second	476.0 (0, 1855.2)			7.5 (0, 26.1)		
Highest	1550.1 (812.9, 2287.4)			14.9 (5.0, 24.8)		
P for trend[c]	0.868			0.425		
Total fish		0.28	0.230		0.23	0.330
Lowest	1355.3 (548.6, 2161.9)			13.7 (3.3, 24.1)		
Second	1059.7 (312.9, 1806.5)			12.8 (3.2, 22.4)		
Highest	1709.9 (963.1, 2456.7)			22.8 (13.2, 32.4)		
P for trend[c]	0.461			0.170		
Total meat and fish		0.66	0.001		0.65	0.002
Lowest	615.1 (0, 1254.2)			8.1 (0, 17.0)		
Second	1317.3 (725.6, 1909.0)			14.4 (6.1, 22.6)		
Highest	2086.7 (1495.1, 2678.4)			26.0 (17.8, 34.2)		
P for trend[c]	0.004			0.008		

[a] 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.
[b] Dietary records.
[c] Mantel extension test for difference.

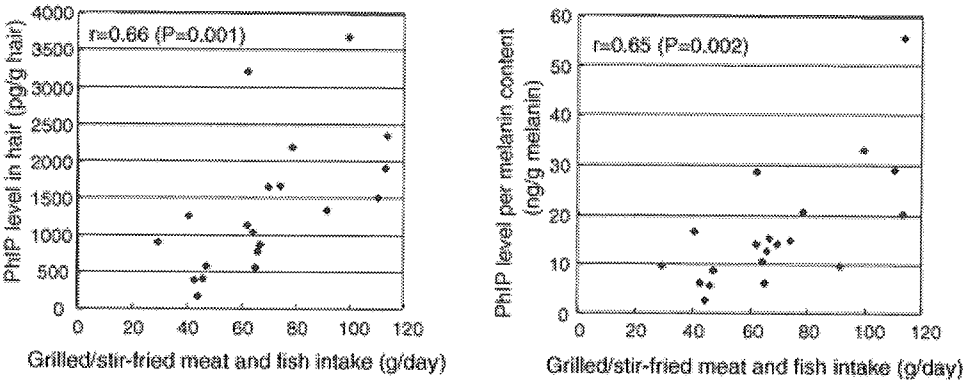

Fig. 1. Scatter plot of PhIP level in hair (crude levels or levels per melanin content) vs. dietary grilled/stir-fried meat and fish intake ($n = 20$).

with DR. As seen in Fig. 1, the PhIP level in hair was highly correlated with intakes of the amount of the grilled/stir-fried meat and fish together ($r = 0.66$). This observation also applies to the PhIP level per melanin content ($r = 0.65$). The PhIP level in hair and the PhIP level per melanin content were also highly correlated with the amount of grilled/stir-fried total meat intake ($r = 0.68$ and 0.69, respectively) but was not correlated with the amount of grilled/stir-fried fish intake ($r = 0.28$ and 0.23, respectively).

The PhIP level in hair and frequency of the burnt portion in fish intake were not correlated ($r = 0.02$). The PhIP level in hair and frequency of preferable doneness level when the meat was grilled or stir-fried were also not correlated ($r = 0.02$).

4. Discussion

The present study is the first to examine the availability of HCAs in hair as a biochemical indicator of dietary intake of HCAs using the grilled/stir-fried meat or fish intake from 28 days DR. Although the PhIP level in hair did not correspond to the amount of grilled/stir-fried fish intake by DR, it corresponded to the amount of grilled/stir-fried meat or grilled/stir-fried meat and fish combined.

In epidemiological studies, biochemical indicators of dietary HCA intake have great roles as an independent measure of HCA intake, which is independent of dietary assessment error and could account for the variability due to foods, cooking method or doneness level. In previous studies, human urine has been investigated as an indicator of HCA intake [12,13]. However, the half-life of the HCA metabolites is <12 h and reflects recent dietary intake [14,15]. The urinary metabolites will therefore not provide an accurate assessment of usual HCA intake. Hair has often been used for tests of drug abuse, because drugs in hair have a long half-life compared with those in urine or blood [22–24]. HCAs in hair samples are reportedly available to determine the HCA contents in regular diet [16]. However, in Reistad's report, HCAs in hair samples were compared with frequency of meat intake and not with quantitative dietary intake.

Foods which contribute to HCA intake differ with the generation or area in Japan. Because subjects in this study were relatively young and lived in the suburbs of Tokyo, they might have consumed more meat and less fish than the general population. Daily meat and fish intake in these subjects were thus compared with those of subjects in a large-scale population-based prospective study in Japan (JPHC study) [25,26]. For instance, daily meat intake was 86.7 g and daily grilled/stir-fried meat intake was 47.7 g in the present study, against daily meat intake of 58.3 g and daily grilled/stir-fried meat intake of 31.4 g in the JPHC study. Daily fish intake was 48.4 g and daily grilled/stir-fried fish intake was 21.8 g in the present study, against daily fish intake of 83.2 g and daily grilled/stir-fried fish intake of 54.7 g in the JPHC study. These low grilled/stir-fried fish intakes inevitably lead to the low HCA intake. Besides, it has been reported that HCA composition differs in terms of the type of fish, and that the HCA level in fish skin is higher than in fish flesh [26]. As possible reasons why the amount of grilled/stir-fried meat intake significantly correlated with the PhIP level in hair whereas the grilled/stir-fried fish intake did not, there was little high grilled/stir-fried meat intake and low grilled/stir-fried fish intake, and especially little dried fish intake in which the cooking method was almost always grilling. It was also possible that subjects were relatively health-conscious and tended not to eat grilled fish skin. However, an association between the PhIP level in hair and combined grilled/stir-fried meat and fish was observed in the present study. It is highly