同前方視的無作為化比較試験を行い，IVIG+PSLが冠動脈病変の抑制，有熱時間の短縮，CRPの早期陰性化に有用であることを報告した[1]．そこで無作為化比較試験に参加した178症例にリスクスコアを適用し，川崎病初期治療の層別化が可能か否かをシミュレーションしてみた．

IVIGのうち29.5%の26症例，IVIG+PSLのうち32.2%の29症例が高リスク患者に分類された．リスク別の転帰を表2に示す．低リスク患者においては治療抵抗例の割合，冠動脈病変の合併頻度いずれにおいても両治療群間で統計学的な有意差を認めなかった．冠動脈病変はIVIGの1例のみに認めたが，1カ月時には正常化した．一方，高リスク患者においてはIVIGに比べIVIG+PSLでは治療抵抗例，ことに初期治療不応例が有意に低頻度であった．また，経過中の冠動脈病変合併頻度もIVIG+PSLで有意に低頻度であり，1カ月時の冠動脈病変合併頻度も低い傾向であった．治療開始後解熱するまでの期間は低リスク患者，高リスク患者ともIVIG+PSLで有意に短縮していた．一方，治療開始後CRP陰性化するまでの日数は低リスク患者ではIVIGとIVIG+PSLで統計学的な有意差を示さなかったのに対し，高リスク患者ではIVIG+PSLが有意に短縮していた．これらの検討の結果，低リスク患者においてはIVIG+PSLによってより早期の解熱効果は得られるものの治療抵抗例や冠動脈病変合併例の頻度低下，CRP陰性化するまでの期間の短縮といった臨床効果が乏しい一方，高リスク群においてはIVIG+PSLは治療抵抗例や冠動脈病変合併例の頻度を有意に減らし，発熱期間，CRP陰性化までの期間が有意に短縮するといった効果があった．これらの結果から治療開始前にリスクスコアを用いて重症度を層別化し，初期治療を変更することによって重症川崎病患者の臨床経過，冠動脈予後を改善させることができる可能性が示された．

おわりに

IVIG無効例に関連する因子と初期治療層別化の可能性について解説した．今後の川崎病初期治療の方向性として，治療を開始する前に重症度に応じた初期治療を選択し，より早い段階で川崎病血管炎を鎮静化させ，冠動脈瘤の発生を少なくするといった治療戦略が必要となるであろう．リスクスコアとIVIG+PSLはその候補としてあげられる．これらを証明するために大規模な多施設共同前方視的無作為化比較試験を行う必要があり，現在重症川崎病患者に対するIVIG+PSLの有用性を検討する臨床研究を計画中である．

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文献

1) Burns JC, Glodé MP: Kawasaki syndrome. Lancet 364: 533-544, 2004.
2) Newburger JW, et al: A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. N Engl J Med 324: 1633-1639, 1991.
3) Burns JC, et al: Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. US/Canadian Kawasaki Syndrome Study Group. Pediatr Infect Dis J 17: 1144-1148, 1998.
4) Kobayashi T, et al: Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. Circulation 113: 2606-2612, 2006.
5) Harada K: Intravenous gamma-globulin treatment in Kawasaki disease. Acta Paediatr Jpn 33: 805-810, 1991.
6) Muta H, et al: Serum sodium levels in patients with Kawasaki disease. Pediatr Cardiol 26: 404-

407, 2005.
7) Nomura Y, et al: Patients diagnosed with Kawasaki disease before the fifth day of illness have a higher risk of coronary artery aneurysm. Pediatr Int 44: 353-357, 2002.
8) Nakamura Y, et al: Use of laboratory data to identify risk factors of giant coronary aneurysms due to Kawasaki disease. Pediatr Int 46: 33-38, 2004.
9) Nakamura Y, et al: Cardiac sequelae of Kawasaki disease in Japan: statistical analysis. Pediatrics 88: 1144-1147, 1991.
10) Muta H, et al: Early intravenous gamma-globulin treatment for Kawasaki disease: the nationwide surveys in Japan. J Pediatr 144: 496-499, 2004.
11) Inoue Y, et al: A multicenter prospective randomized trial of corticosteroids in primary therapy for Kawasaki disease: clinical course and coronary artery outcome. J Pediatr 149: 336-341, 2006.
12) Wooditch AC, Aronoff SC: Effect of initial corticosteroid therapy on coronary artery aneurysm formation in Kawasaki disease: a meta-analysis of 362 children. Pediatrics 116: 989-995, 2005.
13) Raman V, et al: Response of refractory Kawasaki disease to pulse steroid and cyclosporin A therapy. Pediatr Infect Dis J 20: 635-637, 2001.
14) Burns JC, et al: Infliximab treatment for refractory Kawasaki syndrome. J Pediatr 146: 662-667, 2005.

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Plasma vitamin D and risk of colorectal cancer: the Japan Public Health Center-Based Prospective Study

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We investigated the association between plasma 25(OH)D and the subsequent colorectal cancer incidence risk by a nested case–control study in The Japan Public Health Center-based Prospective Study, covering 375 newly diagnosed cases of colorectal cancer from 38 373 study subjects during a 11.5-year follow-up after blood collection. Two controls were matched per case on sex, age, study area, date of blood draw, and fasting time. In a conditional logistic regression model with matched pairs adjusted for smoking, alcohol consumption, body mass index, physical exercise, vitamin supplement use, and family history of colorectal cancer, plasma 25(OH)D was not significantly associated with colorectal cancer in men or in women. However, the lowest category of plasma 25(OH)D was associated with an elevated risk of rectal cancer in both men (odds ratio (OR), 4.6; 95% confidence interval (CI), 1.0–20) and women (OR, 2.7, 95% CI, 0.94–7.6), compared with the combined category of the other quartiles. Our results suggest that a low level of plasma 25(OH)D may increase the risk of rectal cancer.
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Ecologic studies have reported that sunlight or solar ultraviolet B exposure is inversely associated with the risk of colorectal cancer incidence and mortality in the United States (Grant and Garland, 2004; Giovannucci, 2005) and in Japan (Mizoue, 2004). This ultraviolet B is involved in the production of vitamin D from 7-dehydrocholesterol in the skin (Holick, 2004). Vitamin D, which is derived from skin and dietary products or supplemental sources, is catalysed to 25-hydroxyvitamin D in the liver, which is the most useful measure of vitamin D status (Hunter, 1998). The 25-hydroxyvitamin D re-enters the circulation and is converted in the kidney by 25-hydroxyvitamin D-1α-hydroxylate to 1,25-dihydroxy-vitamin D, which regulates calcium metabolism through its interaction with its major target tissues, bone and intestine; 25-hydroxyvitamin D is also metabolised in colorectal mucosa for regulation of cellular growth.

Several prospective studies reported that 25-hydroxyvitamin D in the blood was inversely associated with colorectal cancer risk (Garland *et al*, 1989; Braun *et al*, 1995; Wactawski-Wende *et al*, 2006), and especially distal colon and rectal cancer (Tangrea *et al*, 1997; Feskanich *et al*, 2004). One cohort was used to analyse a small number of cases (Garland *et al*, 1989; Braun *et al*, 1995), while others were from specific populations such as Finnish male smokers (Tangrea *et al*, 1997) and US nurses (Feskanich *et al*, 2004). Further confirmation is needed from general populations, especially with a different sunlight exposure and skin pigmentation reducing the cutaneous synthesis of vitamin D (Clemens *et al*, 1982; Matsuoka *et al*, 1991).

We investigated the association between plasma 25-hydroxy-vitamin D and the subsequent risk of colorectal cancer in a nested case-control study in a large general population cohort in Japan.

MATERIALS AND METHODS

The Japan Public Health Centre-based Prospective Study (JPHC study) is an ongoing cohort study investigating cancer, cardiovascular disease, and other lifestyle-related diseases. The first group (Cohort I) of the JPHC study started in 1990 and the second group (Cohort II) in 1993 (Watanabe *et al*, 2001). Study subjects were mainly residents living in several municipalities in each area administered by a Public Health Center, aged 40–59 years for Cohort I and 40–69 years for Cohort II. Two more subcohorts of health check-up examinees and random samples from one city aged 40–69 were added to Cohort II. The study subjects were identified by the population registry in each municipality. We studied a cohort of 65 803 men and 67 520 women. Our study was approved by the institutional review board of the National Cancer Centre, Tokyo, Japan.

Using a self-administered questionnaire, study subjects were asked to provide information about their personal and familial medical histories, smoking, alcohol consumption, frequency of physical exercise, dietary habits, and other lifestyle factors. Their dietary habits were assessed by a food-frequency questionnaire of

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44 items for Cohort I (Tsubono *et al*, 2003) and 52 items for Cohort II. A total of 50 456 men (77%) and 55 909 women (83%) filled out and returned the questionnaire. Among the study subjects, 15 258 men (23%) and 26 703 women (40%) donated 10-ml of venous blood, drawn into vacutainer tubes containing heparin, collected at the time of their health check-ups (1990–1992 for Cohort I, and 1993–1995 for Cohort II), and divided into plasma and buffy layers, and then preserved at −80°C until analysis.

Follow-up

We followed study subjects until 31 December, 2003, obtaining mortality details from the Ministry of Health, Labour and Welfare as necessary. Subjects moving to other municipalities were identified annually through residential registries in their Public Health Center areas; 9.9% moved away, and 0.2% were lost to follow-up.

Incidence data on colorectal cancer were collected for the JPHC cancer registry through local major hospitals, and population-based cancer registries. Indicators of the completeness of colorectal cancer case ascertainment conformed to the international standard (Parkin *et al*, 2002) as follows: 5.5% of incident cases were notified by death certificates (Death Certificate Notification, DCN); 2.2% did not have detailed information except death certificates (Death Certificate Only, DCO); and 94.7% were verified by histological examination (Histological Verification, HV). We identified 375 cases (196 men and 179 women) of colorectal cancer up to 31 December, 2003 from among the 38 373 subjects (14 004 men and 24 369 women) who had returned the baseline questionnaire, did not report diagnosis of any cancer, and provided the blood samples. All 375 cases were pathologically confirmed as adenocarcinoma, after excluding 18 cases of unknown pathology and seven non-adenocarcinoma cases. Of these, 256 subjects had cancer of the colon (International Classification of Diseases for Oncology, Third edition (ICD-O-3) (World Health Organisation, 2000) code C180–C189) and 119 had cancer of the rectum (ICD-O-3 code C199 and C209). Colon cancers were classified into proximal (ICD-O-3 code C180–C185) or distal colon (ICD-O-3 code C186 and C187). Information on tumour depth was available in 370 of the 375 cases, with 120 tumors of the intramucosal type corresponding to Tis in TNM classification (International Union Against Cancer, 1997) and 250 of the invasive type corresponding to T1 or more.

For each case, two controls were selected, using incidence density sampling (Clayton and Hills, 1993), matched on sex, age (within 3 years), date of blood draw (within 3 months), time since last meal (within 4 h), and study location (each Public Health Centre area) from subjects who had no history of colorectal cancer when the case was diagnosed.

Plasma 25-hydroxyvitamin D concentrations were measured by the competitive protein-binding assay of Haddad and Chyu (1971). A modified method using Gc-globulin (Sigma-Aldrich Co., St Louis, MO, USA) instead of a tissue extract was adopted. All samples were assayed by one commercial laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo, Japan). Samples from matched sets were assayed together. All laboratory personnel were blinded with respect to case or control status. The intra-assay coefficient of variation from the quality control samples was 8.4% ($n=9$).

Statistical analysis

Adjusted means for cases and controls were calculated using least square means in analysis of covariance by the PROC GLM procedure in SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA). Percentages of baseline characteristics were unadjusted crude values. We used the extensions of the Mantel–Haenszel procedure (Mantel, 1963) with matched pairs for a comparison of the baseline characteristics and plasma 25-hydroxyvitamin D between cases and controls, using the PROC FREQ procedure with CMH option. We tested the linear trend of covariates among controls by quartiles of plasma 25-hydroxyvitamin D, also using the extensions of the Mantel–Haenszel procedure (Mantel, 1963). The odds ratios (OR) and 95% confidence intervals (CI) for plasma 25-hydroxyvitamin D divided into quartiles based on control distribution were calculated by a conditional logistic regression model adjusted for pack-years of smoking (continuous), alcohol consumption (continuous), body mass index (continuous), physical exercise (less than once a week, or once a week or more), vitamin supplement use (any vitamin supplements, i.e., B-vitamins, vitamin C, E, A, multivitamins, or other), and family history of colorectal cancer as well as using matched pairs. The linear trend of OR was tested using the logarithmic-transformed median value of plasma 25-hydroxyvitamin D in the category, since the plasma value was log-normally distributed. The heterogeneity over quartiles of plasma 25-hydroxyvitamin D levels was tested by the Wald χ^2 statistic. *P*-values for the trend were two-sided, with 0.05 as the significance level. We estimated the ORs for colorectal cancer as a whole, and also colon and rectal cancers separately with assessment of difference between these two cancers using a test for heterogeneity (Greenland, 1998). To examine whether the plasma 25-hydroxyvitamin D levels affected the risk of colorectal cancer differently between periods of high (July to November) and low (December to June) blood levels, we conducted a seasonally stratified analysis of blood drawn. Additionally, we tried to estimate the risk for hypovitaminosis D (15 ng ml^{-1} (37.5 nmol l^{-1})) (Nesby-O'Dell *et al*, 2002).

RESULTS

Colorectal cancer cases in men smoked more, consumed more alcohol beverages, and had a higher body mass index than their controls, but also consumed less dietary fiber than controls (Table 1). Female cases consumed more diet-origin vitamin D than their controls.

No potential confounding factors correlated with plasma 25-hydroxyvitamin D among controls (Table 2). Female controls in the lowest quartile of plasma 25-hydroxyvitamin D consumed more alcoholic beverages than other quartiles. Food and nutrient intakes were not associated with plasma 25-hydroxyvitamin D except *n*-3 polyunsaturated fatty acid intake in women, which is contained in fish, a major source of dietary vitamin D in the Japanese population (Nakamura *et al*, 2002). Dietary vitamin D did not correlate with plasma 25-hydroxyvitamin D in either men or women.

Plasma 25-hydroxyvitamin D was lower in rectal cancer cases than their controls (Table 3). Median plasma levels were 24.3 ng ml^{-1} in men and 26.6 ng ml^{-1} in their controls ($P=0.0051$); 20.6 ng ml^{-1} in women and 22.6 ng ml^{-1} in their controls ($P=0.093$). There was no difference between colon cancer cases and their controls.

Plasma 25-hydroxyvitamin D was not associated with the risk of colorectal cancer in men or women, although there was a suggestion of an inverse relationship in men (Table 4), with ORs (95% CI) of 0.76 (0.42–1.4) for the second, 0.76 (0.39–1.5) for the third, and 0.73 (0.35–1.5) for the highest quartile, compared to the lowest quartile (*P* for trend, 0.39). It appeared that only the lowest quartile had a somewhat higher risk than other quartiles for rectal cancer risk, although again the trend test was not significant. The ORs were 1.0 for the lowest, 0.17 for the second, 0.25 for the third, and 0.075 for the highest quartile for men (*P* for trend, 0.06); colon cancer did not show a similar association. In women, a similar association was observed for rectal cancer risk, with ORs of 0.26, 0.46, and 0.33 for the second, third and highest quartiles respectively (*P* for trend, 0.17). Heterogeneity tests between colon

Table 1 Baseline characteristics of cases and controls

Characteristics	Men			Women		
	Cases	Controls	P	Cases	Controls	P
n	196	392		179	358	
Age (years), mean	56.9	56.9	0.69	56.5	56.4	0.35
Smoking (pack–years), mean[a]	27.5	23.8	0.020	0.458	0.657	0.54
Alcohol consumption (g week^{-1} ethanol), mean[a]	236	175	<0.0010	9.63	5.70	0.37
Body mass index (kg m^{-2}), mean[a]	23.8	23.2	0.027	23.5	23.6	0.68
Physical exercise, *n* (%)[b]	49 (26)	75 (20)	0.12	33 (19)	54 (15)	0.28
Vitamin supplement use, *n* (%)	35 (20)	53 (15)	0.11	24 (15)	54 (17)	0.43
Family history of colorectal cancer, *n* (%)	5 (2.6)	4 (1.0)	0.16	4 (2.2)	4 (1.1)	0.32
Total energy intake (kcal day^{-1}), mean[c]	2021	2064	0.34	1277	1265	0.67
Dietary fibre intake (g day^{-1}), mean[d]	7.76	8.05	0.047	7.83	7.58	0.16
Folate intake (μg day^{-1}), mean[d]	328	329	0.60	298	291	0.31
Calcium intake (mg day^{-1}), mean[d]	441	463	0.15	452	423	0.074
Vitamin D intake (μg day^{-1}), mean[d]	6.58	6.67	0.54	5.57	5.16	0.047
n-3 fatty-acid intake (μg d^{-1}), mean[d]	1.32	1.38	0.28	1.19	1.13	0.060
Red meat intake (g d^{-1}), mean[d]	17.0	17.2	0.70	13.8	12.7	0.17
Fish intake (g d^{-1}), mean[d]	62.2	62.0	0.88	46.7	43.6	0.15

[a]Adjusted for age. [b]Number (percentage) of subjects doing physical exercise once a week or more. [c]Adjusted for age and cohort. [d]Adjusted for age, cohort, and energy intake.

Table 2 Association between plasma 25-hydroxyvitamin D and covariates among controls at baseline

Variables	Quartiles of plasma 25-hydroxyvitamin D									
	Men					Women				
	Lowest	Second	Third	Highest	P for trend	Lowest	Second	Third	Highest	P for trend
Range, ng ml^{-1}	(<22.9)	(22.9–27.5)	(27.6–32.0)	(32.1+)		(<18.7)	(18.7–22.2)	(22.3–26.9)	(27.0+)	
Median, ng ml^{-1}	19.9	25.0	29.6	35.6		16.6	20.6	24.1	31.4	
Age, mean	57.6	55.1	56.9	58.0	0.45	55.3	57.9	56.8	55.6	0.92
Smoking, mean[a]	25.9	23.9	21.4	23.9	0.44	0.8	1.3	0.4	0.2	0.22
Alcohol consumption, mean[a]	172	172	183	174	0.40	11.2	3.6	4.6	3.5	0.062
Body mass index, mean[a]	23.5	23.1	23.3	23.1	0.53	23.5	23.4	23.7	23.8	0.34
Physical exercise, *n*	18	21	16	20	0.70	11	16	12	15	0.83
Vitamin supplement use, *n*	15	9	21	8	0.30	14	15	12	13	0.74
Family history of colorectal cancer, *n*	0	1	1	2	0.15	0	2	1	1	0.47
Dietary fibre intake, mean[b]	7.53	8.56	8.39	7.64	0.84	7.13	7.60	7.62	7.80	0.96
Folate intake, mean[b]	338	340	331	312	0.091	279	288	292	300	0.48
Calcium intake, mean[b]	451	463	497	443	0.57	429	419	437	399	0.24
Vitamin D intake, mean[b]	6.30	6.84	7.14	6.47	0.92	5.00	5.19	4.84	5.54	0.067
n-3 fatty-acid intake, mean[b]	1.30	1.44	1.47	1.31	0.81	1.07	1.13	1.08	1.20	0.026
Red meat intake, mean[b]	17.8	17.8	16.2	17.0	0.50	10.6	13.8	13.4	12.9	0.49
Fish intake, mean[b]	59.5	63.9	66.2	58.9	0.70	42.2	44.0	39.1	48.4	0.087

[a]Adjusted for age. [b]Adjusted for age, cohort, and energy intake.

Table 3 Plasma 25-hydvroxyvitamin D between cases and controls

	Plasma 25-hydroxyvitamin D, (ng ml^{-1})		
	Cases	Controls	P[a]
Men			
Colorectal cancer, *n*	196	392	
Median [interquartile range]	27.3 [22.2–32.8]	27.6 [22.9–32.1]	0.67
Colon cancer, *n*	141	282	
Median [interquartile range]	28.3 [23.0–35.0]	28.0 [23.0–32.3]	0.25
Rectal cancer, *n*	55	110	
Median [interquartile range]	24.3 [20.2–29.1]	26.6 [22.9–31.2]	0.0051
Women			
Colorectal cancer, *n*	179	358	
Median [interquartile range]	22.5 [18.5–27.1]	22.3 [18.7–27.0]	0.91
Colon cancer, *n*	115	230	
Median [interquartile range]	23.3 [19.1–27.3]	22.0 [18.5–27.5]	0.25
Rectal cancer, *n*	64	128	
Median [interquartile range]	20.6 [17.8–25.3]	22.6 [19.1–26.5]	0.093

[a]Tested by extensions of Mantel–Haenszel procedure with matched pairs.

and rectal cancer risk were borderline ($P=0.06$ in men; 0.04 in women), using estimates from the statistical model for the trend test.

In addition to the main results, we calculated the rectal cancer risk of the lowest quartile compared with the combined category of other quartiles in men and women. These ORs were 4.6 (95% CI, 1.0–20) in men and 2.7 (95% CI, 0.94–7.6) in women. The fact that the low plasma levels were associated with rectal cancer risk did not substantially change when the first 2-year cases were excluded, although the OR in men was attenuated (OR, 2.2 (95% CI, 0.44–11) in men; OR, 2.7 (95% CI, 0.92–7.8) in women). Further adjustment for *n*-3 polyunsaturated fatty acid or fish intake did not change the main results (data not shown).

We repeatedly examined the association after stratifying data by the season of blood collection, that is, that with high plasma levels (July to November) or low plasma levels (December to June), and by study location (northern or southern Japan), but the results did not substantially change. These further analyses could not be applied to rectal cancer separately because the numbers were too small; similarly the risk of hypovitaminosis D could not be investigated because of the small numbers (six men cases and six men controls; 10 women cases and 28 women controls).

Table 4 Odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer for plasma 25-hydroxyvitamin D

	Quartiles of plasma 25-hydroxyvitamin D					
	Lowest	**Second**	**Third**	**Highest**	***P* for heterogeneity**	***P* for trend**
Men						
Range, ng ml^{-1}	(<22.9)	(22.9–27.5)	(27.6–32.0)	(32.1+)		
Median, ng ml^{-1}	19.9	25.0	29.6	35.6		
Colorectal cancer, n^a	43/74	40/85	36/85	44/80		
Unadjusted OR[b] (95% CI)	1.0[c]	0.81 (0.49–1.3)	0.64 (0.36–1.1)	0.91 (0.50–1.7)	0.37	0.53
Adjusted OR[d] (95% CI)	1.0[c]	0.76 (0.42–1.4)	0.76 (0.39–1.5)	0.73 (0.35–1.5)	0.78	0.39
Colon cancer, n^a	25/54	27/55	29/66	38/62		
Unadjusted OR[b] (95% CI)	1.0[c]	1.0 (0.55–1.8)	0.89 (0.46–1.7)	1.5 (0.73–3.0)	0.41	0.37
Adjusted OR[d] (95% CI)	1.0[c]	0.98 (0.48–2.0)	1.0 (0.48–2.3)	1.2 (0.51–2.7)	0.97	0.70
Rectal cancer, n^a	18/20	13/30	7/19	6/18		
Unadjusted OR[b] (95% CI)	1.0[c]	0.39 (0.15–1.0)	0.26 (0.084–0.82)	0.16 (0.036–0.69)	0.055	0.0066
Adjusted OR[d] (95% CI)	1.0[c]	0.17 (0.024–1.2)	0.25 (0.051–1.3)	0.075 (0.0057–0.99)	0.19	0.06
Women						
Range, ng ml^{-1}	(<18.7)	(18.7–22.2)	(22.3–26.9)	(27.0+)		
Median, ng ml^{-1}	16.6	20.6	24.1	31.4		
Colorectal cancer, n^a	41/77	34/73	44/71	41/76		
Unadjusted OR[b] (95% CI)	1.0[c]	0.92 (0.54–1.6)	1.1 (0.61–1.8)	0.98 (0.51–1.9)	0.96	0.96
Adjusted OR[d] (95% CI)	1.0[c]	1.0 (0.55–1.9)	1.2 (0.65–2.3)	1.1 (0.50–2.3)	0.92	0.74
Colon cancer, n^a	21/53	27/48	27/41	31/53		
Unadjusted OR[b] (95% CI)	1.0[c]	1.3 (0.68–2.5)	1.8 (0.88–3.6)	1.9 (0.83–4.3)	0.38	0.10
Adjusted OR[d] (95% CI)	1.0[c]	1.7 (0.78–3.6)	2.1 (0.90–4.7)	2.1 (0.78–5.6)	0.34	0.12
Rectal cancer, n^a	20/24	7/25	17/30	10/23		
Unadjusted OR[b] (95% CI)	1.0[c]	0.39 (0.14–1.1)	0.39 (0.15–1.0)	0.28 (0.087–0.88)	0.14	0.04
Adjusted OR[d] (95% CI)	1.0[c]	0.26 (0.069–1.0)	0.46 (0.15–1.4)	0.33 (0.084–1.3)	0.22	0.17

[a]Numbers of cases/controls. [b]ORs estimated using matched pairs without any adjustment. [c]Reference category. [d]ORs estimated using matched pairs with adjustment for pack-years of smoking (continuous), alcohol consumption (g/week ethanol, continuous), body mass index (continuous), physical exercise (less than once a week, or once a week or more), vitamin supplement use, and family history of colorectal cancer.

DISCUSSION

Our results suggest that a low level of plasma 25-hydroxyvitamin D is associated with rectal cancer risk in both men and women but not colon cancer. A significant inverse association between 25-hydroxyvitamin D and colorectal cancer, especially of the distal colon and rectum has been reported (Tangrea *et al*, 1997; Feskanich *et al*, 2004; Wactawski-Wende *et al*, 2006), whereas another prospective study showed no such association with colon cancer risk (Braun *et al*, 1995).

Comparison of the ranges of 25-hydroxyvitamin D levels in the above studies is relevant. A Finnish cohort (Tangrea *et al*, 1997) covered the lower and narrower range, that is 9.8 or less to more than 19.2 ng ml^{-1}, as did the Women's Health Initiative Study, a randomised, double-blind, placebo-controlled trial among US women, from less than 12.4–23.4 ng ml^{-1} or more (Wactawski-Wende *et al*, 2006). A Nurses' Health Study among US women (Feskanich *et al*, 2004) covered a higher and wider range (14.9, the median for the lowest category to 35.3 ng ml^{-1}, the median for the highest category). On the other hand, the small Washington County Study covered a higher and narrower range (less than 17.2–30.1 ng ml^{-1} or more) (Braun *et al*, 1995).

Our study similarly showed no association between colon cancer and 25-hydroxyvitamin D in the higher and narrower range, that is, below 22.9–32.1 ng ml^{-1} or more in men and below 18.7–27.0 ng ml^{-1} or more in women. However, an effect of a low level of 25-hydroxyvitamin D was found for rectal cancer in our subjects. In short, populations covering the lower range of 25-hydroxyvitamin D may show a preventive effect against colorectal cancer even with a narrower variation of 25-hydroxyvitamin D. The higher range presumably shows the protective effect only if they have a sufficiently wide variation of 25-hydroxyvitamin D. Differences in our findings from the Washington County Study may be due to the different characteristics of the colon and rectum.

Additionally, this inconsistency may reflect assay differences. Some studies used radioimmunoassay with an iodine-125 labelled tracer (Braun *et al*, 1995; Tangrea *et al*, 1997; Feskanich *et al*, 2004); others used a chemiluminescent radioimmunoassay (Wactawski-Wende *et al*, 2006) or competitive protein-binding assay (our study). If assay methodology differs among laboratories, even the same samples may show different measurements (Binkley *et al*, 2004).

Differences between colon and rectum may derive from 1,25-dihydroxyvitamin D receptor (Vitamin D receptor, VDR) expression. VDR also has some differences in genetic polymorphisms by ethnic group. *Bsm*I B and short poly A alleles, for example, are more prevalent in Caucasians than in Japanese (Tokita *et al*, 1996; Ingles *et al*, 1997), and may be protective against colorectal cancer (Slatter *et al*, 2001). Japanese may be more vulnerable to rectal cancer due to the low prevalence of this protective VDR genotype.

The Women's Health Initiative study reported that calcium plus vitamin D supplementation did not decrease the subsequent risk of colorectal cancer over a follow-up of 7 years (Wactawski-Wende *et al*, 2006). However, the supplementation might influence only the group with low plasma levels, colorectal cancer risk among the group with the lowest level of plasma 25-hydroxyvitamin D being slightly, though not significantly, decreased.

It is not surprising that bioavailable vitamin D status did not correlate with dietary vitamin D intake. Holick (2004) indicated that over 90% of the vitamin D requirement comes from casual exposure to sunlight. Of course, blood levels of 25-hydroxyvitamin D partly reflect dietary intake of vitamin D (Nakamura *et al*, 2000, 2002), although our results did not show a positive correlation between dietary and plasma vitamin D levels, partly due to the low validity of the food frequency questionnaire. Spearman's correlation coefficients between dietary records and estimates from the food frequency questionnaire were 0.26 for men and 0.38 for women in Cohort I; 0.32 for men and 0.28 for women in Cohort II,

assessed by volunteers from our cohorts (Ishihara *et al*, 2006). In addition, skin pigmentation is one of the determinants of 25-hydroxyvitamin D levels. Oriental people show lower cutaneous synthesis of vitamin D than white people (Matsuoka *et al*, 1991). In addition to sunlight, dietary intake, and skin pigmentation, season of blood sampling and subjects' body mass index may influence blood levels (Giovannucci, 2005; Giovannucci *et al*, 2006). Thus, 25-hydroxyvitamin D measurement is more appropriate way to assess entirely vitamin D status than dietary intake.

An active form of vitamin D, 1,25-dihydroxyvitamin D, was not investigated in the present study, as its half-life in the circulation (less than 4 h) is shorter than that of 25-hydroxyvitamin D (approximately 2 weeks) and normal levels are maintained even with vitamin D deficiency and low 25-hydroxyvitamin D levels, making it unsuitable for studies of colorectal cancer risk (Braun *et al*, 1995; Tangrea *et al*, 1997; Feskanich *et al*, 2004; Holick, 2004).

Although blood samples were collected before cancer diagnosis, measurement errors may exist because only one blood sample for each subject was used to measure plasma 25-hydroxyvitamin D concentrations, causing random misclassification to attenuate statistical associations. Long-term storage is recommended only at temperatures of less than −18°C (Hunter, 1998) and our plasma storage (−80°C) satisfied this condition. Furthermore, this plasma biomarker has a seasonal variation, declining in winter and rising in summer, reflecting sunlight exposure (Holick, 2004). In fact, our study controls' levels were slightly lower among blood samples drawn in winter (mean values from November to March; 26.3 ng dl^{-1} for men and 22.8 ng dl^{-1} for women) than those drawn at other times (mean values from April to October; 28.4 ng dl^{-1} for men and 23.5 ng dl^{-1} for women). To minimise bias, we matched date of blood collection between cases and controls (within 3 months), differing by less than 1 month among 63% of the case–control pairs, only by 1–2 months among 14% of them, and by 2–3 months among 17%. The proportion of case–control pairs where both were drawn at high-level seasons of plasma 25-hydroxyvitamin D (April to October) was 72% of all pairs, so difference in timing of blood draw would minimally impact on risk estimates.

In conclusion, a low plasma 25-hydroxyvitamin D level may be associated with a subsequent risk of rectal but not colon cancer.

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REFERENCES

Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, DeLuca HF, Drezner MK (2004) Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. *J Clin Endocrinol Metab* 89: 3152–3157

Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW (1995) Colon cancer and serum vitamin D metabolite levels 10–17 years prior to diagnosis. *Am J Epidemiol* 142: 608–611

Clayton D, Hills M (1993) 16.5 Incidence density sampling. In *Statistical Models in Epidemiology*, Clayton D, Hills M. (eds) pp 161–162. New York: Oxford University Press

Clemens TL, Adams JS, Henderson SL, Holick MF (1982) Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet* 1: 74–76

Feskanich D, Ma J, Fuchs CS, Kirkner GJ, Hankinson SE, Hollis BW, Giovannucci EL (2004) Plasma vitamin D metabolites and risk of colorectal cancer in women. *Cancer Epidemiol Biomarkers Prev* 13: 1502–1508

Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED (1989) Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet* 2: 1176–1178

Giovannucci E (2005) The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). *Cancer Causes Control* 16: 83–95

Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, Willett WC (2006) Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst* 98: 451–459

Grant WB, Garland CF (2004) A critical review of studies on vitamin D in relation to colorectal cancer. *Nutr Cancer* 48: 115–123

Greenland S (1998) Basic statistical analysis of heterogeneity. In *Modern Epidemiology*, Rothman KJ, Greenland S. (eds) 2nd edn. pp 662–664. Philadelphia, PA: Lippincott Williams & Wilkins

Haddad JG, Chyu KJ (1971) Competitive protein-binding radioassay for 25-hydroxycholecalciferol. *J Clin Endocrinol Metab* 33: 992–995

Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 80: 1678S–1688S

Hunter D (1998) Biochemical indicator of dietary intake. Vitamin D. In *Nutritional Epidemiology*, Willet W. (ed) 2nd edn. Chapter 9 pp 197–199. New York, Oxford: Oxford University Press

Ingles SA, Haile RW, Henderson BE, Kolonel LN, Nakaichi G, Shi CY, Yu MC, Ross RK, Coetzee GA (1997) Strength of linkage disequilibrium between two vitamin D receptor markers in five ethnic groups: implications for association studies. *Cancer Epidemiol Biomarkers Prev* 6: 93–98

International Union Against Cancer (1997) *TNM Classification of Malignant Tumours*, 5th edn. New York: Wiley

Ishihara J, Inoue M, Kobayashi M, Tanaka S, Yamamoto S, Iso H, Tsugane S (2006) Impact of the revision of a nutrient database on the validity of a self-administered food frequency questionnaire (FFQ). *J Epidemiol* 16: 107–116

Mantel N (1963) Chi-square tests with one degree of freedom; extensions of the Mantel-Haenszel Procedure. *J Am Stat Assoc* 58: 690–700

Matsuoka LY, Wortsman J, Haddad JG, Kolm P, Hollis BW (1991) Racial pigmentation and the cutaneous synthesis of vitamin D. *Arch Dermatol* 127: 536–538

Mizoue T (2004) Ecological study of solar radiation and cancer mortality in Japan. *Health Phys* 87: 532–538

Nakamura K, Nashimoto M, Hori Y, Yamamoto M (2000) Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. *Am J Clin Nutr* 71: 1161–1165

Nakamura K, Nashimoto M, Okuda Y, Ota T, Yamamoto M (2002) Fish as a major source of vitamin D in the Japanese diet. *Nutrition* 18: 415–416

Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, Allen C, Doughertly C, Gunter EW, Bowman BA (2002) Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 76: 187–192

Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB (2002) *Cancer Incidence in Five Continents*, vol. VIII No. 155. Lyon: International Agency for Research on Cancer

Slatter ML, Yakumo K, Hoffman M, Neuhausen S (2001) Variants of the VDR gene and risk of colon cancer (United States). *Cancer Causes Control* 12: 359–364

Tangrea J, Helzlsouer K, Pietinen P, Taylor P, Hollis B, Virtamo J, Albanes D (1997) Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. *Cancer Causes Control* 8: 615–625

Tokita A, Matsumoto H, Morrison NA, Tawa T, Miura Y, Fukamauchi K, Mitsuhashi N, Irimoto M, Yamamori S, Miura M, Watanabe T, Kuwabara Y, Yabuta K, Eisman JA (1996) Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. *J Bone Miner Res* 11: 1003–1009

Tsubono Y, Kobayashi M, Sasaki S, Tsugane S (2003) 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

Wactawski-Wende J, Kotchen JM, Anderson GL, Assaf AR, Brunner RL, O'Sullivan MJ, Margolis KL, Ockene JK, Phillips L, Pottern L, Prentice RL, Robbins J, Rohan TE, Sarto GE, Sharma S, Stefanick ML, Van Horn L, Wallace RB, Whitlock E, Bassford T, Beresford SA, Black HR, Bonds DE, Brzyski RG, Caan B, Chlebowski RT, Cochrane B, Garland C, Gass M, Hays J, Heiss G, Hendrix SL, Howard BV, Hsia J, Hubbell FA, Jackson RD, Johnson KC, Judd H, Kooperberg CL, Kuller LH, LaCroix AZ, Lane DS, Langer RD, Lasser NL, Lewis CE, Limacher MC, Manson JE (2006) Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med* 354: 684–696

Watanabe S, Tsugane S, Sobue T, Konishi M, Baba S (2001) Study design and organization of the JPHC study. *J Epidemiol* 11: S3–S7

World Health Organization (2000) *International Classification of Diseases for Oncology*, 3rd edn. Geneva: WHO

Appendix A

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ORIGINAL PAPER

Plasma folate and risk of colorectal cancer in a nested case-control study: the Japan Public Health Center-based prospective study

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Abstract
Objective There is some evidence that folate may prevent colorectal cancer by stabilizing DNA sufficiently and methylating DNA appropriately. Plasma folate is a good marker to assess folate status in the body, but it has not been adequately examined in prospective epidemiologic studies. We investigated the association between plasma folate and the risk of colorectal cancer in a nested case-control study.
Methods During a 11.5-year follow-up, 375 newly diagnosed colorectal cancers were identified in a cohort of 38,373 adults who had returned their baseline questionnaires and provided blood samples. Two controls for each case were selected from the cohort. The odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer for plasma folate was estimated using the conditional logistic regression model adjusted for potential confounding factors.
Results Plasma folate was not associated with the risk of colorectal cancer in either men or women, although a small reduction of OR in men was observed in the second (OR, 0.70; 95% CI, 0.37–1.3), the third (OR, 0.72; 95% CI, 0.38–1.3), and the highest quartiles (OR, 0.86; 95% CI, 0.45–1.6) without a dose–response relationship (P for trend 0.88). A similar association was observed in the risk of colon or rectal cancer. No statistical interaction with the risk of colorectal cancer was observed between plasma folate and alcohol consumption.
Conclusion Our results did not support the hypothesis that a folate-rich status may prevent colorectal cancer, though that finding may be due to an insufficient number of folate-deficient subjects in our study population.

Keywords Plasma folate · Colorectal cancer · Nested case-control study

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Introduction

Folate provides one-carbon groups in the synthesis of thymidylates and the methylation of DNA and protein [1, 2]. Folate deficiency is associated with DNA instability [3] and hypomethylation [4], resulting in human carcinogenesis including the large bowel.

Many epidemiologic studies have shown an inverse association between dietary folate and colorectal cancer [5, 6]. Although some populations have exhibited an independent effect of dietary folate intake in multivariate-adjusted statistical models [7–9], other populations have not, because the association with colorectal cancer risk disappeared when adjusted for potential confounding factors including dietary fiber [10–12].

Plasma or serum folate is considered a sensitive indicator of dietary folate [1]. This indicator is similar to erythrocyte folate concentration, an indicator of folate's long-term status, in terms of association with colorectal neoplasms [13]. Folate absorption in the intestine [14] and its availability in the body [15] can be modified by alcohol consumption. In short, plasma folate can be a good marker of folate status in the body, especially among those populations in which it is difficult to separate folate intake from other confounding nutrients.

Although some prospective studies have investigated the association between plasma or serum folate and the risk of colorectal cancer [16–19], the evidence is insufficient to date. We investigated the association between plasma folate and colorectal cancer as well as the interaction between plasma folate and alcohol consumption in relation to the colorectal cancer risk in a nested case-control study of a prospective cohort.

Subjects and methods

Study population

The Japan Public Health Center-based prospective study (JPHC study) is an ongoing cohort study investigating cancer, cardiovascular disease, and other lifestyle-related diseases. The first group (Cohort I) of the JPHC study was started in 1990, and the second (Cohort II) in 1993 [20]. Study subjects were mainly residents of several municipalities in areas administered by a Public Health Center, aged 40–59 years for Cohort I and 40–69 years for Cohort II. Moreover, a sub-cohort of health check-up examinees was added to Cohort I, while two more sub-cohorts of examinees and random samples aged 40–69 from one city were added to Cohort II. The study subjects were identified by the population registry in each municipality. Since cancer incidence data were not available, the additional health check-up examinee sub-cohort of Cohort I were excluded from this report. Thus, we were left with a cohort of 65,803 men and 67,520 women. Our study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Questionnaire survey

Using a self-administered questionnaire, study subjects were asked to provide information about their personal and familial medical histories, smoking, alcohol consumption, frequency of physical exercise, dietary habits, and other lifestyle factors. Their dietary habits were assessed by a food-frequency questionnaire of 44 items for Cohort I [21] and 52 items for Cohort II. A total of 50,456 men (77%) and 55,909 women (83%) filled out and returned the questionnaires.

Blood collection

Among the study subjects, 15,258 men (23%) and 26,703 women (40%) donated 10-ml samples of venous blood that was drawn into vacutainer tubes containing heparin. Samples were collected at the time of their health check-ups, which extended from 1990 to 1992 for Cohort I and 1993 to 1995 for Cohort II, and were divided into plasma aliquots and buffy layers, and then preserved at –80°C until analysis.

Follow-up

We followed study subjects until December 31, 2003. Those who had died or moved to other municipalities were identified annually through residential registries in their Public Health Center areas. To confirm their causes of death, we used mortality data from the Ministry of Health, Labor and Welfare. Among study subjects, 9.9% had moved away, and 0.2% were lost to follow-up during the study period.

Selection of cases and controls

Incidence data on colorectal cancer were collected for the JPHC cancer registry through two data sources: local major hospitals, and population-based cancer registries. The following indicators of the completeness of colorectal cancer case-ascertainment conformed to the international standards [22]: information on 5.5% of incident cases first by way of death certificates (Death Certificate Notification, DCN); 2.2% lacked any detailed information other than death certificates (Death Certificate Only, DCO); and 94.7% were verified by histological examination (Histological Verification, HV). We identified 375 cases (196 men and 179 women) of colorectal cancer until December 31, 2003 from among the 38,373 subjects (14,004 men and 24,369 women) who had returned the baseline questionnaire, reported no diagnosis of any cancer, and provided blood samples. All 375 cases were pathologically confirmed as adenocarcinoma, after excluding 18 cases of unknown pathology and seven non-adenocarcinoma cases. Of these, 256 subjects had cancer of the colon (International Classification of Diseases for Oncology, Third edition (ICD-O-3) [23) code C180–C189], and 119 had cancer of the rectum (ICD-O-3 code C199 and C209). Colon cancers were classified into those of the proximal (ICD-O-3 code C180–C185) or distal colon (ICD-O-3 code C186 and C187). Information on tumor depth was available in 370 of the 375 cases, with 120 tumors of the intramucosal type corresponding to Tis in the TNM classification [24], and 250 of the invasive type corresponding to T1 or more. The mean time from blood draw to colorectal cancer diagnosis was 5.3 (SD, 3.5) years for men and 5.3 (SD, 3.4) years for women.

For each case, two controls were selected using incidence density-sampling [25] from those who had no prior history of colorectal cancer when the case was diagnosed. Controls were matched for each case on sex, age (within 3 years), date of blood drawn (within 3 months), time since last meal (within 4 h), and study location (Public Health Center area).

Laboratory assays

Plasma folate concentrations were measured by chemiluminescence immunoassay using a reagent from Bayer HealthCare LLC (East Walpole, MA), and assayed at a commercial laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo, Japan). Samples from matched sets were assayed together. All laboratory personnel were blinded with respect to case or control status. The intra-assay coefficient of variation from the quality control samples was 2.4% ($n = 20$).

Statistical analysis

Adjusted means for cases and controls were calculated by least square means in an analysis of covariance by the PROC GLM procedure using SAS software (version 9.1; SAS Institute Inc., Cary, NC). Percentages of baseline characteristics were unadjusted crude values. We used extensions of the Mantel–Haenszel procedure [26] with matched pairs for a comparison of the baseline characteristics and plasma folate between cases and controls, using the PROC FREQ procedure with the CMH option. We also tested the linear trend of covariates by quartiles of plasma folate using extensions of the Mantel–Haenszel procedure [26]. The odds ratios (OR) and 95% confidence intervals (CI) for plasma folate divided into quartiles based on controls' distribution were calculated by the conditional logistic regression model adjusted for pack-years of smoking (continuous), alcohol consumption (g/week ethanol, continuous), body mass index (continuous), physical exercise (less than once a week, or once a week or more), any vitamin supplement use, and family history of colorectal cancer as well as by using matched pairs. The linear trend of OR was tested using the logarithmic-transformed median value of plasma folate in each category, since the measurements were log-normally distributed. *P*-values for the trend were evaluated using the two-sided test with 0.05 as the significant level. We also estimated the OR of cancer cases stratified by site or depth of tumor. The statistical interactions between plasma folate and alcohol consumption were examined, since alcohol consumption modifies folate absorption and availability [14, 15]. The cut-off point on alcohol consumption is whether or not subjects consumed 150 g/week ethanol, since such heavy drinking was significantly associated with the risk of colorectal cancer in this cohort [27]. SAS software was used for all statistical analyses.

Results

In men, pack-years of smoking, alcohol consumption, and body mass index were higher in cases than in their matched controls (Table 1), and dietary fiber intake was slightly lower. In women, vitamin D intake was higher in cases than in their controls. Other characteristics did not substantially differ between the two groups.

The lower the quartiles of plasma folate were among controls, the older they were and the more they smoked (Table 2). Plasma folate was not correlated with other potential covariates including any dietary intakes except dietary fiber intake in men.

Plasma folate did not differ between cases and controls. Median values were 6.9 ng/ml in male cases and 6.8 ng/ml in male controls; 8.1 ng/ml in female cases and 8.4 ng/ml in female controls. There was no site-specific difference between colon cancer pairs and rectal cancer pairs (data not shown in tables).

Plasma folate was not associated with the risk of colorectal cancer either in men or women, although in men, a small reduction in OR without a dose–response relationship was observed in the second and third quartiles (Table 3). Among men, the ORs (95% CI) were 0.70 (0.37–1.3) for the second quartile, 0.72 (0.38–1.3) for the third quartile, and 0.86 (0.45–1.6) for the highest quartile (*P* for trend, 0.88). Stratification by alcohol consumption did not alter the ORs where statistical interaction was not significant (*P* for interaction, 0.53 in men and 0.97 in women; data not shown in tables). The same association was observed for the risk of colon or rectal cancer.

Since only the lowest category appeared to show a high risk, we repeatedly calculated the OR for the lowest quartile compared to the combined category among the second to highest quartiles. The ORs (95% CI) were 1.3 (0.78–2.3) in men and 0.80 (0.48–1.3) in women. Similar results were observed in the lowest octile category (i.e., eight groups). There was no statistical interaction between alcohol consumption and such low levels of plasma folate (*P* for interaction, 0.65 in men and 0.63 in women). A further adjustment for dietary fiber intake, correlating with plasma folate concentration, did not substantially alter either the main or subsequently analyzed results (data not shown in tables). Furthermore, no substantial change was observed by a further analysis adjusted for dietary vitamin D intake or hormone use history, a

Table 1 Baseline characteristics of cases and controls

Characteristics	Cases	Controls	*P*
Men			
n	196	392	
Age, years, mean	56.9	56.9	0.69
Smoking, pack-years, mean[a]	27.5	23.8	0.020
Alcohol consumption, g/week ethanol, mean[a]	236	175	<0.0010
Body mass index, kg/m[b], mean[a]	23.8	23.2	0.027
Physical exercise, *n* (%)[b]	49 (26)	75 (20)	0.12
Vitamin supplement use, *n* (%)	35 (20)	53 (15)	0.11
Family history of colorectal cancer, *n* (%)	5 (2.6)	4 (1.0)	0.16
Total energy intake, kcal/day, mean[c]	2,021	2,064	0.34
Dietary fiber intake, g/day, mean[d]	7.76	8.05	0.047
Folate intake, μg/day, mean[d]	328	329	0.60
Vitamin B_6 intake, mg/day, mean[d]	1.23	1.23	0.47
Vitamin B_{12} intake, μg/day, mean[d]	7.53	7.45	0.98
Calcium intake, mg/day, mean[d]	441	463	0.15
Vitamin D intake, μg/day, mean[d]	6.58	6.67	0.54
Red meat intake, g/day, mean[d]	17.0	17.2	0.70
Women			
n	179	358	
Age, years, mean	56.5	56.4	0.35
Smoking, pack-years, mean[a]	0.458	0.657	0.54
Alcohol consumption, g/week ethanol, mean[a]	9.63	5.70	0.37
Body mass index, kg/m[b], mean[a]	23.5	23.6	0.68
Physical exercise, *n* (%)[b]	33 (19)	54 (15)	0.28
Vitamin supplement use, *n* (%)	24 (15)	54 (17)	0.43
Family history of colorectal cancer, *n* (%)	4 (2.2)	4 (1.1)	0.32
Total energy intake, kcal/day, mean[c]	1,277	1,265	0.67
Dietary fiber intake, g/day, mean[d]	7.83	7.58	0.16
Folate intake, μg/day, mean[d]	298	291	0.31
Vitamin B_6 intake, mg/day, mean[d]	0.88	0.85	0.098
Vitamin B_{12} intake, μg/day, mean[d]	5.80	5.63	0.42
Calcium intake, mg/day, mean[d]	452	423	0.074
Vitamin D intake, μg/day, mean[d]	5.57	5.16	0.047
Red meat intake, g/day, mean[d]	13.8	12.7	0.17
Hormone use history,[e] *n*, (%)	17 (9.5)	43 (12)	0.38

[a] Adjusted for age

[b] Number (percentage) of subjects doing physical exercise once a week or more

[c] Adjusted for age and cohort

[d] Adjusted for age, cohort, and energy intake

[e] Past or current oral contraceptive use or hormone replacement therapy

potential preventive factor for colorectal cancer, in women. Additionally, the association did not change after excluding cases that occurred for 2 years after blood was drawn.

Discussion

Our results did not confirm the hypothesis that a good folate status may prevent colorectal neoplasms [5, 6]. Kato et al. [17] reported that an inverse linear association existed between serum folate and the risk of colorectal cancer in a nested case-control study among New York City women, who showed a wider range of folate levels than that in our study. Ma et al. [18], in the Physicians' Health Study, found that folate deficiency (less than 3 ng/ml; [28]) was marginally but significantly associated with colorectal cancer (OR, 1.78, 95% CI, 0.93–3.42 for the group with folate deficiency). The preventive effect of plasma or serum folate may be observable only in populations that include folate-deficient subjects. Our study subjects of the present analysis included only one man with folate deficiency. We failed to show any interaction between plasma folate and high alcohol consumption leading to methyl-poor status, which could result in DNA instability [3] or hypomethylation [4]. This may also be due to the low number of our analytic subjects with folate deficiency. However, a clear association between plasma folate and colorectal cancer cannot be observed among only subjects

Table 2 Association between plasma folate and covariates among controls at baseline

Variables	Quartiles of plasma folate				
	Lowest	Second	Third	Highest	*P* for trend
Men					
Range, ng/ml	(<5.6)	(5.6–6.7)	(6.8–8.5)	(8.6+)	
Median, ng/ml	4.9	6.0	7.4	10.4	
Age, mean	58.5	56.4	57.2	55.5	0.013
Smoking, mean[a]	27.9	25.5	20.1	22.1	0.031
Alcohol consumption, mean[a]	202	166	168	170	0.24
Body mass index, mean[a]	23.2	23.5	23.3	22.9	0.24
Physical exercise, *n*	14	21	27	13	0.72
Vitamin supplement use, *n*	13	18	11	11	0.19
Family history[b], *n*	0	1	1	2	0.14
Dietary fiber intake, mean[c]	7.68	8.09	8.01	8.49	0.011
Folate intake, mean[c]	319	328	346	328	0.86
Vitamin B_6 intake, mean[c]	1.19	1.23	1.25	1.25	0.13
Vitamin B_{12} intake, mean[c]	7.85	7.43	7.75	6.93	0.28
Vitamin D intake, mean[c]	6.96	6.70	6.55	6.60	0.33
Women					
Range, ng/ml	(<6.6)	(6.6–8.3)	(8.4–10.5)	(10.6+)	
Median, ng/ml	5.7	7.4	9.2	13.0	
Age, mean	58.3	55.8	55.3	56.6	0.25
Smoking, mean[a]	0.3	0.6	0.8	0.9	0.26
Alcohol consumption, mean[a]	3.9	6.0	9.6	3.3	0.97
Body mass index, mean[a]	23.6	23.3	23.6	23.9	0.53
Physical exercise, *n*	13	8	11	22	0.065
Vitamin supplement use, *n*	15	16	13	10	0.14
Family history[b], *n*	0	0	2	2	0.16
Dietary fiber intake, mean[c]	7.44	7.29	7.59	7.93	0.093
Folate intake, mean[c]	284	283	289	303	0.25
Vitamin B_6 intake, mean[c]	0.85	0.83	0.85	0.87	0.19
Vitamin B_{12} intake, mean[c]	5.32	5.54	5.82	5.74	0.41
Vitamin D intake, mean[c]	4.90	4.84	5.28	5.55	0.067
Hormone use history[a]	9	9	12	13	0.62

[a] Adjusted for age

[b] Family history of colorectal cancer

[c] Adjusted for age, cohort, and energy intake

with folate deficiency. It was also reported that serum folate was not associated with colorectal cancer in a nested case-control study among Finnish male smokers [16]. They mainly showed low serum folate levels, a median value of 3.8 ng/ml in control subjects.

We cannot entirely rule out a sampling bias as the reason for such a small proportion of our subjects with folate deficiency. A low percentage of men donated blood samples (23%). In addition, most of them seemed to have favorable lifestyles and to be health conscious [29]. In fact, there was a statistically significant difference between the control sample in the present study and the entire cohort population regarding folate intake in men: 373.7 μg/day for the mean of present male controls versus 343.1 μg/day for the mean of male cohort subjects in the first cohort; 288.2 μg/day for the mean of present male controls versus 261.7 μg/day for the mean of male cohort subjects in the second cohort. In our entire cohort, dietary folate was not associated with the risk of colorectal cancer [30].

In addition to the lack of individuals with folate deficiency, the timing of assessment of folate status may have been inappropriate to examine the association with the risk of colorectal cancer. Jacobs et al. [31] reported that past (10 years before baseline), but not recent, multivitamin use containing folate was associated with a reduced risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort. In a murine model, dietary folate supplementation protected against small intestine and colonic carcinogenesis if it was provided before the establishment of neoplastic foci. If provided after the establishment, dietary folate supplementation increased the risk of carcinogenesis [32]. A recently reported randomized clinical

Table 3 OR and 95% CI of colorectal cancer for plasma folate

	Quartiles of plasma folate				
	Lowest	Second	Third	Highest	*P* for trend
Men					
Range, ng/ml	(<5.6)	(5.6–6.7)	(6.8–8.5)	(8.6+)	
Median, ng/ml	4.9	6.0	7.4	10.4	
Colorectal cancer, *n*[a]	37/64	40/86	41/90	45/84	
OR (95% CI)	1.0[b]	0.70 (0.37–1.3)	0.72 (0.38–1.3)	0.86 (0.45–1.6)	0.88
Colon cancer, *n*[a]	27/43	28/63	29/67	35/64	
OR (95% CI)	1.0[b]	0.65 (0.29–1.5)	0.66 (0.31–1.4)	0.82 (0.39–1.7)	0.91
Rectal cancer, *n*[a]	10/21	12/23	12/23	10/20	
OR (95% CI)	1.0[b]	0.32 (0.085–1.2)	0.58 (0.14–2.5)	0.63 (0.11–3.6)	0.73
Women					
Range, ng/ml	(<6.6)	(6.6–8.3)	(8.4–10.5)	(10.6+)	
Median, ng/ml	5.7	7.4	9.2	13.0	
Colorectal cancer, *n*[a]	33/75	56/74	33/76	38/72	
OR (95% CI)	1.0[b]	1.7 (0.93–3.0)	0.98 (0.53–1.8)	1.0 (0.56–1.9)	0.63
Colon cancer, *n*[a]	27/51	33/44	22/56	24/44	
OR (95% CI)	1.0[b]	1.3 (0.66–2.7)	0.75 (0.36–1.6)	0.97 (0.47–2.0)	0.68
Rectal cancer, *n*[a]	6/24	23/30	11/20	14/28	
OR (95% CI)	1.0[b]	3.3 (1.0–11)	2.1 (0.60–7.5)	1.5 (0.38–5.5)	0.81

Notes: ORs estimated using matched pairs with adjustment for pack-years of smoking (continuous), alcohol consumption (g/week ethanol, continuous), body mass index (continuous), physical exercise (less than once a week, or once a week or more), vitamin supplement use, and family history of colorectal cancer

[a] Numbers of cases/controls

[b] Reference category

trial failed to confirm a beneficial effect of folate supplementation against adenoma formation in the large intestine among individuals with previously removed adenomas [33]. Its folate supplementation group showed a borderline-significantly increased risk of advanced lesions. Those individuals with adenoma history might have already had the neoplastic foci at the time they enrolled in the study. In short, when folate status in the body was assessed in the adenoma–carcinoma sequence may influence the result of examining the association between folate status and the risk of colorectal cancer.

Since plasma or serum folate is considered a sensitive indicator of dietary folate, it is difficult to judge a low folate status caused by a transitory reduction in folate intake or a chronic folate deficiency accompanied with depleted folate stores [1]. Erythrocyte folate concentration is an indicator of long-term status due to erythrocyte's 120-day life span. However, Bird et al. [13] showed a similar inverse association between plasma or erythrocyte folate and colorectal adenoma in a case-control study. Nevertheless, we cannot completely exclude the possibility that our present results, i.e., no association of plasma folate with the risk of colorectal cancer, were due to a random misclassification of plasma folate measurements. Still another reason may be a residual confounding due to other lifestyle factors, we did not have information on. In addition, a stratified analysis by tumor site may have reduced the statistical power to detect a significant association because of the small number of cases.

Some enzymes are well-known to catalyze folate metabolism in the body. Genetic polymorphisms in these enzymes have been investigated in the association between plasma folate and colorectal cancer. Folate deficiency was strongly associated with the risk of colorectal cancer in subjects having the homozygous variant genotype of methylenetetrahydrofolate reductase, in whom this enzyme shows a more reduced activity than in those having two wild-type alleles [18]. A suggestive interaction was found in the combination between plasma folate and a genetic polymorphism of methionine synthase, an enzyme contributing to DNA methylation in the one-carbon metabolism of folate [19]. We were unable to examine interactions with genetic polymorphisms catalyzing the one-carbon pathway due to the lack of such information to date.

In conclusion, our results did not support the hypothesis that a folate-rich status may prevent colorectal cancer, a finding which may be due to the insufficient number of folate-deficient subjects in our study population.

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Appendix

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References

1. Institute of Medicine (1998) 8. Folate. In: Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. National Academy Press, Washington, pp 196–305
2. Molloy AM, Scott JM (2001) Folates and prevention of disease. Public Health Nutr 4:601–609
3. Duthie SJ, Narayanan S, Brand GM, Grant G (2000) DNA stability and genomic methylation status in colonocytes isolated from methyl-donor-deficient rats. Eur J Nutr 39:106–111
4. Goelz SE, Vogelstein B, Hamilton SR, Feinberg AP (1985) Hypomethylation of DNA from benign and malignant human colon neoplasms. Science 228:187–190
5. Giovannucci E (2002) Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr 132:2350S–2355S
6. Sanjoaquin MA, Allen N, Couto E, Roddam AW, Key TJ (2005) Folate intake and colorectal cancer risk: a meta-analytical approach. Int J Cancer 113:825–828
7. Giovannucci E, Stampfer MJ, Colditz GA et al (1998) Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann Intern Med 129:517–524
8. Su LJ, Arab L (2001) Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. Ann Epidemiol 11:65–72
9. Konings EJ, Goldbohm RA, Brants HA, Saris WH, van den Brandt PA (2002) Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. Cancer 95:1421–1433
10. Michels KB, Fuchs CS, Giovannucci E et al (2005) Fiber intake and incidence of colorectal cancer among 76,947 women and 47,279 men. Cancer Epidemiol Biomarkers Prev 14: 842–849
11. Bingham SA, Norat T, Moskal A et al (2005) Is the association with fiber from foods in colorectal cancer confounded by folate intake? Cancer Epidemiol Biomarkers Prev 14:1552–1556
12. Otani T, Iwasaki M, Hanaoka T et al (2005) Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. Nutr Cancer 53:42–50
13. Bird CL, Swendseid ME, Witte JS et al (1995) Red cell and plasma folate, folate consumption, and the risk of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 4: 709–714
14. Halsted CH, Robles EA, Mezey E (1971) Decreased jejunal uptake of labeled folic acid (3 H-PGA) in alcoholic patients: roles of alcohol and nutrition. N Engl J Med 285:701–706
15. Shaw S, Jayatilleke E, Herbert V, Colman N (1989) Cleavage of folates during ethanol metabolism: role of acetaldehyde/xanthine oxidase-generated superoxide. Biochem J 257:277–280
16. Glynn SA, Albanes D, Pietinen P et al (1996) Colorectal cancer and folate status: a nested case-control study among male smokers. Cancer Epidemiol Biomarkers Prev 5:487–494
17. Kato I, Dnistrian AM, Schwartz M et al (1999) Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. Br J Cancer 79:1917–1922

18. Ma J, Stampfer MJ, Giovannucci E et al (1997) Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 57:1098–1102
19. Ma J, Stampfer MJ, Christensen B et al (1999) A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocyst(e)ine, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 8:825–829
20. Watanabe S, Tsugane S, Sobue T, Konishi M, Baba S (2001) Study design and organization of the JPHC study. J Epidemiol 11:S3–7
21. Tsubono Y, Kobayashi M, Sasaki S, Tsugane S (2003) Validity and reproducibility of a self-administered food frequency questionnaire used in the baseline survey of the JPHC Study Cohort I. J Epidemiol 13:S125–133
22. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB (eds) (2002) Cancer incidence in five continents, vol VIII no. 155. International Agency for Research on Cancer, Lyon
23. World Health Organization (2000) In: Fritz A, Percy C, Jack A et al. (eds) International classification of diseases for oncology, 3rd edn. WHO, Geneva
24. International Union Against Cancer (1997) In: Sobin LH, Wittekind Ch (eds) TNM classification of malignant tumours, 5th edn. Wiley, New York
25. Clayton D, Hills M (1993) 16.5 Incidence density sampling. In: Clayton D, Hills M (eds) Statistical models in epidemiology. Oxford University Press, New York, pp 161–162
26. Mantel N (1963) Chi-square tests with one degree of freedom; extensions of the Mantel–Haenszel procedure. J Am Stat Assoc 58:690–700
27. Otani T, Iwasaki M, Yamamoto S et al (2003) Alcohol consumption, smoking, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan Public Health Center-based prospective study. Cancer Epidemiol Biomarkers Prev 12:1492–1500
28. Herbert V, Zalusky R (1962) Interrelations of vitamin B12 and folic acid metabolism: folic acid clearance studies. J Clin Invest 41:1263–1276
29. Iwasaki M, Otani T, Yamamoto S et al (2003) Background characteristics of basic health examination participants: the JPHC Study Baseline Survey. J Epidemiol 13:216–225
30. Ishihara J, Otani T, Inoue M, Iwasaki M, Sasazuki S, Tsugane S (2007) Low intake of vitamin B-6 is associated with increased risk of colorectal cancer in Japanese men. J Nutr 137:1808–1814
31. Jacobs EJ, Connell CJ, Chao A et al (2003) Multivitamin use and colorectal cancer incidence in a US cohort: does timing matter? Am J Epidemiol 158:621–628
32. Song J, Sohn KJ, Medline A, Ash C, Gallinger S, Kim YI (2000) Chemopreventive effects of dietary folate on intestinal polyps in ApcMsh2-/- mice. Cancer Res 60:3191–3199
33. Cole BF, Baron JA, Sandler RS et al (2007) Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 297:2351–2359

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Plasma C-peptide, insulin-like growth factor-I, insulin-like growth factor binding proteins and risk of colorectal cancer in a nested case-control study: The Japan public health center-based prospective study

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The physical inactivity and obesity involved in hyperglycemia and hyperinsulinemia is supposed to lead to an increased bioavailability of insulin-like growth factor-I (IGF-I). The carcinogenic effect of IGF-I may be influenced by IGF binding proteins. We investigated the association between plasma levels of C-peptide, a surrogate biomarker of insulin, IGFBP-1, IGF-I or IGFBP-3, and the risk of colorectal cancer in a nested case-control study. During an 11.5-year follow-up, 375 newly diagnosed colorectal cancers were identified in a cohort of 38,373 adults who had returned the baseline questionnaire and provided blood samples. Two matched-controls for each case were selected from the cohort. The odds ratio (OR) of colorectal cancer for plasma levels of each protein was estimated using the conditional logistic regression model adjusted for potential confounding factors. We observed a statistically significant association of plasma C-peptide with colorectal cancer only in men. The ORs were 1.0, 2.3, 2.8 and 3.2 along with quartiles (p trend, 0.0072). The association was stronger in colon cancer (p trend, 0.025) than in rectal cancer (p trend, 0.24). Other peptides were not associated with the risk in either men or women. The results did not change when repeatedly analyzed by tumor invasion levels, tumor sites or follow-up periods. In conclusion, a higher plasma C-peptide may indicate a subsequent risk of colorectal cancer in Japanese men.

Key words: C-peptide; insulin-like growth factor I; insulin-like growth factor binding proteins; colorectal cancer; nested case-control study

Physical inactivity and obesity are prominent risk factors for colorectal cancer.[1] Hyperglycemia and hyperinsulinemia involved in these factors may underlie the biological mechanism of colorectal cancer development.[2] Such elevated insulin suppresses the production of insulin-like growth factor binding protein (IGFBP)-1, which inhibits insulin-like growth factor (IGF)-I-stimulated cell growth and differentiation, leading to elevated IGF-I bioactivity.[3] In addition, acromegalic population having an increased risk of colon cancer[4] shows a high blood level of IGF-I, a growth-hormone-dependent peptide. The IGF-I is supposed to have a potent antiapoptotic and mitogenic properties in both normal and neoplastic cells.[5] Growth hormone also increases insulin-like growth factor binding protein 3 (IGFBP-3), which has IGF-I-dependent and independent antiproliferative effects.[3,6] Both IGF-I and IGFBP-3 are circulating hormones and local paracrine factors with potential influences on cell growth.[5,6]

Many nested case-control studies in prospective cohorts have been conducted on these peptides such as insulin[7–9] or C-peptide,[10–12] an indicator of an endogenous insulin secretion, IGF-I[10,12–16] and IGFBPs.[8–10,12–16] The evidence on C-peptide, which is better suited to assessing insulin secretion, is insufficient so far. Although the IGF-I findings have accumulated and are consistent, as meta-analyses showed,[17,18] those of IGFBP-3 are inconsistent.[10,12–16] There are few investigations regarding IGFBP-1 and their findings are inconsistent.[9,10,12]

We investigated the association between plasma levels of C-peptide, IGFBP-1, IGF-I or IGFBP-3 and the risk of colorectal cancer in a nested case-control study.

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TABLE I – BASELINE CHARACTERISTICS OF CASES AND CONTROLS

Characteristics	Men			Women		
	Cases	Controls	*P*	Cases	Controls	*p*
N	196	392		179	358	
Age, years, mean	56.9	56.9	0.69	56.5	56.4	0.35
Smoking, pack-years, mean[1]	27.5	23.8	0.020	0.458	0.657	0.54
Alcohol consumption (g/week), ethanol, mean[1]	236	175	<0.0010	9.63	5.70	0.37
Body mass index[2] (kg/m), mean[1]	23.8	23.2	0.027	23.5	23.6	0.68
Body height (cm), mean[1]	162.3	161.9	0.45	150.0	150.8	0.065
Physical exercise, *n* (%)[2]	49 (26)	75 (20)	0.12	33 (19)	54 (15)	0.28
Family history of colorectal cancer, *n* (%)	5 (2.6)	4 (1.0)	0.16	4 (2.2)	4 (1.1)	0.32
Past medical history of diabetes, *n* (%)	15 (7.7)	21 (5.4)	0.28	8 (4.5)	17 (4.8)	0.94
Vitamin supplement use, *n* (%)	35 (20)	53 (15)	0.11	24 (15)	54 (17)	0.43
Total energy intake (kcal/day), mean[3]	2,021	2,064	0.34	1,277	1,265	0.67
Dietary fiber intake (g/day), mean[4]	7.76	8.05	0.047	7.83	7.58	0.16
Folate intake (μg/day), mean[4]	328	329	0.60	298	291	0.31
Calcium intake (mg/day), mean[4]	441	463	0.15	452	423	0.074
Vitamin D intake (μg/day), mean[4]	6.58	6.67	0.54	5.57	5.16	0.047
Red meat intake (g/day), mean[4]	17.0	17.2	0.70	13.8	12.7	0.17
Fasting blood samples,[5] *n* (%)	110 (56)	219 (56)	0.90	103 (58)	204 (57)	0.79
Hormone use,[6] *n* (%)				17 (9.5)	43 (12)	0.38
Postmenopausal diagnosis,[7] *n* (%)				170 (95)		

[1]Adjusted for age.–[2]Number (percentage) of subjects doing physical exercise once a week or more.–[3]Adjusted for age and cohort.–[4]Adjusted for age, cohort and energy intake.–[5]Four or more hours after meal.–[6]Past or current oral contraceptive use or hormone replacement therapy.–[7]A diagnosis at age 50 years or more was defined as postmenopausal diagnosis.

Material and methods

Study population

The Japan Public Health Center-based Prospective Study (JPHC study) is an ongoing cohort study investigating cancer, cardiovascular disease and other lifestyle-related diseases. The first group (Cohort I) of the JPHC study was started in 1990 and the second group (Cohort II) in 1993.[19] Study subjects were mainly residents living in several municipalities in each area administered by a Public Health Center, regarding in age from 40 to 59 years in Cohort I and 40 to 69 years in Cohort II. Moreover, a sub-cohort of health check-up examinees was added to Cohort I, while 2 more subcohorts of examinees and random samples aged 40–69 from 1 city were added to Cohort II. The study subjects were identified by the population registry in each municipality. The sub-cohort of health check-up examinees in Cohort I mentioned earlier were excluded from this report, since their cancer incidence data were not available. Thus, we were left with a cohort of 65,803 men and 67,520 women. Our study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Questionnaire survey

Using a self-administered questionnaire, study subjects were asked to provide information about their personal and familial medical histories, smoking, alcohol consumption, frequency of physical exercise, dietary habits and other lifestyle factors. Their dietary habits were assessed by a food-frequency questionnaire of 44 items for Cohort I[20] and 52 items for Cohort II. A total of 50,456 men (77%) and 55,909 women (83%) filled out and returned the questionnaires.

Blood collection

Among the study subjects, 15,258 men (23%) and 26,703 women (40%) donated 10 ml samples of venous blood that was drawn into vacutainer tubes containing heparin. Samples were collected at the time of their health check-ups, which extended from 1990 to 1992 for Cohort I and 1993 to 1995 for Cohort II, and were divided into plasma and buffy layers, then preserved at −80°C until analysis. Of these blood samples, 57% were fasting samples (4 hr or more since last meal, Table I).

Follow-up

We followed study subjects until December 31, 2003. Those having died or moving to other municipalities were identified annually through residential registries in their Public Health Center areas. To confirm their causes of death, we used mortality data from the Ministry of Health, Labor and Welfare. Among study subjects, 9.9% had moved away, and 0.2% was lost to follow-up during the study period.

Selection of cases and controls

Incidence data on colorectal cancer were collected for the JPHC cancer registry through 2 data sources: local major hospitals and population-based cancer registries. Indicators of the completeness of colorectal cancer case-ascertainment conformed to the international standard[21] as follows: information on 5.5% of incident cases first came by way of death certificates (Death Certificate Notification, DCN); 2.2% lacked any detailed information other than death certificates (Death Certificate Only, DCO); and 94.7% were verified by histological examination (Histological Verification, HV). We identified 375 cases (196 men and 179 women) of colorectal cancer up to December 31, 2003 from among the 38,373 subjects (14,004 men and 24,369 women) who had returned the baseline questionnaire, reported no diagnosis of any cancer and provided blood samples. All 375 cases were pathologically confirmed as adenocarcinoma, after excluding 18 cases of unknown pathology and 7 non-adenocarcinoma cases. Of these, 256 subjects had cancer of the colon [International Classification of Diseases for Oncology, Third edition (ICD-O-3)[22] code C180 to C189] and 119 had cancer of the rectum (ICD-O-3 code C199 and C209). Colon cancers were classified into those of the proximal (ICD-O-3 code C180 to C185) or distal colon (ICD-O-3 code C186 and C187). Information on tumor depth was available in 370 of the 375 cases, with 120 tumors of the intramucosal type corresponding to Tis in the TNM classification,[23] and 250 of the invasive type corresponding to T1 or more.

For each case, 2 controls were selected using incidence-density sampling[24] from subjects who had no prior history of colorectal cancer when their case was diagnosed. Controls were matched for each case on sex, age (within 3 years), date of blood drawn (within 3 months), time since last meal (within 4 hr) and study location (Public Health Center area).

Laboratory assays

Plasma C-peptide was measured by radioimmunoassay using a reagent from Shionogi, Osaka, Japan. Plasma IGF-I was measured by immunoradiometric assay using a reagent from Mitsubishi Kagaku Iatron, Tokyo, Japan. This commercial assay measured total not bioavailable IGF-I. Plasma IGFBP-1 and IGFBP-3 were

TABLE II – BASELINE CONCENTRATIONS OF PLASMA C PEPTIDE, INSULIN LIKE GROWTH FACTOR-I (IGF-I) AND INSULIN LIKE GROWTH FACTOR BINDING PROTEINS (IGFBP-1 AND -3) BETWEEN CASES AND CONTROLS

	Men			Women		
	Cases	Controls	*p*	Cases	Controls	*p*
C-peptide (ng/ml)	2.9 (1.8–4.9)	2.5 (1.5–4.5)	0.014	2.2 (1.5–3.8)	2.2 (1.6–3.8)	0.86
Fasting[1]	2.0 (1.5–2.8)	1.6 (1.2–2.5)	0.046	1.7 (1.2–2.7)	1.8 (1.3–2.4)	0.64
Nonfasting	4.9 (3.4–5.9)	4.3 (3.0–5.6)	0.14	3.5 (2.3–4.6)	3.4 (2.4–4.8)	0.78
Past medical history of diabetes	2.3 (1.6–2.8)	3.6 (2.2–5.1)	0.32	2.9 (1.9–3.3)	2.1 (1.8–3.2)	[2]
IGFBP-1 (ng/ml)	16.4 (8.10–31.3)	19.3 (8.50–33.2)	0.29	14.2 (6.40–35.8)	16.4 (6.50–32.4)	0.66
IGF-I (ng/ml)	172 (137–206)	164 (136–204)	0.27	160 (129–190)	159 (121–197)	0.99
IGFBP-3 (ng/ml)	4,520 (3,995–5,170)	4,450 (3,895–5,050)	0.22	4,870 (4,320–5,490)	4,885 (4,260–5,440)	0.28

Median (interquartile range). *p* values show statistical results of comparison between cases and controls using extension of Mantel-Haenszel procedure with matched pairs.
[1]Four or more hours after meal.–[2]*p* value was not available because of much smaller number of subjects to analyze.

measured by immunoradiometric assay using a reagent from Diagnostic Systems Laboratories, Webster, TX. All analyses were assayed at Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan. Samples from matched sets were assayed together. All laboratory personnel were blinded with respect to case or control status. The intra-assay coefficients of variation from the quality control samples were 5.3% for C-peptide, 6.2% for IGFBP-1, 2.4% for IGF-I and 3.4% for IGFBP-3 ($n = 20$).

Statistical analysis

Adjusted means for cases and controls were calculated using least square means in an analysis of covariance by the PROC GLM procedure in SAS software (version 9.1; SAS Institute, Cary, NC). Percentages of baseline characteristics were unadjusted crude values. Baseline plasma levels of peptides were compared between cases and control using medians and interquartile ranges of each group because those levels were not normally distributed. We used extensions of the Mantel-Haenszel procedure[25] with matched pairs for a comparison of the baseline characteristics and the baseline plasma levels of peptides between cases and controls, using the PROC FREQ procedure with the CMH option. Spearman correlation coefficients were calculated among plasma peptides and factors of body size and lifestyle. These coefficients were adjusted for sex, age and time elapsed since last meal at blood collection. The odds ratios (OR) and 95% confidence intervals (CI) for plasma levels of peptides divided into quartiles based on control distribution were calculated by a conditional logistic regression model adjusted for pack-years of smoking (continuous), alcohol consumption (continuous), body mass index (continuous), physical exercise (less than once a week, or once a week or more) and family history of colorectal cancer as well as by using matched pairs. The linear trend of OR was tested using the median values of IGF-I and IGFBP-3, or the logarithmic-transformed median values of C-peptide, IGFBP-1. *p*-values for the trend were evaluated using the 2-sided test with 0.05 as the significant level. We estimated the OR of cancer cases stratified by site or depth of tumor as well as the OR of all colorectal cancer cases. SAS software was used for all statistical analyses.

Results

Pack-years of smoking or alcohol consumption were higher in cases than in matched controls in men but not in women (Table I). Other characteristics, including dietary factors, did not substantially differ between the 2 groups except for dietary fiber in men and vitamin D in women.

Plasma C-peptide was higher among cases than controls (Table II) in men but not in women. Except for C-peptide, there was no statistically significant difference between cases and controls in any peptides. Plasma levels correlated between C-peptide and IGFBP-1 [partial Spearman rank correlation coefficients adjusted for age and time since last meal (r_s), −0.44 in men; −0.56 in women], and between IGF-I and IGFBP-3 (r_s, 0.66 in men; 0.68 in women). A modest correlation was found between IGFBP-1 and body mass index (r_s, −0.32 in men; −0.34 in women). Correlation coefficients were 0.19 in men and 0.24 in women between C-peptide and body mass index. Dietary factors were not correlated with plasma peptides. Those correlation coefficients were around 0.2 or less (see Supplementary Tables).

Plasma C-peptide had a statistically significant association with colorectal cancer in men (Table III). The ORs were 2.3 (95% CI; 1.2, 4.5) for the second quartile, 2.8 (95% CI; 1.3, 6.1) for the third quartile and 3.2 (95% CI; 1.4, 7.6) for the highest quartile compared to the lowest (*p* trend, 0.0072). Other peptides were not associated with the risk of colorectal cancer. In women, there were no associations between plasma peptides and colorectal cancer.

In addition, we examined ORs by tumor sites, *i.e.*, colon or rectum (Table IV); by intramucosal or invasive type of tumor; or by the first follow-up period of less than 6 years or the last one of 6 years or more, conducted under a concept derived from some other studies.[13,15] The site-specific association of plasma C-peptide was stronger in colon cancer (*p* trend, 0.025) than in rectal cancer (*p* trend, 0.24). We also calculated subsite-specific ORs of proximal or distal colon cancer. The results seemed to show a stronger association in distal than in the proximal colon in men, although they were unstable. The OR estimates for cancers by tumor depth or follow-up period were not substantially different from those for all colorectal cancers (see Supplementary Tables).

No further analyses show remarkable findings when compared with the results mentioned earlier. We did analyses with further adjustments for past medical history of diabetes in our multivariate-adjusted model. In addition, we limited analytic subjects to those without diabetes or those who provided fasting blood samples. We also conducted a further adjustment for hormone use history and an analysis limited to women diagnosed at postmenopause (aged 50 or more). Furthermore, we examined whether our multivariate-adjusted model was over-adjusted using a less adjusted model, *i.e.*, a model not adjusted for body mass index and physical exercise. Moreover, a model not mutually adjusted for C-peptide and IGFBP-1, or IGF-I and IGFBP-3, was also examined. However, these efforts produced no substantial change in our results (see Supplementary Tables).

Discussion

Plasma C-peptide, a biomarker indicating endogenous insulin secretion, was clearly associated with a subsequent risk of colorectal cancer in men. This result supported the finding that hyperinsulinemia caused by a high body mass index or low physical activity is involved in colorectal cancer development.[1–3] Our study confirmed the previous reports showing association between hyperinsulinemia and colorectal cancer.[7,11] Nevertheless, it remains unclear why the association in our study was found only in men but not in women. Wei *et al.*[12] examined this association in a nested case-control study derived from a US Nurses' cohort, where the association was positive but not statistically significant. However, Kaaks *et al.*[10] reported a positive association between

TABLE III – ODDS RATIO (OR) AND 95% CONFIDENCE INTERVAL (CI) OF COLORECTAL CANCER FOR BASELINE CONCENTRATIONS OF PLASMA C-PEPTIDE, INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGFBP-1 AND -3)

	Men					Women				
	Quartile				*p* trend	Quartile				*p* trend
	1	2	3	4		1	2	3	4	
C-peptide, median	1.1	1.9	3.3	5.6		1.1	1.9	2.8	4.8	
n	25/86	49/92	50/93	56/88		51/80	32/77	46/93	46/88	
OR[1] (95% CI)	1.0 (reference)	2.3 (1.2–4.5)	2.8 (1.3–6.1)	3.2 (1.4–7.6)	0.0072	1.0 (reference)	0.71 (0.39–1.3)	0.75 (0.40–1.4)	0.78 (0.38–1.6)	0.49
IGFBP-1, median	5.3	12.9	25.6	47.2		4.2	10.9	23.3	47.2	
n	49/90	59/86	33/91	39/92		44/87	52/84	27/81	52/86	
OR[1] (95% CI)	1.0 (reference)	1.3 (0.74–2.3)	0.83 (0.41–1.7)	1.1 (0.52–2.5)	0.84	1.0 (reference)	1.1 (0.64–2.0)	0.56 (0.28–1.1)	1.1 (0.49–2.4)	0.68
IGF-I, median	115	149	181	231		96	139	176	233	
n	44/87	36/89	51/91	49/92		38/77	46/86	52/89	39/86	
OR[2] (95% CI)	1.0 (reference)	0.70 (0.38–1.3)	0.78 (0.40–1.5)	0.83 (0.40–1.7)	0.91	1.0 (reference)	1.0 (0.57–1.8)	1.2 (0.59–2.2)	0.83 (0.38–1.8)	0.60
IGFBP-3, median	3,440	4,160	4,740	5,460		3,745	4,560	5,170	5,850	
n	34/89	50/89	43/88	53/93		40/82	48/85	38/82	49/89	
OR[2] (95% CI)	1.0 (reference)	1.4 (0.74–2.5)	1.1 (0.58–2.2)	1.4 (0.65–2.8)	0.60	1.0 (reference)	1.1 (0.61–2.0)	0.88 (0.45–1.7)	1.1 (0.53–2.3)	0.74

n: Numbers of cases/controls. ORs estimated using matched pairs with adjustment for pack-years of smoking (continuous), alcohol consumption (g/week ethanol, continuous), body mass index (continuous), physical exercise (less than once a week, or once a week or more), family history of colorectal cancer and following plasma measurements mutually:
[1]IGFBP-1 or C-peptide.–[2]IGFBP-3 or IGF-I.

TABLE IV – ODDS RATIO (OR) AND 95% CONFIDENCE INTERVAL (CI) OF COLON OR RECTAL CANCER FOR BASELINE CONCENTRATIONS OF PLASMA C-PEPTIDE, INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGFBP-1 AND -3)

	Men					Women				
	Quartile				*p* trend	Quartile				*p* trend
	1	2	3	4		1	2	3	4	
Colon										
C-peptide[1]	1.0 (reference)	2.1 (0.96–4.6)	2.6 (1.0–6.3)	3.5 (1.2–10)	0.025	1.0 (reference)	0.65 (0.29–1.4)	0.92 (0.41–2.0)	0.72 (0.28–1.8)	0.54
IGFBP-1[1]	1.0 (reference)	1.5 (0.75–3.1)	1.3 (0.57–3.0)	1.6 (0.62–4.0)	0.47	1.0 (reference)	1.2 (0.57–2.5)	0.73 (0.31–1.7)	1.3 (0.47–3.5)	0.95
IGF-I[2]	1.0 (reference)	0.69 (0.33–1.4)	0.73 (0.33–1.6)	0.82 (0.36–1.9)	0.89	1.0 (reference)	0.99 (0.48–2.1)	1.1 (0.48–2.5)	0.64 (0.24–1.7)	0.43
IGFBP-3[2]	1.0 (reference)	1.6 (0.74–3.3)	1.4 (0.64–3.1)	1.6 (0.67–3.7)	0.41	1.0 (reference)	1.3 (0.61–2.7)	0.88 (0.37–2.1)	1.4 (0.54–3.5)	0.52
Rectum										
C-peptide[1]	1.0 (reference)	1.8 (0.40–8.0)	3.8 (0.83–18)	2.2 (0.47–10)	0.24	1.0 (reference)	0.88 (0.34–2.3)	0.46 (0.14–1.5)	0.76 (0.23–2.5)	0.82
IGFBP-1[1]	1.0 (reference)	0.86 (0.16–2.8)	0.21 (0.045–0.94)	0.30 (0.053–1.7)	0.056	1.0 (reference)	0.95 (0.37–2.5)	0.28 (0.075–1.1)	0.79 (0.21–2.9)	0.45
IGF-I[2]	1.0 (reference)	0.74 (0.20–2.8)	1.1 (0.25–5.1)	0.96 (0.16–5.7)	0.92	1.0 (reference)	1.1 (0.40–2.9)	1.4 (0.39–5.2)	1.4 (0.31–6.0)	0.75
IGFBP-3[2]	1.0 (reference)	1.0 (0.31–3.4)	0.98 (0.21–4.7)	0.89 (0.19–4.2)	0.82	1.0 (reference)	0.83 (0.32–2.2)	0.67 (0.20–2.2)	0.77 (0.20–3.0)	0.73

ORs estimated using matched pairs with adjustment for pack-years of smoking (continuous), alcohol consumption (g/week ethanol, continuous), body mass index (continuous), physical exercise (less than once a week, or once a week or more), family history of colorectal cancer and the following plasma measurements mutually:
[1]IGFBP-1 or C-peptide.–[2]IGFBP-3 or IGF-I.