Determination of Serum Carotenoids, Retinol, and Tocopherols

All the samples were analyzed by trained staff blinded to case-control status. Serum total cholesterol was determined using an autoanalyzer. Serum concentrations of carotenoids, retinol, and tocopherols were measured by high-performance liquid chromatography, as described elsewhere (14), using the same equipment for all specimens. The ranges of repeatability and day-to-day variation (coefficients of variation) were 4.6% to 6.9% and 6.3% to 20.0%, respectively, for the assays of carotenoids, retinol, and tocopherols. We could not separately measure serum levels of zeaxanthin and lutein or β- and γ-tocopherols and therefore report the combined levels as zeaxanthin/lutein and β-/γ-tocopherols, respectively. We calculated total carotenes as the sum of α- and β-carotenes and lycopene, total xanthophylls as the sum of β-cryptoxanthin, canthaxanthin, and zeaxanthin/lutein, and total provitamin A as the sum of α- and β-carotenes and β-cryptoxanthin. Total carotenoids were calculated as total carotenes plus total xanthophylls.

To assess the degradation of serum components in stored sera, we previously compared serum levels of carotenoids, retinol, and tocopherols at the time of collection and after 9 yr of storage at −80°C (Ito Y, et al., unpublished data; $n = 46$). The mean decrease in serum components was less than 15% for α- and β-carotenes and less than 20% for retinol, α-tocopherol, lycopene, β-cryptoxanthin, and zeaxanthin/lutein.

Statistical Analysis

We first analyzed the data by sex because the associations of some carotenoids with colorectal cancer risk were considerably different between men and women. The associations of micronutrients with the risk in men and women combined were examined only for the compounds without a substantial effect modification by sex. Analyses limited to colon or rectal cancer cases were not made due to the small number of cases.

Body mass index (BMI) at baseline was calculated from reported height and weight: BMI = (weight in kg)/(height in m)^2. We compared background characteristics between cases and controls by the chi-square test or the Mantel test. Mean differences between cases and controls were examined by analysis of covariance (ANCOVA) allowing for the matching after converting serum levels of carotenoids, retinol, and tocopherols to logarithmic values. Adjusted as possible confounding factors were education (age at completion of education: <16, 16–18, or ≥19 yr), family history of colorectal cancer in parents or siblings (yes or no), BMI (as a continuous variable), smoking (never smokers, ex-smokers, or current smokers), alcohol drinking (never drinkers, ex-drinkers, or current drinkers), walking time (≤30 or ≥30 min/day), sedentary work (yes or no), consumption of beef (≤2 times/month, 1–2 times/wk, or ≥3 times/wk), and serum total cholesterol level (as a continuous variable).

In the study questionnaire, subjects reported the intake frequency of beef with five possible responses: almost never, 1–2 times/mo, 1–2 times/wk, 3–4 times/wk, or almost every day. If the intake frequency of a subject did not exactly fit any category, the participant chose the category he or she regarded as most appropriate. We then classified participants into three groups, that is, ≤2 times/mo, 1–2 times/wk, or ≥3 times/wk, according to the response. For walking time, the questionnaire included four possible responses: ≥1 hr/day, 30–60 min/day, about 30 min/day, or almost never. If a subject walked for 1 h or 30 min per day, the response he or she considered more appropriate was selected. The response was then dichotomized into ≤30 min/day (about 30 min/day or almost never) and ≥30 min/day (≥1 h/day or 30–60 min/day).

Whether the case-control difference was modified by sex was tested by ANCOVA, including the previously mentioned confounding variables and the product term for interaction between case-control status and sex.

Conditional logistic models were applied to calculate odds ratios (ORs) for the incidence of colorectal cancer (20). Cases and controls were categorized into three groups, according to tertile levels of carotenoids, retinol, and tocopherols among controls. However, control subjects were not precisely divided into three equal groups because some controls had identical serum values. ORs were calculated for the middle and highest tertiles vs. the lowest one, considering only matching variables (sex, age, and participating institution), or matching factors, education (age at completion of education: <16, 16–18, or ≥19 yr), family history of colorectal cancer in parents or siblings (yes or no), BMI (<20.0, 20.0–24.9, or ≥25.0 kg/m^2), smoking (never smokers, ex-smokers, or current smokers), alcohol drinking (never drinkers, ex-drinkers, or current drinkers), walking time (≤30 or ≥30 min/day), sedentary work (yes or no), consumption of beef (≤2 times/mo, 1–2 times/wk, or ≥3 times/wk), and serum total cholesterol level (<4.0, 4.0–4.9, 5.0–5.9, or ≥6.0 mmol/l). We did not adjust for supplement use because we intended to assess overall bioavailability of the micronutrients using serum concentration as an indicator.

To test for linear trends in OR over tertiles, we coded each tertile as 0, 1, or 2, and then incorporated it into the logistic model as a single variable. We further statistically tested whether the effects of serum carotenoids, retinol, and tocopherols on colorectal cancer risk were modified by sex by including product terms between sex and each of the tertiles in the logistic models (21). We have also attempted to determine whether the effect modification is due to sex or due to smoking or drinking habit by multivariate analysis. Included were product terms between smoking or drinking habit (never or ever) and each of the tertiles of serum micronutrients in the conditional logistic models, in addition to those between sex and each of the tertiles.

All P values were two-sided, and all analyses were performed using the Statistical Analysis System, release 8.2 (SAS Institute Inc., Cary, NC; 22). In ANCOVA or the conditional logistic regression analysis, missing values in each cat-

egorical covariate were treated as an additional category in the variable and were included in the model.

Results

Table 1 compares baseline characteristics of colorectal cancer cases with those of controls by sex. Age distribution was quite comparable between cases and controls due to the matching: mean ages ± SD were 61.2 ± 8.8 yr in male cases, 60.5 ± 8.4 yr in male controls, 62.0 ± 7.6 yr in female cases, and 61.4 ± 7.3 yr in female controls. Compared with controls, cases were likely to have a family history of colorectal cancer and to be engaged in sedentary work in both sexes. Particularly in men, the serum level of total cholesterol tended to be higher in cases than in controls. Female case subjects tended to be leaner than control participants. All the case-control differences, however, did not reach statistical significance. Smoking and alcohol drinking habits were similarly distributed between cases and controls.

The proportion of users of multivitamin or vitamin E supplement was comparable between cases and controls except that a higher percentage of female case subjects used a vitamin E supplement than controls without a significant difference: The percentages of multivitamin use were 15.0% in male cases, 15.8% in male controls, 10.2% in female cases, and 10.6% in female controls, while those of vitamin E use were 5.1%, 5.3%, 16.3%, and 11.4%, correspondingly.

In men, the geometric means of serum concentration were significantly lower in colorectal cancer cases than in controls for zeaxanthin/lutein (by 11%), canthaxanthin (by 6%), and lycopene (by 18%; Table 2; $P < 0.05$). In women, the geometric mean was higher in cases than in controls for α-carotene by 21% ($P = 0.005$).

Of interest, the mean serum level of total carotenoids was lower in cases than in controls among men (geometric mean, 1.59 μmol/l in cases vs. 1.79 μmol/l in controls), while it was higher in cases among women (2.55 μmol/l in cases vs. 2.33 μmol/l in controls). A highly significant interaction was detected between case-control status and sex (P for interaction = 0.002). Such effect modifications by sex were found also for zeaxanthin/lutein, canthaxanthin, α-carotene, β-carotene, total carotenes, total xanthophylls, and provitamin A (P for interaction <0.05).

In men, the highest tertiles of serum canthaxanthin, total carotenes, and total carotenoids were associated with a 60–70% decreased risk compared with the lowest tertiles (Table 3): The multivariate-adjusted ORs (OR2) were 0.36 (95% confidence interval [CI] = 0.11–1.16; trend P over tertiles = 0.089) for canthaxanthin, 0.40 (95% CI = 0.14–1.17; trend $P = 0.10$) for total carotenes, and 0.34 (95% CI = 0.11–1.00; trend $P = 0.040$) for total carotenoids. In women, on the contrary, the higher levels of α- and β-carotenes and total carotenoids were related to a somewhat increased risk: The OR2 for the highest vs. the lowest tertile was 4.72 (95% CI = 1.29–17.3; trend $P = 0.007$) for α-carotene, 2.00 (95% CI = 0.70–5.73; trend $P = 0.040$) for β-carotene, and 2.47 (95% CI = 0.73–8.34; trend $P = 0.064$) for total carotenoids. The risk modification by sex for the highest tertile was statistically significant for zeaxanthin/lutein (P for interaction = 0.048), α-carotene ($P = 0.024$), total carotenes ($P = 0.037$), and total carotenoids ($P = 0.022$; data not shown in the table). Similar effect modifications by sex (i.e., low OR in men but high OR in women for the highest tertiles) were observed also for canthaxanthin (P for interaction = 0.055), β-carotene ($P = 0.056$), total xanthophylls ($P = 0.070$), and provitamin A ($P = 0.082$).

Among the carotenoids with possible effect modification by sex, all but α-carotene failed to show independent and significant ($P < 0.10$) effect modification by sex after adjustment for risk modification by smoking or drinking habit. For α-carotene, the risk modification by sex seemed to be independent of that by alcohol drinking (P for interaction between α-carotene and sex for the highest tertile = 0.096). On the other hand, an interaction was suggested between canthaxanthin and smoking ($P = 0.033$) or drinking habit ($P = 0.084$), which was independent of effect modification by sex; that is, the potential protective effects appeared to be stronger among smokers or drinkers. No significant interaction was found between carotenoids other than canthaxanthin and smoking or drinking habit after adjustment for the interaction between carotenoids and sex.

We also found a somewhat decreasing risk with increasing concentrations of serum retinol and α-tocopherol in men: The multivariate ORs (OR2) across tertiles were 1.00, 0.56 (95% CI = 0.20–1.52), and 0.31 (95% CI = 0.07–1.34) with a P for trend of 0.099 for retinol, and 1.00, 0.23 (95% CI = 0.07–0.80), and 0.29 (95% CI = 0.07–1.17) (trend $P = 0.098$) for α-tocopherol.

When we combined men and women for the substances without a substantial effect modification by sex (Table 4), the serum retinol level was inversely correlated with colorectal cancer risk. The OR2 (95% CI) over tertiles was 1.00, 0.51 (0.27–0.99), and 0.29 (0.11–0.78; trend $P = 0.010$). Subjects with a higher value of serum lycopene tended to show a lower OR (OR2 for the highest tertile = 0.48; 95% CI = 0.20–1.15; trend $P = 0.096$).

Findings for OR considering only matching variables (OR1) were generally in line with those for multivariate OR (OR2), but the OR1 tended to approach unity compared with OR2 (Tables 3 and 4). Excluding subjects without at least a 2-yr follow-up did not essentially alter the association of serum carotenoids, retinol, and tocopherols with the risk of colorectal cancer (data not shown).

Discussion

In the present study, we found that the higher serum total carotenes and total carotenoids tended to be associated with a decreased risk of colorectal cancer in men. On the contrary, women with higher levels of α- and β-carotenes and total carotenoids showed an increased risk. The female predominance in OR for the highest tertiles of serum levels was statistically or

Table 1. Distribution of Baseline Characteristics in Cases of Colorectal Cancer and Controls by Sex[a]

	Men					Women				
	Cases		Controls			Cases		Controls		
	N	%	*N*	%	*P* for Difference	*N*	%	*N*	%	*P* for Difference
Total number	54	100.0	141	100.0		62	100.0	157	100.0	
Age (yr)										
40–49	8	14.8	23	16.3	0.65	4	6.5	10	6.4	0.53
50–59	14	25.9	38	27.0		19	30.6	55	35.0	
60–69	20	37.0	53	37.6		30	48.4	74	47.1	
70–79	12	22.2	27	19.1		9	14.5	18	11.5	
Age at completion of education (yr)										
<16	15	27.8	39	27.7	0.57	14	22.6	43	27.4	0.30
16–18	17	31.5	49	34.8		32	51.6	77	49.0	
19–	9	16.7	16	11.3		7	11.3	12	7.6	
Unknown	13	24.1	37	26.2		9	14.5	25	15.9	
Family history of colorectal cancer in parents or siblings										
Yes	3	5.6	3	2.1	0.22	6	9.7	7	4.5	0.14
No	51	94.4	138	97.9		56	90.3	150	95.5	
Body mass index (kg/m^2)										
<20.0	7	13.0	19	13.5	0.42	13	21.0	20	12.7	0.16
20.0–24.9	34	63.0	93	66.0		32	51.6	83	52.9	
25.0–	13	24.1	24	17.0		16	25.8	49	31.2	
Unknown	0	0.0	5	3.5		1	1.6	5	3.2	
Smoking										
Nonsmokers	10	18.5	27	19.1	0.94	57	91.9	141	89.8	0.84
Ex-smokers	17	31.5	41	29.1		2	3.2	4	2.5	
Current smokers	25	46.3	68	48.2		2	3.2	3	1.9	
Unknown	2	3.7	5	3.5		1	1.6	9	5.7	
Alcohol drinking										
Nondrinkers	10	18.5	26	18.4	0.86	48	77.4	118	75.2	0.43
Ex-drinkers	2	3.7	8	5.7		0	0.0	4	2.5	
Current drinkers	41	75.9	105	74.5		13	21.0	29	18.5	
Unknown	1	1.9	2	1.4		1	1.6	6	3.8	
Walking time (min/day)										
≤30	10	18.5	28	19.9	0.70	9	14.5	36	22.9	0.20
≥30	30	55.6	71	50.4		40	64.5	94	59.9	
Unknown	14	25.9	42	29.8		13	21.0	27	17.2	
Sedentary work										
Yes	15	27.8	22	15.6	0.12	13	21.0	23	14.6	0.16
No	25	46.3	69	48.9		26	41.9	82	52.2	
Unknown	14	25.9	50	35.5		23	37.1	52	33.1	
Consumption of beef										
≤2 times/mo	22	40.7	53	37.6	0.66	22	35.5	61	38.9	0.85
1–2 times/wk	12	22.2	40	28.4		18	29.0	38	24.2	
≥3 times/wk	4	7.4	10	7.1		11	17.7	30	19.1	
Unknown	16	29.6	38	27.0		11	17.7	28	17.8	
Serum total cholesterol (mmol/l)										
<4.0	7	13.0	25	17.7	0.22	2	3.2	8	5.1	0.36
4.0–4.9	20	37.0	58	41.1		16	25.8	45	28.7	
5.0–5.9	17	31.5	36	25.5		25	40.3	62	39.5	
6.0–	9	16.7	18	12.8		18	29.0	38	24.2	
Unknown	1	1.9	4	2.8		1	1.6	4	2.5	

a: See text for details on the categories for walking time and consumption of beef.

Table 2. Geometric Means and 5-95 Percentiles (μmol/l) of Serum Levels of Retinol, Tocopherols, and Carotenoids in Cases of Colorectal Cancer and Controls by Sex

	Men			Women			
	Geometric Mean (5–95 percentile)			Geometric Mean (5–95 percentile)			
	Cases ($N = 54$)	Controls ($N = 141$)	P for Difference[a]	Cases ($N = 62$)	Controls ($N = 157$)	P for Difference[a]	P for Interaction Between Case-Control Status and Sex[b]
Retinol (μmol/l)	2.79 (1.37–5.45)	2.86 (1.78–4.52)	0.14	2.25 (1.47–3.89)	2.30 (1.43–4.36)	0.24	0.99
β-/γ-Tocopherols (μmol/l)	3.02 (1.32–8.51)	2.86 (1.21–6.75)	0.21	3.40 (2.05–6.63)	3.32 (1.57–6.27)	0.35	0.68
α-Tocopherol (μmol/l)	17.47 (8.80–30.13)	17.40 (6.99–30.81)	0.66	20.94 (9.38–34.82)	20.96 (9.82–34.95)	0.90	0.73
Zeaxanthin/lutein (μmol/l)	0.78 (0.32–1.77)	0.87 (0.38–2.07)	0.030	1.00 (0.46–1.76)	0.93 (0.38–1.88)	0.087	0.002
Canthaxanthin (μmol/l)	0.021 (0.008–0.057)	0.023 (0.010–0.053)	0.046	0.026 (0.013–0.055)	0.024 (0.011–0.051)	0.15	0.011
β-Cryptoxanthin (μmol/l)	0.20 (0.03–1.15)	0.18 (0.03–1.25)	0.62	0.32 (0.09–1.19)	0.32 (0.05–0.88)	0.74	0.99
Lycopene (μmol/l)	0.11 (0.02–0.48)	0.14 (0.03–0.75)	0.035	0.20 (0.02–1.10)	0.22 (0.05–1.06)	0.47	0.14
α-Carotene (μmol/l)	0.047 (0.003–0.176)	0.052 (0.010–0.160)	0.33	0.091 (0.024–0.234)	0.076 (0.014–0.211)	0.005	0.003
β-Carotene (μmol/l)	0.25 (0.04–1.21)	0.32 (0.07–1.49)	0.077	0.65 (0.10–2.38)	0.55 (0.08–1.69)	0.086	0.0009
Total carotenes (μmol/l)	0.45 (0.09–1.68)	0.56 (0.13–2.12)	0.051	1.00 (0.16–3.05)	0.91 (0.18–2.43)	0.22	0.004
Total xanthophylls (μmol/l)	1.09 (0.39–3.22)	1.16 (0.44–3.18)	0.25	1.43 (0.57–2.88)	1.34 (0.57–2.72)	0.15	0.017
Provitamin A (μmol/l)	0.54 (0.09–3.58)	0.59 (0.11–2.47)	0.46	1.13 (0.19–3.37)	1.00 (0.20–2.50)	0.10	0.020
Total carotenoids (μmol/l)	1.59 (0.52–4.87)	1.79 (0.64–4.68)	0.096	2.55 (0.80–5.88)	2.33 (0.75–5.00)	0.065	0.002

a: P value for difference of the geometric mean between cases and controls adjusted for education (age at completion of education: <16, 16–18, or ≥19 yr), family history of colorectal cancer in parents or siblings (yes or no), body mass index (as a continuous variable), smoking (never smokers, ex-smokers, or current smokers), alcohol drinking (never drinkers, ex-drinkers, or current drinkers), walking time (≤30 or ≥30 min/day), sedentary work (yes or no), consumption of beef (≤2 times/mo, 1–2 times/wk, or ≥3 times/wk), and serum total cholesterol level (as a continuous variable) by analysis of covariance.

b: Adjusted for education (age at completion of education: <16, 16–18, or ≥19 yr), family history of colorectal cancer in parents or siblings (yes or no), body mass index (as a continuous variable), smoking (never smokers, ex-smokers, or current smokers), alcohol drinking (never drinkers, ex-drinkers, or current drinkers), walking time (≤30 or ≥30 min/day), sedentary work (yes or no), consumption of beef (≤2 times/mo, 1–2 times/wk, or ≥3 times/wk), and serum total cholesterol level (as a continuous variable) by analysis of covariance. See text for details on the categories for walking time and consumption of beef.

Table 3. Odds Ratios (OR) and 95% Confidence Intervals (CI) for Colorectal Cancer Risk by Serum Levels of Carotenoids, Retinol, and Tocopherols by Sex[a]

	Men							Women						
	Category (μmol/l)	Cases	Controls	OR1[b]	95% CI	OR2[c]	95% CI	Category (μmol/l)	Cases	Controls	OR1[b]	95% CI	OR2[c]	95% CI
Retinol	<2.47	24	46	1.00		1.00		<1.93	22	51	1.00		1.00	
	2.47–3.35	14	48	0.45	0.18–1.14***	0.56	0.20–1.52	1.93–2.58	21	53	0.89	0.41–1.93	0.87	0.33–2.27
	3.36–	16	47	0.41	0.14–1.25	0.31	0.07–1.34	2.59–	19	53	0.53	0.17–1.71	0.49	0.10–2.43
					trend $P = 0.095$		trend $P = 0.099$					trend $P = 0.34$		trend $P = 0.44$
β-/γ-Tocopherols	<2.46	15	46	1.00		1.00		<2.81	16	52	1.00		1.00	
	2.46– 3.48	21	46	1.51	0.65–3.54	2.07	0.67–6.36	2.81– 3.97	26	50	1.60	0.76–3.35	1.85	0.76–4.47
	3.49–	18	48	1.36	0.51–3.62	1.85	0.50–6.87	3.98–	20	55	1.30	0.57–2.96	1.58	0.54–4.61
					trend $P = 0.59$		trend $P = 0.41$					trend $P = 0.51$		trend $P = 0.34$
α-tocopherol	<16.51	23	46	1.00		1.00		<19.53	22	51	1.00		1.00	
	16.51–22.52	12	48	0.40	0.15–1.03***	0.23	0.07–0.80**	19.53–25.42	18	53	0.75	0.31–1.83	0.71	0.23–2.21
	22.53–	19	47	0.61	0.20–1.87	0.29	0.07–1.17***	25.43–	22	53	1.03	0.42–2.54	0.70	0.20–2.46
					trend $P = 0.41$		trend $P = 0.098$					trend $P = 0.82$		trend $P = 0.62$
Zeaxanthin/lutein	<0.71	22	45	1.00		1.00		<0.79	21	51	1.00		1.00	
	0.71–1.02	16	49	0.64	0.28–1.47	0.66	0.23–1.89	0.79–1.11	12	52	0.60	0.25–1.45	0.81	0.29–2.28
	1.03–	16	47	0.62	0.26–1.51	0.48	0.17–1.39	1.12–	29	54	1.53	0.68–3.44	1.96	0.72–5.28
					trend $P = 0.29$		trend $P = 0.18$					trend $P = 0.20$		trend $P = 0.15$
Canthaxanthin	<0.017	21	44	1.00		1.00		<0.021	16	52	1.00		1.00	
	0.017–0.029	17	49	0.68	0.30–1.57	0.48	0.17–1.33	0.021–0.031	23	48	1.64	0.69–3.91	1.54	0.56–4.22
	0.030–	16	48	0.58	0.23–1.46	0.36	0.11–1.16***	0.032–	23	57	1.49	0.63–3.53	1.83	0.62–5.35
					trend $P = 0.25$		trend $P = 0.089$					trend $P = 0.44$		trend $P = 0.28$
β-cryptoxanthin	<0.11	17	46	1.00		1.00		<0.28	26	51	1.00		1.00	
	0.11–0.32	20	48	1.11	0.45–2.73	1.02	0.34–3.03	0.28–0.46	16	53	0.57	0.25–1.31	0.43	0.15–1.22
	0.33–	17	47	0.92	0.35–2.47	0.95	0.28–3.23	0.47–	20	53	0.71	0.30–1.67	0.50	0.18–1.44
					trend $P = 0.84$		trend $P = 0.93$					trend $P = 0.49$		trend $P = 0.24$
Lycopene	<0.09	20	43	1.00		1.00		<0.13	23	52	1.00		1.00	
	0.09–0.20	17	50	0.70	0.30–1.63	0.77	0.28–2.16	0.13–0.34	16	52	0.76	0.34–1.71	0.71	0.27–1.88
	0.21–	17	48	0.73	0.29–1.87	0.57	0.19–1.71	0.35–	23	53	1.23	0.46–3.32	1.12	0.32–3.95
					trend $P = 0.51$		trend $P = 0.32$					trend $P = 0.76$		trend $P = 0.93$
α-carotene	<0.038	16	41	1.00		1.00		<0.070	17	51	1.00		1.00	
	0.038–0.079	24	53	0.99	0.46–2.16	1.10	0.41–2.93	0.070–0.102	14	51	1.03	0.39–2.73	1.36	0.38–4.87
	0.080–	14	47	0.63	0.25–1.57	0.73	0.24–2.20	0.103–	31	55	2.70	1.05–6.95**	4.72	1.29–17.3**
					trend $P = 0.30$		trend $P = 0.54$					trend $P = 0.019$		trend $P = 0.007$

(continued)

Table 3. *(Continued)*

	Men							Women						
	Category (μmol/l)	Cases	Controls	OR1[b]	95% CI	OR2[c]	95% CI	Category (μmol/l)	Cases	Controls	OR1[b]	95% CI	OR2[c]	95% CI
β-carotene	<0.21	21	45	1.00		1.00		<0.50	20	52	1.00		1.00	
	0.21–0.53	20	49	0.79	0.34–1.82	0.69	0.25–1.90	0.50–0.75	8	52	0.42	0.15–1.18***	0.24	0.06–0.89**
	0.54–	13	47	0.48	0.19–1.18	0.39	0.12–1.23	0.76–	34	53	1.88	0.84–4.22	2.00	0.70–5.73
					trend $P = 0.11$		trend $P = 0.10$					trend $P = 0.029$		trend $P = 0.040$
Total carotenes	<0.42	25	46	1.00		1.00		<0.80	21	52	1.00		1.00	
	0.42–0.87	15	48	0.46	0.18–1.17	0.28	0.08–1.02***	0.80–1.36	14	52	0.77	0.32–1.81	0.59	0.21–1.71
	0.88–	14	47	0.45	0.19–1.05***	0.40	0.14–1.17***	1.37–	27	53	1.78	0.74–4.28	1.96	0.61–6.34
					trend $P = 0.070$		trend $P = 0.10$					trend $P = 0.16$		trend $P = 0.21$
Total xanthophylls	<0.95	22	46	1.00		1.00		<1.20	21	52	1.00		1.00	
	0.95–1.41	15	47	0.64	0.28–1.46	0.50	0.17–1.50	1.20–1.63	12	52	0.66	0.28–1.55	0.68	0.24–1.92
	1.42–	17	48	0.66	0.29–1.53	0.60	0.21–1.69	1.64–	29	53	1.62	0.72–3.64	2.01	0.71–5.68
					trend $P = 0.34$		trend $P = 0.34$					trend $P = 0.17$		trend $P = 0.14$
Provitamin A	<0.38	19	46	1.00		1.00		<0.93	18	52	1.00		1.00	
	0.38–0.93	22	48	1.12	0.46–2.69	1.07	0.36–3.18	0.93–1.38	15	51	1.07	0.44–2.59	0.98	0.33–2.88
	0.94–	13	47	0.59	0.23–1.55	0.46	0.14–1.55	1.39–	29	54	1.91	0.84–4.37	1.98	0.68–5.71
					trend $P = 0.23$		trend $P = 0.20$					trend $P = 0.078$		trend $P = 0.12$
Total carotenoids	<1.48	28	46	1.00		1.00		<2.06	20	52	1.00		1.00	
	1.48–2.20	10	48	0.29	0.12–0.72*	0.19	0.06–0.66*	2.06–2.95	12	52	0.75	0.29–1.92	0.62	0.19–2.03
	2.21–	16	47	0.45	0.19–1.04***	0.34	0.11–1.00***	2.96–	30	53	1.96	0.79–4.87	2.47	0.73–8.34
					trend $P = 0.054$		trend $P = 0.040$					trend $P = 0.057$		trend $P = 0.064$

a: $*P < 0.01$; $**P < 0.05$; $***P < 0.10$. Controls were not precisely divided into three even groups because of identical measurement values.

b: Considering only matching variables (age and participating institution) by using conditional logistic models.

c: Adjusted for education (age at completion of education: <16, 16–18, or ≥19 yr), family history of colorectal cancer in parents or siblings (yes or no), body mass index (<20.0, 20.0–24.9, or ≥25.0 kg/m^2), smoking (never smokers, ex-smokers, or current smokers), alcohol drinking (never drinkers, ex-drinkers, or current drinkers), walking time (≤30 or ≥30 min/day), sedentary work (yes or no), consumption of beef (≤2 times/mo, 1–2 times/wk, or ≥3 times/wk), and serum total cholesterol level (<4.0, 4.0–4.9, 5.0–5.9, or ≥6.0 mmol/l) by using conditional logistic models. See text for details on the categories for walking time and consumption of beef.

Table 4. Odds Ratios (OR) and 95% Confidence Intervals (CI) for Colorectal Cancer Risk by Serum Levels of Carotenoids, Retinol, and Tocopherols in Men and Women Combined[a]

	Category (μmol/l)	Cases	Controls	OR1[b]	95% CI	OR2[c]	95% CI
Retinol	<2.11	45	95	1.00		1.00	
	2.11–3.01	37	103	0.63	0.34–1.14	0.51	0.27–0.99*
	3.02–	34	100	0.42	0.18–0.98*	0.29	0.11–0.78*
					trend $P = 0.039$		trend $P = 0.010$
β-/γ-Tocopherols	<2.65	32	98	1.00		1.00	
	2.65–3.61	44	89	1.53	0.87–2.69	1.48	0.77–2.81
	3.62–	40	110	1.21	0.65–2.26	1.17	0.56–2.44
					trend $P = 0.57$		trend $P = 0.65$
α-Tocopherol	<17.86	42	96	1.00		1.00	
	17.86–23.85	33	101	0.75	0.41–1.38	0.60	0.29–1.22
	23.86–	41	101	0.96	0.49–1.89	0.61	0.27–1.39
					trend $P = 0.99$		trend $P = 0.30$
β-Cryptoxanthin	<0.18	40	99	1.00		1.00	
	0.18–0.40	39	98	0.95	0.52–1.72	0.89	0.45–1.74
	0.41–	37	101	0.90	0.46–1.76	0.78	0.36–1.70
					trend $P = 0.75$		trend $P = 0.53$
Lycopene	<0.10	38	85	1.00		1.00	
	0.10–0.27	42	112	0.83	0.47–1.45	0.70	0.37–1.31
	0.28–	36	101	0.76	0.35–1.64	0.48	0.20–1.15**
					trend $P = 0.46$		trend $P = 0.096$

a: $*P < 0.05$; $**P < 0.10$. Analysis was restricted to antioxidants with no substantial effect modification by sex. Controls were not precisely divided into three even groups because of identical measurement values.

b: Considering only matching variables (sex, age, and participating institution) by using conditional logistic models.

c: Adjusted for education (age at completion of education: <16, 16–18, or ≥19 yr), family history of colorectal cancer in parents or siblings (yes or no), body mass index (<20.0, 20.0–24.9, or ≥25.0 kg/m^2), smoking (never smokers, ex-smokers, or current smokers), alcohol drinking (never drinkers, ex-drinkers, or current drinkers), walking time (≤30 or ≥30 min/day), sedentary work (yes or no), consumption of beef (≤2 times/mo, 1–2 times/wk, or ≥3 times/wk), and serum total cholesterol level (<4.0, 4.0–4.9, 5.0–5.9, or ≥6.0 mmol/l) by using conditional logistic models. See text for details on the categories for walking time and consumption of beef.

marginally significant for several carotenoids. Such effect modifications by sex were also detected when we compared geometric means of serum concentrations between cases and controls. In addition, we found a somewhat decreasing trend in risk with increasing serum levels of retinol in men or men and women combined, and of α-tocopherol in men.

There is little evidence of any relationship between blood levels of carotenoids and colorectal cancer risk. Malila et al. (11) reported no association of serum β-carotene with the risk of colorectal cancer in an 8-yr prospective study of male smokers. For colorectal adenomas, the precursors of colorectal cancers, Erhardt et al. (23) found that the plasma lycopene level was significantly lower in the adenoma group than in the control group. They also reported a lower plasma β-carotene level, although not significant, in adenoma cases. Their findings are, in part, consistent with ours that the geometric mean of serum lycopene concentration was significantly lower in male cases of colorectal cancer than in corresponding controls, and subjects with a higher level of serum lycopene were at a somewhat lower risk in the analysis with men and women combined. Shikany and coworkers (24), however, revealed no associations between any individual carotenoid or total carotenoids in plasma and adenomatous polyps in a case-control study. The study, however, included only adenomas of the distal colon and rectum, while subjects in the study by Erhardt et al. (23) underwent a total colonoscopy.

In line with some previous studies on α-tocopherol and retinol, we found a somewhat decreasing trend in risk with increasing serum levels of α-tocopherol and retinol in men. A pooled analysis of data from five cohorts revealed a 30% reduction in colorectal cancer risk for the highest quartile of serum α-tocopherol concentration compared with the lowest after adjustment for serum cholesterol level (25). Ingles et al. (26) found that a high α-tocopherol to γ-tocopherol ratio was associated with a decreased risk of large colorectal adenomas. Furthermore, in a controlled trial, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, α-tocopherol supplementation conferred a modest preventive effect against colorectal cancer in older male smokers (27). Breuer-Katschinski et al. (28) found an inverse association between serum concentration of vitamin A and colorectal adenoma in a case-control study, although the risk was not correlated with the serum vitamin E level.

Malila and colleagues (11), however, did not support the possible protective effects of α-tocopherol or retinol against colorectal cancer in a prospective study using serum samples before the intervention of the ATBC study. More data, particularly from prospective studies, should be accumulated to assess the relationship between blood levels of tocopherols or

retinol and the risk of not only colorectal adenoma but also cancer of the colorectum.

The low risk in men and the high risk in women associated with higher serum levels of some carotenoids can be interpreted in several ways. First, men and women may biologically differ in the effects of carotenoids on colorectal cancer. In women, blood carotenoid levels seem to be regulated by sex hormones to some extent and thus may have implications different from those of men for cancer risk (29). Murtaugh et al. (30) detected no association between dietary β-carotene and rectal cancer risk in a case-control study in the analysis by sex. Among female subjects, however, they found an increased risk associated with combination of lower presumed estrogen status (postmenopausal without hormone replacement therapy) and low intake of β-carotene. This suggests that sex hormones may modify the action of carotenoids. However, the authors also reported a negative association of dietary lycopene with rectal cancer risk only in women, which may be somewhat inconsistent with our findings, that is, the elevated risk in women with higher serum levels of selected carotenoids. Further investigations on possible interactions between sex hormones and carotenoids are warranted.

Second, lifestyles more prevalent in Japanese men than in women, such as smoking or alcohol drinking, may interact with the effect of carotenoids. Smoking and drinking habits have been related to decreased blood levels of carotenoids (31,32), and the lowered levels may not be enough to exert protective effects against colorectal cancer. Although it was not feasible to examine the interaction between these lifestyle factors and carotenoids in the present study due to the limited number of nonsmoking or nondrinking men, the greater risk reduction by intake of vegetables and fruits in smokers has been observed for cancer of the lung (33) and stomach (34). Supplementation of β-carotene, however, conferred a modest increase in the risk of colorectal adenoma recurrence in smokers while decreasing the risk in nonsmoking and nondrinking subjects (35). Although the effect modification by sex for carotenoids might be confounded by smoking or drinking habit, the very strong correlations between sex and these lifestyles prevented us from drawing clear conclusions, even with the multivariate analysis; for example, the female subjects were almost all nonsmokers. Studies in nonsmokers or nondrinkers would be required to address this issue.

On balance, the greater risk of colorectal cancer in women with higher serum concentrations of carotenes cannot be explained by the two interpretations mentioned previously. The third hypothesis is that blood levels of some carotenoids may have a U-shaped association with colorectal cancer risk regardless of sex. Too much carotene intake could increase the risk of malignancy (36). β-Carotene has not only antioxidant activity but also prooxidant actions, especially at high concentrations and/or under high oxygen tension (37). Although the colon is in an anaerobic environment, higher oral intake of β-carotene can lead to its accumulation in the colonic mucosa (38), and its tissue concentration may reach a level at which β-carotene acts as a prooxidant. In the present study, women generally demonstrated higher levels of serum carotenoids than men. A part of female subjects may have had such high blood levels that it increased their risk of colorectal cancer, while men with relatively higher levels may have shown a lower risk.

The strength of our study derives principally from its prospective design in that blood samples were collected before diagnosis of colorectal cancer. Using serum samples allowed objective measurements of dietary factors, considering inter-individual variations of their bioavailability. Some methodological limitations, however, need elucidation.

First, the sample size was relatively small to examine the sex-specific effects of carotenoids, retinol, and tocopherols on colorectal cancer risk. These significant effect modifications by sex for some carotenoids, therefore, must be confirmed by larger studies.

Second, the serum levels of carotenoids, retinol, and tocopherols varied widely between study areas: The coefficients of variation computed by one-way analysis of variance ranged from 69.6% (total xanthophylls) to 285.3% (lycopene) in men and from 87.4% (canthaxanthin) to 290.9% (lycopene) in women. These variations between areas may partly be due to not only the difference in dietary intake but also to the difference in procedures after drawing blood. The cases and controls, however, are still comparable because of the matching for participating institutions. Further, even when excluding a study area with the values furthest from the means, the overall directions of associations for some carotenoids, namely, inverse associations in men and positive ones in women, were not altered.

Finally, we could not include all the potentially confounding factors. For example, the limitation of samples prevented us from considering serum folate. Folate has been linked to the reduced risk of colorectal cancer in alcohol drinkers (39) and is rich in green leafy vegetables that also contain much carotenoid. Adjustment for consumption of green leafy vegetables, however, strengthened the inverse associations of some carotenoids with colorectal cancer risk in men: The multivariate-adjusted ORs for the middle and highest tertiles were 0.42 (95% CI = 0.15–1.20) and 0.20 (95% CI = 0.06–0.72) for canthaxanthin (trend $P = 0.014$), 0.23 (95% CI = 0.06–0.90) and 0.22 (95% CI = 0.06–0.81) for total carotenes (trend $P = 0.023$), and 0.17 (95% CI = 0.05–0.60) and 0.24 (95% CI = 0.07–0.82) for total carotenoids (trend $P = 0.011$), respectively. Moreover, the positive associations of serum levels of α- and β-carotenes and total carotenoids with colorectal cancer risk in women cannot be ascribed to the confounding by folate.

In conclusion, the effect of some carotenoids on colorectal cancer risk may be modified by sex or by factors associated with sex, including lifestyle factors such as smoking and drinking habits. The male low risk and female high risk associated with the higher blood levels, if confirmed, could provide another interpretation to the relationship between vegetable and fruit consumption and the risk of colorectal cancer. The observed decreasing trend in risk with an elevating se-

rum retinol (in men or men plus women) and α-tocopherol (in men) may support the possible protective effects of these substances against colorectal cancer.

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Rare *Helicobacter pylori* infection as a factor for the very low stomach cancer incidence in Yogyakarta, Indonesia

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Abstract

To elucidate factors associated with the very low risk of gastric neoplasia in Yogyakarta, Indonesia, approximately 1/50 of the level in Japan, we recruited 52 male and 39 female participants from the general populace in the city of Yogyakarta in October 2003. *Helicobacter pylori* IgG antibodies were found in only 5% (0–13) (95% confidence interval) and 4% (0–9) for Javanese males and females, respectively, and were statistically lower than the 62% (58–65) and 57% (53–60), respectively, in Japanese. Furthermore, positive findings of pepsinogen test were only 0 and 2% (0–6) for males and females, in Yogyakarta, and were again significantly lower than the 23% (22–25) and 22% (20–23), in Japan. The very low incidence of stomach cancer in Yogyakarta may be due to a low prevalence of *H. pylori* infection and chronic atrophic gastritis.

Keywords: *Helicobacter pylori*; Yogyakarta; Stomach cancer; Ecological study

1. Introduction

Since 2002, collaborative epidemiologic studies on host and environmental factors for stomach and colorectal cancer have been underway in a number of Southeast Asian countries. Ecological and case-control studies are now being performed in Hanoi,

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Viet Nam; Khon Kaen, Thailand; and Yogyakarta, Indonesia in order to take advantage of the major variation in incidence rates among these geographical locations and also with data for Japan. Such international comparisons clearly have potential for providing clues for epidemiology and prevention of neoplastic development. Stomach cancer incidences in Hanoi, Khon Kaen and Yogyakarta are approximately 1/2, 1/10 and 1/50 of the level in Japan, respectively: that is, those for Yogyakarta are $1.3/10^5$ for males and $0.7/10^5$ for females during 1994–1996, and for Japan $67/10^5$ for males and $27/10^5$ for females in 1995 [1–3].

We here report results of an ecological study of stomach cancer with reference to the prevalence of *Helicobacter pylori* (*H. pylori*) infection, a definite and necessary carcinogen for the stomach [4,5], and chronic atrophic gastritis (CAG) markers along with sodium and potassium excretions in Yogyakarta, compared with those intakes in Japan. Analysis of *H. pylori* in the feces is also underway, but results are not yet available for inclusion in this report.

2. Subjects and methods

In October 2003, we randomly recruited 52 male and 39 female participants from the general populace in the city of Yogyakarta. Mean ages were 48.0 ± 9.0 (SD) for males and 46.6 ± 8.5 for females. Written informed consent was obtained from the study participants. The protocol was submitted to the Internal Review Boards of Nagoya City University and Gadjah Mada University, and approved. The subjects were requested to respond to lifestyle and food frequency questionnaires, which had also been adopted for a case-control study, and were interviewed by health nurses at a local health center. Body weight and height were measured, and overnight-fasting blood, breath, second morning voiding urine (SMVU) and feces were sampled from each participant.

Serum antibodies for *H. pylori* were examined by enzyme immunoassay (EIA) (Kyowa Medics, Co., Tokyo, Japan) and values ≥ 2.3 were defined as positive. Serum pepsinogen (PG) I and PGII were measured by chemical luminescence enzyme immunoassay (CLEIA) (Eiken Chemicals Co., Tokyo, Japan) with cut-off points of PGI ≤ 70 ng/ml and PGI/PGII ≤ 3.0 [6]. For the urea breath test (UBT), UBiT-IR300 kits (Otsuka Pharmaceutical Co., Tokyo, Japan) were employed with $\geq 2.5‰$ as positive. Because the values differed by sex and age, age-adjustment was made for the rates, adopting the world population [1] as standard, for comparison with the figures for Japan. Using SMVU, excretions of sodium, as a marker of intake of salt and salty foods, and potassium, as a marker of consumption of vegetables and fruit, were analyzed by electrode assay and creatinine by an enzymatic method. Daily excretions of salt (sodium chloride) and potassium were then estimated with adjustment for creatinine [7], which were compared with those intakes in Japanese after adjustment for age.

3. Results

As shown in Table 1, age-adjusted *H. pylori* IgG antibodies were found in only 5% (0–13) and 4% (0–10) of males and females, respectively, in Yogyakarta, and were significantly lower than

Table 1
H. pylori-related markers in Javanese vs. Japanese

	Javanese		Japanese	
	Male ($n=52$)	Female ($n=39$)	Male	Female
Serum *H. pylori* IgG (+)	5% (0–13)[a]	4%(0–9)	62%(58–65)[b]	57%(53–60)
Urea breath test (+)	4%(0–10)	0%	NA[c]	NA
Pepsinogen test[d] (+)	0%	2%(0–6)	23% (22–25)[e]	22% (20–23)

[a] Age-adjusted prevalence (95% confidence interval).
[b] The values were cited from Ref. [8].
[c] Values of urea breath test by sex and age in the Japanese populace were not available.
[d] Positive test was defined as pgI $\leqq 70$ ng/ml and pgI/pgII $\leqq 3.0$.
[e] The values were cited from Ref. [9].

Table 2
Urinary excretions of salt and potassium in Javanese vs. consumption in Japanese

	Javanese		Japanese	
	Male	Female	Male	Female
Salt (g/day)	11.0 (10.0–12.1)[a]	9.4 (8.5–10.3)	12.9 (12.7–13.1)[b]	11.2 (11.1–11.4)
Potassium (g/day)	2.1 (1.9–2.2)	2.2 (2.0–2.3)	2.5 (2.5–2.5)[b]	2.4 (2.3–2.4)

[a] Age-adjusted mean (95% confidence interval).
[b] The values were cited from Ref. [10].

the 62% (58–65) for males and 57% (53–60) for females in Japan [8]. Positive rates for UBT were 4% (0–10) for Javanese males and 0% for females. Positive findings of the PG test were 0 and 2% (0–6) for Javanese males and females, respectively, and again were significantly lower than the 23% (22–25) and 22% (20–23) reported for Japan [9]. Salt excretions were calculated to be 11.0 g/day (10.0–12.1) for males and 9.4 g/day (8.5–10.3) for females in Yogyakarta, and were significantly/marginally lower than the consumption of 12.9 g/day (12.7–13.1) for males and 11.2 g/day (11.1–11.4) for females, Japan [10] (Table 2). Potassium excretions were 2.1 g/day (1.9–2.2) for males and 2.2 g/day (2.0–2.3) for females, and were again significantly lower than the consumption of 2.5 g/day (2.5–2.5) and 2.4 g/day (2.3–2.4) in Japan.

4. Discussion

This is the first report to assess the association between prevalence of *H. pylori* infection and risk of stomach cancer in the Javanese, in Yogyakarta. Prevalence of *H. pylori* IgG antibodies were only 5% (0–13) and 4% (0–9) for males and females, respectively, in Yogyakarta, and were statistically lower than the 62% (58–65) and 57% (53–60), respectively, in Japan. Positive rates for the urea breath test were 4% (0–10) for Javanese males and 0% for females. Furthermore, positive findings of pepsinogen testing were only 0 and 2% (0–6) for males and females, in Yogyakarta and were again significantly lower than the 23% (22–25) and 22% (20–23), in Japan. The very low incidence of stomach cancer in Yogyakarta seems to be ascribed not only to a low prevalence of *H. pylori* infection but also chronic atrophic gastritis.

Serum *H. pylori* IgG was assayed by EIA using HM-CAP, which may give rise to false negatives [11], but this was offset by use of the UBT, which detects all bacteria with urease activity and thus can yield false positives. *H. pylori* IgG seroprevalence is ordinarily lower than that of UBT, but this was here not the case. The precise reason remains, however, unclear. Whatever the case, it would appear that the *H. pylori* infection rate is very low in the Javanese, which is in line with the very low seropositivity of *H. pylori* reported for Malay people [12–14]. The findings, however, seem contradictory to the hypothesis of so-called 'African/Asian paradox/enigmas,' in which the prevalence rates of *H. pylori* are high but incidence rates of stomach cancer are low in certain African and Asian countries [14,15].

H. pylori is well established to be a major factor for causing CAG, a precursor of stomach cancer [4,16]. Because results of the PG test correlate well with the Sydney classification of CAG [6,17], it can be utilized as a non-invasive surrogate for histopathological evidence. The implied low prevalence of CAG in Yogyakarta is very plausible given the present findings for *H. pylori*, which is again compatible with the observations: that is, both prevalence of gastric ulcer and incidence of stomach cancer are low in Malay people [14,18]. Host genetic polymorphisms associated with cellular immunity for bacterial infection and chronic inflammation [19], along with differences of *H. pylori* DNA [11,20], may make a certain contribution.

While consumption of salt and salty foods is another factor for stomach cancer risk [21,22], a high-salt diet and *H. pylori* infection act synergistically on the development of stomach cancer in human [23] and in animal models [24]. But the differences between values for Javanese and Japanese would suggest that consumption of salt and salty foods as well as

vegetables and fruit is less important as an explanation for the very low incidence of gastric tumors in Indonesia, where the definite and necessary carcinogen, *H. pylori*, scarcely exists. Furthermore, compared with Japan, there may be reduced exposure to exogenous carcinogens, including pyrolysate chemicals and components of tobacco smoke, and sustained yield of endogenous carcinogens, including nitrosamines generated in the stomach from nitrite and amine precursors.

We should admit that ecological studies are generally regarded as providing low-rank evidence and the number of recruited subjects was not sufficiently large to be representative of the Yogyakarta populace. Furthermore, variation in diagnostic techniques and cancer registration could have a bearing since the data for the Javanese are hospital rather than population-based. However, it seems obvious that the incidence of stomach cancers is very low and the present observations for prevalence of *H. pylori* infection and CAG markers are very suggestive that *H. pylori* is a definite and necessary factor for stomach cancer, and we may conclude that the disease has an infection-dependent etiology.

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Serum Levels of Polyunsaturated Fatty Acids and Risk of Colorectal Cancer: A Prospective Study

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To examine the relation between serum fatty acids and risk of colorectal cancer, the authors conducted a nested case-control study of 169 colorectal cancer cases and 481 controls matched by age and enrollment area as part of the Japan Collaborative Cohort Study. Serum samples were donated by subjects at baseline (between 1988 and 1990) and were stored at −80°C until 2002. Serum fatty acid levels were measured by using gas chromatography and were expressed as the weight percentage of total lipids. Conditional logistic regression analyses adjusted for lifestyle factors revealed that total ω-3 polyunsaturated fatty acids (odds ratio = 0.24, 95% confidence interval: 0.08, 0.76), α-linolenic acid (odds ratio = 0.39, 95% confidence interval: 0.16, 0.91), docosapentaenoic acid (odds ratio = 0.30, 95% confidence interval: 0.11, 0.80), and docosahexaenoic acid (odds ratio = 0.23, 95% confidence interval: 0.07, 0.76) all showed a significantly decreased risk for the highest versus the lowest quartile levels for colorectal cancer in men. For women, a weak negative association was observed between docosapentaenoic acid and colorectal cancer risk, although it was not statistically significant. No adverse effects of high serum levels of ω-6 polyunsaturated fatty acids on colorectal cancer risk were detected.

alpha-linolenic acid; chromatography; colorectal neoplasms; docosahexaenoic acids; eicosapentaenoic acid; fatty acids; prospective studies; serum

Abbreviations: CI, confidence interval; JACC Study, Japan Collaborative Cohort Study for the Evaluation of Cancer Risk; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid; Q, quartile.

A number of experimental studies have reported an association between specific fatty acids and colorectal cancer risk. In particular, protective effects of ω-3 polyunsaturated fatty acids (PUFAs) (1–5) and adverse effects of ω-6 PUFAs (6–8) have been observed. However, the evidence from epidemiologic studies is limited and inconsistent (9). One major problem is the difficulty of measuring fatty acids accurately. The fatty acid composition of serum lipids is considered a reliable index reflecting dietary intake of fatty acids over periods of weeks or months (10, 11). Nevertheless, because of the high cost of measuring serum fatty acid levels, this procedure is performed in only those studies with relatively small numbers of subjects. Here, we report the results of a nested case-control study conducted as part of a nationwide

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cohort study, in which the association between serum fatty acid levels and risk of colorectal cancer was examined prospectively.

MATERIALS AND METHODS

Subjects

Details of the Japan Collaborative Cohort Study for the Evaluation of Cancer Risk (JACC Study), sponsored by Monbukagakusho (the Ministry of Education, Culture, Sports, Science and Technology of Japan), have been reported elsewhere (12, 13). The JACC Study involved 110,792 healthy residents who were aged 40–79 years at baseline and were recruited from 45 areas throughout Japan between 1988 and 1990. The subjects for the present study were restricted to 65,184 persons who lived in 24 study areas in which cancer registries were available. On enrollment, the participants completed a self-administered questionnaire that assessed demographic characteristics, lifestyle, and medical history. Blood samples were donated by 36.6 percent of the total study group (n = 23,863). Participants who reported a previous history of cancer (n = 409) were excluded from the analysis. Written informed consent for participation was obtained individually from most subjects, with the exception of those in study areas in which informed consent was provided at the group level after the aim of the study and the confidentiality of the data had been explained to community leaders. The study protocol was approved by the Ethics Committee of Medical Care and Research of the Fujita Health University School of Medicine, Japan.

Case ascertainment and control selection

Cases were defined as subjects who developed colorectal cancer (according to the *International Statistical Classification of Diseases and Related Health Problems*, Tenth Revision, codes C18–20) during the follow-up period (mean, 7.1 years; standard deviation, 2.3), which ran until the end of 1997. We ascertained the incidence of cancer from population-based cancer registries, supplemented by a systematic review of death certificates (14). For each case, two or three controls with no previous history of cancer were selected from the population at risk. The controls were matched to each case by sex, age (±3 years, as close as possible), and participating institution. A total of 169 colorectal cancer cases (83 men and 86 women) and 481 controls (241 men and 240 women) were involved in the analysis. The numbers of colon cancer cases and matched controls were 119 (52 men and 67 women) and 336 (151 men and 185 women), respectively.

Serum fatty acid analysis

Serum was separated from the blood samples at local laboratories in or near the surveyed municipalities and was stored for 11–14 years at −80°C. All samples were analyzed in November 2002 in one laboratory by trained staff blinded to case-control status. Samples were organized in batches of up to 50, which included two samples from a standard pool for quality control. Lipids in 0.2 ml of serum were extracted with Folch's solution under a nitrogen atmosphere (15). After methyl esterification by 0.4 M potassium methoxide and 14 weight percentage boron trifluoride methanol, fatty acids were measured by using a gas chromatograph (model GC17A; Shimazu, Kyoto, Japan) equipped with an Omegawax 250 capillary column (30-m × 0.25-mm inside diameter; 0.25-μm thickness; Supelco, Bellefonte, Pennsylvania). Peaks were determined by using a flame-ionization detector and were quantified with an electric integrator (model CR-7A; Shimazu) using pure standard mixtures (Sigma, St. Louis, Missouri).

A total of 24 fatty acids were identified from 12:0 to 24:1 ω-9. The serum level of each fatty acid was expressed as the composition, by weight percentage, of total lipids. The limit of detection for the assay was 0.02 weight percent. The respective repeatability and day-to-day variation of the standard sample coefficients of variation were as follows: 5.5 percent for both measurements for 16:0; 6.2 percent and 5.6 percent for 18:2; 4.9 percent and 6.5 percent for 20:3; and 2.9 percent and 4.5 percent for 24:1.

In particular, four ω-3 PUFAs and six ω-6 PUFAs were measured: α-18:3 ω-3 (α-linolenic acid), 20:5 ω-3 (eicosapentaenoic acid), 22:5 ω-3 (docosapentaenoic acid), 22:6 ω-3 (docosahexaenoic acid), 18:2 ω-6 (linoleic acid), γ-18:2 ω-6 (γ-linolenic acid), 20:2 ω-6 (eicosadienoic acid), 20:3 ω-6 (dihomo-γ-linolenic acid), 20:4 ω-6 (arachidonic acid), and 22:4 ω-6 (docosatetoraenoic acid). In addition, we calculated the content of total saturated fatty acids (12:0 + 14:0 + 16:0 + 18:0 + 20:0 + 22:0 + 24:0), monounsaturated fatty acids (MUFAs; 16:1 ω-7 + 18:1 ω-9 + 20:1 ω-9 + 22:1 ω-9 + 24:1 ω-9), and total ω-3 and ω-6 PUFAs. The ratio of ω-6 to ω-3 PUFAs was also determined.

Statistical methods

Background characteristics were compared between cases and controls by using the Cochran-Mantel-Haenszel test (16) and analysis of covariance (17), with adjustment for matching factors (age and area of enrollment) by sex. Spearman's correlation coefficients were calculated to determine the association between fatty acids. Conditional logistic regression models were used to calculate odds ratios for the incidence of colorectal cancer (18) for the serum level of each specific fatty acid. The odds ratios for colon cancer risk were also examined separately from those for rectal cancer. Cases and controls were divided into four groups according to the level of fatty acids in controls. Odds ratios were calculated for the second quartile ((Q)2), third quartile (Q3), and highest quartile (Q4) versus the lowest quartile (Q1). To test for linear trends in odds ratios over quartiles, we coded each quartile as 0, 1, 2, or 3 and incorporated these data into the logistic model as a single variable.

We adjusted for the following factors by including them in the logistic models (19): age at completing final education (≤18 years or ≥19 years); history of colorectal cancer in parents or siblings (yes or no); body mass index (weight (kg)/height (m)^2; <20.0 kg/m^2, 20.0–24.9 kg/m^2, or ≥25.0 kg/m^2) calculated from reported height and weight at baseline; smoking status (never, former, or current); daily alcohol

TABLE 1. Baseline characteristics of colorectal cancer cases and controls from the Japan Collaborative Cohort Study for the Evaluation of Cancer Risk, 1988–1997

Variable	Men		Women	
	Cases (n = 83)	Controls (n = 241)	Cases (n = 86)	Controls (n = 240)
Age in years (mean (standard deviation))	61.0 (8.0)	60.5 (7.7)	62.7 (7.8)	62.4 (7.8)
Body mass index† (mean (standard deviation))	23.1 (2.7)	22.8 (2.7)	22.9 (3.5)	23.3 (3.2)
History of colorectal cancer in parents or siblings: yes (%)	4.8	1.7	9.3	2.9*
Smoking history (%)				
Current smoker	44.6	46.1	3.5	2.9
Former smoker	34.9	27.8	2.3	2.1
Never smoker	16.9	21.6	87.2	86.7
Unreported	3.6	4.6	7.0	8.3
Alcohol drinking history (%)				
Current drinker	79.5	75.5	23.3	18.8
Former drinker	2.4	3.7	0.0	1.7
Never drinker	16.9	17.5	73.3	74.2
Unreported	1.2	3.3	3.4	5.3
Intake frequency of green leafy vegetables (e.g., spinach): almost every day (%)	56.6	58.5	53.5	56.3
Physical exercise ≥3 hours per week (%)	15.7	16.2	8.1	12.1
Education: age ≥19 years at completion of full-time education (%)	15.7	12.9	9.3	8.3

* $p < 0.05$ by the Cochrane-Mantel-Haenzel test adjusted for age and area of enrollment.
† Weight (kg)/height $(m)^2$.

consumption (never, former, or current); frequency of intake of green leafy vegetables (almost every day or ≤3–4 days per week), and time spent exercising (<3 hours per week or ≥3 hours per week) (20). The results were not significantly affected by adjustment for potential confounding factors; therefore, only the multivariate-adjusted odds ratios are presented in the tables in this paper. To eliminate the influence of undiagnosed colorectal cancers at baseline, the analyses were repeated by excluding men and women who developed colorectal cancer within the first 2 or 5 years of follow-up, respectively, along with their matched controls.

All analyses were performed by using SAS software, release 8.2 (SAS Institute, Inc., Cary, North Carolina). In the conditional logistic regression analysis, missing values for each categorical covariate were treated as an additional category of the variable and were included in the model. Two-tailed probability (p) values of <0.1 were considered marginally significant, and p values of <0.05 were considered statistically significant.

RESULTS

Table 1 gives the baseline characteristics of all colorectal cancer cases and controls by sex. For both men and women, the age distributions of the cases and controls were well matched. Cases were more likely than controls to have a family history of colorectal cancer. There were no significant differences between cases and controls regarding body mass index, educational level, smoking and alcohol drinking habits, or frequency of green leafy vegetable intake or physical exercise.

Spearman's correlation coefficients between the fatty acids were computed (data not shown). The directions of the associations were not affected by sex, and all correlation coefficients were statistically significant. For both men and women, ω-3 PUFAs were mildly inversely correlated with ω-6 PUFAs ($r = -0.24$ and $r = -0.12$, respectively), MUFAs ($r = -0.30$ and $r = -0.44$, respectively), and saturated fatty acids ($r = -0.18$ and $r = -0.27$, respectively). In men and women, ω-6 PUFAs were moderately inversely correlated with MUFAs ($r = -0.68$ for both sexes) and saturated fatty acids ($r = -0.72$ and $r = -0.77$, respectively). In addition, MUFAs and saturated fatty acids were mildly positively correlated in both men and women ($r = 0.35$ and $r = 0.46$, respectively).

Table 2 shows the associations between the serum levels of each group of fatty acids and the risk of colorectal cancer by sex. For men, total saturated fatty acids and ω-6 PUFAs failed to show significant associations with colorectal cancer risk. A marginally significant positive trend was observed between serum level of total MUFAs and colorectal cancer risk (p for linear trend = 0.06). Total ω-3 PUFAs were inversely associated with colorectal cancer risk, showing a 76 percent risk reduction when Q4 and Q1 were compared (odds ratio = 0.24, 95 percent confidence interval (CI): 0.08, 0.76; p for linear trend = 0.08). For the ω-6/ω-3 ratio, Q2 showed a marginally significant association, with a 2.36-fold increased risk of colorectal cancer relative to Q1 (95 percent CI: 0.99, 5.66), although the dose-response relation was

TABLE 2. Associations of serum level of fatty acids with colorectal cancer risk, by sex, Japan Collaborative Cohort Study for the Evaluation of Cancer Risk, 1988–1997

Q*	Men						Women					
	Value†	Cases (no.)	Controls (no.)	OR*,‡	95% CI*	*p* trend	Value†	Cases (no.)	Controls (no.)	OR‡	95% CI	*p* trend
						Saturated fatty acids						
Q1	<31.91	18	60	1.00		0.36	<31.40	24	60	1.00		0.17
Q2	31.91–33.73	20	60	1.22	0.51, 2.91		31.40–33.00	27	60	1.10	0.53, 2.32	
Q3	33.74–36.04	20	59	1.12	0.47, 2.64		33.01–35.40	16	60	0.56	0.24, 1.30	
Q4	≥36.05	25	62	1.71	0.66, 4.47		≥35.41	19	60	0.59	0.23, 1.52	
						Monounsaturated fatty acids						
Q1	<20.78	13	50	1.00		0.06	<20.44	24	56	1.00		0.51
Q2	20.78–22.49	13	51	1.04	0.40, 2.74		20.44–22.14	23	57	0.96	0.45, 2.02	
Q3	22.50–24.67	17	50	1.48	0.59, 3.72		22.15–24.12	16	56	0.70	0.30, 1.65	
Q4	≥24.68	28	52	2.05	0.86, 4.89		≥24.13	19	57	0.83	0.36, 1.92	
						ω-3 polyunsaturated fatty acids						
Q1	<7.74	24	60	1.00		0.08	<7.84	24	60	1.00		0.96
Q2	7.74–9.639	19	59	0.76	0.34, 1.72		7.84–9.379	18	60	0.53	0.23, 1.20	
Q3	9.64–12.03	31	61	1.09	0.49, 2.44		9.38–10.96	21	60	0.75	0.35, 1.63	
Q4	≥12.04	9	61	0.24	0.08, 0.76		≥10.97	23	60	0.85	0.38, 1.91	
						ω-6 polyunsaturated fatty acids						
Q1	<28.89	26	60	1.00		0.36	<31.90	23	60	1.00		0.32
Q2	28.89–32.77	19	60	0.79	0.38, 1.64		31.90–34.30	10	59	0.44	0.17, 1.11	
Q3	32.78–36.10	18	60	0.67	0.30, 1.47		34.31–37.53	27	61	1.28	0.58, 2.82	
Q4	≥36.11	20	61	0.69	0.30, 1.61		≥37.54	26	60	1.15	0.48, 2.75	
						ω-6/ω-3 ratio						
Q1	<2.59	14	60	1.00		0.33	<3.07	22	60	1.00		0.90
Q2	2.59–3.369	27	60	2.36	0.99, 5.66		3.07–3.749	20	60	0.84	0.37, 1.92	
Q3	3.37–4.389	19	60	1.76	0.67, 4.64		3.75–4.579	19	60	0.76	0.35, 1.65	
Q4	≥4.39	23	61	2.05	0.78, 5.40		≥4.58	25	60	1.08	0.50, 2.36	

* Q, quartile; OR, odds ratio; CI, confidence interval.

† Values are expressed as the weight percentage of total serum lipids.

‡ Odds ratios were derived from a conditional logistic analysis model adjusted for family history of colorectal cancer in first-degree relatives, body mass index, education, smoking and alcohol drinking history, green leafy vegetable intake, and physical exercise. For men, 83 cases and 241 controls and, for women, 86 cases and 240 controls matched on age and participating institution were involved in the analyses.

unclear. In the analysis of colon cancer risk alone (data not shown), there was no statistically significant association with the serum levels of any of the fatty acids. However, the directions of the nonsignificant trends were similar to those observed in the combined analyses. The odds ratios for Q4 versus Q1 of each group of fatty acids for the risk of colon cancer in men were as follows: 1.00 for saturated fatty acids (95 percent CI: 0.28, 3.52; *p* for linear trend = 0.84), 1.19 for MUFAs (95 percent CI: 0.34, 4.22; *p* for linear trend = 0.60), 0.40 for ω-3 PUFAs (95 percent CI: 0.10, 1.55; *p* for linear trend = 0.43), and 1.04 for ω-6 PUFAs (95 percent CI: 0.34, 3.16; *p* for linear trend = 0.86).

For women, the association between the levels of all groups of fatty acids, except saturated fatty acids, and colorectal cancer risk tended to be U- or J-shaped, although any associations failed to reach the statistically significant level (table 2). Similar results were obtained when the analyses were repeated for colon cancer cases alone (data not shown). The odds ratios for Q4 versus Q1 of groups of fatty acids were as follows: 0.56 for saturated fatty acids (95 percent CI: 0.20, 1.59; *p* for linear trend = 0.12), 0.70 for MUFAs (95 percent CI: 0.26, 1.84; *p* for linear trend = 0.53), 0.90 for ω-3 PUFAs (95 percent CI: 0.37, 2.20; *p* for linear trend = 0.46), and 1.01 for ω-6 PUFAs (95 percent CI: 0.36, 2.86; *p* for linear trend = 0.46).

To exclude the influence of undiagnosed colorectal cancer at baseline, the analyses were repeated by excluding men and women who developed colorectal cancer during the first 2 or 5 years of follow-up, respectively, along with their matched controls (table 3). For men, excluding those who developed colorectal cancer within the first 5 years strengthened both the positive association of total MUFAs and the inverse association of total ω-3 PUFAs with colorectal cancer risk. For women, excluding those who developed colorectal cancer within the first 2 years revealed a significant association for ω-3 PUFAs, with a 70 percent decreased risk of colorectal cancer at the Q2 compared with the Q1 level (odds ratio = 0.30, 95 percent CI: 0.11, 0.79).

Table 4 shows the association of levels of specific ω-3 fatty acids with colorectal cancer risk. For men, of the four

TABLE 3. Associations of serum level of fatty acids with colorectal cancer risk, by length of cases' follow-up period, Japan Collaborative Cohort Study for the Evaluation of Cancer Risk, 1988–1997

Q*,†	Follow-up period for men						Follow-up period for women					
	>2 years (*n* = 64 cases; *n* = 186 controls)			>5 years (*n* = 40 cases; *n* = 115 controls)			>2 years (*n* = 69 cases; *n* = 195 controls)			>5 years (*n* = 30 cases; *n* = 84 controls)		
	OR*,‡	95% CI*	*p* trend	OR‡	95% CI	*p* trend	OR‡	95% CI	*p* trend	OR‡	95% CI	*p* trend
						Saturated fatty acids						
Q1	1.00		0.84	1.00		0.45	1.00		0.31	1.00		0.58
Q2	1.74	0.6, 4.44		1.23	0.32, 4.68		0.59	0.25, 1.39		0.89	0.10, 7.90	
Q3	1.02	0.3, 2.75		1.09	0.23, 5.10		0.43	0.17, 1.09		1.52	0.21, 11.0	
Q4	1.04	0.3, 3.15		1.72	0.33, 9.07		0.69	0.23, 2.02		4.02	0.23, 71.7	
						Monounsaturated fatty acids						
Q1	1.00		0.18	1.00		0.03	1.00		0.72	1.00		0.71
Q2	1.36	0.4, 4.25		2.92	0.51, 16.6		0.99	0.44, 2.20		2.05	0.36, 11.6	
Q3	1.80	0.5, 5.56		1.88	0.33, 10.7		0.67	0.26, 1.73		1.35	0.19, 9.64	
Q4	1.90	0.6, 5.23		8.85	1.37, 57.4		1.00	0.38, 2.60		0.70	0.07, 6.87	
						ω-3 polyunsaturated fatty acids						
Q1	1.00		0.35	1.00		0.09	1.00		0.33	1.00		0.80
Q2	0.82	0.32, 2.08		0.38	0.10, 1.48		0.30	0.11, 0.79		0.04	0.00, 0.63	
Q3	1.46	0.58, 3.68		0.79	0.21, 3.00		0.68	0.29, 1.61		0.01	0.00, 0.51	
Q4	0.30	0.08, 1.16		0.26	0.05, 1.32		0.47	0.18, 1.20		0.59	0.08, 4.52	
						ω-6 polyunsaturated fatty acids						
Q1	1.00		0.68	1.00		0.24	1.00		0.18	1.00		0.66
Q2	1.07	0.45, 2.55		2.03	0.56, 7.33		0.35	0.12, 1.05		0.25	0.01, 5.28	
Q3	0.89	0.36, 2.22		0.75	0.18, 3.12		1.28	0.51, 3.20		3.82	0.33, 43.8	
Q4	0.83	0.32, 2.19		0.54	0.10, 2.78		1.36	0.52, 3.54		1.01	0.13, 8.12	
						ω-6/ω-3 ratio						
Q1	1.00		0.38	1.00		0.36	1.00		0.38	1.00		0.66
Q2	2.57	0.91, 7.29		2.50	0.59, 10.5		1.07	0.41, 2.81		0.95	0.14, 6.62	
Q3	2.07	0.69, 6.23		1.10	0.23, 5.19		0.69	0.27, 1.75		0.60	0.10, 3.79	
Q4	2.12	0.69, 6.54		2.64	0.57, 12.2		1.73	0.71, 4.23		1.74	0.30, 10.2	

* Q, quartile; OR, odds ratio; CI, confidence interval.

† Quartiles were determined by the distribution of each fatty acid, expressed as the weight percentage of total serum lipids, in controls.

‡ Odds ratios were derived from a conditional logistic analysis model adjusted for family history of colorectal cancer in first-degree relatives, body mass index, education, smoking and alcohol drinking history, green leafy vegetable intake, and physical exercise. Cases and controls were matched on age and participating institution.

ω-3 PUFAs examined, all except eicosapentaenoic acid showed significant or marginally significant inverse associations with colorectal cancer risk. Eicosapentaenoic acid narrowly failed to show a significant inverse association with colorectal cancer risk (Q4 vs. Q1 odds ratio = 0.44, 95 percent CI: 0.18, 1.08; *p* for linear trend = 0.13). The inverse associations between serum levels of specific ω-3 PUFAs and colorectal cancer risk became more significant when cases were restricted to only those followed up for more than 5 years. For men, the odds ratios for Q4 versus Q1 were as follows: 0.10 for α-linoleic acid (95 percent CI: 0.01, 0.86; *p* for linear trend = 0.23), 0.44 for eicosapentaenoic acid (95 percent CI: 0.10, 1.94; *p* for linear trend = 0.13), 0.24 for docosapentaenoic acid (95 percent CI: 0.05, 1.09; *p* for linear trend = 0.07), and 0.07 for docosahexaenoic acid (95 percent CI: 0.01, 0.70; *p* for linear trend = 0.10).

For women, a significantly increased risk for Q3 compared with Q1 was observed for α-linolenic acid (table 4). For the other ω-3 PUFAs, the odds ratios for the highest versus the lowest quartiles were less than 1.0. When those participants who developed colorectal cancer within the first 5 years of follow-up were excluded from the analyses, all ω-3 PUFAs showed a decreased risk at the highest level (data not shown). The odds ratios for Q4 versus Q1 for women were as follows: 0.64 for α-linolenic acid (95 percent CI: 0.11, 3.75; *p* for linear trend = 0.51), 0.55 for eicosapentaenoic acid (95 percent CI: 0.10, 3.11; *p* for linear trend = 0.40), 0.86 for docosapentaenoic acid (95 percent CI: 0.17, 4.45; *p* for linear trend = 0.58), and 0.53 for docosahexaenoic acid (95 percent CI: 0.07, 3.98; *p* for linear trend = 0.80). No significant linear trend was detected between the serum levels of any ω-6 PUFAs and colorectal cancer risk for men or women (table 5). Only eicosadienoic acid showed a significant association with a decreased risk of colorectal cancer at the Q3 versus Q1 level for men (odds ratio = 0.18, 95 percent CI: 0.06, 0.56).