表7

著者	Y. Suwazono, et al
論文名	The factors related to undergoing stomach examination and through medical examination in Japanese male workers
発表雑誌	J Occup Health, 42(3), 144-146, 2000
目的（受診率向上）は明確か？	受診の要因調査
解析方法（RCT／観察的）は？	観察的研究
対象集団の定義？　人数？	自動車工場勤務の男性466人
介入群と対照群は明確に定義されているか？	定義されていない
評価指標は明確に定義されているか？	胃がん検診受診のレセプト（症状受診も含まれていると思われる）
結果	多変量解析で差が出たのは、技術系職種・飲酒歴・時間の束縛・検査の知識。
研究の限界が記載されているか？ Bias は？	研究対象者数が少ない。後ろ向き研究。一般集団には応用できない。
結論	時間の束縛を改善させるために、ペプシノゲン法の導入も必要かもしれないという結論。
結論は適切に評価されているか？	受診関連要因であり、直接受診率向上につながるものはない。
日本において適用可能か？	日本の研究である。

表8

著者	加藤育子、他
論文名	無既往・無自覚症状者の生活習慣の特徴
発表雑誌	日本公衆衛生学会誌、35(10)、556-561、1988
目的（受診率向上）は明確か？	ベースラインコホートの解析であり、無自覚無症状一般集団の特性分析である。
解析方法（RCT／観察的）は？	観察的研究（コホート研究）
対象集団の定義？　人数？	40歳以上の全住民33,538例中、有効回答者30,070人（有効回答率92.8%）
介入群と対照群は明確に定義されているか？	観察的研究で、介入群は設けられていない。
評価指標は明確に定義されているか？	無自覚・無症状者の割合
結果	無症状・無自覚の健康群では、がん検診への参加が少ない。
研究の限界が記載されているか？ Bias は？	断面調査のため疾病や症状の結果も含まれる。
結論	何らかの疾患や自覚症状があるものが検診に参加しやすい。
結論は適切に評価されているか？	受診関連要因であり、直接受診率向上につながるものはない。
日本において適用可能か？	日本の研究である。

考　察

今回の検討対象となった8論文はすべて日本での研究論文であったが、胃がん検診を老人保健法という社会システムの中で実施している唯一の国であるのでこれも当然のことである。日本からの研究論文であるので、「日本における胃がん検診受診率を向上させるにはどうすれば良いか？」という Key Question にはそのまま回答を与えることができるという点で有用ではあったが、無作為化比較対照試験による研究は皆無であり、質的に満足できるものではなかった。

受診率向上対策についての3論文から示唆されるのは、1）チケット制を含む個別検診は多様な住民の利便に応えることによって受診者数を増加させるかも知れないが、個別検診は既に

全国的に導入されつつある。2）基本検診や他のがん検診等と併せて複合化（総合化）することは、多様な住民の利便に応えることによって受診者数を増加させるかも知れないが、これも既に全国的に導入されつつある。ということで新たな知見ではなかった。

受診者の要因調査についての5論文からは受診率向上に直接言及するものはなかったが以下のことが示唆された。すなわち、1）胃がん検診受診率に関連する因子として、家族のがん既往歴が正の相関を示したが、一方無自覚・無症状の「健常者」の受診率は低く、検診の本来の対象者を受診につなげる動機付けができていなかった。2）受診者の心理学的分析によれば、胃癌の深刻さの認識を増長することは、受診率低下を来す可能性が示唆された。禁煙指導のような心理学的アプローチが今後必要となるのかもしれない。

このように、胃がん検診の受診率向上対策としては、1）個別検診、2）他の検診・健診と一緒に実施すること、3）心理学的アプローチを行うことが有用と考えられた。

おわりに

以上のような点を地域における胃がん検診システムに取り込んで行く必要があると考える。

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Laboratory/Clinical Translational Research

Oncology

Oncology 2005;68:454–461
DOI: 10.1159/000086988

Received: August 25, 2004
Accepted after revision: December 12, 2004
Published online: July 14, 2005

Leptin Is Associated with an Increased Female Colorectal Cancer Risk: A Nested Case-Control Study in Japan

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Key Words

Colorectal cancer · Epidemiology · Japanese women · Leptin · Nested case-control study

Abstract

Objective: To elucidate whether leptin is involved in the etiology of female colorectal cancer. ***Methods:*** A case-control study nested in the Japan Collaborative Cohort Study. We compared serum leptin levels in 58 cases of female colorectal cancer with those in 145 controls matched for study area and age. Data were analyzed using a conditional logistic regression model with adjustments for known risk factors for the development of colorectal cancer. Quintile cutoff points were determined on the distribution of leptin levels in cases and controls combined. ***Results:*** Serum geometric mean levels of leptin were 6.88 ng/ml in cases and 6.00 ng/ml in controls. The odds ratios of female colorectal cancer risk were 1.40 (95% confidence interval, CI: 0.41–4.78) for the category of the second and third quintiles combined, and 4.84 (CI: 1.29–18.1) for the category of the fourth and fifth quintiles combined relative to the first quintile after adjustment for body mass index (BMI), life-style factors, reproductive factors, and hormonal variables including insulin-like growth factor and its binding protein. ***Conclusion:*** Our results suggest that leptin most likely increases the risk of female colorectal cancer substantially independent of BMI.

Introduction

Leptin, the adipocyte-derived hormone, regulates satiety and energy expenditure by carrying information to the brain concerning the size of energy stores [1]. Circulating levels of leptin are elevated in obesity [2] and are

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increased by high-energy food intake [3]. Leptin is also implicated in the regulation and function of reproductive hormones [4–6], and circulating leptin levels have been negatively associated with smoking and positively associated with alcohol intake [7]. Furthermore, leptin has been shown to regulate growth hormone (GH) secretion [8], and serum leptin has been negatively associated with circulating insulin-like growth factor-I (IGF-I)/IGF-binding protein-3 (IGFBP-3) ratio in normal and growth-hormone-deficient humans [9].

The disorders associated with insulin resistance syndrome, obesity [10] and hyperinsulinemia [11], and reproductive factors such as parity, age at menarche, and menopausal status [12, 13] are implicated in the etiology of female colorectal cancer. IGFs and their binding protein, IGFBP-3, also play an important role in the pathogenesis of colorectal cancer [14–16]. Recent experimental studies have shown that leptin stimulates the proliferation and invasiveness of human colon cancer cells [17–20]. In view of these data, the possibility exists that leptin might be directly related to the etiology of female colorectal cancer by underlying the associations between the above risk factors and colorectal cancer.

Therefore, we have conducted a nested case-control study among Japanese women to assess the role of leptin in relation to female colorectal cancer after adjusting for potential confounding effects, including body mass index (BMI), life-style factors, reproductive factors, and IGFs and their binding protein.

Subjects and Methods

Study Population and Serum Sample

We conducted a nested case-control study as a part of the Japan Collaborative Cohort (JACC) Study sponsored by the Monbukagakusho (the Ministry of Education, Culture, Sports, Science and Technology of Japan). The details of the JACC study are described elsewhere [21]. Briefly, our study was initiated in 1988, and enrollment continued until the end of 1990. A total of 45 areas were involved in this prospective study, and 127,477 apparently healthy inhabitants of these areas were enrolled upon completion of the questionnaire. Among them, 110,792 (46,465 men and 64,327 women), aged 40–79 years, were followed up. Of the 64,327 women, 38,720 lived in 24 study areas where cancer registries were available. In addition to filling out the questionnaire, the participants who lived in the area where health check-ups were conducted, were asked to donate fasting blood samples during the same period as the questionnaire survey. Eventually, 16,070 women (41.5% of 38,720 female respondents to the questionnaire survey) provided blood samples. Sera were separated from the samples as soon as possible. Each serum was separated into three to five tubes (100–500 μl per tube) and stored in deep freezers at –80°C until analyzed.

Informed consent was obtained from all participants. The present study protocol was approved by the Ethics Committee of the Fujita Health University, Toyoake, Japan.

Case Identification and Control Selection

We used population registries in local municipalities to determine the vital and residential status of the subjects. Registration of death is required by the Family Registration Law in Japan and is enforced nationwide. For logistical reasons, we discontinued the follow-up of subjects who had moved out of their study areas.

Diagnosis of colorectal cancer was defined by the C18, C19, and C20 codes in the International Statistical Classification of Diseases and Related Health Problems, 10th Revision. We ascertained the incidence of cancer by consulting the records of population-based cancer registries, supplemented by a systematic review of death certificates. In some areas, medical records of major local hospitals were also reviewed. The follow-up was conducted from the time of the baseline survey through the end of 1997 except for three areas where it ended in 1994, 1995, and 1996. The incidence/death ratio for female colorectal cancer was 2.26 in the cohort covered by cancer registries. This figure is comparable with those in acceptably accurate population-based cancer registries in Japan (1.89–3.70) [22], indicating that a reasonably high proportion of female colorectal cancer cases were identified.

During the mean follow-up of 7.9 years, 97 incident cases of colorectal cancer were documented among the women who had provided serum samples. Two or three controls for each case were randomly selected from the subjects without incident cancer or a previous history of any cancer by matching for sex, age, and study area. Of 97 cases, 32 were excluded because of insufficient samples for measurement. We excluded 7 more because appropriate controls were not available. Finally, 58 cases of female colorectal cancer (including 44 cases of colon cancer) and 145 controls were involved in the present analysis.

Biochemical Analysis

All samples were analyzed at a single laboratory by the trained staff blinded to the case-control status. Serum leptin concentrations were determined by an immunometric sandwich enzyme immunoassay (Cayman) that permits leptin measurements within the range of 1–50 ng/ml, typically with a detection limit of 1 ng/ml. The inter-assay coefficient of variation (CV) was 8.5% and the intra-assay CV range was 2.3–6.1%. There were no significant differences in the means of leptin levels among study areas.

Serum levels of IGF-I, IGF-II, and IGFBP-3 were measured by immunoradiometric assay, using commercially available kits (Daiichi). Serum insulin was quantitated by a two-step sandwich enzyme immunoassay (Eiken).

Statistical Analysis

Logarithmic transformation (ln) with reduced skewness was used for leptin concentrations. Geometric means and 95% confidence intervals (CIs) of serum leptin levels are presented. Baseline characteristics of cases and controls were compared by χ^2 test and two-way analysis of variance (ANOVA) allowing for the matching. The cross-sectional relationships among study variables were examined by the Spearman correlation coefficient. Odds ratios (ORs) and 95% CI for disease were calculated using conditional logistic regression by quintile of leptin levels, matching for age and study area. Quintile cutoff points were determined on the distribution of

Table 1. Baseline characteristics for excluded and study cases with colorectal cancer and control subjects

	Excluded cases		p value[1]	Study cases		p value[2]	Controls	
	n	%		n	%		n	%
Individuals	39			58			145	
Age								
40–49 years	0			4	6.9		10	6.9
50–59 years	8	20.5	0.09	20	34.5	0.99	50	34.5
60–69 years	19	48.7		24	41.4		64	42.1
70–79 years	12	30.8		10	17.2		24	16.6
BMI at baseline								
<22 kg/m^2	15	38.5		23	39.7		51	35.2
22–25 kg/m^2	10	25.6	0.53	21	36.2	0.33	45	31.0
≥25 kg/m^2	12	30.8		11	19.0		45	31.0
Unknown	2	5.1		3	5.2		4	2.8
Smoking status								
Nonsmoker or ever smoker	32	82.1		52	89.7		130	89.7
Current smoker	1	2.6	0.40	2	3.4	0.57	2	1.4
Unknown	6	15.4		4	6.9		13	9.0
Alcohol drinking status								
Nondrinker or ever drinker	27	69.2		45	77.6		117	80.7
Current drinker	8	20.5	0.65	9	15.5	0.56	23	15.9
Unknown	4	10.3		4	6.9		5	3.4
Exercise								
Seldom	24	61.5		34	58.6		89	61.4
≥1–2 h/week	14	35.9	0.10	15	25.9	0.78	31	21.4
Unknown	1	2.6		9	15.5		25	17.2
Consumption of beef								
≤2 times/month	18	46.2		15	25.9		40	27.6
1–2 times/week	7	17.9	0.21	17	29.3	0.91	42	29.0
≥3 times/week	7	17.9		14	24.1		29	20.0
Unknown	7	17.9		12	20.7		34	23.4
Consumption of green leafy vegetables								
≤2 times/month	7	17.9		4	6.9		6	4.1
1–2 times/week	9	23.1	0.12	9	15.5	0.22	40	27.6
≥3 times/week	20	51.3		33	56.9		79	54.5
Unknown	3	7.7		12	20.7		20	13.8
Family history of colorectal cancer								
Yes	4	10.3	0.34	3	5.2	0.69	5	3.4
No	35	89.7		55	94.8		140	96.6
Parity								
0–1	5	12.8		6	10.3		7	4.8
2–3	21	53.8	0.61	39	67.2	0.18	91	62.8
≥4	9	23.1		9	15.5		40	27.6
Unknown	4	10.3		4	6.9		7	4.8
Age at menarche								
≤13 years	5	12.8		8	13.8		26	17.9
14–15 years	9	23.1	0.13	26	44.8	0.79	58	40.0
≥16 years	23	59.0		21	36.2		56	38.6
Unknown	2	5.1		3	5.2		5	3.4
Menopause								
Yes	36	92.3	0.88	54	93.1	0.78	132	91.0
No	3	7.7		4	6.9		13	9.0
Leptin levels, ng/ml (geometric mean and 95% CI)				6.88 (5.84–8.12)		0.21	6.00 (5.32–6.77)	

[1] p for difference between excluded and available cases.
[2] p for difference between available cases and controls.

leptin levels in controls. Since the analysis of leptin consistently showed similar ORs for quintiles 2 and 3 and quintiles 4 and 5, these quintiles were analyzed in combination. In multivariate analysis, further adjustments were made for smoking status (never or ever, current), alcohol consumption (never or ever, current), exercise: 'How often do you engage in exercise or sport per week?' (≥ 1–2 h, seldom), green leafy vegetable intake (≤ 2 times/month, 1–2 times/week, ≥ 3 times/week), beef intake (≤ 2 times/month, 1–2 times/week, ≥ 3 times/week), family history of colorectal cancer in parents and/or siblings (yes, no), BMI (weight in kilograms/height in meters squared: <22, 22–25, ≥ 25 kg/m^2), parity (0–1, 2–3, ≥ 4 times), age at menarche (≤ 13, 14–15, ≥ 16 years), and menopausal status (yes, no). Both BMI and parity were categorized according to the results of our previous studies [10, 23]. As hormone replacement therapy was not popular at baseline (in 1988) in Japan, the questionnaire on hormone replacement therapy was not included in our study. Using additional multivariate analysis, insulin, IGF-I, IGF-II, and IGFBP-3 were adjusted as continuous variables. These variables were assessed by the baseline questionnaire or using serum samples, and were selected as covariates since they were known or suspected to modify the risk of female colorectal cancer. Missing values for categorical variables were treated as a separated category in the analysis, whereas missing continuous values in the logistic regression analysis were replaced by the mean value representing each reference. To test for linear trends in ORs over quintiles combined into three categories, we coded them as 1, 2, and 3, and then incorporated the coded number into the logistic model as a single variable. All calculations were made with the statistical program SPSS (version 10.0; SPSS, Chicago, Ill., USA). All p values were based on two-sided tests in which $p < 0.05$ was considered statistically significant.

Results

The distributions of the 39 excluded cases, the 58 study cases, and the 145 controls by age, potential colorectal cancer risk factors, reproductive factors and serum leptin levels are shown in table 1. There were no significant differences between excluded and study cases in these variables. No significant differences between cases and controls were observed, too. The controls were likely to have higher but non-significant BMI than study cases. The controls seemed to show less parity in agreement with the risk profile for colorectal cancer. The geometric mean level of leptin was 6.88 ng/ml among cases and 6.00 ng/ml among controls.

Since blood samples had been collected before diagnosis, we combined the data of cases and controls for an analysis of interrelationships between leptin and other variables (table 2). Serum leptin was correlated to factors associated with the insulin resistance syndrome, i.e. BMI and serum insulin level. Serum leptin was also correlated to IGF-I and IGFBP-3 but not to IGF-II.

Table 2. Spearman correlation coefficients between leptin and selected variables

Variable	Correlation coefficients	p value
Age	0.04	0.62
BMI	0.42	<0.01
Parity	0.10	0.18
Age at baseline	–0.05	0.51
Insulin	0.26	<0.01
IGF-I	0.16	0.03
IGF-II	0.10	0.17
IGFBP-3	0.24	<0.01

Table 3 presents the above-mentioned variables by leptin quintiles. BMI values increased significantly with increasing levels of leptin. There were no significant differences in other variables by leptin quintiles.

In logistic regression analysis, the unadjusted ORs of colorectal cancer and 95% CIs according to the tertiles of leptin were 1.0, 0.80 (95% CI: 0.34–1.87), and 1.58 (0.78–3.31) (p for trend = 0.175). The unadjusted ORs by the quintiles of leptin were 1.0, 1.38 (95% CI: 0.47–4.10), 0.95 (0.31–2.92), 2.66 (0.99–7.20), and 1.87 (0.69–5.12) (p for trend = 0.08). Since similar ORs were seen for quintiles 2 and 3 and quintiles 4 and 5, these quintiles were combined into two groups (Q_{2-3}, Q_{4-5}).

The ORs, compared to Q_1, were 1.16 (0.44–3.05) for Q_{2-3} and 2.07 (0.83–5.18) for Q_{4-5} (p for trend = 0.060; table 4). Simultaneous adjustments for BMI at baseline, potential colorectal cancer risk factors, and reproductive factors strengthened the association of colorectal cancer risk with elevated leptin levels. Further adjustments for insulin, IGF-I, IGF-II, and IGFBP-3 did not change the association. As the association of colorectal cancer risk with elevated leptin levels was affected by BMI at baseline, we calculated the ORs, stratified by BMI 25 kg/m^2. The ORs adjusted for BMI compared to Q1 were 0.74 (0.15–3.78) for Q_{2-3} and 2.20 (0.42–11.8) for Q_{4-5} (p for trend = 0.099) among subjects with BMI <25 kg/m^2 and the respective ORs were 1.54 (0.26–9.02) and 3.15 (0.44–22.5) (p for trend = 0.021) among subjects with BMI ≥ 25 kg/m^2. The association almost showed the same trend although a significant trend was observed among subjects with BMI ≥ 25 kg/m^2. Furthermore, after adjustment for continuous BMI at baseline instead of categorical variable, the association of colorectal cancer risk with elevated leptin levels as above mentioned did not change.

Table 3. Age, BMI at baseline, life-style factors, and reproductive factors for quintiles of serum leptin levels

	Q1		Q2		Q3		Q4		Q5		p value
	n	%	n	%	n	%	n	%	n	%	
Range of leptin levels, ng/ml	1.0–3.4		3.5–5.3		5.4–7.5		7.6–11.5		11.7–28.0		
Individuals	37		38		37		48		43		
Age	60.7 ± 1.35		62.1 ± 1.33		63.6 ± 1.35		61.4 ± 1.18		61.9 ± 1.25		0.65
40–49 years	4	10.8	1	2.6	3	8.1	4	8.3	2	4.7	0.28
50–59 years	16	43.2	14	36.8	6	16.2	17	35.4	17	39.5	
60–69 years	9	24.3	18	47.4	19	51.4	22	45.8	17	39.5	
70–79 years	8	21.6	5	13.2	9	24.3	5	10.4	7	16.3	
BMI at baseline	21.6 ± 0.43		21.6 ± 0.42		23.7 ± 0.43		23.7 ± 0.38		25.0 ± 0.39		0.01
<22 kg/m^2	21	56.8	20	52.6	13	35.1	13	27.1	7	16.3	0.01
22–25 kg/m^2	10	27.0	15	39.5	11	29.7	14	29.2	16	32.5	
≥25 kg/m^2	5	13.5	2	5.3	11	29.7	19	39.6	56	27.6	
Unknown	1	2.7	1	2.6	2	5.4	2	4.2	7	3.4	
Smoking status											
Nonsmoker or ever smoker	34	91.9	35	92.1	33	89.2	43	89.6	37	86.0	0.86
Current smoker	0		1	2.6	0		1	2.1	2	4.7	
Unknown	3	8.1	3	5.3	4	10.8	4	8.3	4	9.3	
Alcohol drinking status											
Nondrinker or ever drinker	30	81.1	31	81.6	29	78.4	36	75.0	36	83.7	0.79
Current drinker	5	13.5	7	18.4	5	13.5	9	18.8	6	14.0	
Unknown	2	5.4	0.00		3	8.1	3	6.3	1	2.3	
Exercise											
Seldom	24	64.9	21	55.3	21	56.8	27	56.3	30	69.8	0.57
≥1–2 h/week	10	27.0	10	26.3	7	18.9	13	27.1	6	14.0	
Unknown	3	8.1	7	18.4	9	24.3	8	16.7	7	16.3	
Consumption of beef											
≤2 times/month	10	27.0	13	34.2	10	27.0	10	20.8	12	27.9	0.91
1–2 times/week	11	29.7	11	28.9	9	24.3	15	31.3	13	30.2	
≥3 times/week	8	21.6	4	10.5	9	24.3	14	29.2	8	18.6	
Unknown	8	21.6	10	26.3	9	24.3	9	18.8	10	22.7	
Consumption of green leafy vegetables											
≤2 times/month	2	5.4	1	2.6	4	10.8	2	4.2	1	2.3	0.91
1–2 times/week	9	24.3	7	18.4	9	24.3	12	25.0	12	27.9	
≥3 times/week	22	59.5	23	60.5	18	48.6	27	56.3	22	51.2	
Unknown	4	10.8	7	18.4	6	16.2	7	14.6	8	18.6	
Family history of colorectal cancer											
Yes	1	2.7	2	5.3	2	5.4	1	2.1	2	4.7	0.91
No	36	97.3	36	94.7	35	94.6	47	97.9	41	95.3	
Parity	2.96 ± 0.20		2.67 ± 0.21		3.35 ± 0.20		3.16 ± 0.18		3.03 ± 0.18		0.08
0–1	3	8.1	5	13.2	2	5.4	1	2.1	2	4.7	0.38
2–3	27	73.0	22	57.9	21	56.8	30	62.5	30	69.8	
≥4	7	18.9	7	18.4	11	29.7	14	29.2	10	23.3	
Unknown	0		4	10.5	3	8.1	3	6.3	1	2.3	
Age at menarche	14.9 ± 0.26		15.6 ± 0.26		15.5 ± 0.25		15.0 ± 0.23		14.8 ± 0.24		0.24
≤13 years	8	21.6	4	10.5	6	16.2	8	16.7	8	18.6	0.69
14–15 years	14	37.8	16	42.1	12	32.4	24	50.0	18	41.9	
≥16 years	14	37.8	16	42.1	19	51.4	13	27.1	15	34.9	
Unknown	1	2.7	2	5.3	0		3	6.3	2	4.7	
Menopause											
Yes	32	86.5	35	92.1	35	94.6	44	91.7	40	93.0	0.77
No	5	13.5	3	7.9	2	5.4	4	8.3	3	7.0	

We also performed the same analysis using 45 colon cancer cases and 114 matched controls. The BMI-adjusted ORs for Q_{2-3} and Q_{4-5} relative to Q_1 were 0.86 (0.29–2.59) and 1.99 (0.64–6.16), respectively (p for trend = 0.12). Moreover, among postmenopausal women (54 colorectal cancer cases and 128 matched controls), the ORs of colorectal cancer, compared to Q_1, were 1.37 (0.45–4.14) for Q_{2-3} and 3.35 (1.07–10.5) for Q_{4-5} (p for trend = 0.016). However, we were unable to elucidate either the association of elevated leptin levels

Table 4. ORs of colorectal cancer for quintiles of serum leptin levels

	Q1	Q2	Q3	Q4	Q5	p for trend
Cases/controls	8/29	10/28	8/29	18/30	14/29	
OR (95% CI)[1]	1.00	1.38 (0.47–4.10)	0.95 (0.31–2.92)	2.22 (0.83–5.97)	1.90 (0.67–5.35)	0.11
OR (95% CI)[2]	1.00	1.29 (0.42–3.90)	1.20 (0.37–3.91)	3.31 (1.13–9.71)	2.84 (0.92–8.77)	0.02
OR (95% CI)[3]	1.00	1.79 (0.49–6.53)	1.56 (0.40–6.18)	4.90 (1.33–18.1)	3.94 (1.04–14.9)	0.02
OR (95% CI)[4]	1.00	1.35 (0.34–5.34)	1.47 (0.34–6.30)	5.07 (1.23–21.0)	4.58 (1.09–20.2)	0.02
OR (95% CI)[1]	1.00	1.16 (0.44–3.05)		2.07 (0.83–5.18)		0.06
OR (95% CI)[2]	1.00	1.25 (0.46–3.39)		3.12 (1.15–8.47)		<0.01
OR (95% CI)[3]	1.00	1.68 (0.53–5.30)		4.50 (1.36–14.8)		<0.01
OR (95% CI)[4]	1.00	1.40 (0.41–4.78)		4.84 (1.29–18.1)		0.01

Since similar ORs were seen for quintiles 2 and 3 and quintiles 4 and 5, these quintiles were combined into two groups.
[1] Conditional logistic regression analyses were matched for age and study area.
[2] Additionally adjusted for BMI at baseline.
[3] Additionally adjusted for smoking status, alcohol consumption, exercise, beef intake, green leafy vegetable intake, family history of colon cancer, parity, age at menarche, and menopausal status.
[4] Additionally adjusted for insulin, IGF-I, IGF-II, and IGFBP-3.

with rectal cancer risk or the risk factors for colorectal cancer among premenopausal women due to the small number of cases.

Discussion

This study is the first nested case-control study reporting a significant increase in the risk of female colorectal cancer with increasing serum levels of leptin. To date, two other case-control studies have been published on the possible association between leptin and colorectal cancer [24, 25]. However, those studies found such an association among men but not among women.

A complex relationship existed between leptin and the identified or suggested risk factors of female colorectal cancer in the background of our study. Leptin is an adipocyte-secreted protein, the circulating levels of which reflect the amount of energy stored in adipose tissue, thus accounting for the elevated leptin levels in obese humans [2]. Although leptin has been viewed as the hormonal signal for the regulation of energy homeostasis as mentioned above, there is much evidence to suggest that leptin plays a broader physiological role. Leptin regulates several neuroendocrine axes, some of which play important roles in the pathogenesis of female colorectal cancer, and it has been implicated in the reproductive function of women. Increasing leptin levels may also signal the onset of puberty in girls [6]. The pulsatile secretion of leptin is also synchronous with the pulsatility of luteinizing hormone and estradiol in normal women [5]. Leptin production may differ with menopausal status [26]. In addition, leptin has been shown to regulate GH secretion and has been associated with circulating IGF-I and IGFBP-3 levels in normal and GH-deficient humans [9]. Finally, serum leptin levels have been associated with certain life-style factors, such as smoking and alcohol consumption [7].

Interestingly, the above endocrine axes (IGF-I, IGF-II, and IGFBP-3), life-style factors (smoking and alcohol consumption), reproductive factors (parity, age at menarche, and menopausal status) have also been implicated in the pathogenesis of female colorectal cancer. In view of the above evidence, taken as a whole, we hypothesized that leptin may explain the association between those identified risk factors and female colorectal cancer risk or may provide a link between both. In the present study, we found that elevated leptin levels were significantly associated with the risk of colorectal cancer independent of the potential risk factors, and that the association became stronger with the number of covariates. These results appear to support our hypothesis.

Several recent studies have reported that leptin can directly stimulate tumor development in several types of cells [17–20]. Leptin receptors have also been demonstrated in human colon cancer cell lines and in specimens of human colonic tumors [21, 22]. In vitro, leptin stimulated the proliferation and invasiveness of human colon

cancer cells [17–19]. In vivo, cell proliferation increased in the colon of mice by the administration of leptin [17, 19]. Leptin has also been demonstrated to induce angiogenesis [20]. These functions of leptin may explain in part the mechanisms underlying the independent association of elevated leptin levels with the risk of colorectal cancer in females.

It is interesting to note that our study showed a distinct threshold effect for the impact of leptin on the colorectal cancer risk. The OR for Q_{4-5} rose remarkably. This phenomenon has been demonstrated in previous studies on the association between leptin and colorectal [19] and prostate cancer [27]. The subjects in Q4 and Q5 showed high BMIs, with a mean of 23.7 kg/m^2 in Q4 and 25.0 kg/m^2 in Q5. It has also been reported that obesity was associated with decreasing levels of the circulating soluble leptin receptors in humans [28]. Speculatively, the fact that levels of circulating free leptin, which is not bound to soluble leptin receptors and is biologically active, may increase dramatically among Q4–Q5 subjects may explain the above-mentioned phenomenon.

There are both strengths and limitations in our study. The former include its prospective design. Both the subjects' serum and data on exposure were collected before diagnosis and prior to any colorectal cancer deaths, which could preclude the effect of cancer on leptin secretion. Moreover, since we collected data on exposures to the many kinds of factors known or suspected to modify the risk of colorectal cancer including insulin and IGFs, we could elucidate the independent effects of leptin by multivariate adjustment. One limitation of this study lies in the absence of information on the specific subsites of origin in the large bowel, since one study has suggested that the influence of leptin should be either greater or restricted to the right side of the large bowel. Secondly, the frequency of missing values in some variables was high since the questionnaires were a little different among study areas. Therefore, we could not help replacing missing values with means of non-missing values or including these subjects into separate categories. Thirdly, since the study cohort comprised exclusively Japanese women, our results may not apply to occidental women with a high BMI; one previous study reported no association between leptin and the colorectal cancer risk among occidental women [23]. Finally, it remains unclear whether leptin levels at baseline reflect its levels over the mean 7.9 follow-up years.

In conclusion, this prospective study demonstrated that elevated leptin levels were associated with the risk of female colorectal cancer. That association remained after further adjustments for potential risk factors. These results suggest that leptin may substantially increase the risk of female colorectal cancer. However, the possibility remains that leptin may also be a vigorous marker of other obesity-related hormonal disorders or yet unidentified risk factors.

Acknowledgments

The authors wish to express their sincere appreciation to Dr. Kunio Aoki, Professor Emeritus of the Nagoya University School of Medicine and former chairman of the JACC Study Group, and also to Dr. Haruo Sugano, former Director of the Cancer Institute of the Japanese Foundation for Cancer Research; both of them greatly contributed to the initiation of this study.

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Masahiro Nakao, Kyoto Prefectural University of Medicine; Dr. Takaichiro Suzuki, Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases; Dr. Tsutomu Hashimoto, Wakayama Medical University; Dr. Teruo Ishibashi, Asama General Hospital, and Dr. Katsuhiro Fukuda, Kurume University School of Medicine.

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (2) (No. 14031222) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The JACC Study has also been supported by Grants-in-Aid for Scientific Research from the same ministry (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, and 11181101).

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Association of Serum Carotenoid Concentration and Dietary Habits among the JACC Study Subjects

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BACKGROUND: We wished to determine the validity of the association between serum carotenoid concentrations and dietary habits obtained from a food frequency questionnaire in the Japan Collaborative Cohort Study (JACC Study) for Evaluation of Cancer Risk sponsored by the Ministry of Education, Science, Sports and Culture of Japan (Monbusho).
METHODS: The subjects were 866 male and 569 female controls in nested case-control studies for evaluating the risk of lung, colorectal, and urothelial cancers as parts of the JACC Study. Dietary habits were assessed using a food frequency questionnaire, and serum samples were obtained at baseline. Serum carotenoid concentrations of frozen-stored sera were measured and compared with the results of the survey.
RESULTS: In males, consumption of dairy products, some oily foods, vegetables, fruits, and boiled beans correlated positively with serum carotenoid concentrations, whereas ingestion of boiled rice and *sansai* (edible wild plants) was negatively correlated with serum carotenoids. In females, only fruit consumption was positively correlated with serum carotenoid concentration, whereas ingestion of butter, *sansai*, and potatoes were negatively correlated. Some specific associations, between serum lycopene and tomato consumption and between serum β-cryptoxanthin and ingestion of oranges, were observed in both sexes.
CONCLUSIONS: In males, serum carotenoid concentrations were slightly associated with intake of foods rich in carotenoids. The lack of associations in females suggests that the food frequency questionnaire did not validly evaluate females' dietary habits concerning carotenoids in the JACC Study.
J Epidemiol 2005; 15: S220-S227.

Key words: Carotenoids, Food Habits, validity, Cohort Studies.

Carotenoids are contained in various vegetables and fruits, especially those colored deep green, yellow, or red. The concentration of some carotenoids is especially high in certain vegetables or fruits, for example, lycopene in tomatoes and cryptoxanthin in oranges.[1] Carotenoids are thought to have anti-carcinogenic properties, which are associated with their anti-oxidant activity as well as other mechanisms.[2] Studies in cancer epidemiology have revealed associations between carotenoid intake or serum concentration and cancer risk.

Epidemiologic studies of the risk of chronic diseases such as cancer typically evaluate the risks associated with long-term, stable lifestyles. Many of these studies use questionnaires to determine the average conditions over long periods of time prior to having the target illness. Serum concentrations of nutrients or

Received December 24, 2004, and accepted February 10, 2005.
This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (2) (No. 12218216, 14031221, 14031222, 15026215) from the Ministry of Education, Science, Sports and Culture of Japan (Monbusho). The JACC Study has been supported by Grants-in-Aid for Scientific Research from the same Ministry (No. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102 and 11181101).

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other constituents at baseline are not good determinants of the average concentration over time, but are merely snapshots taken at a single time point. For example, data from a single ingestion of β-carotene showed that its half life was about 10 days,[3] suggesting that serum carotenoid concentrations reflect their intake in daily life. To assess the validity of questionnaire surveys, however, it is necessary to compare their results with those from serum measurements.[4-6]

During the Japan Collaborative Cohort Study (JACC Study) for Evaluation of Cancer Risk sponsored by the Ministry of Education, Science, Sports and Culture of Japan (Monbusho), a food frequency questionnaire was used to assess dietary habits of the subjects at baseline survey. In addition, blood samples were collected from all subjects at baseline,[7] and serum concentrations of carotenoids were measured to evaluate cancer risk at lung,[8] colon and rectum,[9] and urothelial system (pelvis, ureter, and bladder).[10] To validate these results, we have compared serum carotenoid concentrations with dietary habits in a sample set of the JACC Study.

METHODS

The subjects of this study were 866 male (mean ± standard deviation of age, 64.4 ± 8.3 years) and 569 female (mean ± SD of age, 64.0 ± 8.3 years) controls in nested case-control studies, conducted as parts of the JACC Study, which examined the risks of lung, colorectal, and urothelial cancers related to serum carotenoid concentrations. Their dietary habits were surveyed from 1988 to 1990 using a self-administered food frequency questionnaire, in which the subjects indicated their average consumption of 32 food items (beef, pork, ham and sausage, chicken, liver, egg, milk, yogurt, cheese, butter, margarine, fried food, fried vegetables, fish (unprocessed), boiled fish paste ('*kamaboko*' in Japanese), dried or salted fish, green-leaf vegetables, carrots and squash, tomatoes, cabbage and lettuce, Chinese cabbage, edible wild plants ('*sansai*'), mushroom, potatoes, seaweed, pickles, food boiled down in soy sauce ('*tsukudani*'), boiled beans, tofu, oranges, fruits other than oranges, and fruit juice). Subjects indicated frequency of consumption as scarcely, 1-2 times a month, 1-2 times a week, 3-4 times a week, and almost every day. Boiled rice was evaluated as the number of bowls consumed per day. Consumption of miso soup was evaluated as scarcely, a few times per month, every other day, almost every day, with the latter indicating the number of bowls consumed.

The subjects donated blood samples at health-screening checks sponsored by municipalities during the same time period as the questionnaire survey. As soon as possible after blood drawing, serum was separated at laboratories in or near the municipalities and stored at -80℃ until analyzed. Individual written or oral consent, or consent from community representatives, was obtained for the subjects, or a poster notification/opting-out system was applied.[7] The Ethical Boards of the Nagoya University School of Medicine, Fujita Health University, and the Kyoto Prefectural University of Medicine approved this study.

We assayed serum concentrations of carotenoids by high-performance liquid chromatography (HPLC).[11] Because we did not separately measure serum zeaxanthin and lutein, we recorded their combined levels as zeaxanthin/lutein. The total carotene concentration was recorded as the sum of α- and β-carotenes and lycopene; total xanthophyll concentration was recorded as the sum of β-cryptoxanthin, canthaxanthin and zeaxanthin/lutein. Total carotenoid concentration was calculated as total carotenes plus total xanthophylls. Serum concentrations of total cholesterol were assayed at the SRL Laboratory (Hachioji, Japan) in 2001-2003.

Associations between serum carotenoid concentrations and lifestyle, including dietary habits (frequency of food consumption), smoking, and drinking, were evaluated using Spearman's correlation coefficients (r_S). Missing values for smoking and drinking were treated as an additional category or replaced with surrogate values to determine the rank used for adjustment. Subjects who did not answer the question whether they were smoking, quit smoking, or did not smoke were treated as a category of 'unknown smoking status.' Their serum carotenoid concentrations were similar to those of non- or ex-smokers. For current smokers who did not indicate the number of cigarettes consumed per day, we assumed that males smoked 18.9 and females smoked 11.4 cigarettes, the mean numbers of cigarettes smoked per day by each sex. Consequently, smoking was ranked in the order non-smokers, unknown smoking status, ex-smokers, and current smokers, with the latter ranked by number of cigarettes consumed per day. Of the 866 males, 396 were current smokers, 247 were ex-smokers, 192 were nonsmokers, and 31 were of unknown smoking status. Of the 569 females, 16 were current smokers, 14 were ex-smokers, 475 were nonsmokers, and 64 were of unknown smoking status.

Alcohol drinking was treated in the same manner, with current drinkers who did not indicate the amount of alcohol consumed per day assigned their respective gender means (45 mL for males and 17 mL for females). Of the 866 males, 604 were current drinkers, 39 were ex-drinkers, 193 were nondrinkers, and 30 were of unknown drinking status. Of the 569 females, 101 were current drinkers, 7 were ex-drinkers, 419 were nondrinkers, and 42 were of unknown drinking status.

RESULTS

The geometric means of serum carotenoid concentrations and the back-transformed values of log-transformed mean ± standard deviation are shown by sex in Table 1. These concentrations were generally higher in females. When we segregated serum carotenoid concentrations by age, smoking, and drinking status, we found that some carotenoids were sporadically correlated, either positively or negatively, with age in both sexes. Almost all the carotenoids were negatively correlated with smoking and drinking in males, but not in females. Serum concentrations of

carotenoids and total cholesterol were correlated in both sexes; the range of r_s was 0.23 to 0.34 for males and 0.19 to 0.38 for females.

The association of serum concentrations of total carotenoids and the frequency of intake of individual food items is shown in Table 2. In males, ingestion of eggs, dairy products, some oily foods, vegetables, fruits, and boiled beans were positively correlated with serum concentrations of carotenoids, whereas boiled rice and sansai were negatively correlated. In females, only intake of fruits was positively correlated to serum concentration of total carotenoids, whereas intake of butter, *sansai*, and potatoes was negatively correlated. These tendencies remained after adjusting for age, smoking, drinking, and serum concentration of total cholesterol (Table 2). After further adjustment for intake of oranges, other fruits, green-leafy vegetables, carrots and squash, and tomatoes, the positive correlation of serum carotenoid concentration with intake of eggs and milk, and the negative correlation with intake of *sansai*, still remained in males. In females, however, there were negative correlations between serum carotenoid concentration and the intake of butter, fish, dried and salted fish, and potatoes.

The associations between individual carotenoid concentrations and food intake are shown in Table 3. Among males, intake of all green-yellow vegetables and fruits on the list were correlated with serum zeaxanthin/lutein concentration; intake of all fruits and vegetables, except tomatoes, was correlated with serum α - and β -carotene concentrations; intake of fruits was correlated with serum β -cryptoxanthin concentration; and intake of tomatoes and fruits was correlated with serum lycopene concentration. Among females, however, most of these correlations were not observed, except that intake of oranges was correlated with serum β -cryptoxanthin concentration and intake of tomatoes with serum lycopene concentration.

Smoking was negatively correlated with intake of eggs, milk, some oily foods, green-yellow vegetables, fruits, and beans in males, and with intake of miso soup, fried foods, fishes, some green-yellow vegetables, potatoes, and tofu in females (Table 2). Drinking in males was negatively correlated with ingestion of milk (r_s = -0.10, p<0.01), boiled beans (r_s = -0.10, p<0.01), oranges (r_s = -0.09, p<0.05), and fruits other than oranges (r_s = -0.10, p<0.01), but was positively correlated with intake of miso soup (r_s = 0.07, p<0.05), liver (r_s = 0.10, p<0.05), cheese (r_s = 0.07, p<0.05), and sansai (r_s = 0.13, p<0.01). In females, drinking was negatively correlated with intake of miso soup (r_s = -0.09, p<0.05), carrots and squash (r_s = -0.09, p<0.05), and tofu (r_s = -0.09, p<0.05), and positively correlated with consumption of cheese (r_s = 0.09, p<0.05), butter (r_s = 0.13, p<0.01), margarine (r_s = 0.12, p<0.01), and fruit juice (r_s = 0.13, p<0.01).

Table 1. Distribution of serum concentrations of carotenoids and vitamins, and Spearman's correlation coefficients with age and smoking.

	Geometric means (μ M) and means ± SD†				Spearman's correlation coefficients					
					Age		Smoking ‡		Alcohol drinking ‡	
	Males (n=866)		Females (n=569)		Males	Females	Males	Females	Males	Females
Zeaxanthin/lutein	0.91	0.52, 1.60	1.02	0.59, 1.76	0.04	-0.00	-0.08**	-0.03	0.01	-0.02
Canthaxanthin	0.03	0.01, 0.05	0.03	0.02, 0.05	0.01	-0.09*	-0.06+	-0.04	-0.06*	0.00
β -Cryptoxanthin	0.17	0.06, 0.50	0.32	0.13, 0.81	-0.02	-0.08+	-0.15**	-0.02	-0.17**	0.05
Total xantophylls	1.18	0.66, 2.12	1.46	0.83, 2.57	0.02	-0.02	-0.12**	0.01	-0.04	0.00
α -Carotene	0.05	0.02, 0.13	0.09	0.04, 0.19	0.02	-0.10*	-0.13**	-0.00	-0.17**	0.04
β -Carotene	0.32	0.11, 0.97	0.64	0.26, 1.55	0.08*	-0.03	-0.17**	-0.04	-0.21**	0.00
Lycopene	0.11	0.04, 0.33	0.20	0.08, 0.52	-0.06*	-0.06	-0.08*	-0.08*	-0.11**	0.01
Total carotenes	0.53	0.20, 1.42	1.00	0.45, 2.22	0.04	-0.06	-0.17**	-0.05	-0.20**	0.01
Total carotenoids	1.78	0.92, 3.46	2.54	1.38, 4.68	0.03	-0.04	-0.16**	-0.01	-0.12**	0.01

** p<0.01, * p<0.05, + p<0.1

†: Backtransformation from log-transformed data

‡: Smoking and drinking were ranked, and missing numbers were supplied, as described in Methods part.

Table 2. Spearman's correlation coefficients of foods with total carotenoids and smoking.

	Total carotenoids†		Total carotenoids‡		Total carotenoids§		Smoking†	
	Males	Females	Males	Females	Males	Females	Males	Females
Boiled rice	-0.11**	-0.04	-0.07*	-0.04	-0.04	-0.03	-0.00	-0.07+
Miso soup	-0.06+	-0.06	-0.00	-0.03	-0.05	-0.02	-0.00	-0.13**
Beef	-0.02	-0.04	-0.02	-0.07	-0.02	-0.10+	0.06+	0.03
Pork	0.03	0.00	0.05	0.00	0.00	0.01	0.04	-0.08+
Ham and sausage	0.04	-0.05	0.04	-0.08+	0.00	-0.05	-0.02	-0.05
Chicken	-0.02	0.05	-0.00	-0.01	-0.06	-0.02	-0.05	-0.08+
Liver	-0.02	-0.02	0.01	-0.00	-0.01	-0.03	0.01	0.09+
Eggs	0.12**	-0.00	0.12**	0.01	0.10*	-0.03	-0.07*	-0.06
Milk	0.27**	0.07	0.20**	0.04	0.16**	0.05	-0.10**	-0.05
Yogurt	0.15**	0.03	0.10*	0.00	0.04	0.02	-0.06+	0.05
Cheese	0.12**	0.01	0.11**	-0.00	0.06	-0.00	-0.02	0.04
Butter	0.07+	-0.12**	0.08*	-0.12*	0.04	-0.15**	0.00	-0.04
Margarine	0.08*	0.01	0.03	0.01	-0.01	0.00	-0.04	0.01
Fried foods	0.07*	-0.03	0.06	-0.02	0.06	-0.05	-0.08*	-0.13**
Fried vegetables	0.03	0.00	0.02	0.03	0.00	0.03	-0.13**	-0.06
Fish (unprocessed)	-0.00	-0.05	-0.01	-0.08+	-0.06	-0.14**	-0.04	-0.09*
Kamaboko#	0.06	-0.03	0.05	-0.00	0.00	-0.00	-0.05	-0.05
Dried or salted fish	0.02	-0.05	0.04	-0.05	-0.00	-0.11*	0.05	-0.09*
Green-leafy vegetables	0.06+	0.00	0.07+	0.02	-	-	-0.11**	-0.01
Carrots and squash	0.07*	0.00	0.08*	-0.01	-	-	-0.08*	-0.15**
Tomatoes	0.08*	0.05	0.10**	0.02	-	-	-0.05	-0.14**
Cabbage and lettuce	0.07*	-0.01	0.02	-0.02	-0.06	-0.03	-0.06+	-0.03
Chinese cabbage	0.08*	-0.04	0.09*	-0.01	0.00	-0.02	-0.06+	0.03
Sansai¶	-0.12**	-0.10*	-0.10**	-0.07	-0.10*	-0.09+	-0.01	-0.01
Mushrooms	0.01	-0.01	0.01	0.01	-0.03	-0.01	0.00	0.00
Potatoes	0.03	-0.10*	0.01	-0.09*	-0.06	-0.13*	-0.08*	-0.13**
Seaweeds	0.03	0.01	0.06	0.02	-0.04	0.03	0.00	-0.03
Pickles	-0.05	0.01	-0.02	0.04	-0.02	0.05	0.04	-0.06
Tsukudani††	-0.01	-0.02	0.00	-0.00	-0.01	0.00	-0.00	-0.06
Boiled beans	0.11**	-0.02	0.07*	0.02	0.02	0.00	-0.07+	-0.02
Tofu	-0.00	-0.07+	0.02	-0.07	-0.07+	-0.06	0.00	-0.10*
Oranges	0.19**	0.03	0.15**	0.03	-	-	-0.12**	0.02
Fruits other than oranges	0.19**	0.09*	0.10**	0.04	-	-	-0.12**	-0.07+
Fruit juice	0.11**	0.03	0.07+	0.01	0.04	0.01	-0.09*	0.01

Analysis of 565-835males and 388-560 females.

** p<0.01, * p<0.05, + p<0.1

†: Crude correlation coefficients

‡: Adjusted for age, smoking, drinking and serum concentration of total cholesterol.

§: Adjusted for age, smoking, drinking, serum concentration of total cholesterol, and dietary intake of oranges, other fruits, green-leafy vegetables, carrots and squash, and tomatoes.

#Boiled fish paste, ¶Edible wild plants,††Food boiled down in soy sauce

Table 3. Spearman's correlation coefficients of specific dietary habits with carotenoids adjusted for age, smoking, and drinking.

	Zeaxanthin/ lutein	Cantha- xanthin	β-Crypto- xanthin	Total xantophylls	α-Carotene	β-Carotene	Lycopene	Total carotenes	Total carotenoids
Males									
Green-leafy vegetables	0.08*	0.05	0.03	0.07+	0.09**	0.06+	0.02	0.05	0.05
Carrots and squash	0.09**	0.00	0.01	0.06+	0.08*	0.08*	0.05	0.07*	0.06+
Tomatoes	0.09**	-0.00	-0.03	0.05	0.05	0.05	0.12**	0.08*	0.07*
Oranges	0.11**	0.09*	0.23**	0.17**	0.16**	0.14**	0.09*	0.14**	0.17**
Fruits other than oranges	0.10**	0.07+	0.16**	0.13**	0.12**	0.15**	0.08*	0.13**	0.13**
Eggs	0.14**	0.10**	0.05	0.13**	0.08*	0.09**	0.03	0.07*	0.11**
Milk	0.18**	0.13**	0.25**	0.22**	0.23**	0.25**	0.13**	0.24**	0.24**
Sansai†	-0.01	-0.09*	-0.15**	-0.06+	-0.09*	-0.13**	-0.10**	-0.14**	-0.11**
Females									
Green-leafy vegetables	0.04	-0.01	-0.00	0.04	-0.00	-0.00	-0.00	-0.00	0.01
Carrots and squash	0.03	-0.00	-0.00	0.01	0.00	-0.01	-0.01	-0.01	0.00
Tomatoes	0.06	0.05	-0.04	0.01	0.00	0.03	0.13**	0.07+	0.05
Oranges	0.02	0.10*	0.19**	0.11**	0.00	0.02	-0.13**	-0.03	0.04
Fruits other than oranges	0.06	0.00	0.09*	0.09+	0.02	0.03	-0.05	0.00	0.05
Eggs	0.06	0.02	0.00	0.03	-0.01	-0.02	-0.01	-0.02	-0.00
Milk	0.07+	0.06	0.08+	0.09*	0.06	0.06	-0.02	0.04	0.06
Sansai†	-0.04	-0.09*	-0.13**	-0.08+	-0.10*	-0.09+	-0.07	-0.09*	-0.10*

** $p<0.01$, * $p<0.05$, + $p<0.1$

† : Edible wild plants.

DISCUSSION

This study was primarily designed to validate the food frequency questionnaire and serum concentrations of carotenoids in the nested case-control studies as parts of the JACC Study.[8-10] Controls of those nested case-control studies were thought to represent the general population of the JACC study, and adequate to the sample set of this study. However, discussion applied in a different situation may be limited; e.g., most subjects of the JACC Study lived in rural areas, where dietary habits may be different from those in urban areas; subjects who donated blood samples were around 30% of whole respondents to the baseline questionnaire survey, therefore, they may be more health-conscious.

Quantitative stability of carotenoids during long-term storage at -80℃ is reviewed in the previous paper;[8] briefly, decreases after 9 years of storage at -80℃ were less than 15-20% for various carotenoids, and those serum levels measured in the JACC Study were comparable to those found in other studies in Japan.

In most comparisons of dietary intake of carotenoids and serum carotenoid concentrations, the amount of each individual carotenoid has been estimated using a semi-quantified food frequency questionnaire and a nutrient database.[4-6] In the JACC study, however, a simple food frequency questionnaire (without portion size) for 32 food items was used. We therefore compared serum carotenoid concentrations with frequency of food intake using five frequency levels, which may explain why Spearman's correlation coefficients were generally low (0.2 or less) in our study.

Serum carotenoid concentrations are lowered by smoking, a decrease attributed to the presence of β-unsaturated aldehydes and large numbers of free radicals in cigarette smoke.[2] Alcohol drinking is also associated with decreased serum carotenoid concentrations.[2] In this study, we found that, in male subjects, both smoking and drinking were negatively associated with the concentrations of most carotenoids. In female subjects, however, these associations were generally not observed, probably a much smaller percentage of females than males were smokers or drinkers. Other factors affecting serum carotenoid concentrations include those inhibiting the intestinal absorption of carotenoids or coexistent lipid-like substances,[2] but these were generally not evaluated in this study. In this study, serum total cholesterol concentration was used to adjust carotenoid concentrations because lipoprotein fraction contains carotenoids and serum lipid fraction affects serum carotenoid concentrations.

Smoking and alcohol drinking are usually associated with other unfavorable lifestyle parameters, a finding confirmed in this study. We found that smoking was negatively associated with the intake of foods regarded in Japan as healthy, but the association between drinking and the intake of these foods was weaker. Serum concentration of total cholesterol could be correlated with serum carotenoid concentrations, as well as with dietary habits. These factors were therefore adjusted when evaluating the correlation between dietary habits and serum carotenoid concentrations.

After adjusting for smoking, drinking, and serum concentration of total cholesterol, the correlation between serum concentration of total carotenoids and intake of various foods was slightly changed in males. Among 192 nonsmoking males, there were positive correlations between serum concentration of total carotenoids and consumption of milk (r_s = 0.24, $p<0.01$) and tomatoes (r_s = 0.18, $p<0.05$), and weaker correlations between total carotenoids and intake of carrots and squash (r_s = 0.12, $p<0.1$), oranges (r_s = 0.09, $p<0.1$), and other fruits (r_s = 0.04, $p<0.1$) (data not shown). Although milk contains some carotenoids, it is not regarded as directly associated with these nutrients. Nevertheless, among nonsmokers, intake of milk showed a higher association with serum carotenoid concentrations than did foods rich in carotenoids. These results suggest that the correlations adjusted for smoking, drinking, and total cholesterol in Table 2 may still contain residual confounding.

Further adjustment for intake of green-yellow vegetables and fruits, and for serum concentration of total cholesterol, was performed to determine the true association between serum carotenoid concentrations and foods, excluding those rich in carotenoids. Positive correlations of serum concentration of total carotenoids with intake of eggs and milk may be caused by confounding, although these foods contain some carotenoids. These foods are regarded as healthy, and their intake may be related to the intake of carotenoid-rich foods not surveyed in the questionnaire. The negative correlations between carotenoid concentrations and intake of *sansai* (edible wild plants) were not clearly interpreted, although individuals who eat large amounts of *sansai* may not eat sufficient vegetables.

Our finding of a lack of association between carotenoid concentrations and intake of green-yellow vegetables and fruit among female subjects was somewhat surprising, inasmuch as it is generally thought that women can recall their dietary habits more easily than men because women are more involved in food preparation than are men. In the studies of lung cancer in the JACC Study, consumption of green-yellow vegetables and fruits showed a clear protective effect against lung cancer deaths in males, but not in females.[12] In contrast, high serum carotenoid concentrations were clearly associated with a decrease in the risk of lung cancer in both sexes[8] and in females alone.[13] These findings suggest that the food frequency questionnaire used in the JACC Study may not have reflected the dietary habits of women, especially in foods containing carotenoids.

There are several explanations for the poor correlations between food intake and serum carotenoid concentrations in females. First, each category in the questionnaire, such as green-leafy vegetables, carrots and squash, and fruits other than oranges, included the names of various kinds of foods. This may have led to some confusion and inter-subject differences in filling out the questionnaire. In addition, portion size was not surveyed. This explanation may be probable because specific relationships between tomatoes and lycopene, or oranges and β-cryptoxanthin were observed even in females. A second possibility is that sub-

jects may have answered the questionnaire in such a way as to record what they thought their lifestyle should be, rather than what it was. The survey questionnaire was administered to the subjects to evaluate the association between lifestyle and illness, and the survey was linked with health checkups in some study areas. This possibility may be common among the subjects who were strongly interested in their health. Third, carotenoids are better absorbed when ingested with fat, and serum carotenoid concentrations correlate with serum lipid concentrations. Lipid metabolism may be unstable in middle-aged or elderly women; for example, serum cholesterol levels increase after menopause. Thus, in these women, serum carotenoid concentrations may be more affected by these lipid metabolic conditions than by dietary intake.

In conclusion, we have shown here a slight association between serum carotenoid concentrations and the intake of foods rich in carotenoids in males. Associations with intake of eggs and milk may be affected by confounding with foods rich in carotenoids that were not evaluated in the questionnaire. In females, the associations between serum carotenoid concentrations and intake of foods rich in carotenoids were scarce, suggesting that the food frequency questionnaire did not validly evaluate the dietary habits of females in the JACC Study. We observed several specific associations in both sexes, including those between serum β-cryptoxanthin concentration and consumption of oranges, and between serum lycopene concentration and ingestion of tomatoes.

MEMBER OF LIST OF THE JACC STUDY GROUP

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ACKNOWLEDGMENTS

The authors sincerely express their appreciation to Dr. Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine and the former chairman of the JACC Study, and Dr. Haruo Sugano, the former Director, Cancer Institute, Tokyo, who greatly contributed to the initiation of the JACC Study, and Dr. Yoshiyuki Ohno, Professor Emeritus, Nagoya University School of Medicine, who was the past chairman of the study. The authors also wish to thank Dr. Tomoyuki Kitagawa, Cancer Institute of the Japanese Foundation for Cancer Research and the former chairman of Grant-in-Aid for Scientific Research on Priority Area 'Cancer', for his full support of this study.

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Association of Serum Phytoestrogen Concentration and Dietary Habits in a Sample Set of the JACC Study

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BACKGROUND: Phytoestrogens may be associated with a reduced risk of hormone dependent neoplasms such as prostate and breast cancers. We tried to determine the validity of the association between serum phytoestrogen concentrations and dietary habits obtained from a food frequency questionnaire used in the Japan Collaborative Cohort Study (JACC Study) for Evaluation of Cancer Risk sponsored by the Ministry of Education, Science, Sports and Culture of Japan (Monbusho).
METHODS: The subjects were 151 male controls who were selected for a nested case-control study for evaluating prostate cancer risk as part of the JACC Study. Dietary habits were determined using a food frequency questionnaire at baseline, and the concentrations of genistein, daidzein, and equol in frozen-stored serum samples assayed in 2002 were compared.
RESULTS: Tofu intake showed a significant association with the serum concentrations of genistein and daidzein (Spearman's correlation coefficients (r_s) =0.30 and 0.27, respectively), and miso soup showed a slight association with serum concentrations of these phytoestrogens. In contrast, serum concentrations of equol were not associated with dietary intake of tofu and miso soup. After adjustment for serum daidzein concentration, serum equol concentration was associated with the intake of foods containing fat, meat, and coffee, but not green tea.
CONCLUSIONS: Serum genistein and daidzein concentrations were significantly associated with dietary intake of tofu, and slightly with intake of miso soup. Consumption of fat, meat, and coffee may be associated with equol production by intestinal microflora in this sample set.
J Epidemiol 2005; 15: S196-202.

Key words: phytoestrogen, Isoflavones, Food Habits, validity, Cohort Studies.

Phytoestrogens are isoflavonoids and lignans of plant origin with estrogen-like activities that may be associated with a reduced risk of hormone dependent neoplasms such as prostate and breast cancers.[1,2] Consumption of large amounts of phytoestrogen-rich foods such as soybeans may be associated with the low incidence of prostate and breast cancers in Japan and other Asian countries. A nested case-control study within the Japan Collaborative Cohort Study (JACC Study) for Evaluation of Cancer Risk sponsored by

Received December 24, 2004, and accepted February 10, 2005.
This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (2) (No. 14031221, 15026215) from the Ministry of Education, Science, Sports and Culture of Japan (Monbusho). The JACC Study has been supported by Grants-in-Aid for Scientific Research from the same Ministry (No. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102 and 11181101).

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the Ministry of Education, Science, Sports and Culture of Japan (Monbusho)[3] found that a high serum concentration of phytoestrogens was associated with a reduced risk of prostate cancer.[4]

Variables evaluated at baseline of a cohort study should reflect the long-term condition of subjects. Turnover of phytoestrogens in serum is thought to be relatively rapid, with half times of about 6-8 hours.[5] The relationship between dietary intake of phytoestrogens and their serum concentrations should therefore be investigated. In addition, equol, a strong phytoestrogen, is thought to be produced from daidzein by intestinal microflora of particular individuals,[2] suggesting that the relationship between dietary intake of daidzein and the serum concentration of equol should therefore be examined to determine the dietary habits related to equol-producing ability.

We therefore examined the association between serum phytoestrogen concentrations and dietary habits obtained from a food frequency questionnaire used in the baseline survey of the JACC Study and tried to validate it. Additionally, some issues about equol production were discussed.

METHODS

The subjects were 151 male controls who were selected for a nested case-control study,[4] performed as part of the JACC Study,[3] in which the risk of prostate cancer was related to serum phytoestrogen concentrations. The mean age of the subjects was 68.7 years old (range, 58 to 83 years).

Dietary habits of the subjects were surveyed using a self-administered food frequency questionnaire at the baseline survey of the JACC Study in 1988-90. Each subject indicated his average frequency of consumption of 32 food items; beef, pork, ham and sausage, chicken, liver, egg, milk, yogurt, cheese, butter, margarine, fried food, fried vegetables, fish (unprocessed), boiled fish paste ('*kamaboko*' in Japanese), dried or salted fish, green-leaf vegetables, carrots and squash, tomatoes, cabbage and lettuce, Chinese cabbage, edible wild plants ('*sansai*'), mushroom, potatoes, seaweed, pickles, food boiled down in soy sauce, boiled beans, tofu (soybean curd), oranges, fruits other than oranges, and fruit juice. There were five frequency categories: scarcely, 1-2 times a month, 1-2 times a week, 3-4 times a week, and almost every day. Boiled rice was evaluated as the number of bowls consumed per day. Miso soup consumption was re-categorized as five levels: every other day or less, one bowl a day, two bowls a day, three bowls a day, and four or more bowls a day. Beverages such as coffee, tea, Japanese tea (including green tea, coarse tea ('*ban-cha*'), and roasted tea ('*hoji-cha*')), and Chinese tea were evaluated by frequency (scarcely, 1-2 times a month, 1-2 times a week, 3-4 times a week, and almost every day), with the number of cups a day recorded for those consuming these beverages almost every day.

Each subject donated blood samples at health-screening checks near the baseline survey.[3] Serum was obtained and stored at -80℃ until analyzed. Individual written or oral consent, or consent from community representatives, was obtained, or poster notification/opting-out system was applied.[3] The Ethical Boards of Nagoya University School of Medicine and the Kyoto Prefectural University of Medicine approved this study.

All serum samples were assayed at a single laboratory (SRL, Hachioji, Japan) in 2002, with the staff blinded to case-control status. Serum levels of daidzein, genistein, and equol were measured as described.[6] Briefly, each sample was hydrolyzed and incubated overnight at 37℃ with β-glucuronidase/sulfatase (Nippon Biotest Laboratories Inc., Kokubunji, Japan) in acetate buffer (pH 4.5). The dimethyl ether extract of the sample was dried under nitrogen flow and redissolved in a 2:1:3 mixture of methanol, acetonitrile and water. Each sample was centrifuge-filtered using Ultrafree-MC, 0.22 μm pore size (Millipore, Bedford, MA), and the concentrations of daidzein, genistein, and equol were measured using an LC/MS/MS system (LC: HP1100 Series, Agilent Technologies, Palo Alto, CA, MS/MS: Quattro-Ultima, Micromass Ltd, Manchester, UK). The ionizing method was electrospray using negative ions, and multiple reaction monitoring was used for mass analysis. Reference genistein, daidzein, and equol were made by Extrasynthese S.A. (Genay Cedex, France). For quality control, we determined the variation of measurements for two samples. We found that the coefficients of variation (CV) were 6.5% and 7.5% for genistein, 6.9% and 8.2% for daidzein, and 8.2% and 9.1% for equol. Equol-producers were defined as subjects whose serum equol was detected (>6.9 nM) in this study.

Geometric means of serum phytoestrogen concentrations were compared with levels of food intake because concentrations of the former were log-normally distributed. Pearson's correlation coefficient (r) between serum phytoestrogen concentrations was calculated for log-transformed values of moles per liter.

The association between serum phytoestrogen concentrations and food intake was examined using Spearman's correlation coefficients (r_s) and regression coefficients (b), which were calculated using integral numbers for food intake levels and log-transformed data. We also attempted to estimate serum phytoestrogen concentration by a multivariate regression model.

Geometric means of daidzein concentration between equol-producers and nonequol-producers were compared using Student's t-test, and their relationship to the intake of various foods was compared by Wilcoxon's rank test. Food intake related to production of equol was examined using Spearman's partial correlation coefficients after adjustment for serum daidzein concentrations, with serum equol concentration set at zero for nonequol-producers. We assumed that p values less than 0.05 as statistically significant, and also mentioned tendencies with p values less than 0.1 for discussion.

RESULTS

The geometric mean concentrations for all subjects were 368 nM for genistein (mean ± standard deviation of log-transformed