表 3 現状において日本人に推奨できるがん予防法

・たばこは吸わない。他人のたばこの煙を可能な限り避ける。
・適度な飲酒。具体的には，1日あたりエタノール量に換算して約 23 g 以内。 飲まない人・飲めない人は無理に飲まない。
・食事は偏らずバランスよく。 塩蔵食品・食塩の摂取は最小限。具体的には，食塩として 1 日 10 グラム未満，特に，塩分濃度が 10%程度の高塩分食品は，週に 1 回以内。 野菜・果物不足にならない。例えば，野菜は毎食，果物は毎日食べて，少なくとも一日 400 g とる。 熱い飲食物，保存・加工肉の摂取は控えめに。
・定期的な運動の継続。例えば，ほぼ毎日合計 60 分程度の歩行などの適度な運動，週に 1 回程度は汗をかくような運動。
・成人期での体重を維持（太り過ぎない，やせ過ぎない）。具体的には，中年期男性の BMI で 27 を超さない，21 を下まわらない。 中年期女性では，25 を超さない，19 を下まわらない。
・肝炎ウイルス感染の有無を知り，感染している場合は，その治療の措置をとる。 がんを引き起こすウイルスへの感染を予防する。

出典: 厚生労働省研究班「生活習慣改善によるがん予防法の開発と評価」
http://epi.ncc.go.jp/can_prev/preventive_measures.html（Accessed October 12, 2008）

② ライフイベント

初経年齢が早いこと，自然閉経年齢が遅いこと，出産経験がないこと，初産年齢が遅い（30 歳以上）ことはすべて乳がんのリスクファクターと考えられている。これらは，内因性エストロゲンに曝露されている期間が長いことを意味している。乳がんの発生や増殖には女性ホルモンであるエストロゲンが重要な働きをしており，月経や妊娠に関連する要因がホルモンレベルに影響して，乳がん発症のリスクを高めると考えられる。また逆に，初経年齢の遅さ，自然閉経年齢の早さ，出産経験があること，初産年齢の早さは乳がんに対して予防的な効果をもつ。

③ 放 射 線

エックス線など，医療で用いられる電離放射線への曝露は，低線量であっても乳がんのリスクファクターである。特に，思春期における曝露はリスクを高める。

④ 薬　　物

ホルモン補充療法は乳がんのリスクファクターである。また，経口避妊薬はエストロゲン単独，エストロゲンとプロゲステロンを併用した場合のいずれにおいても乳がんのリスクを高めるが，リスクは比較的小さい。これらは外因性ホルモンであり，月経や妊娠に関連する内因性ホルモンと同様に，乳がんのリスクを上昇させる。

3．日本におけるエビデンス

日本人に関する乳がんリスクのエビデンスとしては，厚生労働省の科学研究費補助金・第 3 次対がん総合戦略研究事業「生活習慣改善によるがん予防法の開発と評価」研究班が日本人を対象にした疫学研究のレビューを行っている。ここでは日本におけるエビデンスとして，研究班の報告を紹介する。なお，研究の詳細については，研究班のサイト[6]で参照できる。また，研究班では，World Health Organization（WHO，世界保健機関）/Food and Agriculture Organization（FAO，国際連合食糧農業機関）が 2003 年に発行した”Diet, Nutrition and the Prevention of Chronic Diseases”の報告や，研究班で行った日本人を対象とするエビデンスの評価をもとに，「現状において日本人に推奨できるがん予防法」を提示している（表 3）。これは，前述の WCRF/AICR による“Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective”の報告も踏まえたものとなっている。

乳がんのリスクファクターについて，研究班の評価をまとめたものを表 4 に示す。なお，研究班の報告では，閉経前乳がんと閉経後乳がんのリスクファクターを分けずに合わせて評価している。

研究班の報告によると，授乳が乳がんのリスクを低下させることは「ほぼ確実」と判定されており，大豆は「可能性あり」と評価されている。また，喫煙がリスクを上昇させることは「可能性あり」である。飲酒，野菜，果物，緑茶，乳製品，脂肪，肉類，運動に関しては，「証拠不十分」とされている。

4．乳がんリスクファクターに関する世界のエビデンスと日本のエビデンスとの比較

WCRF/AICR により評価された世界のエビデンスと，厚生労働省研究班による日本のエビデンスとを比較すると，全般的に，日本人においては食事や栄養，身体活動と乳がんとの関連を示すエビデンスレベルが低い傾向がみられた。これには，日本人を対象とした研究はいまだ

表 4　厚生労働省研究班による日本における食事，栄養，身体活動と乳がんとの関連

	リスクを減少させるもの	リスクを上昇させるもの
Convincing (確実)		
Probable (ほぼ確実)	授乳	
Limited-suggestive (可能性あり)	大豆	喫煙
Limited-no conclusion (証拠不十分)	飲酒，野菜，果物，緑茶，食物脂肪，肉類，乳製品，運動	
Substantial effects on risk unlikely (大きな関連なし)		

厚生労働省科学研究費補助金・第3次対がん総合戦略研究事業「生活習慣改善によるがん予防法の開発に関する研究」http://epi.ncc.go.jp/can_prev/(Accessed October 12, 2008) をもとに作成した

に少なく，乳がんとの関連を判定するのに十分なエビデンスが得られていないことが理由として考えられる。また，肥満やアルコール摂取との関連が日本人では弱いことについては，日本人女性では肥満の人の割合や，飲酒習慣がある，あるいは大量に飲酒する人の割合が小さいため，対象集団内でのばらつきが小さいことが影響している可能性も考えられる。

大豆や大豆製品に多く含まれるイソフラボンは内因性エストロゲンに対して抗エストロゲン作用をもつと考えられている。また大豆や大豆製品は日本では非常に多く食べられているが，欧米における摂取量は極めて少ない。これらのことから，日本人の乳がん罹患率は増加を続けているものの，欧米に比べると依然としてはるかに低いことの要因として，大豆や大豆製品と乳がんとの関連が注目されてきた。しかし，大豆については，肥満やアルコール摂取などとは逆に，WCRF/AICR の報告では閉経前乳がん，閉経後乳がんともに「証拠不十分」と判定されているが，厚生労働省研究班による判定では「可能性あり」とされている。これには，欧米では対象集団における大豆の摂取量が一律少なく，コホート内でのばらつきが小さいことが原因として考えられている。しかし，日本人を対象とした研究についても，一定の結論が得られているとは言えず，さらなる検討が必要である。

また，喫煙に関しては，WCRF/AICR の報告では扱われていないが，これまでに乳がんのリスク上昇と関連がみられた疫学研究は少ない。IARC の" IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 83, Tobacco smoke and involuntary smoking" でも，喫煙と乳がんとの関連を示唆する証拠はないと判定されている。また，受動喫煙についても，乳がんのリスク上昇との関連について一貫した結果は得られていない[7]。一方日本では，厚生労働省研究班のレビューの結果，「可能性あり」と判定されている。結果の違いが生じた理由については，関連する遺伝子型の分布の違いや，食事などの生活習慣の違いの影響が考えられているが，明確な理由は明らかになっていない。

日本人の乳がんは近年増加しており，予防対策の重要性も高まっている。乳がんのリスクファクターとして確立されているもののうち，遺伝や月経，妊娠など生理・生殖に関するものは，個人の努力でリスクを低減することはできないが，アルコール摂取や肥満，身体活動などは，予防に向けた努力が可能である。しかし，そのような要因に関して，現時点では，日本人に関するエビデンスは少ない。日本人の乳がん予防を考えるためにも，今後，日本人を対象とした研究の蓄積が待たれる。

また，がんサバイバーについては，食事や栄養，身体活動と，乳がんの再発などとの関連を検討する研究が，日本のみならず世界的にも不足している。そのため，WCRF/AICR の報告書でも明確な推奨も行われていない。アメリカを中心にいくつか乳がんサバイバーを対象とするコホート研究が開始されており，日本においても厚生労働省科学研究費補助金がん臨床研究事業による「生活習慣や支持療法等が乳がん患者の QOL に与える影響を調べる多目的コホート研究」をわれわれが開始した。がんサバイバーに有益な情報を提供するため，今後研究が蓄積されることが望まれる。

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Effect of Physical Activity on Breast Cancer Risk: Findings of the Japan Collaborative Cohort Study

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Abstract

Purpose: **This study aimed to examine prospectively the association between physical activity and breast cancer risk in a non-Western population.**

Methods: **We analyzed data from the Japan Collaborative Cohort Study, which included 30,157 women, ages 40 to 69 years at baseline (1988-1990), who reported no previous history of breast cancer, and provided information on their walking and exercise habits. The subjects were followed prospectively from enrollment until 2001 (median follow-up period, 12.4 years). Breast cancer incidence during this period was confirmed using records held at population-based cancer registries. The Cox proportional hazards model was used to estimate the hazard ratio (HR) for the association of breast cancer incidence with physical activity.**

Results: **During the 340,055 person-years of follow-up, we identified 207 incident cases of breast cancer. The most physically active group (who walked for ≥1 hour per day and exercised for ≥1 hour per week) had a lower risk of breast cancer (HR, 0.45; 95% confidence interval, 0.25-0.78) compared with the least active group after adjusting for potential confounding factors. The inverse association of exercise on breast cancer was stronger among those who walked for ≥1 hour per day than those who walked for <1 hour per day ($P = 0.042$). These results were not significantly modified by menopausal status or body mass index (BMI).**

Conclusions: **Our analysis provided evidence that physical activity decreased the risk of breast cancer. Walking for 1 hour per day and undertaking additional weekly exercise both seemed to be protective against breast cancer, regardless of menopausal status or BMI.** (Cancer Epidemiol Biomarkers Prev 2008;17(12): 3396–401)

Introduction

Since the early 1990s, breast cancer has been the most commonly diagnosed cancer, even among Japanese women (1). The continuous increase in breast cancer incidence during recent decades has been an important public health concern in Japan, and there has been growing interest in physical activity as a means of primary prevention. Worldwide, numerous epidemiologic studies have reported associations between physical activity and cancer risk, with most observing a protective effect. Reviews published in 2002 concluded that there was sufficient evidence to support the role of physical activity in preventing breast cancer (2, 3). A systematic review published in 2007 (4) showed a decreased relative risk (<0.8) associated with leisure activities in 8 of 17 cohort studies (5-12), whereas the remaining 9 reported no association (13-21). Three more-recent cohort studies supported the risk reduction (22-24), whereas one found no evidence of a protective effect of physical activity on breast cancer (25). In addition to the 20% to 40% overall risk reduction of breast cancer among the more physically active women (2), the effects of menstrual characteristics, obesity, use of sex hormones, hormone-receptor status, and immune function have also been discussed in previous reports (24, 26, 27). However, these have been based on data from Western populations, and to our knowledge there have been no prospective reports from Asia. Different factors might influence Asian populations, as their characteristics (such as breast cancer incidence, physical activity, and body size) tend to differ from those of Western populations. Here, we analyzed data from a large-cohort study, the Japan Collaborative Cohort (JACC) Study, to examine the relationship between physical activity and breast cancer with a particular emphasis on the interactions with other risk factors, such as menopausal status and obesity.

Materials and Methods

Study Population. The present analysis was based on data from the JACC Study. This prospective cohort study evaluated the cancer risk associated with lifestyle factors

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among the Japanese population. At baseline (1988-1990), 110,792 subjects (46,465 men and 64,327 women), ages 40 to 79 y, were enrolled from 45 areas throughout Japan. All of the participants were subsequently followed up for all-cause mortality. Of the women in the baseline cohort, 34,086 lived in the 22 areas where data on both cancer incidence and physical activity were available. The JACC Study has been described in detail elsewhere (28, 29). Informed consent for participation was obtained from all individuals, with the exception of those in a few study areas where informed consent was provided at the group level, after the aims and data confidentiality had been explained to community leaders. The Ethics Board of Nagoya University School of Medicine, Japan, approved the JACC Study protocol.

Physical Activity Assessment. A self-administered questionnaire was used to obtain information on physical activity at baseline (30). The items covered included amount of time spent walking, amount of time spent exercising, and physical activity at the work place. Time spent walking daily was classified into three categories (<30 min, 30-59 min, and ≥1 h), as was time spent exercising (never or seldom, 1-2 h/wk, ≥3 h/wk). The validity of the estimates of time spent participating in sports and leisure activities was examined in a sample of the baseline subjects, suggesting that measuring physical activity level with the single-item question may be appropriate for establishing baseline data that reflect long-term physical activity in a large-scale cohort study (30, 31). We did not analyze the metabolic equivalent intensity, because of a lack of information on the strength of the exercise.

Other Variables. Information on additional potential breast cancer risk factors, such as family history, body mass index (BMI), tobacco and alcohol use, age at menarche, marital status, parity, age at birth of first child, menopausal status, and hormone use, was collected via the baseline questionnaire.

Follow-up and Identification of Breast Cancer Cases. The study participants were followed up from the time of enrollment until 2001, excluding five areas in which they were halted earlier. During this period, population registry data from each municipality were used to ascertain the residential and vital status of the participants. In Japan, the registration of death is required by the Family Registration Law, theoretically providing complete mortality data. Breast cancer incidence was confirmed mainly through the records of the population-based cancer registries in each area. During the study period, only 1,189 (3.9%) of the subjects were lost to follow-up due to moving out of the given study areas. The proportion of death-certificate-only cases was 6.3% (13 of 207). The mortality-to-incidence ratio for breast cancer was 0.26 (50 of 194) in the cohort covered by the cancer registries, which was within the range calculated using the available data from population-based cancer registries in Japan (0.20-0.30; ref. 32). We expect 37.4 breast cancer incidence cases who cannot be found from the cancer registries. The present analysis excluded 246 women who reported a previous diagnosis of breast cancer and 3,683 women who did not provide information on physical activity at baseline. Thus, a total of 30,157 women were included in the present analysis.

Statistical Analysis. For all participants, the person-years of follow-up was calculated as the time from enrollment until the diagnosis of breast cancer, death from any cause, moving out of the study area, or the end of follow-up, whichever occurred first. For the breast cancer cases ascertained by death-certificate-only, the person-years of follow-up were calculated from the time of enrollment until the time of death from breast cancer. Those individuals who died from causes other than breast cancer (n = 2,518) or who moved out of the study areas were treated as censored cases. We used Cox proportional hazards models to estimate the hazard ratios (HR) and 95% confidence intervals (95% CI) for the association of breast cancer incidence with physical activity. We evaluated the relationship using two models: an age-adjusted model (using 5-y age groups), and a multivariable model with adjustments for age, BMI (<22.0 kg/m^2, 22.0-23.9 kg/m^2, 24.0-25.9 kg/m^2, ≥26.0 kg/m^2, or unknown), alcohol drinking (never, past, current, or unknown), age at menarche (<15 y, 15-16 y, ≥17 y, or unknown), education level (attended school until the age of <16 y, 16-18 y, ≥19 y, or unknown), parity (nulliparous, 1 birth, 2-3 births, ≥4 births, or unknown), age at birth of first child (<22 y, 22-23 y, 24-25 y, ≥26 y, or unknown), use of exogenous female hormone (yes, no, or unknown), family history of breast cancer in a first-degree relative (yes, no, or unknown), menopausal status (premenopausal or postmenopausal), and menopausal age for postmenopausal women (<45 y, 45-49 y, ≥50 y, or unknown). In this study, those who provided menopausal age or who were at the average age at menopause, i.e., ≥49 y at baseline, were treated as postmenopausal women, and only those who were <49 y without information of menopausal age were treated as premenopausal women. Each "unknown" category included 5% to 9% of all women. All analyses were stratified by six study areas (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku, and Kyushu). Trend tests were done for category-based scores, which were assessed by allocating values ranging from 1 to 3 to each individual according to the selected physical activity variables.

To estimate the interaction of time spent walking and time spent exercising, we recategorized the subjects into four groups using the following cutoff points for physical activity: daily walking for <1 h or ≥1 h, and weekly exercising for <1 h or ≥1 h. Furthermore, the HR for the most active group (those who walked for ≥1 h/d and exercised for ≥1 h/wk) compared with the other groups was estimated according to menopausal status and BMI (<24 or ≥24 kg/m^2), and we examined the interaction between physical activity and these factors (Table 5). We used a BMI of 24 kg/m^2 instead of 25 kg/m^2 as a cutoff point for overweight. That was because there were only 47 cases for BMI ≥24 kg/m^2, which were too few to discuss interaction. For instance, we estimated the two HRs for physical activity among women who were premenopausal and postmenopausal at baseline, and then the P value for the interaction term of menopausal status and physical activity was calculated to test the difference between these HRs. We repeated the analysis after excluding the initial 2 y of follow-up, during which 37 cases of breast cancer were diagnosed. All of the P values were two-sided, with $P < 0.05$ indicating statistical significance. All of the analyses were done with SAS version 9.1 (SAS Institute, Inc.).

Table 1. Baseline characteristics associated with age in the JACC Study

Characteristics	Age group				Total
	40-49 y	50-59 y	60-69 y	70-79 y	
Number, *n* (row %)	7,561 (25.1)	9,361 (31.0)	9,098 (30.2)	4,137 (13.7)	30,157 (100.0)
Time spent walking per day					
Never or seldom, *n* (%)	868 (11.5)	1,013 (10.8)	807 (8.9)	403 (9.7)	3,091 (10.2)
Around 30 min, *n* (%)	1,393 (18.4)	1,650 (17.6)	1,794 (19.7)	876 (21.2)	5,713 (18.9)
30-59 min, *n* (%)	1,584 (20.9)	1,989 (21.2)	1,945 (21.4)	956 (23.1)	6,474 (21.5)
≥1 h, *n* (%)	3,716 (49.1)	4,709 (50.3)	4,552 (50.0)	1,902 (46.0)	14,879 (49.3)
Time spent exercising per wk					
Never or seldom, *n* (%)	5,890 (77.9)	7,365 (78.7)	6,591 (72.4)	2,842 (68.7)	22,688 (75.2)
1-2 h, *n* (%)	1,176 (15.6)	1,298 (13.9)	1,412 (15.5)	617 (14.9)	4,503 (14.9)
3-4 h, *n* (%)	338 (4.5)	399 (4.3)	572 (6.3)	306 (7.4)	1,615 (5.4)
≥5 h, *n* (%)	157 (2.1)	299 (3.2)	523 (5.7)	372 (9.0)	1,351 (4.5)

NOTE: Mean (SD) or %, calculated for participants with complete physical activity data.

Results

The average age at baseline was 57.6 ± 10.1 years, and the median follow-up time was 12.4 years. During the 340,055 person-years of follow-up, we identified 207 incident cases of breast cancer. The annual incidence of breast cancer in the cohort per 1,000 women was 0.61. Table 1 shows the distributions of physical activity according to age. Time spent walking was distributed similarly in the four age groups, with ~50% of the subjects walking for ≥1 hour per day. By contrast, for time spent exercising and physical activity at the work place, the older the subjects, the more physically active they tended to be. Regardless of the age group, more than two thirds of the participants never or seldom exercised.

Table 2 presents the risk of breast cancer in relation to physical activity. After adjusting for potential confounding factors, the HR was marginally decreased among those who walked for ≥1 hour per day (HR, 0.73; 95% CI, 0.53-1.01). However, those who exercised for ≥3 hours per week were not statistically decreased (HR, 0.85; 95% CI, 0.51-1.40). The *P* value for the linear trend of time spent walking was 0.043, which indicated that the dose-response effect of time spent walking and breast cancer risk was significant. The adjusted HR for those who walked for ≥1 hour compared with the rest of the women was significantly different (HR, 0.70; 95% CI, 0.53-0.93), although that for those who exercised for ≥3 hours per week was not significant (HR, 0.83; 95% CI, 0.59-1.16).

To investigate the joint effect of walking and exercise, we recategorized the data using the following cutoff points for physical activity: daily walking for <1 hour and exercising for <1 hour per week. Table 3 shows the mean values and distributions of risk factors for breast cancer according to the walking and exercise time categories. The subjects who walked and exercised more tended to be older and to drink more alcohol. The BMI values did not significantly differ between categories (range, 22.7-22.8 kg/m^2).

Table 4 shows the HRs of breast cancer associated with the joint effect of time spent walking and time spent exercising. The most physically active group (those who walked for ≥1 hour per day and exercised for ≥1 hour per week) had a lower risk of breast cancer (HR, 0.45; 95% CI, 0.25-0.78) compared with the least active group after adjusting for potential confounding factors. A significant interaction ($P = 0.042$) was observed between time spent walking and time spent exercising, meaning that the combined effect of exercise and walking on breast cancer was significant.

The HR of the most physically active group compared with the rest of the women was estimated for the subgroups according to menopausal status and BMI in Table 5, to examine the effects modification of these factors on the association between physical activity and breast cancer onset. The marginal inverse association was

Table 2. HR of breast cancer associated with physical activity in the JACC study

Physical activity	Cases	Person-years	Age adjusted	Multivariate*
			HR (95% CI)	HR (95% CI)
Time spent walking per day				
<30 min	69	96,752	1.00 (Reference)	1.00 (Reference)
30–59 min	56	71,411	1.14 (0.71 – 1.84)	1.13 (0.80 – 1.61)
≥1 h	82	171,892	0.70 (0.51 – 0.97)	0.73 (0.53 – 1.01)
P for trend			0.021	0.043
Time spent exercising per week				
Never or seldom	161	255,829	1.00 (Reference)	1.00 (Reference)
1–2 h	29	51,043	0.87 (0.59 – 1.30)	0.83 (0.56 – 1.23)
≥3 h	17	33,183	0.87 (0.53 – 1.45)	0.85 (0.51 – 1.40)
P for trend			0.45	0.33

*Adjusted for age, BMI, alcohol drinking, age at menarche, education level, parity, age at birth of first child, use of exogenous female hormone, family history of breast cancer in a first-degree relative, menopausal status, and menopausal age for postmenopausal women.

Table 3. Baseline characteristics associated with physical activity in the JACC study

Characteristics	Time spent exercising <1 h/wk		Time spent exercising ≥1 h/wk	
	Time spent walking per day		Time spent walking per day	
	<1 h	≥1 h	<1 h	≥1 h
Number, *n* (row %)	11,864 (39.3)	10,824 (35.9)	3,414 (11.3)	4,055 (13.4)
BMI, mean ± SD (kg/m^2)	22.8 ± 3.2	22.7 ± 3.0	22.8 ± 3.0	22.7 ± 2.9
Age at baseline, mean ± SD, y	57.5 ± 10.3	56.8 ± 9.6	58.5 ± 10.3	59.2 ± 10.4
Age at menarche, mean ± SD, y	14.8 ± 1.8	14.9 ± 1.8	14.8 ± 1.8	14.9 ± 1.8
Age at birth of first child, mean ± SD, y	25.2 ± 3.3	25.0 ± 3.3	25.1 ± 3.2	24.9 ± 3.1
Age at menopause, mean ± SD, y	48.7 ± 4.8	48.6 ± 4.6	48.8 ± 4.7	48.9 ± 4.5
Age of the end of education, mean ± SD, y	16.6 ± 2.1	16.5 ± 2.1	16.9 ± 2.2	16.7 ± 2.1
Postmenopausal, *n* (%)	8,946 (75.4)	8,176 (75.5)	2,657 (77.8)	3,225 (79.5)
Nulliparous, *n* (%)	612 (5.2)	387 (3.6)	142 (4.2)	163 (4.0)
Not married, *n* (%)	223 (2.0)	120 (1.2)	65 (2.1)	42 (1.1)
Exogenous female hormone use, *n* (%)	580 (5.4)	474 (4.8)	191 (6.2)	207 (5.7)
Family history of breast cancer,* *n* (%)	191 (1.6)	159 (1.5)	63 (1.9)	65 (1.6)
Current smoker, *n* (%)	606 (5.6)	556 (5.7)	133 (4.3)	183 (5.0)
Current drinker, *n* (%)	2,594 (23.1)	2,447 (24.0)	906 (27.9)	1,122 (29.4)

NOTE: Mean (SD) or %, calculated for participants with complete physical activity data.
*In a first-degree relative.

observed in each subgroup, and no significant interaction was observed. This suggests that the inverse association was not modified by these factors. Similar results were found after excluding the initial 2 years of follow-up, during which 37 cases of breast cancer were diagnosed.

Discussion

Our prospective analysis of the relationship between physical activity and breast cancer in Japanese women revealed a significant inverse association. In particular, the combined effect of walking and exercise was stronger than that expected based on the individual effects. Moreover, the combined protective effect of walking and exercise was not modified significantly by menopausal status or BMI. This suggests that physical activity has a protective effect regardless of menopausal status or weight. Previous studies of Western populations have provided convincing evidence of an inverse association between physical activity and breast cancer risk (2, 3), as supported by a recent systematic review (4). Adding more recent cohort studies (22-25), 10 of 21 showed a significantly decreased breast cancer risk associated with physical activity. Despite the comparatively lower incidence of breast cancer in Japan (1), an inverse association between physical activity and breast cancer incidence has also been observed, which was consistent with the findings of previous case-control studies in Japan (33-35).

The present study showed an interactive effect of walking and exercise. This could be explained in several ways. For instance, multiple types of exercise might work more effectively than a single type of exercise, the effect of physical activity might be quadratic, or walkers might tend to exercise more intensely. Whatever the reason, our results indicate that walking for ≥1 hour per day should initially be recommended, and additional weekly exercise should be undertaken to improve the protective effect against breast cancer.

In the present study, menopausal status and BMI did not affect the relationship between physical activity and breast cancer. Of the two, the modifying effect of menopausal status is the more controversial. Among the previous cohort studies that have analyzed this association according to menopausal status, only two have observed a significantly decreased breast cancer risk among premenopausal women (11, 22), and the evidence is weaker among premenopausal women (5, 10, 17). This difference might be partly due to the way in which menopause has been treated in the analyses. All of the studies, including the present one, reporting a protective effect of physical activity among premenopausal women have used only baseline menopausal status and have not updated this measure. By contrast, a study that found no association did update the menopausal status (19), and menopause was included as one of its end points.

Compared with menopausal status, the effect modification of BMI on the association between physical

Table 4. HR of breast cancer associated with physical activity in the JACC study

Physical activity		Cases	Person-years	Age adjusted	Multivariate*
Time spent walking (h/d)	Time spent exercising (h/wk)			HR (95% CI)	HR (95% CI)
<1	<1	93	130,279	1.00 (Reference)	1.00 (Reference)
≥1	<1	68	125,550	1.18 (0.79 – 1.77)	1.13 (0.75 – 1.69)
<1	≥1	32	37,885	0.76 (0.56 – 1.04)	0.82 (0.60 – 1.12)
≥1	≥1	14	46,342	0.42 (0.24 – 0.74)	0.45 (0.25 – 0.78)
P for interaction				0.035	0.041

*Adjusted for age, BMI, alcohol drinking, age at menarche, education level, parity, age at birth of first child, use of exogenous female hormone, family history of breast cancer in a first-degree relative, menopausal status, and menopausal age for postmenopausal women.

Table 5. HR of breast cancer among the most physically active group compared with the rest of the women by subgroup of menopausal status and BMI in the JACC study

Subgroup	Age adjusted	Multivariate*
	HR (95% CI)	HR (95% CI)
Menopausal status		
Premenopausal	0.14 (0.02 – 0.97)	0.13 (0.02 – 0.91)
Postmenopausal	0.53 (0.29 – 0.96)	0.53 (0.29 – 0.96)
P for interaction	0.524	0.528
BMI (kg/m^2)		
<24	0.43 (0.20 – 0.91)	0.42 (0.19 – 0.90)
≥24	0.45 (0.18 – 1.10)	0.44 (0.18 – 1.09)
P for interaction	0.940	0.949

*Adjusted for age, BMI, alcohol drinking, age at menarche, education level, parity, age at birth of first child, use of exogenous female hormone, family history of breast cancer in a first-degree relative, menopausal status, and menopausal age for postmenopausal women.

activity and breast cancer risk has been more consistent, as previous studies have failed to show general effects (5, 6, 8-10, 13, 14, 16, 18, 19, 21). These findings suggest that the effect of physical activity is independent of menopausal status (despite the possibility of a less precise effect among premenopausal women) and BMI. Therefore, the recommendation to undertake physical activity to prevent breast cancer does not need to be altered according to differences in these factors.

A major strength of the present study is its prospective design, which might avoid the recall bias that is possible in case-control studies. Moreover, information on other risk factors for breast cancer was included, and potential confounding factors were controlled for in the analyses when examining the association.

Our study had some limitations that should be considered when interpreting the results. First, because we used only a simple questionnaire at baseline, neither metric equivalent nor updated values were available to evaluate physical activity. In general, assessing physical activity in epidemiologic studies is difficult, which might explain the heterogeneous results observed across studies of its association with breast cancer (36). Although it is possible that the reported levels might have overestimated or underestimated the actual physical activity, the information was collected before diagnosis and should not have differed according to the end point status. Thus, the misclassification of physical activity in the present study for both reasons is nondifferential. It means the estimated HRs tend to be close to the null, and true HRs should be smaller due to the misclassification. In addition, because more than two thirds of the women in our cohort never or seldom exercised, we expect less serious misclassification. Second, updated information on menopausal status was lacking, which could modify the relationship between physical activity and breast cancer. Thus, from an etiologic viewpoint, the misclassification of menopausal status at the onset of breast cancer should be important. However, from the viewpoint of cancer prevention, the menopausal status at cancer onset is comparatively less important, and the HR could be naturally interpreted for premenopausal women at baseline. Third, misclassification of menopausal status at baseline should also be considered. However, the point estimate of the HR among premenopausal women was smaller than that among postmenopausal women, which could not be explained from misclassification. In addition, the results were not essentially changed when we removed women who were 47 to 50 years old from the premenopausal group. More studies are needed of premenopausal women in larger subjects.

In summary, our analysis provided evidence that physical activity decreased the risk of breast cancer among Japanese women. Another encouraging finding of this study is that the effect of physical activity on breast cancer risk is not modified by menopausal status and BMI. We recommend walking for 1 hour per day along with additional weekly exercise to protect against breast cancer, regardless of menopausal status and BMI.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Appendix 1. The Japan Collaborative Cohort Study Group

The present members of the JACC Study and their affiliations are as follows: Dr. Akiko Tamakoshi (present chairman of the study group), Aichi Medical University School of Medicine; Dr. Mitsuru Mori, Sapporo Medical University School of Medicine; Dr. Yutaka Motohashi, Akita University School of Medicine; Dr. Ichiro Tsuji, Tohoku University Graduate School of Medicine; Dr. Yosikazu Nakamura, Jichi Medical School; Dr. Hiroyasu Iso, Institute of Community Medicine, University of Tsukuba; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Yutaka Inaba, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, University of Human Arts and Sciences Graduate School; Dr. Hiroshi Suzuki, Niigata University Graduate School of Medical and Dental Sciences; Dr. Hiroyuki Shimizu, Gifu University School of Medicine; Dr. Hideaki Toyoshima, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, Nagoya City University Graduate School of Medical Sciences; Dr. Yoshinori Ito, Fujita Health University School of Health Sciences; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Kenji Wakai, Nayoga University Graduate School of Medicine; Dr. Akio Koizumi, Graduate School of Medicine and Faculty of Medicine, Kyoto University; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Dr. Yoshiyuki Watanabe and Dr. Tsuneharu Miki, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Chigusa Date, Faculty of Human Environmental Sciences, Mukogawa Women's University; Dr. Kiyomi Sakata, Wakayama Medical University; Dr. Takayuki Nose, Tottori University Faculty of Medicine; Dr. Norihiko Hayakawa, Research Institute for Radiation Biology and Medicine, Hiroshima University; Dr. Takesumi Yoshimura, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan; Dr. Akira Shibata, Kurume

University School of Medicine; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; Dr. Hideo Shio, Moriyama Municipal Hospital; Dr. Yoshiyuki Ohno (former chairman of the study group), Asahi Rosai Hospital; Dr. Tomoyuki Kitagawa, Cancer Institute of the Japanese Foundation for Cancer Research; Dr. Toshio Kuroki, Gifu University; and Dr. Kazuo Tajima, Aichi Cancer Center Research Institute.

The past investigators of the study group were listed in ref. 28 except for the following eight members (affiliations are those at the time they participated in the study): Dr. Takashi Shimamoto, Institute of Community Medicine, University of Tsukuba; Dr. Heizo Tanaka, Medical Research Institute, Tokyo Medical and Dental University; Dr. Shigeru Hisamichi, Tohoku University Graduate School of Medicine; Dr. 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.

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RESEARCH COMMUNICATION

Sex and Seasonal Variations of Plasma Retinol, α-Tocopherol, and Carotenoid Concentrations in Japanese Dietitians

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Abstract

Aim: To clarify sex and seasonal variations of plasma antioxidant concentrations among middle-aged Japanese. Subjects and Methods: We investigated sex and seasonal variations of plasma antioxidant concentrations, including retinol, α-tocopherol, and carotenoids (α-carotene, β-carotene,β-cryptoxanthin, lutein and lycopene), in 55 middle-aged dietitians (46 women and 9 men) in Aichi Prefecture, Central Japan, who took no supplements from autumn 1996 to summer 1997. Reversed-phase high performance liquid chromatography was used to measure plasma antioxidant concentrations in overnight-fasting blood samples. Results: Plasma levels of α-tocopherol, α-/β-carotene, β-cryptoxanthin and lutein were significantly influenced by sex, being significantly higher for women than men in each corresponding season; retinol and lycopene, however, showed no such difference. For women, winter values of α-tocopherol, α-/β-carotene, lutein and lycopene were significantly lower than corresponding summer values, and had reached their annual lowest. Retinol failed to show any significant seasonal variation, whereas the winter value of β-cryptoxanthin had reached its annual highest. For men, β-cryptoxanthin exhibited significant seasonal changes and was also highest in winter. Winter values of α-tocopherol, α-/β-carotene and lycopene were lower compared with other seasons, but not statistically significant, probably due to the small sample size. Conclusions: The findings indicate that sex and seasonal variations of plasma antioxidant concentrations should be taken into account in nutritional epidemiologic studies.

Key Words: Sex - season - variation - plasma antioxidants - retinol - α-tocopherol - carotenoids

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Introduction

According to epidemiologic observations, many antioxidants are inversely associated with the risk or prevalence of chronic diseases (Goodman, 1984; Cutler, 1991; Mobarhan et al., 1991; Byers et al., 1992). Antioxidants are thought to play an important role in disease prevention, in particular, such as vitamin E in the prevention of heart disease (Rimm et al., 1993; Stampfer et al., 1993), and retinoids, tocopherols and carotenoids in the prevention of cancer (Moon et al., 1976; Suda et al., 1986; Bertram et al., 1991; Knekt et al., 1991; Rimm et al., 1993).

Plasma concentrations of antioxidants may fluctuate according to host and environmental factors. Sex (Shibata et al., 1989), smoking/drinking habits (Stryker et al., 1988) and season (Cantilena et al., 1992) have also been observed to impact on plasma antioxidant levels. However, in the Asian countries, including Japan, where host and environmental factors are known to be fundamentally different from those in the Western world, few studies have been conducted to investigate the effects of sex and seasonal changes on plasma antioxidant concentrations.

Here we examined the influence of sex (a host factor) and season (an environmental factor) on variations in plasma antioxidant levels in Japanese dietitians living in Aichi Prefecture, Central Japan who participated in our previous nutritional studies (Tokudome et al., 2001; Imaeda et al., 2002; Kuriki et al., 2002; Tokudome et al., 2002).

Materials and Methods

Study Subjects

One hundred and six middle-aged dietitians (85 women, 21 men), who were members of the Aichi

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Prefectural Dietitians' Association residing in Aichi Prefecture, Central Japan voluntarily participated in this study, as reported elsewhere (Tokudome et al., 2001). In brief, 97 subjects were enrolled after excluding those with chronic diseases. Among them, 86 whose four-season blood samples (Autumn 1996 - Summer 1997) were available, entered the current investigation. Information on demographic characteristics, drinking and smoking habits, regular exercise, and intake of supplements, including vitamins, was obtained from a self-administered questionnaire. Finally, 55 subjects (46 women and 9 men) who regularly took no supplements were eligible for in-depth analysis.

This protocol was approved by the Ethics Committee of the Nagoya City University Graduate School of Medical Sciences, and written informed consent was secured from each study participant.

Methods

Overnight-fasting venous blood was sampled using a tube with EDTA-2Na, and separated into plasma, buffy coat, and RBC clot. The samples were stored at -80° C until analysis.

Seven plasma antioxidants, including retinol, α-tocopherol and five carotenoids (α-/β-carotene, β-cryptoxanthin, lutein and lycopene) were assessed by reverse-phase high performance liquid chromatography (HPLC) according to a modification of Talwar's method (Talwar et al., 1998). At the time of analysis, samples were thawed at room temperature. As an internal standard, 0.1 ml retinol acetate and 0.1 ml tocopherol acetate were added to 0.1 ml plasma. The total volume was raised to 1.3 ml with distilled water. After protein was precipitated by adding 0.9 ml ethanol, fat-soluble substances were extracted twice with 1.2 ml hexane.

Samples were mixed and centrifuged for 15 min. The hexane layer was gathered and evaporated under a nitrogen atmosphere. The residue was reconstituted with tetrahydrofuran making up to 0.3 ml with a mobile phase, and 0.2 ml was injected into Shimadzu HPLC with a Nucleosil C18 column. The mobile phase, which consisted of methanol, acetonitrile and tetrahydrofuran in the ratio of 75 : 20 : 5, flowed at 0.6 ml/min. Using an SPD-M10 Avp Shimadzu diode array detector, retinol and retinol acetate were detected at 325 nm, α-tocopherol and tocopherol acetate at 292 nm, α-/β-carotene, β-cryptoxanthin and lutein at 450 nm, along with lycopene at 473 nm.

Laboratory accuracy and precision in the measurements of seven plasma antioxidants (retinol, α-tocopherol, and five carotenoids) were maintained and assessed by using pooled plasma. Concentrations of antioxidants in the plasma were calculated with the aid of commercially available lyophilized serum from NIST (the National Institute of Standards and Technology). Two pooled plasma samples were analyzed at the beginning and the end of each run. Coefficients of variation for the antioxidants ranged from 3.3% (α-tocopherol) to 13.7% (β-crytoxanthin) for the same day determination, and from 5.0% (retinol) to 14.6% (β-crytoxanthin) for day-to-day reproducibility.

Table 1. Baseline Demographic and Lifestyle Characteristics of 55 Japanese Dietitians Not Taking Supplements

Characteristics	Women (n=46)		Men (n=9)		p value[a]
Age(y) mean(SD)	46.4 (7.8)		47.7 (10.2)		0.67
BMI(kg/m²)[b] n (%)					0.11
<19.8	12	26.1	1	11.1	
19.8-24.2	30	65.2	5	55.6	
>24.2	4	8.7	3	33.3	
Smoking habit					<0.001
Current	1	2.2	2	22.2	
Ceased	1	2.2	3	33.3	
Never	44	95.7	4	44.4	
Drinking habit					<0.05
Current	9	19.6	6	66.7	
Ceased	1	2.2	0	0	
Never	36	78.3	3	33.3	
Regular exercise					0.3
With	29	63.0	4	44.4	
Without	17	37.0	5	55.6	

[a]By Chi-square test or Student t-test, [b]Categorization was made according to obesity assessment standards set by Japan Obesity Association.

Table 2. Plasma Concentrations of Retinol, α-Tocopherol, and Carotenoids in Japanese Dietitians Not Taking Supplements

		Women (n=46)	Men (n=9)
Retinol	Autumn	2.12 (1.18-3.60)	2.33 (1.47-2.86)
(umol/L)	Winter	2.25 (0.77-3.45)	2.41 (1.82-2.75)
	Spring	2.16 (1.23-4.18)	2.80 (1.86-2.97)
	Summer	2.25 (1.34-3.88)	2.37 (1.66-3.11)
α-Tocopherol	Autumn	31.8 (18.4-65.3)[e]	21.8 (18.2-39.1)
(umol/L)	Winter	28.2 (17.0-49.0)[c,b,e]	21.7 (18.3-41.6)
	Spring	33.3 (23.2-68.9)[e]	27.2 (19.2-42.9)
	Summer	33.1 (21.6-60.1)[e]	26.0 (18.4-41.9)
α-Carotene	Autumn	0.30 (0.11-0.71)[b]	0.20 (0.10-0.72)
(umol/L)	Winter	0.25 (0.09-0.58)[b,e]	0.13 (0.08-0.40)
	Spring	0.26 (0.11-0.63)[b,e]	0.14 (0.07-0.59)
	Summer	0.36 (0.12-1.17)[e]	0.16 (0.09-1.04)
β-Carotene	Autumn	1.33 (0.42-2.53)[e]	0.60 (0.27-2.08)
(umol/L)	Winter	1.10 (0.21-3.12)[c,b,e]	0.55 (0.26-1.60)
	Spring	1.27 (0.47-3.16)[e]	0.67 (0.13-1.61)
	Summer	1.40 (0.48-3.74)[e]	0.60 (0.30-1.92)
β-Crypto-	Autumn	0.55 (0.10-1.70)[a,b,e]	0.24 (0.11-0.56)
xanthin	Winter	1.06 (0.14-3.20)[c,b]	0.73 (0.13-2.10)[d]
(umol/L)	Spring	0.49 (0.10-2.02)[b,e]	0.18 (0.11-0.57)
	Summer	0.39 (0.08-1.07)[e]	0.22 (0.08-0.32)
Lutein	Autumn	0.85 (0.36-1.21)[a,e]	0.50 (0.32-0.74)
(umol/L)	Winter	0.73 (0.30-1.09)[c,b,e]	0.53 (0.31-0.70)
	Spring	0.81 (0.39-1.66)[e]	0.61 (0.31-0.76)
	Summer	0.87 (0.37-1.71)[e]	0.57 (0.26-1.50)
Lycopene	Autumn	0.49 (0.12-1.49)[b]	0.53 (0.10-1.08)
(umol/L)	Winter	0.32 (0.11-1.67)[c,b]	0.33 (0.10-0.71)
	Spring	0.51 (0.09-1.83)[b]	0.49 (0.11-0.70)
	Summer	0.71 (0.18-2.17)	0.55 (0.12-0.97)

*Medians; ranges (minimum-maximum) in parentheses.Significantly different:[a]from women in winter, p<0.01,[b]from women in summer, p<0.05, [c]from women in spring, p<0.05, [d]from men in summer, p<0.05, [e]between women and men in the same season, p<0.05

Statistical analysis

The normality of distribution of seasonal antioxidant measurements in each sex group was determined by the Shapiro-Wilk test. If not normally distributed, distribution-free (non-parametric) methods were adopted for further

statistical analyses. The Kruskal-Wallis test was adopted for an analysis of variance with the seasons by sex, and the Wilcoxon 2-sample test for variance between men and women. Procedures of FREQ, UNIVARIATE, or NPAR1WAY in the SAS package were used for statistical calculations (SAS Institute, Inc., 1990). Differences were considered to be statistically significant at $p<0.05$.

Results

Baseline demographic and lifestyle characteristics of 55 Japanese dietitians (46 women and 9 men) who took no supplements are summarized in Table 1. There were no statistical differences between women and men in age, BMI or physical exercise. However, smoking and drinking rates were significantly higher in men.

The Shapiro-Wilk test showed that few distributions of plasma antioxidant values in each sex and season appeared normally distributed. Therefore, non-parametric methods were adopted for statistical analyses. Table 2 shows sex- and season-specific medians and ranges of respective antioxidants. Retinol and lycopene did not significantly differ between men and women. α-Tocopherol was significantly higher in women than men in all seasons, and reached its highest in spring for all subjects. The Kruskal-Wallis test showed that there were statistically significant seasonal variations in α-/β-carotene, β-cryptoxanthin, lutein and lycopene in women, as well as β-cryptoxanthin in men. Both α-carotene and β-carotene were higher in women than men in each corresponding season. A higher level was noted in women in summer, and in men in autumn or spring. The concentrations of β-cryptoxanthin were higher in women than men in each corresponding season with the highest value appearing in winter for both groups. Lutein was highest in summer in women, while it was highest in spring in men. Women showed higher lutein than men, irrespective of the season.

Discussion

Plasma levels of α-tocopherol, α-/β-carotene, β-cryptoxanthin and lutein were significantly higher for women than men in each corresponding season; but no sex difference was observed for retinol and lycopene. For women, winter concentrations of α-tocopherol, α-/β-carotene, lutein and lycopene were significantly lower than their corresponding summer values, and were the lowest of the entire year. Retinol demonstrated no significant seasonal variation; however, the winter level of β-cryptoxanthin was found to be the highest of the whole year. For men, β-cryptoxanthin showed significant seasonal changes and was also highest in winter. Winter concentrations of α-tocopherol, α-/β-carotene and lycopene tended to be lower compared with other seasons. Plasma concentrations of α-tocopherol, α-/β-carotene, β-cryptoxanthin and lutein in women were higher than in men throughout the year.

The present findings are in harmony with the observations of healthy Japanese students, university staff and general local inhabitants (Ito et al., 1990), a Spanish clinical staff (Olmedilla et al., 1994), and the US general population (Krasinski et al., 1989), as well as in a US hospital-based study (Stacewicz-Saquntzakis et al., 1987). That may be explained by the fact that Japanese women consume more fruit and vegetables (Inoue et al., 1997), smoke less and drink less alcohol than Japanese men (Tsubono et al., 1997; Health Promotion and Nutrition Division 1998). It has been proven that fruit and vegetables are major sources of these micronutrients (Resources Council 1982; Willett, 1990; Resources Council 1992; Tokudome et al., 1998), while smoking and drinking risked diminishing the reserves of antioxidants (Russell-Briefel et al., 1985; Stryker et al., 1988). Interestingly, however, our results are also consistent with the reports that the retinol level in men may be slightly higher than in women (Stacewicz-Saquntzakis et al., 1987; Krasinski et al., 1989; Ito et al., 1990; Olmedilla et al., 1994). Although it is still impossible to fully elucidate the mechanism underlying why plasma antioxidant concentrations differ by sex, this variation should be taken into account in the assessment as well as the planning of any nutritional epidemiologic research; otherwise it could impose a bias due to the sex predominance in the study population.

In the present study, the seasons did not significantly affect retinol in either men or women. However, there were statistically significant seasonal changes of α-tocopherol, α-/β-carotene, β-cryptoxanthin, lutein and lycopene in women; but statistical power did not appear sufficient to detect such seasonal variations in men. For all study subjects, higher α-tocopherol occurred in spring, and higher β-cryptoxanthin in winter, whereas, α-/β-carotene and lycopene tended to be higher in summer or in autumn. This could be attributable to dietary variations by season, since all study participants come from central Japan (longitude around 136°~137° E., latitude around 35° N.), where there are distinct seasonal changes in the temperature and hours of sunlight. It is noteworthy that from September to later October Japanese enjoy an abundance of agricultural products, including fruit, and green and yellow vegetables. Our previous study of the same dietitian cohort showed that the dietary intakes of selected vitamins and minerals measured by weighed diet records were greater in autumn and winter than those in spring and summer (Tokudome et al., 2002). Similar seasonal changes have also been observed by Olmedilla (Olmedilla et al., 1994), and in studies on α-carotene (Van Staveren et al., 1986; Ziegler, 1989; Rautalahti et al., 1993). In the case of retinol, seasonal variations are not as remarkable as with other micronutrients, compatible with other studies (Olmedilla et al., 1994; Cooney et al., 1995); however, the reason remains obscure, suggesting that adjustments for seasonal effects should be allowedat least for α-tocopherol and the above-mentioned carotenoids in nutritional epidemiologic studies.

In conclusion, although mechanisms of sex and seasonal variations in plasma retinol, α-tocopherol and five carotenoids need to be further elucidated in different ethnic groups and areas, such changes in blood antioxidant levels should also be taken into account to determine any associations with disease prevention and health promotion in nutritional epidemiologic research.

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Eicosapentaenoic Acid Has a Preventive Effect on the Recurrence of Nephrolithiasis

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Key Words
Eicosapentaenoic acid · Nephrolithiasis · Calcium oxalate · Recurrent stone formation

Abstract
Background: With a westernized diet the oxygenated products of renal prostaglandin synthesis are metabolites of the n–6 series and these are known to play important roles in several pathophysiological processes involved in calcium stone formation. As eicosapentaenoic acid (EPA) administration is effective for the prevention of hyperlipidemia and athelosclerosis, we suggest the use of EPA as a possible method of preventing calcium stone formation. ***Methods:*** We administered a highly purified preparation of 1,800 mg/day EPA to 29 patients for 36.4 ± 22.0 months after therapy for nephrolithiasis. We observed the urinary stone recurrence in these patients over the course of 8 years (before, during and after medication) and studied the preventive effect on the recurrence of nephrolithiasis. We analyzed the effect of EPA administration and compared the findings to those before and after administration. ***Results:*** The incidence rates of nephrolithiasis (times/year) before, during and after the administration of EPA were 0.2283, 0.0693 and 0.1742, respectively. The incidence rate of nephrolithiasis during the administration of EPA was significantly lower compared the findings to those before and after its administration ($p < 0.05$). ***Conclusion:*** The results suggest that EPA might reduce the risk of calcium stone formation.

Introduction

Nephrolithiasis is a common, costly and painful disease. Although modification of the diet remains a mainstay in the treatment of nephrolithiasis [1], the effect of many dietary factors on the development of kidney stones is unknown.

Evidence that fatty acid intake may modulate the urinary excretion of calcium and oxalate has led to speculation that increased consumption of n–3 polyunsaturated fatty acids (PUFAs), found in fish or fish oil supplements, may reduce the risk of kidney stone formation [2, 3]. The Greenland Eskimos and coastal Japanese are reported to have a very low incidence of diseases common to Western man, including renal stones [4, 5]. This has been attributed to their diet, which is rich in eicosapentaenoic acid (EPA, one of the main n–3 PUFAs in fish oil) [6]. Recently in Japan, with the adoption of a more Western diet, the incidence of urolithiasis has increased [7]. Buck et al. [6] showed that urinary calcium and urinary oxalate excretion in recurrent, hypercalciuric stone formers was significantly reduced with fish oil treatment. From these results, they suggested that the incorporation of EPA into the diet could be an effective way to correct hypercalciuria.

We have reported that the administration of EPA 1,800 mg/day reduces the urinary excretion of calcium in the long term [8]. It was reported that n–3 PUFAs, for example EPA, may reduce the risk of kidney stone formation [6, 8], but there are few reports of clinical studies. We ex-

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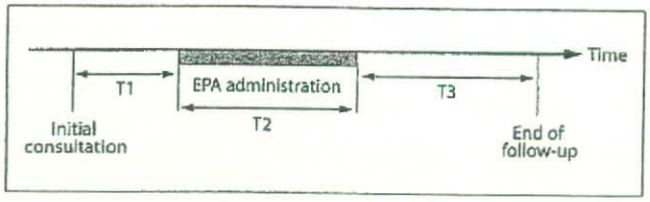

Fig. 1. The study period was divided into the following three phases: the period between the initiation of the study (time of initial consultation at the outpatient clinic) and the initiation of EPA administration (T1); the period during the administration of EPA (T2), and the period between discontinuation of EPA administration and the end of the follow-up study (T3). The frequencies of stone formation during T1, T2 and T3 are indicated by number of stone events/year, individually.

amined the preventive effect of EPA toward nephrolithiasis in Japanese patients in whom we confirmed the stone recurrence incidents before, during and after the administration of EPA.

Methods

The subjects were Japanese patients with calcium oxalate nephrolithiasis who had been treated with EPA for more than 24 months since January 1995. These 29 patients (22 males and 7 females) had a mean age of 53.3 ± 13.6 years at the initiation of this study and were attending our Stone Clinic due to idiopathic calcium oxalate stone disease. The EPA preparation applied was highly purified EPA (Epadel®; Mochida Pharmaceutical Co., Tokyo, Japan). A dose of 600 mg was taken orally immediately after each meal, totaling 1,800 mg/day. Although the diet of the patients was not controlled, they were encouraged to maintain the recommended fat-reduced diet, avoid medication if possible, and drink plenty of fluids. Patients who demonstrated a plasma total cholesterol of ≥220 mg/dl or >150 mg/dl of plasma triglycerides were classified as hyperlipemic. This was the case for 12 patients, 8 men and 4 women. None of the patients had primary hyperparathyroidism, renal tubular acidosis or other conditions known to cause abnormal metabolism. Individuals with urinary tract infection or renal insufficiency were excluded. All gave their informed consent to this study, according to the Helsinki Declaration.

Patients' history of medical treatment was investigated, and those who fulfilled the following inclusion criteria were enrolled for retrospective evaluation: having received ambulatory treatment in our hospital since the time before the administration of EPA, confirmation of nephrolithiasis treated with EPA for more than 24 months, and the presence or absence of stone formation confirmed during the observation period after discontinuation of EPA administration. The development of nephrolithiasis was investigated before, during and after the administration of EPA to evaluate the preventive effect of EPA on stone formation.

The study period was divided into the following three phases: the period between the initiation of this study (time of initial consultation at the outpatient clinic) and the initiation of EPA administration (T1), the period during the administration of EPA (T2), and the period between discontinuation of EPA administration and the end of the follow-up study (T3) (fig. 1). This studythe initiated first episode of stone event and the retrospective analysis was based solely on medical records. All patients were radiologically confirmed to be completely stone-free after spontaneous drainage of the stone or after treatment of each stone event. The patients with residual fragment were excluded from the study. Most of the medications approved for patients in this study were not standard agents and none of the patients were administered any other stone-preventing medication during T1 or T3. Patients were evaluated every 3 months for stone recurrence by plain X-ray and stone passage episode. The number of stone formations during T1, T2 and T3 was N1, N2 and N3, respectively. Stone events involved both radiological detection of stone and renal colic in this study. An initial episode of nephrolithiasis during T1 was not counted as N1. The frequency of stone formation was calculated as follows: between the onset of nephrolithiasis and the initiation of EPA administration, R1 = N1/T1 (times/year), during EPA administration, R2 = N2/T2 (times/year) and between the discontinuation of EPA administration and the end of a follow-up study, R3 = N3/T3 (times/year).

The effects of EPA administration were evaluated based on the comparison between R1 and R2 or between R2 and R3. In addition, the effects of age and period were evaluated by comparing R1 and R3. Considering the observation period and the frequency of recurrence stone formation, we calculated the incidence of nephrolithiasis without counting the initial discovery of nephrolithiasis.

For the comparison of the incidence rate of each period, the incidence rate ratio with a 95% confidence interval was calculated applying the Poisson regression model. Proc Logist in SAS Version 8.2 (SAS Institute, Inc., Cary, N.C., USA) was used for Poisson regression.

Results

The mean duration of T1, T2 and T3 were 47.8 ± 69.0, 36.4 ± 22.0 and 50.6 ± 29.5 months. The number of stone events (N1, N2 and N3) during T1, T2 and T3 in all patients was 26, 6 and 21, respectively.

Incidence rates (R1, R2 and R3) of nephrolithiasis (times/year) before, during, and after the administration of EPA (T1, T2, and T3) were 0.2283, 0.0693 and 0.1742, respectively (table 1). The incidence rate ratios of nephrolithiasis before and after the administration of EPA (T1 and T3) to that of nephrolithiasis during EPA administration (T2) were 3.294 and 2.515, respectively. Compared to the incidence rates of nephrolithiasis before and after the administration of EPA (T1 and T3), the incidence rate of nephrolithiasis during the administration of EPA (T2) was significantly lower ($p < 0.05$). The incidence rate ratio of nephrolithiasis after the administration of EPA (T3) to

Table 1. Incidence rate of nephrolithiasis and EPA administration (n = 29)

	Before EPA administration (T1)	During EPA administration (T2)	After EPA administration (T3)
Incidence of nephrolithiasis	0.2283	0.0693	0.1742
RR[a] compared with T2 (95% CI)	3.294 (1.356–8.001)	reference	2.515 (1.015–6.232)
RR[b] compared with T1 (95% CI)	reference	–	0.763 (0.429–1.356)

RR = Relative risk; 95% CI = 95% confidence interval.
[a] Relative risks are for the risk for stone formation compared with T2.
[b] Relative risks are for the risk for stone formation compared with T1.

Table 2. Effect of EPA administration on urinary and serum parameters (n = 29)

	Before EPA administration (T1)	During EPA administration (T2)	After EPA administration (T3)
Urinary parameters			
Urine volume, l	1.65 ± 0.78	1.68 ± 0.82[NS]	1.75 ± 0.92[NS]
Calcium, mg/day	194 ± 92	184 ± 117[NS]	199 ± 102[NS]
Magnesium, mg/day	87.4 ± 49.8	74.3 ± 42.3[NS]	88.2 ± 50.2[NS]
Phosphorus, g/day	0.863 ± 0.343	0.767 ± 0.329[NS]	0.892 ± 0.322[NS]
Urea nitrogen, g/day	8.95 ± 3.01	7.99 ± 3.23[NS]	8.63 ± 3.45[NS]
Creatinine, g/day	1.21 ± 0.40	1.18 ± 0.38[NS]	1.20 ± 0.43[NS]
Uric acid, mg/day	672 ± 270	612 ± 298[NS]	665 ± 263[NS]
Plasma lipids			
Total cholesterol, mg/dl	188 ± 40	178 ± 41[NS]	194 ± 45[NS]
Triglycerides, mg/dl	135 ± 75	122 ± 69[NS]	134 ± 77[NS]

NS = No significant difference compared to T1.

that of nephrolithiasis before EPA administration (T1) was 0.763. The incidence rate of nephrolithiasis after the administration of EPA (T3) tended to be lower than that before EPA administration (T1), although there was no significant difference.

There were no overall changes in urine volume, urinary calcium, magnesium, phosphorus, urea nitrogen, creatinine or uric acid among T1, T2 and T3. Serum total cholesterol and triglycerides displayed a non-significant trend toward reduction (table 2). In the hyperlipemia group, reduction of triglycerides (226 ± 61, 175 ± 54 and 198 ± 54 mg/dl before (T1), during (T2) and after administration of EPA (T3), respectively) was significant during administration of EPA.

No serious side effects were observed. One patient suffered stomach discomfort after 2 months of EPA administration but could continue the treatment for 36 months.

Discussion

In our study the nephrolithiasis incidence rate during EPA administration (T2) was lower compared to before (T1) or after (T3) its administration. These findings suggest that EPA may be useful for preventing the development of nephrolithiasis. The frequency of stone formation after the administration of EPA (T3) tended to be lower than that before the administration of EPA (T1). This was probably due to the advanced ages of the subjects and an effect of the stone clinic. The mean age of patients before the administration of EPA was 7 years younger and it is difficult to consider that this factor alone decreased the frequency of stone formation 0.76-fold. It is speculated that attending the Stone Clinic had an effect; the instruction to drink water during the long-term ambulatory treatment may be particularly important [9]. The hypothesis was explored in reference to

phospholipid metabolism in the light of the following considerations: (1) phospholipids are recognized as second messengers, and may influence membrane properties and membrane protein functions [10]; (2) an alternation in the urine excretion of prostaglandin E_2 (PGE_2), a phospholipid metabolite, has been described in calcium nephrolithiasis [11]; (3) dietary n–3 PUFAs have been shown to be potent modulators of tissue phospholipid fatty acid composition and eicosanoid production in vitro and in vivo [12] and to reduce urine calcium and oxalate excretion [6]; (4) nephrolithiasis is rare in Eskimos [4].

Several study designs may account for the results of the fish oil supplementation trial that showed a reduction in the excretion of urinary calcium and oxalate. Clinical and experimental investigations seem to underline the important role of fatty acids in the pathogenesis of hypercalciuria, a well-known risk factor for lithogenesis [13]. None of these studies used a placebo-controlled design to ensure that fish oil, rather than changes in the diet, accounted for the difference observed in urine composition [6, 8]. Although our clinical study showed that EPA caused long-term reduction of urinary calcium excretion in those with hypercalciuria [8], we did not examine the resistance effect of EPA on clinical stone recurrence with the control study. In this study, we examined the preventive effect of EPA toward stone recurrence, compared to the duration without EPA administration as the control. In this study, administration of EPA reduced urinary calcium excretion, but not significantly. It is suggested that appropriate selection of patients is not limited to those with hypercalciuria and the number of patients is not extensive.

Recently, a prospective study using a food-frequency questionnaire was reported by Taylor et al. in which the intake of n–3 fatty acid reduced the stone recurrence [14]. However, their study could not indicate that the increased intake of dietary n–3 PUFAs reduced the risk of kidney stone formation. They were unable to rule out the possibility that very high doses of n–3 fatty acids in the form of fish oil supplements provided some benefit. Our study suggests that EPA has the effect of preventing nephrolithiasis recurrence compared to that in the control period without administration of EPA. A larger prospective trial may be needed to confirm whether n–3 PUFA supplementation reduces the risk of calcium stone formation.

In conclusion, in this study, the EPA administration reduced the urinary stone recurrence in calcium stone formers compared to that in the same individuals during an equivalent period without EPA administration. These findings suggest that EPA administration may reduce the risk of recurrent calcium stone formation.

Conclusions

In this study, the EPA administration reduced the urinary stone recurrence in calcium stone formers compared to that in the same individuals during an equivalent period without EPA administration. These findings suggest that EPA administration may reduce the risk of recurrent calcium stone formation.

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