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The Effect of Eicosapentaenoic Acid on Prostate-specific Antigen

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Abstract. *The "Study of EPA Effects on Prostate Cancer" (SEEPC) Group has been conducting a clinical trial with patients who underwent radical prostatectomy. The main purpose of the SEEPC is to evaluate whether eicosapentaenoic acid (EPA) prevents prostate cancer (PC) recurrence. As the surrogate marker of recurrence, the prostate-specific antigen (PSA) level was measured. However, if EPA affects the PSA values independently of PC, PSA may not be a good marker of recurrence in the event of EPA treatment. Thus, in the present study, whether EPA affected the PSA values was investigated using non-PC volunteers. Twenty men, of at least 50 years of age, were recruited, mostly from hospital staff. The volunteers were randomly allocated either to the EPA group or the control. The subjects in the EPA group were administered EPA-ethyl ester a dose of 2400 mg/day for 12 weeks, whereas the controls were administered none. Fasting blood samples were obtained before the start of EPA administration and 4 and 12 weeks later. The EPA concentrations in erythrocytes increased in all the subjects in the EPA group (174±96%) with no significant changes in the control group (8.5±14.0%). There were no significant differences between the two groups in the serum PSA levels, allowing the conclusion that the PSA is an appropriate surrogate marker of recurrence in prostate cancer.*

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Prostate cancer (PC) is one of the most common male cancers in Western countries. It is the leading cause of male malignancy and the second leading cause of cancer mortality among men in the United States (1). The incidence of prostate cancer is apparently lower in Asian countries such as Japan, China, Korea and the Philippines than in the United States (2). The mortality from PC in 1999 was not high in Japan and ranked eighth among all cancer types (3). However, PC mortality in Japan has increased more than other types of cancers during the previous two decades (3). This trend might be explained by the Westernization of the diet and the aging of the Japanese population.

Fleshner *et al.* (4) reviewed 33 published case-control and cohort studies that examined the relationship between PC and dietary fat and concluded that fat intake was significantly associated with PC, even in light of the inherent biases in the methodology. A number of authors recently reported the association between fish consumption and the risk of PC. Their results were equivocal [some showed inverse associations (5-13), while others showed no associations (14-24)], with one exceptional report (25) showing the increased risk of PC associated with a high consumption of fish. Mishina *et al.* (6) reported a case-control study with Japanese PC patients by matched-pair analysis. The healthy control was

matched with the cancer cases by age, to within 1 year, and by residence in the same prefecture. They found that the PC risk in those who ate seafood was occasionally or never significantly higher (RR=2.33) than in those who ate more (6). Akazaki *et al.* (26) found that the age-adjusted prevalence of latent PC did not differ significantly between native and Hawaiian Japanese, the latter eating less fish. Nevertheless, latent PC tended to proliferate and invade more in Hawaiian Japanese than in native Japanese and the prevalence of the proliferative type of latent PC was higher in the immigrant group (26). Severson *et al.* (14) prospectively followed 7999 Hawaiian Japanese men for 17.5 years on average and found that there was no association between the risk of PC and fish intake. Dewailly *et al.* (27) conducted an autopsy study of 61 male Greenlanders, whose fish intake was 10 times higher than that of Japanese, and found only one case with invasive PC. His omega-6/omega-3 fatty acid ratio in the adipose tissue was exceptionally high (12.1) compared with 4.56 (95% CI: 1.98-10.5) for the entire group. Terry *et al.* (11) studied the association between fish consumption and PC in a population-based prospective cohort study of 6272 Swedish men for 30 years. They found that men who ate no fish had a two- to three-fold higher frequency of PC than did those who ate moderate or large amounts of fish (11).

Our research group (the "Study of EPA Effects on Prostate Cancer" or SEEPC Group) has been investigating the effects of eicosapentaenoic acid (EPA) on PC recurrence in patients who underwent radical prostatectomy. Two consecutive elevations of PSA values are used as the surrogate marker of recurrence of PC. However, if EPA affects the PSA levels independently of PC, PSA may not be a good marker of recurrence in cases receiving EPA treatment. Thus, whether EPA affected the PSA values was evaluated in our study using non-PC volunteers.

Materials and Methods

Subjects. Twenty men, >50 years old (55±5; range of ages 50-68) were recruited from local hospitals and companies (Table I). They had not been taking any lipid-lowering medications or supplements during the previous 3 months.

Study design. The subjects were randomly assigned to two groups (EPA and control groups). The subjects of the EPA group (n=10) took 2400 mg of EPA ethyl ester (Epadel-S, purity >98%, Mochida Pharmaceutical Co. Ltd., Tokyo, Japan) per day for 12 weeks. The control subjects (n=10) took none. All the subjects were asked to maintain their body weights and physical activity levels and to consume their normal diets during the study. Fasting blood samples were taken at weeks 0, 4 and 12. At weeks 0 and 12, the subjects were asked to complete a food frequency questionnaire for the previous 4 weeks. The study was approved by the ethics committee of each participating university hospital, and written informed consent was obtained from each participant.

Table I. *Characteristics of the subjects.*

	Group	
	Control (n=10)	EPA (n=10)
Age	56±6	55±4
BMI	23.9±2.8	24.9±3.4
Hypertension	2	2
Diabetes mellitus	1	0
Hyperlipidemia	1	2
Hyperuricemia	2	0
Angina pectoris	1	0
BPH	0	1

Values are means±SD. BMI: body mass index; BPH: benign prostatic hyperplasia.

Fatty acid analysis. Packed red blood cells (RBCs) were obtained from EDTA-anticoagulated blood, washed twice with saline and frozen at –80˚C until analysis. The fatty acid composition of the total phospholipid fraction of the washed RBCs was determined as follows: total lipids were extracted by the method of Bligh and Dyer (28); the total phospholipid fraction was separated by thin-layer chromatography; after transmethylation with HCl-methanol, the fatty acid composition was analyzed by gas chromatography (GC14A Shimadzu Corporation, Kyoto, Japan) with a capillary column DB-225 (0.25 mm, 30 m length id., 0.25 μm; J&W Scientific, Folsom, CA, USA); the column temperature was kept at 170˚C for the first 1 min, raised to 220˚C at the rate of 4˚C/min and kept at this temperature for 22 min; the whole system was controlled with the gaschromatography software, CLASS-GC10 ver. 1.3 (Shimadzu Corporation).

Food analysis. Food intake was calculated with Eiyokun ver 3.0 (Kenpakusha Co. Ltd., Tokyo) using a food frequency questionnaire.

PSA and hormone analyses. The PSA was measured by two-site immunoradiometric assay (29). Testosterone (30) and luteinizing hormone (31) were measured by radioimmuno-assay.

Statistical analysis. The results are expressed as means±SD. StatView (ver. 5.0) was used for the statistical analysis. The fatty acid composition, PSA, testosterone and luteinizing hormone were analyzed parametrically (the paired *t*-test for intragroup comparison and the analysis of covariance for intergroup comparison). The Chi-square test was used for the comparison of the subjects' lifestyle disease. $P<0.05$ was considered to be significant.

Results

The baseline characteristics of both the EPA and control groups are provided in Table I. The age and body mass index did not differ between the two groups, nor did diseases associated with lifestyle and coronary heart disease.

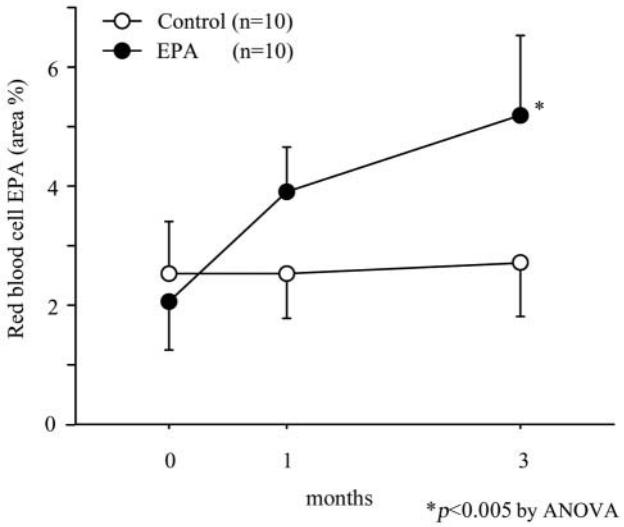

Figure 1. *The EPA concentrations in the RBCs were significantly increased in the EPA group (174±96%) during the study period, with no significant changes in the control group (8.5±14.0%).*

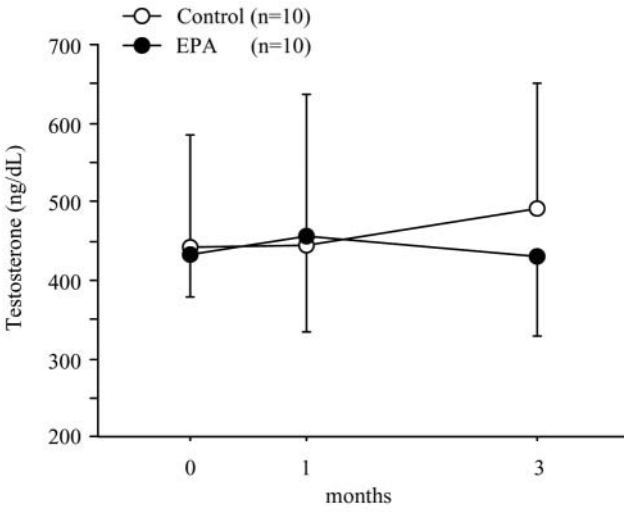

Figure 3. *There were no significant differences in the serum levels of testosterone between the two groups.*

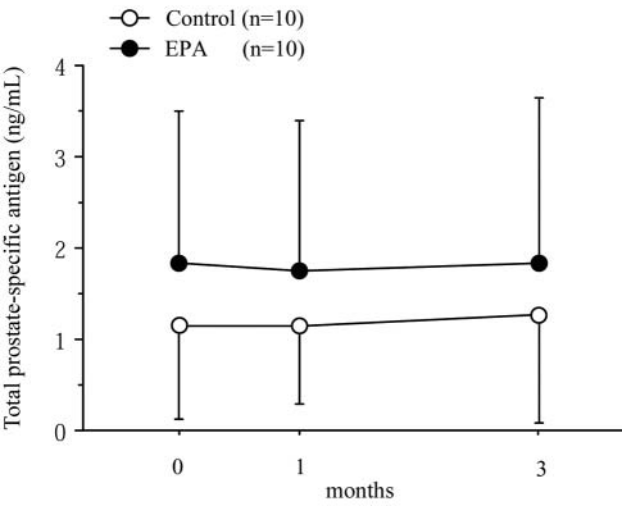

Figure 2. *There were no significant differences in the serum levels of PSA between the two groups.*

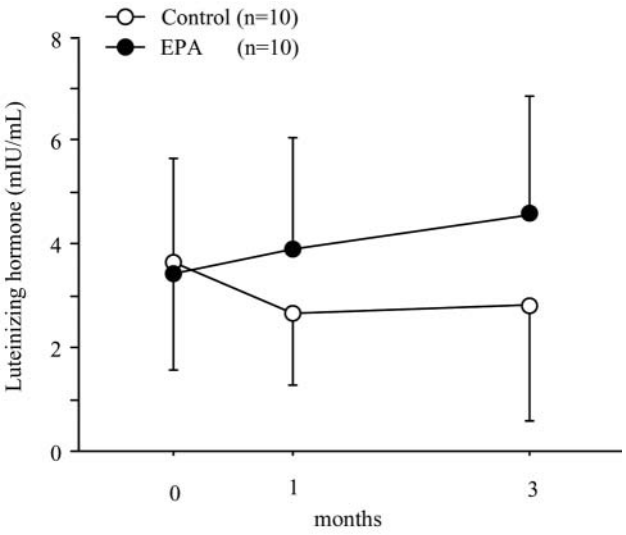

Figure 4. *There were no significant differences in the serum levels of luteinizing hormone between the two groups.*

One subject in the EPA group was using an α1-blocker for benign prostatic hyperplasia (BPH). No subject in either group dropped out of the trial and no adverse effects were seen in the EPA group.

The EPA concentrations in the RBCs (Figure 1) were significantly increased in the EPA group (174±96%) during the study period, with no significant changes in the control group (8.5±14.0%). There were no significant differences in the serum levels of PSA, testosterone and luteinizing hormone between the two groups (Figures 2, 3 and 4, respectively). Food analyses indicated that there were no significant differences in the average intakes of macronutrients, n-3 fatty acids (DHA+EPA: 1.4±1.2 g/day and 1.1±0.5 g/day in the control and EPA groups, respectively) or the other fatty acids (data not shown) between the two groups.

Discussion

Rose *et al.* (33) reported the inhibiting effects of EPA and DHA on the growth of the human PC lines PC-3 and DU-145. Fish oil-containing diets inhibited the growth of

DU-145 cells transplanted into athymic nude mice compared with diets containing corn oil (33, 34). Moreover, as mentioned earlier, several studies showed significant inverse correlations between fish or omega-3 fatty acid consumption and the incidence of PC. However, a recent review by Terry *et al.* (35) did not support the inverse correlations and, to date, there have not been any primary or secondary prevention studies. Aronson *et al.* (36) reported an interesting intervention study. Nine men with untreated PC (cT1c to cT3a) were asked to consume a low-fat (reduced to 15% of total calories) diet and fish oil capsules (1.8 g EPA+1.2 g DHA/day) for 3 months. Such intervention caused a significant increase in the omega-3/omega-6 fatty acid ratio in their plasma and gluteal adipose tissue. Cyclooxygenase-2 (COX-2) expression in the prostate tissue, quantified in seven out of the nine patients, was decreased in four out of the seven patients. This study also prompted us to investigate the effects of EPA on PC recurrence.

There were some limitations to our present study, including the fact that the number of study subjects was not large. In addition, since the patients currently participating in SEEPC are essentially elderly and likely to suffer from complications, study subjects with lifestyle disease were not excluded. The inclusion of such subjects in the present study might also have confounded the results. However, the fact that no subject in the EPA group showed marked increases or decreases in the PSA values (see Figure 2) indicated that these confounding factors did not exert sizable effects.

Although commonly considered to be treatment failure, the presence of detectable PSA after radical prostatectomy does not inexorably portend clinically significant cancer recurrence (37-39). Pound *et al.* (37) reported that the median actual time from biochemical recurrence until progression to metastases was 5 years (mean: 8 years). Nevertheless, the PSA is generally used as a useful surrogate recurrence maker of PC (40, 41). We are currently measuring testosterone and luteinizing hormone in addition to PSA as possible explanatory factors in SEEPC. We confirmed here that these parameters were also stable during EPA administration.

In conclusion, EPA (2400 mg/day for 3 months) had no effect on the serum level of PSA in non-PC subjects. No evidence was found against using PSA as a surrogate marker in the case of EPA treatment. Consequently, it seems appropriate to use PSA as the surrogate marker of recurrence in the SEEPC trial.

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