

Beef Tallow, but Not Perilla or Corn Oil, Promotion of Rat Prostate and Intestinal Carcinogenesis by 3,2'-Dimethyl-4-aminobiphenyl

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The modifying effects of three kinds of fat (corn oil, beef tallow or perilla oil, each at 20% in the diet) on F344 rat prostate carcinogenesis induced by 3,2'-dimethyl-4-aminobiphenyl (DMAB) were investigated. Non-invasive carcinomas of the ventral prostate were induced by DMAB alone and invasive carcinomas of the other prostate lobes and seminal vesicles by DMAB and testosterone propionate (TP). Eight groups of F344 rats were initiated with 50 mg/kg body weight of DMAB at 2-week intervals for the first 20 weeks, four also receiving TP, extended until week 60. The animals received basal chow powder diet or one of three high fat diets throughout the experiment (60 weeks). One further group served as a non-carcinogen-treated control maintained on basal chow powder diet. Beef tallow significantly increased the development of ventral prostate carcinomas with DMAB alone (from 15 to 45%, $P < 0.05$), while perilla oil reduced the incidence of prostatic intraepithelial neoplasia (PIN) in the ventral lobe of rats given DMAB + TP (from 70 to 10%, $P < 0.01$), but not in those given DMAB alone. No other effects of high fats were observed regarding PIN or invasive cancers of the dorsolateral and anterior prostate or seminal vesicles. A satellite experiment demonstrated that all high fat diets for 4 weeks increased the 5-bromo-2-deoxyuridine (BrdU) labeling index of prostate epithelial cells, suggesting that a high fat intake, irrespective of the fatty acid composition, may accelerate cell kinetics in the prostate. Of the three high fat diets, beef tallow was also found to increase intestinal carcinogenesis. Thus, the present data revealed carcinogenesis in the prostate and intestine to be promoted by beef tallow.

Key words: High fat diets — Prostate cancer — Rat

Epidemiological and experimental studies have shown that the diet contains very important environmental risk factors for various types of cancer.¹⁻⁷ With regard to prostate cancer development, energy intake might be important,⁸⁻¹¹ and a high fat intake in particular has been reported to predispose to carcinogenesis in the prostate as well as the colon, pancreas and breast.¹²⁻¹⁴ Experimentally, Pollard and Luckert first demonstrated a slight promotional effect of dietary high fat on rat prostate carcinogenesis under specific conditions.¹⁵ However, a subsequent study by Pour *et al.* did not reveal any enhancing effects of 24.6% corn oil on N-nitrosobis(2-oxopropyl)amine-induced rat prostate carcinogenesis.¹⁶ We similarly obtained negative data with our 3,2'-dimethyl-4-aminobiphenyl (DMAB) rat model.¹⁷ On the other hand, in a two-generation experiment Kondo *et al.* found that a corn oil-based high fat diet significantly increased the appearance of prostatic intraepithelial neoplasia (PIN) in ACI/Seg rats, a strain with a high incidence of spontaneous prostate cancer.¹⁸ Thus, unlike the colon and mam-

mary gland cases, data for promotional effects of a high fat diet on experimental prostate carcinogenesis are equivocal.

Recently much attention has been paid to the roles of $n-3$ and $n-6$ polyunsaturated fatty acids as beneficial and risk factors, respectively, for several kinds of malignancies.^{14,19-22} Experimental studies have shown that $n-6$ polyunsaturated fatty acid may exert promotional effects on prostate carcinogenesis,²³ while $n-3$ polyunsaturated fatty acid-rich oils such as fish oil, and perilla oil were demonstrated to suppress tumorigenesis of the colon²⁴ and mammary glands.²⁵ Perilla oil is produced from *Perilla frutescens*, popular among Japanese people as an edible plant. $n-3$ Polyunsaturated fatty acid-rich oils also suppress the growth of human prostate cancer cells,^{26,27} and an inverse association between their serum levels and prostate cancer was demonstrated in epidemiological studies.^{28,29} In the present investigation, the effects of three different high fat diets (corn oil, beef tallow and perilla oil) were examined, using our DMAB rat prostate carcinogenesis model.^{30,31} These were chosen to respectively be rich in linoleic acid ($n-6$), stearic acid and oleic acid ($n-9$) and linolenic acid ($n-3$).

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MATERIALS AND METHODS

Animals A total of 180 five-week-old male F344 rats were obtained from Charles River Japan, Inc., Atsugi. They were housed in plastic cages on hardwood chips for bedding in an air-conditioned room at a temperature of $22\pm 2^\circ\text{C}$ with a 12-h light, 12-h dark cycle. They were maintained on Oriental MF basal chow diet (Oriental Yeast Co., Tokyo) and tap water *ad libitum*.

Chemicals DMAB was purchased from the NARD Institute, Ltd., Osaka and testosterone propionate (TP) from Tokyo Kasei Kogyo, Tokyo. The three fats (corn oil, beef tallow and perilla oil) were obtained as follows; corn oil from Nihon Shokuhin Kako Co., Shizuoka, beef tallow from Wako Co., Tokyo, and perilla oil from Sugiyama Yakuhin Co., Nagoya. The fatty acid composition of each is shown in Table I. They were incorporated into basal diet at the concentration of 20%, batches being prepared every week and stored in a cold dark stock room until use. Oriental MF chow was formulated as the control diet with corn at 5.1%. The caloric value of the basal MF diet was 357 kcal/100 g while that of all high fat diets was calculated to be 465.6 kcal/100 g.

Prostate carcinogenesis study The experimental protocol is shown in Fig. 1. The animals were randomly allocated to 9 groups of 20 rats each. Rats in 8 groups were subcutaneously (s.c.) injected with DMAB dissolved in corn oil at a dose of 50 mg/kg body weight 10 times at 2-week intervals. The day following the first DMAB injection, animals in groups 1 to 4 also received s.c. implants of 2-cm-long Silastic tubes (3 mm outer diameter and 2 mm inner diameter) containing 40 mg TP, these then being replaced at 6-week intervals until the termination of the experiment, as previously described.^{30, 32, 33} From the beginning of the experiment, rats were given basal MF diet or one of the high fat diets; corn oil diet for groups 1 and 5, beef tallow diet for groups 2 and 6 and perilla oil diet for groups 3 and 7. Groups 4 and 8 received MF basal diet. All surviving animals were killed under ether anesthesia at week 60. Animals that died earlier or were sacrificed upon becoming moribund were also autopsied. All organs were exam-

ined for gross abnormalities, and the prostate, seminal vesicles, testes and lesions detected macroscopically were taken and fixed in 10% buffered formalin. For tissue preparation of the accessory sex organs, two sagittal slices of the ventral prostate, sagittal samples of the dorsolateral prostate, including the urethra, and transverse samples from each side of the seminal vesicle, including the anterior prostate, were routinely processed for embedding in paraffin, cut and stained with hematoxylin and eosin for histopathological examination.

Investigation of DNA synthesis in the prostate and seminal vesicles A total of 32 ten-week-old male F344 rats were randomly allocated to 4 groups of 8 rats each, given basal or one of the high fat diets for 4 weeks. One hour before sacrifice, three rats in each group were intraperitoneally injected with 100 mg/kg b.w. 5-bromo-2-deoxyuridine (BrdU) solution (Sigma Chemical Co., St. Louis, MO). The animals were killed by euthanasia under ether anesthesia, and each prostate was fixed in 10% buffered formalin. Immunohistochemical staining of incorporated BrdU using the avidin-biotin peroxidase complex method with a monoclonal antibody against BrdU was performed on routinely prepared paraffin-embedded sections. The

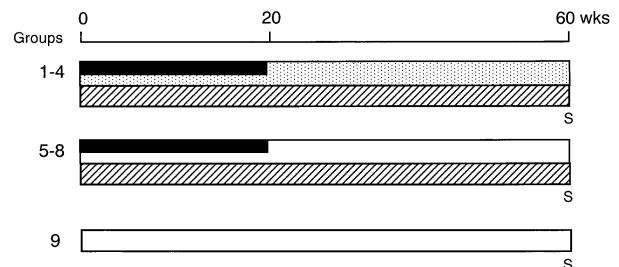


Fig. 1. Experimental design for examination of effects of high fat on rat prostate carcinogenesis. Animals used were male F344 rats, 6 weeks old at the commencement. ■ DMAB, 3,2'-dimethyl-4-aminobiphenyl, 50 mg/kg b.w. s.c. biweekly; ▨ TP, testosterone propionate, Silastic tube (40 mg); ▩ diets: groups 1 and 5, 20% corn oil; groups 2 and 6, 20% beef tallow; groups 3 and 7, 20% perilla oil; groups 4 and 8, basal diet (4% corn oil); S, animals killed.

Table I. Fatty Acid Composition

	Fatty acid (%)				
	Palmitic acid 16:0	Stearic acid 18:0	Oleic acid 18:1	Linoleic acid 18:2	Linolenic acid 18:3
Corn oil	10.0	3.5	34.0	48.0	1.5
Beef tallow	26.2	31.0	36.0	2.0	—
Perilla oil	5.7	1.8	15.7	14.9	61.0
Basal diet ^{a)} (Corn oil)	14.9	2.4	24.0	45.9	3.1

a) Oriental MF diet.

numbers of cells with positively stained nuclei per 2000 cells were counted and labeling indices were expressed as percentage values.

The present experiments were approved by the Institutional Animal Care and Use Committee of Nagoya City University Medical School.

Lipid analysis The five remaining rats in each group in the satellite experiment were used for measurement of fat composition in the prostate. Samples of the ventral, dorso-lateral and anterior prostate, each about 50–150 mg, were rinsed in saline. They were homogenized in phosphate-buffered saline (PBS) and 1 ml of chloroform and 2 ml of methanol were added to each sample. Ten minutes after adding a further 1 ml of chloroform and 0.9 ml of distilled water to each sample, they were centrifuged at 3000 rpm for 10 min and stored at 20°C until measurement of fat composition. Total lipids in the homogenates were extracted with chloroform-methanol according to the method of Bligh and Dyer.³⁴⁾ Phospholipids in total lipids were separated by thin-layer chromatography on silica gel plates (Merck 60, Merck, Darmstadt, Germany), and the fatty acid composition in the phospholipid fractions was analyzed by gas-liquid chromatography-mass spectrography, as detailed previously.^{35, 36)}

Statistical analysis The significance of differences between groups in body and organ weights, values for DNA synthesis and fatty acid composition levels was analyzed by using Student's test according to Welch. The significance of differences in lesion incidences between different groups was examined by using Fisher's exact probability test.

RESULTS

Table II summarizes final body, average food consumption, calorie intake and relative organ weight data for the

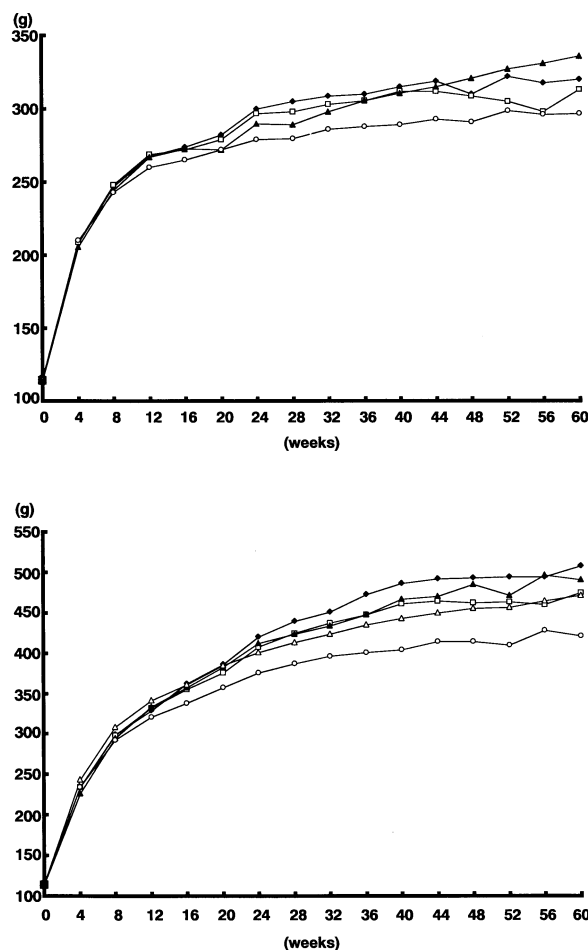


Fig. 2. Growth curves of animals. The upper frame shows data for the groups receiving DMAB, TP and a high fat diet and the lower frame the groups receiving DMAB and high fat, as well as the complete control group. ◆ corn oil, □ beef tallow, ▲ perilla oil, ○ basal diet, △ complete non-treated.

Table II. Final Mean Body and Relative Organ Weights and Food Consumption Data

Group	DMAB	TP	High fat	No. of rats	Food consumption ^{a)}	Calorie intake ^{b)}	Mean final b.w. (g)	Relative organ weight (%)	
								Liver	Kidneys
1	+	+	20% Corn oil	7	14	65.1	320	2.6	0.81 ^{d)}
2	+	+	20% Beef tallow	8	16	74.5	313	2.5	1.01
3	+	+	20% Perilla oil	10	12	55.9	336 ^{c)}	2.6	0.79 ^{d)}
4	+	+	Basal diet	10	19	67.8	297	2.5	1.02
5	+	-	20% Corn oil	15	11	51.2	508 ^{d)}	2.4	0.51 ^{c)}
6	+	-	20% Beef tallow	16	13	60.5	475 ^{c)}	2.6	0.53 ^{c)}
7	+	-	20% Perilla oil	16	10	46.6	491 ^{d)}	2.5	0.52 ^{d)}
8	+	-	Basal diet	16	14	50.0	421	2.4	0.57
9	-	-	Basal diet	20	17	60.7	471	2.1	0.55

a) g/animal/day.

b) kcal/animal/day.

c), d) Significantly different from Basal diet group at P<0.05, 0.01, respectively.

prostate carcinogenesis study. All animals receiving high fat diets, irrespective of TP-administration, had greater body weights than their counterparts receiving the MF basal diet (Fig. 2), although food consumption and calorie intake was greater with the latter (Table II). DMAB without TP induced non-invasive small carcinomas in the ven-

tral lobe, while DMAB with TP produced invasive carcinomas in the dorsolateral and anterior prostate and seminal vesicles. In the groups given DMAB alone with the MF diet (group 8), the incidence of ventral carcinomas was low compared to background data (13–15%) obtained under the same experimental conditions.^{17, 30, 31} As shown

Table III. Incidences (%) of Proliferative Lesions of the Prostate and Seminal Vesicles

Group	DMAB	TP	High fat	Effective No. of rats	Prostate						Seminal vesicles	
					Ventral		Dorsolateral		Anterior		PIN	CA
					PIN	CA	PIN	CA	PIN	CA		
1	+	+	Corn oil	20	14 (70)	0	3 (15)	3 (15)	13 (65)	8 (40)	16 (80)	5 (25)
2	+	+	Beef tallow	20	8 (40)	0	0	7 (35)	15 (75)	11 (55)	14 (70)	10 (50)
3	+	+	Perilla oil	20	2 (10) ^{a)}	1 (5)	5 (25)	5 (25)	13 (65)	10 (50)	17 (85)	9 (45)
4	+	+	Basal diet	18	11 (61)	1 (6)	2 (11)	2 (11)	14 (78)	7 (39)	18 (100)	9 (50)
5	+	-	Corn oil	20	17 (85)	3 (15)	3 (15)	0	3 (15)	0	16 (80)	0
6	+	-	Beef tallow	20	16 (80)	9 (45) ^{b)}	1 (5)	0	2 (10)	0	17 (85)	0
7	+	-	Perilla oil	20	12 (60)	3 (15)	1 (5)	0	2 (10)	0	17 (85)	0
8	+	-	Basal diet	20	12 (60)	2 (10)	2 (10)	0	2 (10)	0	18 (90)	0
9	-	-	Basal diet	20	0	0	0	0	0	0	0	0

PIN, prostate intraepithelial neoplasia; CA, carcinoma.

a), b) Significantly different from the basal diet group at $P < 0.01$, 0.05 , respectively.

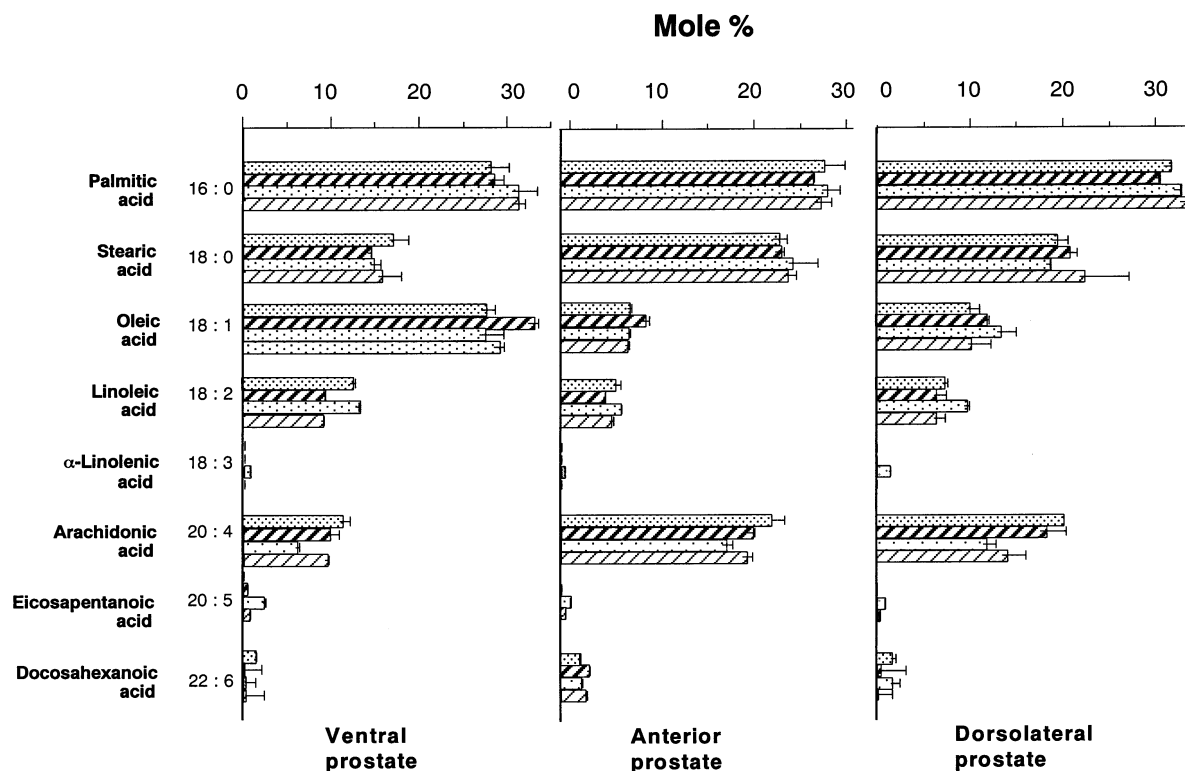


Fig. 3. Data for fatty acid composition of the prostate lobes of rats given high fat diets. \square corn oil, ▨ beef tallow, ▩ perilla oil, \square control.

in Table III, when compared with group 8, beef tallow increased the incidence of ventral prostate carcinomas (45% vs. 10%, $P < 0.05$). Neither corn oil nor perilla oil diets in groups given DMAB without TP had any influence on the incidence of PIN or carcinomas. None of the high fat diets was found to modify development of invasive carcinomas in groups given DMAB and TP, except for a significant suppression of PIN by perilla oil diet. No modification was also noted when only the incidence of large carcinomas occupying large parts of the accessory sex organs or the overall incidence of carcinomas was analyzed.

Spectra of fatty acid contents in the prostate differed among the lobes and between diets used (Fig. 3). Lobe-specific patterns in fatty acid contents were evident. Oleic acid content in the ventral prostate was much greater than in the anterior and dorsolateral prostate but greater con-

tents of stearic acid and arachidonic acid were found in the ventral prostate. The significance of these lobe-specific patterns in the contents of fatty acids remains unclear. Corn oil and perilla oil slightly increased linoleic acid and arachidonic acid in all lobes. Perilla oil increased α -linolenic acid but suppressed arachidonic acid. α -Linolenic acid was detected only in animals receiving perilla oil.

Cell kinetic data showed all of the diets to increase BrdU incorporation in the prostate and seminal vesicles (Fig. 4). In the high fat treatment groups, significant differences were seen in the ventral and dorsolateral prostate and seminal vesicles as compared with basal diet group, with the exception of the ventral lobe in the beef tallow group. Tendencies for increase were also evident in the anterior prostate.

DMAB is a multi organ carcinogen,^{37, 38)} and tumors were also noted in the intestine (Table IV). Among the

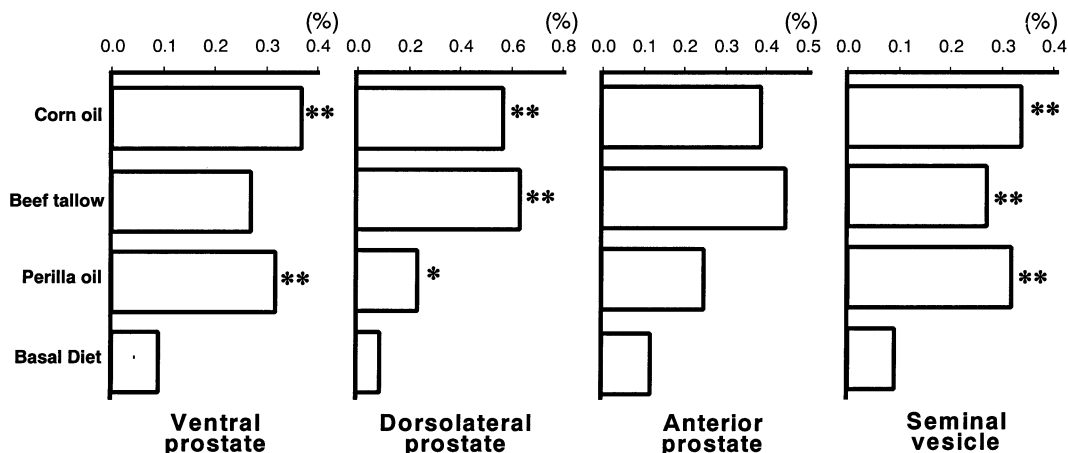


Fig. 4. BrdU labeling indices for prostate lobes and seminal vesicles. *, ** Significantly different from the basal diet group at $P < 0.05$, 0.01, respectively.

Table IV. Incidences (%) of Small Intestine and Colon Tumors

Groups	DMAB	TP	High fat	Effective No. of rats	Small intestine			Colon		
					Total	Adenoma	Carcinoma	Total	Adenoma	Carcinoma
1	+	+	Corn oil	20	2 (10)	1 (5)	1 (5)	1 (5)	1 (5)	0
2	+	+	Beef tallow	20	2 (10)	1 (5)	1 (5)	3 (15)	3 (15)	0
3	+	+	Perilla oil	20	1 (5)	0	1 (5)	0	0	0
4	+	+	Basal diet	18	0	0	0	0	0	0
5	+	-	Corn oil	20	1 (5)	0	1 (5)	3 (15)	1 (5)	2 (10)
6	+	-	Beef tallow	20	8 (40) ^{a)}	4 (20)	4 (20)	8 (40) ^{a)}	3 (15)	6 (30)
7	+	-	Perilla oil	20	3 (15)	0	3 (15)	3 (15)	1 (5)	2 (10)
8	+	-	Basal diet	20	1 (5)	1 (5)	0	1 (5)	0	1 (5)
9	-	-	Basal diet	20	0	0	0	0	0	0

a) Significantly different from the basal diet group value at $P < 0.05$.

three high fat diets, beef tallow increased the tumor incidence (adenomas and adenocarcinomas) in the small intestine and colon of rats given DMAB alone. Such modification was not observed in rats given DMAB plus TP. No significant alteration by the high fat diets of tumor incidences in other organs such as the lung, salivary gland, liver, skin/subcutis, Zymbal's gland, mammary gland or preputial gland was noted (data not shown).

DISCUSSION

The reasons for the greater body weights observed in the animals given a high fat diet compared to the counterparts receiving the MF diet were not clear, because calorie intake can not explain the differences. The slight decreases in relative organ weight of the kidney observed in animals given high fat diet seem to be due to increased body weight.

The present experiment demonstrated beef tallow, but not corn oil or perilla oil diet to significantly enhance the development of ventral carcinomas of the prostate in our protocol with DMAB without TP. This enhancement is not due to a higher calorie intake, because rats given beef tallow diet had similar growth rates to those receiving corn oil and perilla oil diet. Beef tallow is largely composed of palmitic, stearic and oleic acids and contains no or little linoleic or linolenic acids (Table I). In contrast, corn oil is rich in oleic acid and linoleic acid, and perilla oil contains large amounts of linolenic acid. Palmitic acid (16:0) and stearic acid (18:0) are both saturated fatty acids and oleic acid is monounsaturated fatty acid. Linoleic acid and α -linolenic acid are both polyunsaturated fatty acids. The present data on fatty acid composition in prostate tissue indicate that the ventral prostate of rats given beef tallow diet contains elevated levels of oleic acid and low or no linoleic or linolenic acid (Fig. 3).

Total fat intake, particularly of animal fats, has been suggested to be linked with a higher incidence and mortality of prostate cancer, although not all epidemiological studies have been consistent in this respect. Pollard and Luckert experimentally demonstrated a slight enhancing effect of dietary high fat on rat prostate carcinogenesis under their specific conditions,¹⁵⁾ but other experiments did not reveal any influence of high corn oil consumption on chemically induced rat prostate carcinogenesis.^{16, 17)} Kondo *et al.*, did, however, demonstrate that a corn oil-based high fat diet significantly increased the appearance of atypical hyperplasias of the prostate in ACI/Seg rats in a two-generation experiment.¹⁸⁾

Recently, much attention has been paid to the roles of $n-3$ and $n-6$ polyunsaturated fatty acids as beneficial and risk factors, respectively, for several kinds of malignancy such as colon, breast and prostate cancers.^{14, 19-21, 28)} $n-6$ Polyunsaturated fatty acids may exert promotional effects

on prostate carcinogenesis,²³⁾ whereas $n-3$ polyunsaturated fatty acid-rich oils, such as fish and perilla oil, have been reported to suppress tumorigenesis in the colon²⁴⁾ and mammary glands.²⁵⁾ They also reduce growth of human prostate cancer cells^{26, 27)} and epidemiological studies have shown an inverse association between serum $n-3$ polyunsaturated fatty acid levels and prostate cancer.²⁸⁾ From the mechanistic point of view, modification of the arachidonic acid cascade has been proposed to be involved in tumor suppression or promotion by fatty acids. There is convincing evidence that $n-6$ polyunsaturated fatty acids and saturated fatty acids accelerate metabolic pathways leading to cyclooxygenase-mediated production of prostaglandins from arachidonic acid.^{39, 40)} Importantly an inverse association between $n-3$ polyunsaturated fatty acids and suppression of this pathway has been shown. Such an alteration of prostaglandin biosynthesis may also be one of reasons for the promoting effects of beef tallow on prostate neoplasia. The significance of the finding that ventral PIN was decreased by perilla oil diet is unclear, because the suppression was observed only in rats given DMAB with TP.

The satellite experiment revealed that BrdU incorporation in the nuclei of prostate epithelial cells in the three high fat diet groups was higher than that in the basal diet group in almost all prostate lobes, suggesting that a high fat diet, regardless of the fatty acid components, has the potential to increase cell proliferation of prostate epithelium. However, there was no direct relationship with prostate carcinogenesis. Since Latham *et al.* has shown that dietary $n-3$ polyunsaturated fatty acids increase the apoptotic response in colonic crypt cells induced by 1,2-dimethylhydrazine,⁴¹⁾ this type of cell death is one complicating factor. This might be a reason for suppression of the development of PIN in the ventral lobe by perilla oil, although no clear apoptotic bodies were detected (data not shown). Another study suggested that diet may alter endogenous sex hormone metabolism in men.⁴²⁾

The enhancement of tumor development in the small and large intestine observed in the present experiment supports the concept that a diet rich in animal fat is a risk factor for colon cancer in man. However, although it was earlier found that an α -linolenic acid ($n-3$)-rich perilla oil diet can inhibit development of mammary gland, colon and/or kidney tumors as compared to linoleic acid ($n-6$)-rich safflower or soybean oil diets,^{43, 44)} no reduction of intestinal tumor development was noted in rats given perilla oil diet in the present study. This is a complex issue and for elucidation of how fatty acids impact on prostate carcinogenesis requires further research, including development of better animal models.

In conclusion, the present study clearly demonstrated that beef tallow diet can promote prostate and intestinal carcinogenesis induced by DMAB, indicating that animal fats rich in stearic acid (a saturated fatty acid) and mono-

unsaturated oleic acid might pose risks for prostate and colon carcinogenesis.

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