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Modulating Effects of Diets High in ω-3 and ω-6 Fatty Acids in Initiation and Postinitiation Stages of Diethylnitrosamine-induced Hepatocarcinogenesis in Rats

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The effects of sardine fish oil or corn oil on diethylnitrosamine (DEN)-induced hepatocarcinogenesis were investigated in male F344 rats. Starting at 5 weeks of age, animals were divided into 11 groups and fed 23.5% corn oil (HCO) (groups 1 and 7) or 5% corn oil (LCO) (groups 2 and 8), 22.5% sardine oil + 1% corn oil (FO) semipurified diet (groups 3 and 9) or basal diet (CE-2) (groups 4–6, 10 and 11). At 6 weeks of age, all animals except the vehicle-treated groups were given DEN (200 mg/kg body weight, i.p. once weekly for 3 weeks). One week after the final exposure to DEN, groups 1–3 were changed to the basal diet, and groups 4–6 were switched to the HCO, LCO or FO diet, respectively. Animals in groups 1–3 and 10 were given drinking water containing 0.05% phenobarbital (PB). Liver sections from the animals at the termination of the experiment (24 weeks) were doubly stained for glutathione *S*-transferase placental form (GST-P) and silver-stained nucleolar organizer regions (AgNORs). The multiplicity of hepatocellular neoplasms of group 1 was significantly larger than that of group 2 or 3. The number of GST-P-positive foci of group 2 or 3 was significantly smaller than that of group 1. Among the groups fed the experimental diets in the postinitiation phase (groups 4–6), no significant difference was found in the incidence of liver tumors. AgNORs values of the enzyme-altered foci in rats of the HCO diet groups were larger than those of the other diet groups. These results indicate that the enhancing effect of a high dose of corn oil in hepatocarcinogenesis is mainly present during the initiation phase but not during postinitiation phase, and fish oil rich in polyunsaturated ω-3 fatty acids could inhibit DEN-induced hepatocarcinogenesis in rats.

Key words: Dietary fat — Hepatocarcinogenesis — Diethylnitrosamine

Epidemiological and experimental studies have demonstrated that high doses of total fat and animal fat are generally associated with an increased risk for cancer development in several organs.[1–6] Studies in rodents have shown that unsaturated fats enhance the occurrence of cancers in several organs, especially colon and breast.[2, 3, 6, 7] On the other hand, epidemiological as well as experimental evidence exists that ω-3 fatty acids reduce the risk of development of colon or breast cancers.[6–12] These results suggest that the fatty acid composition of dietary fat is one of the determining factors for carcinogenesis.

Modifying effects of dietary fat on hepatocarcinogenesis have been also examined. High fat diet was reported to enhance the development of 2-acetylaminofluorene (AAF), *p*-dimethylaminoazobenzene, aflatoxin B_1 (AfB_1), or diethylnitrosamine (DEN)-induced liver neoplasms or γ-glutamyltranspeptidase (GGT)-positive foci in rats.[13–16] Ramesh and Das reported that fish oil or ground nut oil suppressed DEN-induced neoplastic nodules in rats.[17] In these studies, however, the fat diets were given at the same time as the carcinogen exposure, so that the effects on the different stages of carcinogenesis were not established.

Newberne *et al.* reported a promoting effect of corn oil on AfB_1-induced hepatocarcinogenesis.[18] Glauert and Pitot, however, found that altering the amount and type of dietary fat was not promotive in DEN-induced hepatocarcinogenesis in female rats.[19] Glauert *et al.* examined the effects of a high saturated fat diet and a high polyunsaturated fat diet on DEN or AAF-induced hepatocarcinogenesis and found no significant difference between the tumor incidences in the various groups.[20] Previously, we reported that administration of menhaden fish oil in the postinitiation stage reduced azoxymethane (AOM)-induced glutathione *S*-transferase placental form (GST-P)-positive foci.[21] Thus, the modifying effects of dietary fats remain to be fully established.

The present study was undertaken to assess the effect of diets containing fish oil and corn oil during the initiation or the postinitiation stage of DEN-induced hepatocarcinogenesis in rats. The frequency of GST-P-positive foci and the area of silver-stained nucleolar organizer regions

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Table I. Percent Composition of Experimental Semipurified Diets Containing Corn Oil or Fish Oil

Diet ingredients[a)]	Corn oil		Sardine oil
	Low fat[b)]	High fat[c)]	High fish oil[b)]
Casein, vitamin-free	20.0	23.50	23.50
DL-Methionine	0.3	0.35	0.35
Corn starch	52.0	32.90	32.90
Dextrose	13.0	8.30	8.30
Alphacel	5.0	5.90	5.90
Corn oil	5.0	23.52	1.00
Sardine oil	0	0	22.52
Mineral, AIN	3.5	4.11	4.11
Vitamin, AIN (revised)	1.0	1.18	1.18
Choline bitartrate	0.2	0.24	0.24

a) AIN: American Institute of Nutrition.
b) This diet was prepared on the basis of the AIN Standard Reference Diet [26, 37] with the modification of varying sources of carbohydrate.
c) Additional corn oil and fish oil were added at the expense of starch and dextrose. The composition of diets was adjusted so that all animals in various dietary groups would consume approximately the same amounts of protein, minerals, vitamins, nonnutritive fiber, and calory intake in animals in the different dietary groups was the same [5].

(AgNORs) proteins, which are recognized parameters of cell proliferation in preneoplastic and neoplastic lesions, were measured.[22–24]

MATERIALS AND METHODS

Animals, diets and carcinogen Weanling male F344 rats were purchased from Shizuoka SLC Co., Shizuoka. DEN was from Nacalai Tesque Inc., Kyoto and phenobarbital (PB) was obtained from Maruishi Pharm. Co., Osaka. Sardine fish oil was provided by Tama Biochemical Co., Ltd., Tokyo. CE-2 (CLEA Japan Inc., Tokyo) was used as a basal diet. All dietary ingredients for semipurified diets were purchased from CLEA Japan Inc.

The experimental diets were based on modified AIN-76 diet and the compositions are shown in Table I.[25, 26] All experimental diets were prepared in our laboratory 3 times weekly and stored in a cold room at 4°C. Corn oil (1%) was added to sardine oil diets to provide linoleic acid and to alleviate essential fatty acid deficiency. Freshly prepared diets and those stored for 2 days in a cold room were analyzed for peroxides using the thiobarbituric acid method. No peroxide was detectable. The percentage composition of all experimental diets was adjusted so that the animals in all dietary groups would consume the same amounts of calories, protein, vitamins, minerals and fiber.[27]

Table II. Fatty Acids in Sardine Oil

Fatty acid	Weight %
C14:0	5.9
C16:0	9.2
C16:1	8.5
C17:0	0.5
C17:1	1.0
C18:0	0.6
C18:1	11.4
C18:2	4.6
C20:0	0.2
C20:1+C18:3	3.5
C18:4	4.4
C20:4+C22:1	3.7
C20:4	1.0
C20:5	28.5
C24:1	1.3
C22:4	0.2
C22:5	3.1
C22:6	12.4
Total	100.0

Table III. Fatty Acids in Fat of CE-2[a)]

Fatty acid	%
C14:0	0.4
C16:0	15.4
C16:1	1.3
C18:0	1.8
C18:1	22.7
C18:2	49.4
C18:3	3.4
C20:1	1.0
C20:4	0.2
C20:5	1.8
C22:0	0.2
C22:1	0.4
C22:6	1.7
Not identified	0.3
Total	100.0

a) Total fat concentration in CE-2 is 4.4%.

All animals were housed in wire cages (4 rats/cage). They had free access to water and diet under controlled environmental conditions of humidity (50±10%), lighting (12 h light/dark cycle) and temperature (23±2°C). The food cups were replenished every day.

The fatty acid composition of sardine oil or CE-2 was analyzed by Tama Biochemical Co., Ltd. or CLEA Japan Inc., and the data are shown in Table II or III. Sardine oil and corn oil contained about 25 and 150 ppm of α-tocopherol, respectively.

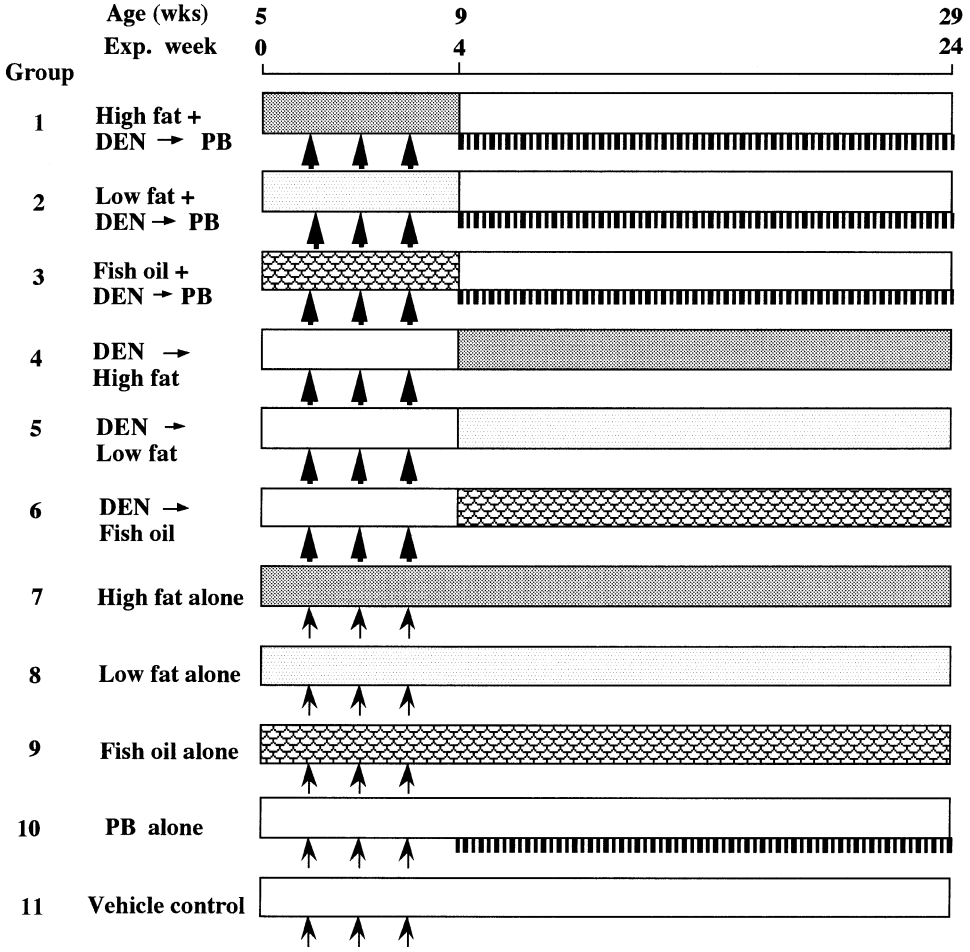

Fig. 1. Experimental design. ▲ DEN 100 mg/kg body weight, once a week for 3 weeks. ▲ Saline. ▥ PB 500 ppm in drinking water. ▭ High corn oil diet (23.5% corn oil). ▭ Low corn oil diet (5% corn oil). ▭ Fish oil diet (22.5% sardine oil+1% corn oil). ▭ Basal diet (CE-2).

Experimental procedure The experimental design is shown in Fig. 1. A total of 161 rats, 5 weeks of age, were divided into 11 groups : group 1, 20 rats for DEN and high corn oil (HCO) diet in the initiation phase and 500 ppm phenobarbital (PB); group 2, 20 rats for DEN and low corn oil (LCO) diet in the initiation phase and 500 ppm PB; group 3, 20 rats for DEN and fish oil (FO) diet in the initiation phase and 500 ppm PB; group 4, 20 rats for DEN and HCO diet in the postinitiation phase; group 5, 20 rats for DEN and LCO diet in the postinitiation phase; group 6, 20 rats for DEN and FO diet in the post-initiation phase; group 7, 8 rats for HCO alone; group 8, 8 rats for LCO alone; group 9, 8 rats for FO alone; group 10, 8 rats for PB alone; and group 11, 14 rats for the vehicle control. Rats in groups 1–3 and 7–9 were given experimental diets from the start of the experiment and animals in the other groups were fed the basal diet. Animals of groups 1 through 6 were given i.p. injections of DEN (100 mg/kg body weight) once a week for 3 weeks from 1 week after the start of the experiment, and groups 7 through 11 received a single i.p. injection of saline (vehicle control). Rats of groups 7–9 were given the experimental diets and groups 10 and 11 were fed the basal diet throughout the experiment (24 weeks). The experimental diets in groups 1–3 were changed to the basal diet, which was then continued to the end of the experiment. Groups 1, 2, 3 and 10 received drinking water containing 500 ppm PB from 1 week after the final injection of carcinogen or vehicle. Groups 4–6 were switched to the experimental diets from 1 week after the end of DEN or vehicle treatment. At the termination of the experiment, all animals were killed under ether anesthesia and complete autopsies were performed. At autopsy, the location, number and size of liver tumors

Table IV. Body and Liver Weights of Rats

Group	Treatment	No. of rats	Body weight (g)	Liver weight (g)	Relative liver weight (%)
1	HCO+DEN → PB	20	305.8±13.0[a)]	14.6±1.0	4.77±0.20
2	LCO+DEN → PB	20	309.4±26.8	15.2±1.9	4.95±0.77
3	FO+DEN → PB	20	315.0±11.5	14.2±1.0	4.52±0.32
4	DEN → HCO	20	345.2±14.3	9.4±0.9	2.84±0.21
5	DEN → LCO	20	309.7±25.1[b)]	8.5±1.1[c)]	2.74±0.28
6	DEN → FO	19	353.8±18.0[d)]	12.0±1.4[b, d)]	3.38±0.31[b, d)]
7	HCO alone	8	332.2±16.6	9.2±0.9	2.79±0.25
8	LCO alone	8	327.0±21.5	9.5±1.0	2.89±0.18
9	FO alone	8	336.4±14.1	11.6±0.5[e, f)]	3.45±0.21[e, f)]
10	PB alone	8	351.8±11.3	15.1±0.8	4.29±0.14
11	Vehicle control	10	346.0±14.5	10.8±0.7[g)]	3.11±0.11[g)]

a) Mean±SD.
b, c) Significantly different from group 4 by Student's *t* test (*b*) $P<0.0001$, *c*) $P<0.01$).
d) Significantly different from group 5 by Student's *t* test ($P<0.0001$).
e) Significantly different from group 7 by Student's *t* test ($P<0.0001$).
f) Significantly different from group 8 by Student's *t* test ($P<0.0001$).
g) Significantly different from group 10 by Student's *t* test ($P<0.0001$).

Table V. Incidence of Liver Tumors in Each Group

Group	Treatment	Incidence (%)			Multiplicity		
		Ad.[a)]	Ca.[b)]	Total	Ad.	Ca.	Total
1	HCO+DEN → PB	100	90	100	4.7±3.7[c)]	2.4±1.4	7.2±4.4
2	LCO+DEN → PB	85	40[d)]	85	2.0±1.8[e)]	0.9±1.3[f)]	2.9±2.7[g)]
3	FO+DEN → PB	90	85[h)]	100	2.0±1.2[f)]	1.8±1.5	3.8±2.0[f)]
4	DEN → HCO	25	25	35	0.3±0.6	0.4±0.7	0.7±1.1
5	DEN → LCO	20	10	25	0.2±0.4	0.1±0.3	0.3±0.6
6	DEN → FO	21	32	47	0.3±0.5	0.5±0.8[i)]	0.7±0.8
7	HCO alone	0	0	0	—	—	—
8	LCO alone	0	0	0	—	—	—
9	FO alone	0	0	0	—	—	—
10	PB alone	0	0	0	—	—	—
11	Vehicle control	0	0	0	—	—	—

a) Hepatocellular adenoma.
b) Hepatocellular carcinoma.
c) Mean±SD.
d) Significantly different from group 1 by Fisher's exact probability test ($P<0.002$).
e–g) Significantly different from group 1 by Student's *t* test (*e*) $P<0.01$, *f*) $P<0.005$, *g*) $P<0.001$).
h) Significantly different from group 2 by Fisher's exact probability test ($P<0.005$).
i) Significantly different from group 5 by Student's *t* test ($P<0.05$).

were recorded. Liver slices were prepared from each lobe. One set of slices was fixed in cold acetone and the other set was fixed in 10% buffered formalin and embedded in paraffin blocks. Two serial sections were sliced from buffered formalin-fixed tissues. One section was processed for routine histological observation with the use of hematoxylin and eosin stain and the other was stained for AgNORs and GST-P. Immunohistochemical staining for GST-P was carried out using the avidin-biotin-peroxidase complex method (Vectastain ABC kit, Vector Lab. Inc., Burlingame, CA). Anti-GST-P antibody was kindly provided by Dr. Kimihiko Satoh, Hirosaki University School of Medicine, Hirosaki, Japan. The areas of GST-P-positive foci and number of foci/cm^2 were measured by means of an

image analyzer with a microscope (IPAP, Sumitomo Chemical Co., Ltd., Osaka). GST-P-positive lesions were defined as foci of more than 0.01 mm^2 in area. The serial sections were stained for AgNORs using a one-step silver colloid method,[23, 24] and then stained immunohistochemically for GST-P. Quantitative analyses of AgNORs were carried out by IPAP.

Statistical analysis Differences of incidence or density of pathological lesions in the liver between groups were analyzed by use of the χ^2-test, Fisher's exact probability test, Student's *t* test or Welch's *t* test.

RESULTS

General observations There was no clear evidence of toxicity of different dietary fats under our experimental conditions (Table IV). One rat in group 6 died of pneumonia before termination of the experiment, but no neoplasm was recognized. The body weight of rats treated with DEN and LCO in the postinitiation phase (group 5) was significantly smaller than that of rats treated with DEN+HCO or FO in the postinitiation phase (group 4 or 6). Liver and relative liver weights were significantly increased by PB and FO.

Tumor incidence Liver tumors were seen only in DEN-treated groups. The neoplasms were of hepatocellular ori-

Table VI. Average Diameter of Hepatocallular Carcinomas in Each Group

Group	Treatment	Average diameter (mm^2)
1	HCO+DEN → PB	5.80±2.32[a)]
2	LCO+DEN → PB	5.11±1.53
3	FO+DEN → PB	5.97±4.46
4	DEN → HCO	5.38±1.30
5	DEN → LCO	9.00
6	DEN → FO	6.50±1.51

a) Mean±SD.

Table VII. Quantitative Analysis of GST-P-positive Foci in Rats of Each Group

Group	Treatment	Density (No./cm^2)	Average area (mm^2)	Unit area ($\times10^{-3}$)
1	HCO+DEN → PB	55.9±12.5[a)]	7.4±1.6	41.4±12.5
2	LCO+DEN → PB	43.6±12.4[b)]	8.5±3.5	36.1±16.0
3	FO+DEN → PB	33.7±9.2[c,d)]	7.3±3.3	24.4±11.0[c,e)]
4	DEN → HCO	22.3±5.5	3.8±1.1	8.5±3.2
5	DEN → LCO	19.5±8.3	4.9±1.9	9.3±5.0
6	DEN → FO	12.7±6.8[f,g)]	9.8±7.8[f,h)]	11.6±9.2

a) Mean±SD.
b, c) Significantly different from group 1 by Student's *t* test (*b*) $P<0.005$, *c*) $P<0.0001$).
d, e) Significantly different from group 2 by Student's *t* test (*d*) $P<0.005$, *e*) $P<0.02$).
f) Significantly different from group 4 by Student's *t* test ($P<0.0001$).
g, h) Significantly different from group 5 by Student's *t* test (*g*) $P<0.01$, *h*) $P<0.0001$).

Table VIII. Average Area of AgNORs in Each Group Treated with DEN

Group	Treatment	Non-lesional area (mm^2/nucleus)	GST-P-positive foci (mm^2/nucleus)	Neoplasia (mm^2/nucleus)
1	HCO+DEN → PB	4.51±0.53[a)]	6.80±0.53	7.11±2.76
2	LCO+DEN → PB	5.45±1.07[b)]	5.97±1.29[b)]	7.54±1.43
3	FO+DEN → PB	4.18±0.09[b,c)]	5.45±0.60[d)]	7.83±1.45
4	DEN → HCO	3.51±0.22	4.98±0.88	6.13±0.92
5	DEN → LCO	3.69±0.54	4.47±0.57[e)]	5.20±0.09[f)]
6	DEN → FO	3.96±0.27[g,h)]	4.52±0.43[e)]	6.60±0.51[e)]

a) Mean+SD.
b) Significantly different from group 1 by Welch's *t* test ($P<0.01$).
c, d) Significantly different from group 2 by Welch's *t* test (*c*) $P<0.005$, *d*) $P<0.002$).
e–g) Significantly different from group 4 by Welch's *t* test (*e*) $P<0.05$, *f*) $P<0.001$) and Student's *t* test (*g*) $P<0.0001$).
h) Significantly different from group 5 by Student's *t* test ($P<0.05$).

gin (Table V). The incidence of carcinoma in group 2 was significantly lower than in groups 1 and 3 ($P<0.002$ and $P<0.005$, respectively). No significant differences in the incidence were found among groups 4–6. The multiplicities of adenoma, carcinoma and total tumors of group 2, and adenoma and total tumors of group 3 were significantly smaller than those of group 1 ($P<0.01$, $P<0.005$ and $P<0.001$, and $P<0.005$ and $P<0.005$, respectively). The multiplicity of carcinoma of group 6 was significantly larger than that of group 5 ($P<0.05$). The average diameter of hepatocellular carcinomas in each group is shown in Table VI. The average diameter of carcinomas in group 2 was slightly smaller than that in group 1 or 3, although no statistically significant differences could be found. The diameter of carcinomas in group 6 was slightly larger than that in group 4, but again this was not significant. Only 2 hepatocellular carcinomas were present in group 5. Therefore, the average diameter of group 5 could not be reliably compared with those of the other groups.

Expression of hepatocellular foci A number of GST-P-positive foci appeared in the groups exposed to DEN. The results of quantitative analysis of the frequency of GST-P-positive foci are summarized in Table VII. The density, average area and unit area of GST-P-positive foci were largest in group 1. The density of foci in groups 2 and 3 was significantly lower than that in group 1, and that in group 3 was significantly lower than that in group 2. The unit area of foci in group 3 was significantly smaller than that in group 1. The density of foci in group 6 was significantly lower than those in groups 4 and 5. The average area of foci in group 6 was significantly larger than those in groups 4 and 5.

Analysis of AgNORs The results of the analysis of AgNORs of liver cell nucleus are summarized in Table VIII. Average area of AgNORs of enzyme-altered foci in the HCO+DEN → PB diet group was larger than that in the other diet groups. The average area of AgNORs in PB-treated groups was larger than in the other groups.

DISCUSSION

In this study, an enhancing effect of a high concentration of corn oil in the diet on hepatocarcinogenesis was seen for exposure in the initiation phase, but not in post-initiation phase, implying that dietary fat has a potent influence on the early stage of carcinogenesis. Another aim of the current study was to investigate the modulating effect of fish oil rich in ω-3 fatty acids as compared with that of corn oil rich in ω-6 fatty acids when fed during the initiation phase of carcinogenesis. To our knowledge, this is the first report to show that a high dose of dietary fish oil effectively reduces hepatocarcinogenesis when given during the initiation phase. These results suggest that high fat intake is a modulator of the initiation of hepatocarcinogenesis and that the fatty acid composition is one of the determinants of the effects of a high fat diet.

The effects of dietary fat on carcinogenesis have been extensively investigated and dietary fat is now considered important in the promotion of cancers in certain organs, such as breast or colon.[6, 7] Reddy demonstrated that a high fat diet containing lard during the initiation or post-initiation phase enhanced the development of colon tumors, whereas feeding of a high corn oil diet had an enhancing effect only during the postinitiation stage of colon carcinogenesis.[6] These results, together with our present data, suggest that this enhancing effect of corn oil is organ-specific.

It is known that increase of fat content in the diet during carcinogenesis enhances the development of hepatic tumors.[13, 18–20, 28] Newberne *et al.* reported a promoting effect of corn oil on AfB_1-induced hepatocarcinogenesis.[18] In contrast, Glauert and Pitot reported that increase of the safflower oil or palm oil content in the diet did not promote DEN-induced carcinogenesis.[19] Furthermore, Glauert *et al.* have suggested that the enhancement of DEN-induced development of GGT-positive foci by corn oil is primarily due to an effect on initiation.[20] In our previous study, the administration of fish oil in the post-initiation stage reduced AOM-induced GST-P-positive foci.[21] Nevertheless, in the present study, fish oil did not suppress DEN-induced tumorigenesis in the liver. It is, thus, suggested that the effect of dietary fat is carcinogen-specific.

Suppressive effects of fish oil on carcinogenesis have been reported in breast, colon and liver.[6, 7, 21] Such effects have been recognized in the case of exposure in the post-initiation phase. However, it remains to be unequivocally determined how corn oil promotes or fish oil suppresses carcinogenesis. Cell proliferation is believed to enhance the frequency of tumor initiation,[29–34] and hepatocytes proliferation is reported to be concerned with several stages of liver cell tumorigenesis.[30, 35, 36] Reddy *et al.* found that dietary fish oil is effective not only in the post-initiation stage, but also in the initiation stage.[37] Rao and Reddy also reported that a high corn oil diet increased liver ornithine decarboxylase (ODC), tyrosine protein kinase (TPK) and prostaglandins, whereas high dietary fish oil suppressed such activities.[38] ODC induction is known to precede cell proliferation in many cells exposed to xenobiotics, including genotoxic carcinogens[39] and the enzyme activity is regarded as a biomarker for cell proliferation.[40, 41] Tyrosine-specific protein phosphorylation is recognized as an important regulatory mechanism of cell proliferation in response to a number of processes.[42] In this work, AgNORs in GST-P-positive foci were measured and the results showed that the area of AgNORs in the foci of the group given the high fat diet

was larger than in the other diet groups. Our results appear to indicate that a high dose of corn oil in the diet enhances the proliferation of initiated cells, but a high dose of fish oil does not.

The difference between fish oil and corn oil when fed during the initiation phase may be related to the action of ω-3 fatty acids on the metabolic activation and detoxification of DEN. DEN is preferentially metabolized by distinct isozymes of cytochrome P-450.[43] It is known that increase of the fat content in the diet enhances the activity of cytochrome P-450 and other enzymes.[28, 44–47] Dietary fat may thus modulate the metabolic activation of DEN. Fish oil was reported to increase the hepatic concentration of cytochrome P-450,[28, 48] and it may also increase NADPH-cytochrome-*c* reductase, aniline hydroxylase, aminopyrine *N*-demethylase, glutathione reductase, glutathione-*S*-transferase, catalase, epoxide hydrolase and UDP-glucuronosyltransferase activities in rats.[48–51] These are antioxidant or phase 2 enzymes, both of which are considered to have roles in the prevention of carcinogenesis.

In this study, no significant differences were found among the results on hepatocellular neoplasms in the groups given the oils in the promotion phase. However, the average area of GST-P-positive foci in the group given the high fish oil diet in the promotion phase was significantly larger than in the other diet groups. This may imply some promoting activity in fish oil. A longer-term experiment is needed to determine whether or not fish oil promotes hepatocarcinogenesis. Previously, we reported that a high fish oil diet in the postinitiation phase reduced AOM-induced hepatocellular foci. These results emphasize that the effects of fish oil appear to be dependent on the types of carcinogens.

In conclusion, the results of this study demonstrate that a high concentration of corn oil in the diet enhances hepatocarcinogenesis at the initiation stage, while fish oil rich in ω-3 fatty acids decreases carcinogenesis at the initiation stage. Although the underlying mechanisms of the modifying effects of corn oil or fish oil in the initiation phase of hepatocarcinogenesis are not clear, the effects of the oils on cell proliferation and metabolic activation may be important factors.

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