# The Effect of Dietary Fatty Acids on the Expression Levels and Activities of Hepatic Drug Metabolizing Enzymes

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Analysis of the fatty acid composition in the microsomes of rats fed the oil rich in  $\alpha$ -linolenic acid (n-3, perilla oil, Per) or linoleic acid (n-6, safflower oil, Saf) showed changes according to those compositions in the diets. The level of phase I enzyme in microsomes prepared from rat liver was measured by the Western blotting method. The levels of CYP1A1, 1A2 and 2E1 were similar between the two oil groups, Per and Saf. Meanwhile, the level of CYP4A1 in the microsomes of rats fed the Per diet was significantly higher than that from rats fed Saf. As a second step, we measured the level and activity of UDP-glucuronosyltransferase (UGT). The results showed that the level of UGT activity in microsomes from rats fed the Per diet was 2.4-fold higher than that from rats fed the Saf diet. The protein level of UGT in microsomes from rats fed the Per diet was also 1.7-fold greater than that from rats fed the Saf diet. The protein level of protein amount and activity of UGT in rats fed Saf was recovered by the administration of the Per diet. Furthermore, the UGT activity in microsomes from rats fed Per oil. These results suggest that the fatty acid composition, n-3 or n-6, in foods influences the level and activity of CYP4A and UGT. Thus, monitoring of the drug level in the blood of patients in consideration of oil intake should be undertaken.

Key words —— cytochrome P-450, UDP-glucuronosyltransferase, safflower oil, perilla oil, CYP4A1

# INTRODUCTION

The drug metabolism reaction proceeds through three steps, activation/detoxification by a phase I enzyme (such as cytochrome P-450, CYP) and conjugation by a phase II enzyme (such as UDPglucuronosyltransferase, UGT). CYP and UGT are the main enzymes of drug metabolism.<sup>1)</sup> These conjugates are excreted by transporters of the 3rd step through the liver and/or kidney. These enzymes metabolize numerous endogenous compounds, such as steroids, bile acids, fatty acids, and biogenic amines, in addition to foreign chemicals including drugs, environmental pollutants, and natural plant products. The factors that influence the level of drugmetabolizing enzymes include alcohol consumption, smoking, and drugs as outer factors, and gender, disease, nutrition, and heredity as inner factors. Among these factors, those based on the fat in food, especially fatty acids, influences the level of drug-metabolizing enzymes, because CYP and UGT appear as insoluble enzymes in microsomes and are buried in lipid bilayers.<sup>2,3)</sup> It was suggested that fatty acid in microsomes is essential for the enzymes to develop activity. CYP, O<sub>2</sub> molecule, NADPH, NADPH-cytochrome P450 reductase and lipids are essential components to produce the phase I reaction with CYP-dependent mixed-function oxidase. The UGT of a major phase II enzyme reacts with endogenous bilirubin or steroids, and with UDP-glucuronic acid (UDPGA) as the glucuronosyl donor. The addition of sugars to fat soluble chemicals is an important process that increases their solubility in water and aids their excretion. In mammals, glucuronic acid is a main sugar that is used to prevent the accumulation of waste products of metabolism and fat-soluble chemicals from the environment to toxic levels in the body, and UGT carries out this reaction.

Fatty acids are important nutrients in foods. Linoleic acid (18 : 2, LA) and arachidonic acid (20 : 4, Ara), n-6 fatty acids, are essential fatty acids to maintain good skin condition, pregnancy and growth.<sup>4)</sup> Meanwhile, docosahexaenoic acid (22 : 6, DHA), n-

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Fig. 1. Metabolism of n-3 and n-6 Series Fatty Acids in Mammals

3 fatty acid, is rich in the retina, brain and muscle,<sup>5–7)</sup> and is important to maintain the good condition of these organs.<sup>8)</sup> Eicosapentaenoic acid (20 : 5, EPA), another n-3 fatty acid, also plays a key role in preventing thrombosis in veins.<sup>9)</sup> In rats fed a non-fat diet, the levels of activity and amount of CYP1A2, CYP2B2, CYP2E1 and UGT were low.<sup>10,11)</sup> The level of some drug-metabolizing enzymes, such as CYP and UGT in the microsomes of rats fed fish oil, were higher than that in rats fed corn oil or olive oil.<sup>12)</sup> It was also reported that the composition of polyunsatulated fatty acids (PUFA) in phospholipids was a key factor in determining the activity of CYP-dependent mixed-function oxidase.<sup>13)</sup>

There are two series of PUFA. The first is n-6 fatty acids, such as LA,  $\gamma$ -linolenic acid (LL) and Ara, and the second is n-3 fatty acids, such as  $\alpha$ -linolenic acid (18 : 3,  $\alpha$ -LNA), EPA and DHA. N-3 oils are fish oil and perilla oil (Per), and a typical n-6 oil is safflower oil (Saf). These two fatty acids are not capable of inter-conversion in mammals, so Ara from LA, and EPA and DHA from  $\alpha$ -LNA are made by the desaturation and elongation reactions shown in Fig. 1, respectively. Recently, the intake of n-6 fatty acids has increased according to the lifestyle trends in food intake, and it is possible that this increase has caused the increase in atherosclerosis and thrombotic diseases such as coronary heart disease and cerebral infarction.<sup>7</sup>

In this study, we examined the influence of high n-6 fatty acids and n-3 fatty acids in food on the fatty acid composition in rat liver microsomes. We also examined the level of drug-metabolizing enzymes, the phase I enzyme of some CYP and the phase II enzyme of UGT in rat liver microsomes.

Table 1.	Comp	osition	of I	Diets
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Ingredient	Composition		
	g/100 g of diets		
Corn starch	48.0		
Milk casein	25.1		
Cellulose	8.2		
Sucrose	5.1		
$Okanol^{a)}$	2.0		
Mineral mixture	6.1		
Vitamin mixture	1.4		
DL-Methionine	0.4		
Choline chloride	0.6		
Oil	3.0		

a) A mixture of carbohydrates.

### MATERIALS AND METHODS

**Materials** — Diets for the rats were prepared according to a previous study.<sup>14)</sup> DHA ethyl ester (95.8% DHA and 4.2% eicosapentaenoate) was a product of Harima Chemicals (Tsukuba, Japan). Oleic acid (OA) ethyl ester was purchased from Wako Pure Chemical Industries (Osaka, Japan). Heptadecanoic acid was purchased from Funakoshi (Tokyo, Japan). These oils were mixed with a semipurified diet (Clea Japan, Tokyo, Japan), as shown Table 1. Vegetable oils (Saf and Per) were purchased from a local market. Fatty acid compositions of the experimental diets are shown in Table 2. Saf (20%), OA ethyl ester (40%), and DHA ethyl ester (40%) were mixed to make Saf-DHA, in which the 18-2n-6 content was adjusted to that of Per.

Animals — All animals used in the experiments were maintained under controlled conditions at  $23 + 2^{\circ}$ C and 50 + 10% humidity. Donryu rats (F<sub>0</sub>) (a conventional specific pathogen-free strain from

Table 2. Faity Reid Composition of Diet				
Fatty Acid	Safflower oil diet	DHA diet <sup>a)</sup>	Perilla oil diet	
		% of total fatty acids		
16:0	6.6	1.3	5.4	
18:0	2.3	0.5	1.4	
18:1	15.8	43.2	15.1	
18 : 2n-6	73.9	14.8	14.8	
18 : 3n-3	0.8	0.2	63.0	
20:0	0.3	0.1	0.1	
20:1	0.2	trace	0.2	
20:5n-3	$N.D.^{b)}$	1.7	$N.D.^{b)}$	
22:6n-3	$N.D.^{b)}$	38.3	$N.D.^{b)}$	
n-6/n-3	90.2	0.37	0.23	

 Table 2. Fatty Acid Composition of Diet

a) DHA diet; 0.6% Safflower oil + 1.2% DHA ethylester + 1.2% oleic acid ethylester. b) N.D.; not detected.



Fig. 2. Two Groups of Rats Fed 2 Oil Diets of F<sub>0</sub>, and 4 Groups of F<sub>1</sub> Rats Fed Oil Diets

SLC, Shizuoka, Japan) at 4 weeks of age were fed the Saf diet (n-3 fatty acid deficient) or the Per diet (n-3 fatty acid sufficient) for 7 weeks. The rats ( $F_0$ ) were mated at 11 weeks of age, and at 3 weeks of age the male pups ( $F_1$ ) from the Saf diet group were weaned and divided randomly into two groups. One group was fed the same Saf diet (Saf-Saf), and the other group was fed a DHA diet (Saf-DHA) or a Per diet (Saf-Per). The male pups ( $F_1$ ) from the Per diet group were fed the same Per diet (Per-Per). A part of these animals was used in learning behavior experiments.<sup>14)</sup> This strategy is shown as a schematic in Fig. 2.

**Preparation of Microsomes from Rat Liver** — Rat livers at 3 weeks of age or 11 weeks of age were extracted with 4 volumes of 0.25 M sucrose at 0°C. The extract was centrifuged for 15 min at  $12500 \times g$ , and the supernatant was further centrifuged for 45 min at  $105000 \times g$ . The supernatant was discarded and the precipitate was washed three times with 0.25 M sucrose and then suspended in 20% glycerol-0.1 M Tris–HCl at pH 7.4 and stored at -80°C. The protein concentration was determined by the method of Bradford.

Analysis of Fatty Acid Composition in Microsomes — Total lipids in the microsomes of  $F_0$  and  $F_1$  rats at the indicated ages were extracted with chloroform-methanol according to the method of Bligh and Dyer.<sup>15)</sup> Fatty acids were converted to their methyl esters by treatment with 5% HCl in methanol and were quantified by capillary column gasliquid chromatography (Shimadzu, Kyoto, Japan)

Fatty Acids	Saf	Per
	%(w/w) of total fatty acids	
14:0	$0.60\pm0.22$	$1.15\pm0.54$
16:0	$18.83 \pm 1.18$	$19.66\pm0.79$
16:1	$0.41\pm0.18$	$2.06 \pm 1.42$
18:0	$16.64\pm2.01$	$15.17\pm3.29$
18:1 (OA)	$10.18\pm2.10$	$11.56\pm2.73$
18 : 2 n-6 (LA)	$16.13\pm2.06$	$8.31\pm0.45$
18 : 3 n-3 (α-LNA)	$0.13\pm0.01$	$6.01\pm3.39$
20 : 3 n-6	$N.D.^{a)}$	$0.70\pm0.20$
20:4 n-6 (AA)	$22.48 \pm 1.97$	$4.29\pm0.71$
20:5 n-3 (EPA)	$N.D.^{a)}$	$9.37 \pm 0.29$
22 : 4 n-6	$1.93\pm0.27$	N.D. <sup><i>a</i>)</sup>
22 : 5 n-6	$8.37 \pm 1.09$	N.D. <sup><i>a</i>)</sup>
22 : 5 n-3	$0.32\pm0.05$	$4.68 \pm 1.40$
22:6 n-3 (DHA)	$1.78\pm0.17$	$14.89\pm2.29$
24:0	$0.57\pm0.04$	$0.47\pm0.10$
24:1	$0.28\pm0.04$	$0.26\pm0.08$
DHA/AA	$0.08\pm0.01$	$3.51\pm0.61$
n-6/n-3	$22.39 \pm 1.62$	$0.39\pm0.04$

Table 3. Fatt Acid Composition of Liver Microsomes (F<sub>1</sub> 3 weeks of Age)

Values are means  $\pm$  S.D. (n = 4). a) N.D.; not detected.

using heptadecanoic acid as an internal standard, as described previously.<sup>14)</sup>

Analysis of Drug-Metabolizing Enzymes by Western Blotting Method —— Rat liver microsomes were mixed with an equivalent volume of the sample solution for electrophoresis, composed of 60 mM Tris-HCl at pH 6.8, 2% sodium dodecyl sulfate (SDS), 20% glycerol, 2% 2-mercaptoethanol and 0.001% bromophenol blue (BPB). The sample mixture was heated at 100°C for 2 min, then electrophoresed on SDS-polyacrylamide gel electrophoresis (PAGE). Then, proteins in the gel were electroblotted onto polyvinylidene difluoride (PVDF) membrane and the membrane was blocked in a 5% skimmed milk-1% bovine serum albumin (BSA) solution. The protein on the membrane was immunoreacted with antiserum (rabbit) for rat CYP1A1, 1A2, 2E1 and 4A1 as the first antibody supplied from Gentests. The antibody used to detect UGT1A was anti-human UGT1A rabbit serum from Gentest. The second antibody used was anti-rabbit goat serum-conjugated peroxidase. The activity on the membrane was detected using ECL<sup>TM</sup> (Amersham-Pharmacia Biotech, U.K.) using LAS 1000 (Fuji Film, Japan) by chemiluminescence. UGT Activity of Phase II Enzyme —— The activ-

ity of UDP-glucuronosyltransferase was determined

by a method reported previously,<sup>16)</sup> as follows. The reaction mixture (50 µl) contained 100 mM Tris-Maleate, 5 mM MgCl<sub>2</sub>, 2 mM *p*-nitrophenol of substrate, 2 mM [<sup>14</sup>C]UDPGA (74 Bq) and 100  $\mu$ g microsomal protein. The reaction mixture was incubated for 60 min at 37°C. The reaction was stopped by the addition of 100  $\mu$ l cold ethanol, and the mixture was centrifuged for 5 min at  $8000 \times g$ . The supernatant was concentrated under a vacuum. The residual was dissolved in 10  $\mu$ l of 70% ethanol and spotted on a silica gel plate (Merck silica gel 60, Merck KGaA, Darmstadt, Germany) and developed in n-butanol-water-acetone-acetic acid-30% ammonia (70:60:50:18:1.5). The radioactivity of the <sup>14</sup>C] product on the thin layer was measured using a radioimage analyzer, BAS 2500 (Fuji Film). Statistical Analysis —— The data were compared by two-way analysis of variance (ANOVA). A difference was considered significant at p < 0.05. Fatty acid compositions at each week of age were compared by one-way ANOVA followed by a Bonferroni multiple comparison test. A difference was considered significant at p < 0.01.

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Fatty Ackis	Saf-Saf	Saf-Per	Saf-DHA	Per-Per	
		%(w/w) of total fatty aci	ds		
14:0	$0.25\pm0.06$	$0.18\pm0.05$	$0.23\pm0.06$	$0.21\pm0.04$	
16:0	$18.96 \pm 1.10$	$20.03 \pm 1.03$	$23.49 \pm 1.94$	$21.77\pm1.27$	
16:1	$3.31\pm0.86$	$2.88\pm0.44$	$3.99 \pm 1.33$	$3.72\pm0.86$	
18:0	$12.89 \pm 1.49$	$15.76\pm1.07$	$14.27\pm1.49$	$15.26\pm1.63$	
18:1 (OA)	$20.00\pm3.71$	$17.41\pm0.62$	$18.12\pm2.81$	$18.69 \pm 1.96$	
18:2 n-6 (LA)	$15.13\pm1.37$	$9.33\pm0.31$	$8.41\pm0.90$	$9.07\pm0.86$	
18 : 3 n-3 (α-LNA)	$0.19\pm0.03$	$3.42\pm0.31$	$0.15\pm0.02$	$2.99\pm0.56$	
20 : 3 n-6	$0.46\pm0.34$	$1.01\pm0.11$	$0.87\pm0.10$	$1.05\pm0.12$	
20:4 n-6 (M)	$21.01\pm2.81$	$6.84\pm0.64$	$3.13\pm0.46$	$5.46\pm0.46$	
20:5 n-3 (EPA)	N.D. <sup><i>a</i>)</sup>	$7.70\pm0.70$	$3.58\pm0.64$	$7.28\pm0.31$	
22 : 4 n-6	$1.14\pm0.24$	$0.11\pm0.06$	$N.D.^{a)}$	$N.D.^{a)}$	
22 : 5 n-6	$3.09\pm0.80$	$0.20\pm0.06$	$0.15\pm0.03$	$0.24\pm0.10$	
22 : 5 n-3	$0.28\pm0.06$	$4.42\pm0.42$	$N.D.^{a)}$	$3.36\pm0.63$	
22:6 n-3 (DHA)	$1.42\pm0.21$	$8.96\pm0.61$	$22.15\pm2.40$	$8.80\pm0.55$	
24:0	$0.46\pm0.10$	$0.59\pm0.07$	$0.53\pm0.10$	$0.53\pm0.09$	
24:1	$0.20\pm0.02$	$0.28\pm0.03$	$0.31\pm0.02$	$0.32\pm0.04$	
DHA/AA	$0.07\pm0.01$	$1.32\pm0.18$	$7.19 \pm 1.13$	$1.62 \pm 0.12$	
n-6/n-3	$22.08 \pm 1.72$	$0.72\pm0.04$	$0.49\pm0.03$	$0.72\pm0.04$	

Table 4. Fatty Acid Composition of Liver Microsomes (F<sub>1</sub> 9 weeks of Age)

Values are means  $\pm$  S.D. (*n* = 4). *a*) N.D.; not detected.

# RESULTS

#### Fatty Acid Composition of Rat Liver Microsomes

Table 3 shows the fatty acid composition of microsomes prepared from F<sub>1</sub> rat fed Saf or Per diets for 3 weeks. The microsomes from rats fed the Saf diet contained high  $\gamma$ -LNA coming from the diets, and a small amount of  $\alpha$ -LNA, EPA and DHA showing deficient n-3 fatty acids. The level of Ara in microsomes from rats fed the Saf diet was approximately 5-fold that of mice fed the Per diet. The fatty acid composition in microsomes from rats fed the Per diet showed a pattern dependent on the fatty acid composition in the diet, such as high EPA and DHA. Table 4 shows the fatty acid composition in microsomes from rats fed 4 kinds of diets, as shown in Fig. 2. Those compositions changed according to the composition in the diets. The fatty acid composition of microsomes from rats fed the Per-Per diet in Table 4 showed the same pattern as rats fed the Per diet, as shown in Table 3. The fatty acid composition of the two groups changed from the Saf diet to the Saf-Per or Saf-DHA diets transformed to a pattern showing high DHA and EPA, similar to those of the Per-Per diet. The fatty acid composition of microsomes from rats fed Saf-DHA showed high DHA (22%), and recovered from low n-3 fatty acids in rats fed the Saf-Saf diet. The oleic acid levels in microsomes from 4 groups of rats at 9 weeks of age were higher than those of rats at 3 weeks of age.

# The Influence of Fatty Acid Composition on the Level of CYP1A1, 1A2, 2E1 and 4A1

We measured the level of phase I drug-metabolizing enzymes by the Western blotting method using each authentic antibody. Figure 3 shows the pattern of the results of CYP1A1 and 1A2, which are known to metabolize and activate some polycyclic aromatic hydrocarbons, such as benzo[a]pyrene, to true carcinogens. CYP1A1 and 1A2 also metabolize some important drugs such as imiplamine, theophylline and (R)-warfarin. CYP1A1 is induced in the lung during smoking. The upper band in Fig. 3 indicates the level of CYP1A1 in rat liver microsomes, and the level is less than that of CYP1A2 of the lower band in the same figure. As shown in Fig. 3, the levels of CYP1A1 and 1A2 in microsomes from rats fed Per and Saf diets were the same level, and we could not find a significant difference between them.

Figure 4 shows the level of CYP2E1, which generally metabolizes benzene, aniline, organic halogen compounds, and acetaminophen of xenobiotics, and is induced by ethanol and acetone. Figure 4



Fig. 3. Effect of Dietary Fatty Acids on the Level of CYP1A1 (A) and 1A2 (B) Detected in Microsomes by Western Blotting Values represent means ± S.D. for four rats. Statistical analysis was performed by two-way ANOVA, and there was no significant effect of the diet on the level of CYP1A1 and 1A2.



Fig. 4. Effect of Fatty Acids on the Level of CYP2E1 Detected in Microsomes by Western Blotting

Values represent means  $\pm$  S.D. for four rats. Statistical analysis was performed by two-way ANOVA, and there was no significant effect of the diet on the level of CYP2E1.

shows no significant difference between the levels of CYP2E1 in microsomes from rats fed the Saf and Per diets. This suggests that the fatty acid composition in diets does not influence the level of CYP2E1 in liver microsomes.

Figure 5 shows a Western blotting pattern of CYP4A1 in microsomes from rats fed the Saf and Per diets at 3 weeks at age, and from 4 groups of rats fed the Saf-Saf, Saf-Per, Saf-DHA, and Per-Per diets at 9 weeks of age. CYP4A1 is specific to rats and relates to the omega-oxidation of laurate and palmitate. In general, CYPs belonging to the CYP4A family play roles to metabolize prostaglandins and leukotrienes. Figure 5A shows the level of CYP4A1 in microsomes from rats fed the Per diet at 3 weeks of age, and the level was significantly higher than those from rats fed the Saf diet. The same pattern of CYP4A1 at 9 weeks of age were found in Fig. 5B, in which the level of CYP4A1 in microsomes from 3 groups of rats, such as Saf-Per, Saf-DHA and Per-Per were significantly higher (1.3-fold) than those from rats fed the Saf-Saf diet. The fatty acid composition of microsomes from the above 3 groups of rats, containing high DHA ad EPA, were changed from the pattern of rats fed Saf to the pattern of rats fed the Per diet, as shown in Tables 3 and 4. These results showed that there was no significant change in the level of CYP1A1, 1A2, and 2E1 between rat liver microsomes fed the Saf and Per diets. Meanwhile, the level of CYP4A1, which relates to the metabolism of fatty acid, was higher in microsomes from rats fed the Per diet, an n-3 fatty acid, than that in rats fed Saf oil of n-6 fatty acid.

# The Influence of the Fatty Acid Composition on the Level of UDP-glucuronosyltransferase

We measured UGT activity in microsomes from rats fed n-3 or n-6 fatty acids, with [<sup>14</sup>C]UDPGA, *p*nitrophenol as a substrate according to the method reported elswhere.<sup>16)</sup> The results obtained from rats at 3 weeks and 9 weeks of age are shown in Figs. 6A and B, respectively. In Fig. 6A, the level of UGT activity in microsomes from rats fed the Per diet at 3 weeks of age was 0.29 nmol/min/mg protein and was significantly higher (2.4-fold) than that from rats fed the Saf diet (0.12 nmol/min/mg protein). The

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Fig. 5. Effect of Dietary Fatty Acids on the Level of CYP4A1 Detected in Microsomes by Western Blotting A, F<sub>1</sub> at 3 weeks of age; B, F<sub>1</sub> at 9 weeks of age. Values represent means ± S.D. for four rats. Statistical analysis was performed by two-way ANOVA. An asterisk indicates significant differences between Saf and Per, or Saf-Saf and the other 3 groups, Saf-Per, Saf-DHA, and Per-Per.



**Fig. 6.** Effect of Dietary Fatty Acids on Liver UGT Activity A, F<sub>1</sub> at 3 weeks of age; B, F<sub>1</sub> at 9 weeks of age. Values represent means ± S.D. for four rats. Statistical analysis was performed by twoway ANOVA. Significant differences are indicated by an asterisk in A, and a, b and c in B (a, Saf-Saf vs the other 3 groups; b, Saf-DHA vs Saf-Per or Per-Per).

same pattern was found in rats at 9 weeks of age, as shown in Fig. 6B. The levels of the Per-Per and Saf-Saf diets were 0.32 and 0.16 nmol/min/mg protein, respectively. Similar patterns were also found in the results of UGT activity in microsomes of 2 groups of rats fed Saf-Per and Saf-DHA (0.32 and 0.37 nmol/min/mg protein, respectively). Furthermore, the level of UGT activity in rats fed the Saf-DHA diet was significantly the highest among the 4 groups of rats in Fig. 6B. This suggests that the DHA content in phospholipids in the membrane plays a key role in promoting UGT activity in the high DHA membrane by high fluidity.

Figure 7 shows the protein levels of UGT in microsomes from rats fed Saf or Per diets. Figure 7A shows the results from rats at 3 weeks of age by Western blotting, and Fig. 7B shows the comparison between the levels of UGT in microsomes from rats fed Saf and Per diets. The protein level of UGT on microsomes from rats fed the Per diet was significantly higher (1.7-fold) than that from rats fed the Saf diet. Figure 7C shows the results of the UGT levels from 4 groups of rats at 9 weeks of age. The UGT levels of rats fed Saf-Per, Saf-DHA and Per-Per were significantly higher than that of rats fed the Saf-Saf diet. This pattern is similar to that shown in Fig. 7B. The pattern of the protein level of UGT in Figs. 7B and C is similar to the activity pattern of UGT shown in Figs. 6A and B. We found a significantly high level of UGT in the Saf-DHA among groups in Fig. 6B, but could not find the high level of UGT in the Saf-DHA among groups in Fig. 7C.

In conclusion, it is suggested that high UGT ac-



Fig. 7. Effect of Dietary Fatty Acids on the Protein Level of UGT Detected in Microsomes by Western Blotting

A, Image of Western blotting of  $F_1$  at 3 weeks of age; B, relative intensity of UGT from rats at 3 weeks of age; C,  $F_1$  at 9 weeks of age. Values represent means  $\pm$  S.D. for four rats. Statistical analysis was performed by two-way ANOVA. Asterisks in B and C indicate a statistical significant difference between Saf and Per in A, and Saf-Saf and the other 3 groups in C.

tivity in microsomes from rats fed the Per diet might mainly have depended on the protein level of UGT. However, it is possible that the lipid environment containing DHA increased the UGT activity. We could not explain the molecular mechanism of n-3 fatty acids (DHA and EPA) in promoting the protein level of UGT, whether it was a high transcription of mRNA and/or low decomposition of UGT enzyme.

# DISCUSSION

There are some studies that reported the influence of the fatty acid composition on the activity of CYP and CYP-dependent mixed function oxidase, and it was suggested that the influence of fatty acids is dispersed according to the CYP isozymes.<sup>17,18)</sup> The level of CYP1A2 in rats fed a non-fat diet was lower than that in rats fed a corn-oil diet.<sup>11)</sup> Ara of n-6 fatty acid inhibits the activity of 7-ethoxycoumarin Odeethylation by CYP1A1 and 1A2 in human liver microsomes.<sup>19)</sup> The present study showed no significant effect of Saf oil containing a high Ara diet, the n-6 fatty acid, on the level of CYP1A1 and 1A2. It is necessary to investigate the direct influence of Ara on this activity. We also showed no effect of fatty acids on the level of CYP2E1. It was reported that a low fat diet did not influence the level of protein and mRNA of CYP2E1, but cholesterol did.<sup>20)</sup> Fish oil increased the aniline hydroxylase by CYP2E1,<sup>21)</sup> and the authors of that study suggested that high activity might be caused by membrane fluidity, following the high reactivity by easily moving mixedfunction oxidase (MFO) enzymes in the membrane.

We showed a high CYP4A1 level in microsomes from rats fed the Per diet. In the case of spontaneously hypertensive rats (SHR) rats, no significant difference was found between the level of CYP4A in the rats fed a corn oil diet and a Per diet.<sup>22)</sup> However, some studies have reported the induction of the oxidation of fatty acids in microsomes and peroxisomes in rats fed PUFA by the mechanisms to promote the transcription by peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) with PUFA.<sup>23)</sup> In the present study, we showed that the influence of n-3 PUFA on the level of CYP4A1 was higher than that of n-6 PUFA. CYP4A is related to  $\omega$ -oxidation of laurate and prostaglandins, and to the hydroxylation of leukotrienes, so the up-regulation of CYP4A1 by n-3 PUFA is reasonable.

We examined the levels of protein and activity of UGT in microsomes from rats fed n-3 or n-6 fatty acids in the present study. We measured UGT activity with *p*-nitrophenol as a substrate (aglycone). In the present study, the enzyme UGT reacting with pnitrophenol might belong to UGT1A6, which is well known to react with planar phenol.<sup>24)</sup> UGT1A6 is an isotype of the UGT family. Meanwhile, the protein level of UGT by Western blotting was shown to contain total UGT. In addition, both the activity level and protein level of UGT were increased, although the increase in the activity was higher than that of the protein level. There are a few studies that reported the influence of fatty acid on UGT. One study reported increases in the level of UGT activity and the UGT protein by administration of fish oil.<sup>25)</sup> However, the present findings showed an increase (2.4-fold) in the UGT activity in rats fed the Per diet, which was higher than the increase in the protein level (1.6-fold). This suggests that high DHA resulting in high membrane fluidity in microsomes. It was reported that Ara-CoA inhibited the UGT activity,<sup>26)</sup> so it is possible that high DHA might decrease the inhibition by Ara-CoA. In the present study, we used a 3% oil concentration in the diets, and this amount is similar to the concentration of oil in normal food. We compared the values obtained with two kinds of oil, Saf and Per, without a control oil. However, the diet containing 3% Saf oil was very similar to the general diet for experimental rats; therefore, the diet containing 3% Saf is a typical control diet.

Finally, we showed that n-3 fatty acids in the diet do not increase the level of CYP1A1, 1A2 and 2E1, but they do increase CYP4A1 and UGT. The change in phase I and phase II drug-metabolizing enzymes must influence the drug level in patients, as well as the drugs effects and toxicity. So, the findings of the present study suggest that care should be taken with the food style given to patients.

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