Enhancement of hepatic drug metabolism in 3-week-old pups by maternal feeding of n-3 polyunsaturated fatty acid diets

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The rates of drug oxidation and glucuronidation in the livers of 3-week-old rats that were born to and nursed by pregnant rats fed diets containing either the n-3 or the n-6 fatty acids throughout gestation and lactation were measured. Pregnant rats were fed diets containing 10% fats composed of: (1) beef tallow and corn oil (32.5% n-6 linoleic acid, control group), (2) corn oil (62.4% n-6 linoleic acid, corn oil group), (3) perilla oil (58.3% n-3 linolenic acid, perilla oil group), and (4) fish oil (35.1% n-3 eicosapentaenoic acid and 17.3% n-3 docosahexaenoic acid, fish oil group). The rates of drug oxidation (ethoxycoumarin O-deethylation) and glucuronidation (glucuronide conjugation of hydroxycoumarin), the reactions carried out by enzymes present in microsomal membranes, were determined by employing the isolated perfused liver system. While the contents of cytochromes P-450 and b_5 were not increased, the determined rates of drug oxidation were significantly higher for the pups of n-3 and n-6 fatty acid diet groups than those of the control group. The rates of both drug oxidation and glucuronidation observed for the n-3 fatty acid diet groups (perilla and fish oil) were higher than those of the n-6 fatty acid diet groups (control and corn oil). In particular, the highest rates of drug oxidation and glucuronidation were observed with the pups of fish oil diet group. The fatty acid compositions of offspring liver microsomes were influenced by the composition present in maternal diets. The n-3 and n-6 fatty acid components in maternal diet converted to their own series of longer polyunsaturated fatty acids and were substitutively incorporated into phospholipids of liver microsomal membranes in 3-week-old rats. Thus, it appeared that the hepatic microsomal drug metabolizing enzymes operated more efficiently when the proportion of n-3 polyunsaturated fatty acid in microsomal membrane phospholipid was increased. However, when the polyunsaturated/monounsaturated/saturated (P/M/S) ratios and double bond indices (an indicator of membrane fluidity) were calculated from the fatty acid composition of 3-week-old rat liver microsomes, although the P/M/S ratios maintained constant values in all groups, the double bond indices of corn, perilla, and fish oil diet groups increased in order. Furthermore, the degree of increases of double bond indices coincided with the increments of the hepatic drug oxidation rate. Therefore, it is concluded that the higher degree of unsaturation rather than the specific kinds (n-3 or n-6) of polyunsaturated fatty acids present in liver microsomal membranes is responsible for the observed increases in the rates of drug metabolism.

Keywords: n-3 and n-6 fatty acid diets; double bond index of liver microsome lipids; drug oxidation and glucuronidation; liver perfusion

Introduction

Most lipophilic drugs, after entering into body, undergo drug metabolism and, as the result, the lipid-soluble compounds are converted into hydrophilic products for easy excretion from the body.[1] In most cases, drug

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metabolism occurs in two successive steps: oxidation involving microsomal mixed function oxidase (phase I) and formation of hydrophilic conjugates (phase II) such as glucuronide and sulfate esters.[1,2] Alternatively, some drugs bearing hydroxyl or other functional groups may undergo direct conjugation.[1,2] Among the variety of hepatic drug metabolizing enzymes, the mixed function oxidase (MFO) system involved in the initial oxidation (phase 1) and the UDP-glucuronyltransferase (UDPGT) involved in glucuronidation (phase 2) are embedded within the microsomal membranes. On the other hand, the sulfotransferase involved in sulfate ester formation is known to be localized strictly in cytoplasm.[2]

Phospholipids of the microsomal membranes are required for efficient interactions among the components of membrane embedded enzymes, and alterations in the character of membrane phospholipids could influence the rates of drug metabolism. Presence of phospholipids in hepatic endoplasmic reticulum is known to be of vital importance in maintaining the maximum activity of membrane-bound enzyme systems.[3-7] Therefore, the activities of drug metabolizing enzymes bound in microsomal membranes (e.g., MFO and UDPGT) could be modulated by the quality and quantity of phospholipids that make up the microsomal membranes and, in turn, modulate the fluidity of membrane matrix.

It has been known that fatty acid composition of microsomal membranes in the liver could be altered by modification of dietary fats.[8-11] Although complicated by other factors, increased incorporation of polyunsaturated fatty acids into the membrane lipid by dietary modification is known to be closely associated with an increase of the membrane fluidity.[12] Consequently, the modification of membrane fluidity of microsomal membranes by altering the composition of dietary fats may be of major importance in regulating the rates of interaction among the component enzymes embedded within the microsomal membranes. Thus, the fluid environment of microsomal membrane matrix may play an important role in facilitating the electron transfer from NADPH to cytochrome P-450, as well as in maintaining the microsomal MFO system in an active conformation.[13-15] In this connection, high catalytic activity of microsomal UDPGT is known to depend on the presence of phosphatidylcholine with longer chain polyunsaturated fatty acids, which in turn, has contributed to higher membrane fluidity.[12]

In this investigation, we attempted to modify the fatty acid composition of hepatic microsomal membranes by selecting corn, perilla, and fish oils as the source of maternal dietary lipids. These dietary lipids were chosen for their specific and characteristic contents of n-3 and n-6 fatty acid compositions. We fed these fatty acid diets to pregnant rats during gestation and lactation periods and then employed the livers of 3-week-old pups in our experiments to ensure that the fatty acid compositions of microsomal membrane were indeed modified. We chose to employ the livers of 3-week-old rats because this is the youngest age at which rats could maintain a stable and measurable rate of drug metabolism in our experimental system. We utilized the isolated perfused liver system (ex vivo) to determine the rates of both drug oxidation and conjugation in a comprehensive manner.

Materials and methods

Animals and diets

Pregnant female Sprague-Dawley rats were divided into four groups and fed experimental fatty acid diets composed of 10% (wt/wt) fats throughout gestation and lactation periods. The fats in the experimental diets were (1) 1:1 mixture of beef tallow and corn oil (control diet), (2) corn oil, (3) perilla oil, and (4) fish oil. The purified corn oil without added antioxidants was donated by the Doo San Grain Co. (Seoul, Korea), and the purified perilla and fish oils without added antioxidants were donated by the Pul Mu Won Foods Co., LTD. (Seoul, Korea). The compositions of experimental diets and their fatty acid contents are given in *Tables 1 and 2*, respectively. The 3-week-old offsprings obtained from dams fed each experimental fatty acid diets were used for all experiments.

Drug metabolism using isolated perfused livers

A pup was anesthetized by an intraperitoneal injection of sodium secobarbital (4 mg/100 g body weight) and the liver was isolated and placed on a perfusion block. The liver was perfused in a noncycled manner at a flow rate of 6 mL/min with a modified Krebs-Henseleit Bicarbonate (KHB) (containing 5.5 mmol/L glucose), which was saturated with 95% oxygen and 5% carbon dioxide at 37° C.

Before infusion with any drug containing KHB, the liver was infused with drug-free KHB buffer for 15 minutes to

Table 1 Composition of experimental diets given to dams (weight %)

Ingredients	Experimental groups			
	Control	Corn oil	Perilla oil	Fish oil
Carbohydrate[a]	65	65	65	65
Protein: Casein	18	18	18	18
DL-Met	0.1	0.1	0.1	0.1
Fat[b]; beef tallow	5	—	—	—
corn oil	5	10	—	—
perilla oil	—	—	10	—
fish oil	—	—	—	10
Salt mixture[c]	4	4	4	4
Vitamin mixture[d]	1	1	1	1
Carboxylmethylcellulose	2	2	2	2

[a]The carbohydrate was a mixture of 80% corn starch and 20% sucrose.

[b]*Table 2* shows the fatty acid composition of dietary fats.

[c]Salt mixture (g per 100 g salt mixture): $CaCO_3$ 29.29; $CaHPO_4 \cdot 2H_2O$ 0.43; KH_2PO_4 34.31; NaCl 25.06; $MgSO_4 \cdot 7H_2O$ 9.98; $Fe(C_6H_5O_7) \cdot 6H_2O$ 0.623; $CuSO_4 \cdot 5H_2O$ 0.156; $MnSO_4 \cdot H_2O$ 0.121; $ZnCl_2$ 0.02; KI 0.0005; $Na_2SeO_3 \cdot H_2O$ 0.0015; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0025.

[d]The vitamin mixture (mg per kg diet): thiamin chloride 5; riboflavin 5; nicotinamide 25; Ca-pantothenate 20; pyridoxine chloride 5; folic acid 0.5; biotin 0.2; vitamin B_{12} 0.03; DL-α-tocopherol acetate 100; retinyl palmitate 2.196; cholecalciferol 10(μg); choline chloride 2000; ascorbic acid 50; menadione 0.5; inositol 100.

Table 2 Fatty acids composition (%) of dietary fats and oils

Fatty acids		Control	Corn oil	Perilla oil	Fish oil
14:0[a]		2.73 ± 0.09	0.09 ± 0.09	0.02 ± 0.03	6.89 ± 3.25
14:1	n-5	0.95 ± 0.33	ND	ND	ND
16:0		24.19 ± 4.50	12.38 ± 1.19	6.85 ± 0.36	10.56 ± 4.57
16:1	n-7	2.57 ± 0.75	0.16 ± 0.14	0.12 ± 0.02	10.84 ± 1.28
18:0		6.50 ± 1.22	1.94 ± 0.45	1.93 ± 0.74	1.19 ± 0.48
18:1	n-9	26.90 ± 1.78	23.00 ± 1.00	16.07 ± 0.48	7.07 ± 1.29
18:1	n-7	3.93 ± 0.71	0.47 ± 0.41	0.95 ± 0.09	2.52 ± 0.31
18:2	n-6	32.46 ± 0.98	62.40 ± 1.29	15.57 ± 0.35	1.69 ± 0.21
18:3	n-3	ND[b]	ND	58.34 ± 1.15	1.02 ± 0.14
20:0		ND	ND	0.14 ± 0.01	ND
20:4	n-6	ND	ND	ND	1.45 ± 0.17
20:5	n-3	ND	ND	ND	35.14 ± 3.22
22:6	n-3	ND	ND	ND	17.25 ± 1.59
P/M/S[c]		0.97/1.03/1	4.33/1.64/1	8.27/1.92/1	3.03/1.10/1
DBI[d]		2.97	10.30	24.98	16.73

Fatty acid compositions are analyzed by GC-MS and data are given as mean ± SD determined from three independent analyses.
[a]Fatty acids are designated by the number of carbon atoms:number of double bond.
[b]Not detectable.
[c]Polyunsaturated/monounsaturated/saturated fatty acids.
[d]Double Bond Index:

$$\frac{\Sigma \text{ (amount unsaturated} \times \text{no. double bonds)}}{\Sigma \text{ amount saturated}}$$

establish equilibration. Depending on the purpose of experiment, the perfusion medium was switched to another KHB solution containing either 200 μmol/L ethoxycoumarin (EC) or 100 μmol/L hydroxycoumarin (HC). Perfusates were collected every 5 minutes and assayed for the amount of free HC (metabolite of EC), and HC glucuronide and sulfate conjugates as described by Cha et al.[16]

Preparation of hepatic microsomes

The hepatic microsomal fractions were prepared from 3-week-old rats by the calcium precipitation method described by Cinti et al.[17] The microsomal pellets were resuspended in 1.15% potassium chloride solution (1 mL/g liver), divided and stored in sealed Eppendorf tubes at −90° C until analysis could be made.

Assays for the contents of cytochrome P-450 and b_5

The concentrations of cytochrome P-450 and cytochrome b_5 in the prepared microsomes were determined by the method of Omura and Sato.[18] Prior to the assay, microsomal preparations were diluted with 0.1 M sodium phosphate buffer (pH 7.4) so that it would contain 1–2 mg protein/mL as determined by the method of Lowry et al.[19]

Fatty acid analysis

Fatty acids present in fats added to the experimental diets and in the prepared hepatic microsomes were extracted and methylated by the procedure of Lepage and Roy.[20] Fatty acid compositions were then determined by gas-liquid chromatography-mass spectrometry (CG-MS) using a Hewlett-Packard (Palo Alto, CA) 5988A mass spectrometer connected to a Hewlett-Packard 5890A gas chromatograph. For the gas chromatographic separation, a bonded fused-silica capillary column (SUPELCOWAX 10, Supelco, USA; 30 m × 0.32 mm ID × 0.25 μm) was used. The initial oven temperature was 150° C with a 0.5 min hold. The oven temperature of gas chromatograph was programmed to reach 235° C at a rate of 3° C/min and then to 250° C at 10° C/min. The temperatures of injection and detector ports were 260° C and 250° C, respectively. Helium was used as the carrier gas and was flowing through the column at a flow rate of 2 mL/min with a split ratio of 20:1. Methyl esters of various fatty acids were identified by comparison with fatty acid methyl ester standards purchased from Supelco (Catalog No. 4-7015) and Nu-Chek-Prep, Inc., USA (GLC-68 A) and then quantified on the basis of added amount of heptadecanoic acid internal standard purchased from Nu-Chek-Prep, Inc. (N-17-A).

Statistics

The results of fatty acid analyses were presented as means ± SD. The results of drug metabolism activities were presented as means ± SEM and analyzed by one-way analysis of variance (ANOVA). The differences in values between control and experimental groups were evaluated by Student t test.

Results

Effects of altering dietary fats on rates of hepatic drug metabolism

When a lipophilic drug like EC is infused through a perfused liver, it is first oxidized to 7 HC and then the HC is conjugated either to HC-glucuronide or HC-sulfate esters. All of these metabolites are found in perfusates. The rate of hepatic drug (EC) oxidation is

determined by measuring the total amount of HC (both free and conjugated) produced per given time then by multiplying that amount with the perfusion flow rate. As the results shown in *Figure 1* indicate, the rates of EC oxidation to HC catalyzed by the offspring hepatic microsomal mixed function oxidase system (shown by the "total" bar), were increased by feeding corn, perilla, and fish oil diets to dams. The results also showed that while the sulfate ester formation catalyzed by sulfotransferase present in hepatic cytoplasm was not increased, the glucuronide conjugation catalyzed by UDP-glucuronyl transferase embedded in microsomal membrane was increased by feeding the corn, perilla, and fish oil diets. Therefore, the results shown in *Figure 1*, obtained by infusing EC, demonstrate comprehensively the effect of feeding various fatty acid diets to dams on the rates of both the hepatic microsomal phase I drug oxidation and phase II glucuronide conjugation in pups.

Results shown in *Figure 2* were obtained by infusing HC, an oxidative metabolite of EC, directly into the isolated perfused liver system to determine the effects of feeding various fatty acid diets solely on the phase-II conjugative enzymes: one embedded in microsomal membrane (UDP-glucuronyl transferase) and another present in cytoplasm (sulfotransferase). Results showed that, while the rate of glucuronidation (microsome) was increased by the corn, perilla, and fish oil diets, the sulfate conjugation rate (cytoplasm) was not increased.

Comparing the results of *Figure 1* and *Figure 2*, it appeared that feeding experimental fatty acid diets to dams had greater enhancing effects on the phase-I oxidation than on the phase-II glucuronidation among the hepatic microsomal drug metabolizing enzymes. This has led us to examine the levels of cytochromes P-450 and b_5 present in various hepatic microsomes of offsprings.

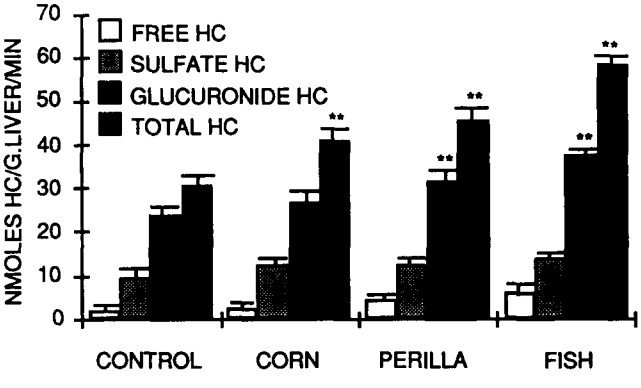

Figure 1 Rates of ethoxycoumarin oxidation and subsequent conjugation in 3-week-old rat livers. Livers were perfused with 200 μmol/L ethoxycoumarin at a rate of 6 mL/min and perfusates were collected and analyzed according to the method described by Cha et al.[16] The results are expressed as nmoles hydroxycoumarin (HC) produced/g liver/min and mean ± SEM determined from 5–10 livers for each diet group. **Significantly different from control at $P < 0.01$.

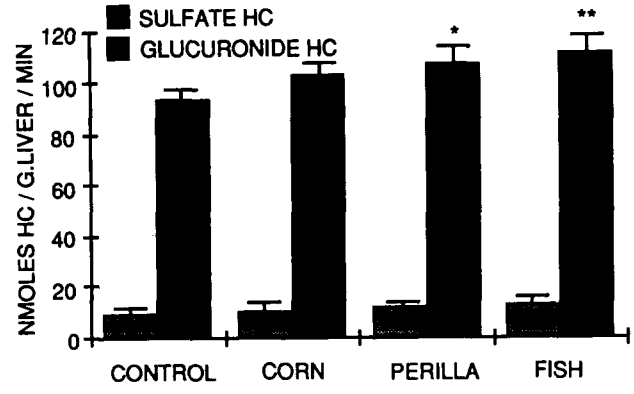

Figure 2 Rates of hydroxycoumarin conjugation in 3-week-old rat livers. The livers were perfused with 100 μmol/L hydroxycoumarin at a rate of 6 mL/min and perfusates were collected and analyzed according to the method described by Cha et al.[16] The results were expressed as nmoles hydroxycoumarin (HC) conjugated/g liver/min and mean ± SEM determined from 5–10 livers for each diet group. *Significantly different from control at $P < 0.05$. **Significantly different from control at $P < 0.01$.

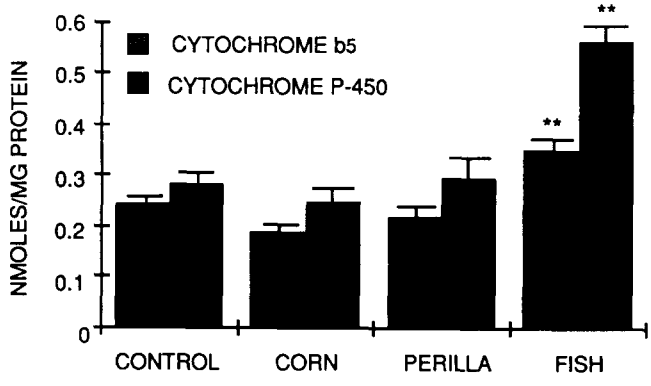

Figure 3 Contents of cytochromes P-450 and b_5 in liver microsomes of 3-week-old rats. Hepatic microsomal fractions were obtained from 3-week-old pups and the contents cytochromes P-450 and b5 were measured by the method of Omura & Sato.[18] Results are expressed in nmoles/mg protein ± SEM determined from 6–12 liver microsomes prepared from each diet group. *Significantly different from the control group at $P < 0.01$.

Effects of altering dietary fats on contents of cytochromes P-450 and b_5 in hepatic microsomes

Results shown in *Figure 3* indicate that, except for the pups obtained from dams fed fish oil diet, there were no significant changes in the amounts of cytochromes P-450 and b_5 present in the isolated hepatic microsomes. These results suggested that factors other than the amounts of cytochrome P-450 and b_5 present in microsomes may have contributed to the observed increases in the rate of hepatic drug oxidation. As the rates of hepatic microsomal mixed function oxidation may be influenced by the rate of interaction between the component enzymes (e.g., NADPH-cytochrome P-450 reductase and cytochrome P-450) within the fluid bilayer matrix of microsomal membrane, and as the fluidity of membrane matrix is modulated by the pro-

portion of unsaturated fatty acids contained, we analyzed the fatty acid components.

Analysis of fatty acid components in dietary fats given to dams and in hepatic microsomes of 3-week-old pups born and reared by the dams

Table 2 shows the results of fatty acid analysis on fats added to the various experimental diets fed to dams. The control and corn oil diets contain primarily the n-6 fatty acids and the perilla and fish oil diets contain primarily the n-3 fatty acids. The corn oil diet has twice the level of linoleic acid (18:2) when compared with that of the control diet. While the perilla oil diet has a high level of linolenic acid (18:3), the fish oil diet has high levels of eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6). Results in *Table 2* also show that the P/M/S ratios as well as the double bond indices (calculated by multiplying the number of double bonds and the amounts of their respective unsaturated fatty acids) vary widely among the experimental diets.

Table 3 shows the results of fatty acid analysis obtained from hepatic microsomes of 3-week-old rats. The results indicate that while the P/M/S ratios were not significantly altered by feeding the experimental fatty acid diets to the dams, the double bond indices were gradually increased in the order of corn, perilla, and fish oil diet-fed groups. The order of increase in the double bond indices of hepatic microsomal lipids of pups obtained from dams fed corn, perilla, and fish oil diets were similar to the increases observed for the rate of hepatic drug oxidation in respective pups.

When the results of *Table 2* (diet) and *Table 3* (microsomes) are compared, the large amount of linoleic acid (18:2) present in the corn oil diet has been converted and incorporated into the liver microsomes of pups as arachidonic acid (20:4). Similarly, the large amount of linolenic acid (18:3) present in the perilla oil diet has been converted and incorporated as EPA (20:5) and DHA (22:6). However, the large amounts of EPA (20:5) and DHA (22:6) contained in the fish oil diet has been converted to and incorporated as DHA (22:6). Thus, the dietary n-3 and n-6 fatty acids have been converted as longer series of their respective polyunsaturated fatty acids.

Discussion

The results show that the composition of fatty acid present in maternal diets played an important modulating role for the overall activities of microsomal mixed function oxidation and glucuronidation that are catalyzed by enzymes embedded in membranes of hepatic endoplasmic reticulum of 3-week-old pups.

It is well known that the fatty acid composition of dietary lipids are closely reflected in the composition of tissue membrane fatty acids.[8,21,22] Confirming this, the n-3 and n-6 fatty acid compositions of maternal diets were reflected in the fatty acid compositions of hepatic microsomal membrane of 3-week-old pups (*Tables 2 and 3*). However, results show that the maternal dietary n-3 and n-6 fatty acids given to dams were transformed and incorporated into their respective series of longer polyunsaturated n-3 and n-6 fatty acids in the liver microsomes of pups. These fatty acids are attached to the C-2 position of phospholipids,[23–25] and the phospholipids are the major constituents of lipid

Table 3 Fatty acid composition (%) of 3-week-old rat liver microsomes

Fatty acids		Control	Corn oil	Perilla oil	Fish oil
14:0[a]		0.91 ± 0.13	0.89 ± 0.41	0.94 ± 0.01	0.56 ± 0.33
16:0		20.00 ± 1.28	21.10 ± 1.92	19.12 ± 1.15	17.70 ± 1.24
16:1	n-7	ND[b]	ND	0.87 ± 0.34	1.04 ± 0.21
18:0		19.02 ± 2.84	15.55 ± 1.79	17.74 ± 0.65	18.98 ± 1.19
18:1	n-9	11.44 ± 1.30	12.74 ± 0.14	11.32 + 1.13	6.00 ± 1.41
18:1	n-7	1.70 ± 0.10	1.37 ± 0.14	1.84 ± 0.08	2.00 ± 0.39
18:2	n-6	14.85 ± 1.68	24.85 ± 2.99	11.35 ± 0.98	3.81 ± 0.39
18:3	n-3	ND	ND	9.45 ± 1.32	0.55 ± 0.96
20:4	n-6	31.53 ± 1.76	23.50 ± 1.86	8.09 ± 0.57	14.93 ± 0.70
20:5	n-3	ND	ND	10.32 ± 1.06	8.82 ± 1.81
22:6	n-3	ND	ND	8.59 ± 0.58	25.23 ± 4.05
24:0		0.59 ± 0.51	ND	0.37 ± 0.64	0.37 ± 0.64
P/M/S[c]		1.14/0.32/1	1.29/0.38/1	1.25/0.37/1	1.42/0.24/1
DBI[d]		4.17	4.20	5.25	7.27

Hepatic microsomal fractions were obtained from 3-week-old pups by feeding experimental diets to their dams. Lipids were extracted from the microsomal fractions and processed by the method of Lepage and Roy.[20] Fatty acid compositions are analysed by GC-MS and data are given as mean ± SD determined from three independent analyses.

[a]Fatty acids are designated by the number of carbon atoms:number of double bond.

[b]Not detectable.

[c]Polyunsaturated/monounsaturated/saturated fatty acid.

[d]Double Bond Index:

$$\frac{\Sigma \text{ (amount unsaturated} \times \text{no. double bonds)}}{\Sigma \text{ amount saturated}}$$

bilayer membranes and their fluidity is known to depend on the compositions of these fatty acids.[12]

Membrane fluidity refers to the physical state of membrane bilayer, and it is generally affected by the physical properties of the C-2 fatty acyl side chains.[23,26] While many complex factors are involved, increasing the degree of unsaturation and the chain length of the fatty acyl side chain have been observed to enhance the membrane fluidity.[26,27] The membrane fluidity can be obtained indirectly by chemical analysis of its fatty acid compositions (*Table 3*). Increased contents of polyunsaturated fatty acids in membrane are known to be causatively associated with increased membrane fluidity and, in this connection, an increased P/M/S ratio used as an indicator of increased membrane fluidity. However, the calculated P/M/S ratio based on the results shown in *Table 3* indicated that the P/M/S ratio of liver microsomal membranes of pups did not differ among the experimental diet groups. Thus, in an attempt to better correlate the changes of fatty acid composition with the changes of membrane fluidity, Farias et al. took the degree of unsaturation of fatty acid components into consideration and obtained the Double Bond Index (DBI).[28] Taking this idea further, Catsuma & Brenner proved that increments of DBI were closely related to the increases of membrane fluidity and the activity of microsomal UDP-glucuronyltransferase.[12] Based on this idea, the increases in calculated DBI for the liver microsomal membranes of experimental animals (*Table 3*) may be because their membrane fluidity increased. The membranes with increased fluidity may then provide a more suitable environment for efficient interactions between component enzymes of MFO and glucuronidation systems, as well as the cooperative interaction between the two microsomal drug-metabolizing enzyme systems (*Figures 1 and 2*).[6]

In an attempt to observe the effects of altering the microsomal membrane fluidity on rates of interaction between the component enzymes in a comprehensive manner, we determined the rates of MFO and glucuronidation, together and separately, by infusing EC (prodrug) and HC (metabolite of EC), respectively, to the isolated livers of 3-week-old rats. Results of corn and perilla oil diet groups shown in *Figure 1*, obtained by infusing EC, confirmed that livers with increased DBI (or increased membrane fluidity) had greater MFO and glucuronidation activity (*Figure 1*) without any changes in the specific contents of cytochrome P-450 (*Figure 3*). These results demonstrate that the rate of electron flow from NADPH via NADPH-cytochrome P-450 reductase to cytochrome P-450 within the microsomal membranes was enhanced in livers with higher DBI, and this supports the hypothesis put forward by Yang et al.[14,15] In addition, the results shown in *Figure 2*, obtained by infusing HC to determine the rate of glucuronidation, demonstrate that livers with increased DBI have increased glucuronidation activity as well. Our results on glucuronidation obtained with isolated perfused liver (ex vivo) support the observations of Singh et al.[6] and Mounié et al.,[29] which were obtained using isolated microsomes (in vitro). Comparing the enhanced rates of MFO (*Figure 1*) and glucuronidation (*Figure 2*) achieved through modifications of microsomal fatty acid compositions, the increased rate of oxidation of EC was more marked than that of the glucuronidation of HC.

The corn oil group, obtained from dams supplied with large amounts of linoleic acid as the only n-6 fatty acid source in the diet, had large amounts of both linoleic and arachidonic acids in its hepatic microsomes. On the other hand, the perilla oil group, which was fed linolenic acid as the sole source of n-3 fatty acid, converted the dietary linolenic acid to EPA and DHA, and incorporated these fatty acids into its hepatic microsomes. Comparing the rates of hepatic drug metabolism of pups obtained from these two dietary groups, the perilla oil group had a slightly increased rate of drug metabolism in the absence of any increase in its microsomal cytochrome P-450 content. The fish oil group, which was supplied with EPA and DHA as the main source of dietary n-3 fatty acids, had high amounts of DHA in its hepatic microsomes. Confirming the results of Mounié et al.,[29] the fish oil group had increased concentrations of cytochrome P-450 and cytochrome b_5 in its hepatic microsomes and had higher rates of mixed function oxidation and glucuronidation, as measured by the isolated perfused liver system.

While the increased rate of hepatic drug metabolism in the fish oil group could be explained by the increased levels of cytochromes P-450 and b_5, it could also be explained by several other factors. Increased DBI in liver microsomes of the fish oil group may have provided a more fluid environment to support the higher activity of drug-metabolizing enzymes. According to Wade et al.,[30] membranes containing large amounts of arachidonic and docosahexaenoic acid could support a higher rate of synthesis or maintain higher activities of hepatic drug metabolizing enzymes. In support of this, studies of Wills[31] and Castuma and Brenner[12] stated that the longer chain polyunsaturated fatty acid components of membrane phospholipids are important in holding the membrane-bound enzymes in their more active forms within the membrane, and thus the enzymes could metabolize drugs more efficiently.

The hepatic microsomal membrane fatty acids of control and corn oil groups consisted of n-6 linoleic and arachidonic acids and those of the perilla and fish oil groups were characterized by the high contents of n-3 fatty acids like EPA and DHA. Although the hepatic microsomal membranes containing only the n-6 polyunsaturated fatty acids (control and corn oil group) could support the oxidation and glucuronidation with moderate efficiencies, the membrane with n-3 polyunsaturated fatty acid carried out the drug metabolism with greater efficiency (*Figures 1 and 2*). Thus, it appears that both the n-3 and n-6 fatty acid membrane matrices could support the hepatic microsomal drug metabolism. In this connection, Wade et al.[30] and Wills[31] stated that the n-3 and n-6 polyunsaturated fatty acids could be interchanged when either is in short supply for supporting the microsomal drug metabo-

lism. Furthermore, rats fed fat-free diets could still carry out drug metabolism, although at a reduced rate.[21]

Therefore, in conclusion, the degree of unsaturation of fatty acids, rather than the specific species (n-3 and n-6) of polyunsaturated fatty acids, making up the phospholipids membrane matrix contribute more to the enhanced rate of hepatic microsomal drug metabolism. The highest rate of drug metabolism observed for the fish oil group (n-3 fatty acids) is most likely due to the high content of longer chain polyunsaturated fatty acids.

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