Rate of alteration of hepatic mixed-function oxidase system in rats fed different dietary fats

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Received August 5, 1993

AMMOUCHE, A., DINH, L., YOUYOU, A., CLÉMENT, M., and BOURRE, J.M. 1993. Rate of alteration of hepatic mixed-function oxidase system in rats fed different dietary fats. Biochem. Cell Biol. **71**: 530–537.

Studies were carried out to evaluate and relate the rate of alteration in mixed-function oxidase system with the changes of the fatty acid composition of rat microsomes induced by different dietary lipids. Male weanling rats were fed from day 21 to 120 with a commercial rat diet or a semisynthetic diet containing no fat or 10% fat consisting of peanut–rapeseed oil, sunflower oil, or salmon oil. In rats fed a fat-free diet, the cytochrome *P*-450 concentration and aniline hydroxylase, aminopyrine *N*-demethylase, and NADPH–cytochrome-*c* reductase activities of liver microsomes at 120 days were, respectively, 26, 16, 10, and 24% lesser than those of rats fed the control diet. However, cytochrome b_5 concentration and NADH–cytochrome-b_5 reductase activity were, respectively, 33 and 43% higher than those of the control group at the same time. When rats were fed the sunflower oil diet, the cytochrome *P*-450 concentration and NADH–cytochrome-b_5 reductase activity at 120 days were, respectively, 11 and 23% lesser than those of control group. But the cytochrome b_5 concentration was 10% higher than that of the control group. In rats fed the fish oil diet, the cytochrome *P*-450 concentration and NADPH–cytochrome-*c* reductase, aniline hydroxylase, and aminopyrine *N*-demethylase activities at 120 days were, respectively, 30, 48, 41, and 31% higher than those of rats fed the control diet. These enzymes were correlated very well ($0.84 < r < 0.93$), $P < 0.05$ with dietary Σ polyunsaturated fatty acids ($n-3$). The fatty acid composition of liver microsomes of rats fed a fat-free diet revealed high levels of 16:1($n-7$), 18:1($n-9$), and 20:3($n-9$) acids. A dramatic decrease in 18:2($n-6$) and 20:4($n-6$) acids was observed. Animals fed a sunflower oil diet showed high levels of 22:4($n-6$) and 22:5($n-6$), and low levels in monounsaturated fatty acids. However when rats were fed a fish oil diet, the liver microsomes were highly enriched in 20:5($n-3$) and 22:6($n-3$) acids and simultaneously there was a decrease in arachidonic acid. From these findings it is concluded that dietary fats induce changes not only in the fatty acid composition of liver microsome lipids, but also in the activities of mixed-function oxidase system. Also, the results suggest that essential fatty acids might play a role in regulating the intrinsic membrane protein activities.

Key words: cytochrome *P*-450, cytochrome b_5, NADH–cytochrome-b_5 reductase, NADPH–cytochrome-*c* reductase, aminopyrine *N*-demethylase, aniline hydroxylase, microsomes, rat liver.

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Dans cette étude, nous avons évalué et établi la relation entre le taux de changement du système d'oxydase à fonction mixte et les modifications de la composition en acides gras des microsomes de rat induites par différents lipides alimentaires. Des rats mâles à peine sevrés ont été nourris du 21^e au 120^e jour avec une diète commerciale pour rat ou avec une diète semi-synthétique sans graisse ou contenant seulement 10% de lipides provenant d'huile d'arachide et de colza, d'huile de tournesol ou d'huile de saumon. Chez les rats nourris avec la diète sans graisse, la concentration du cytochrome *P*-450 et les activités de l'aniline hydroxylase, de l'aminopyrine *N*-déméthylase et de la NADPH–cytochrome-*c* réductase dans les microsomes de foie au 120^e jour sont respectivement 26, 16, 10 et 24% moindres que celles des rats nourris avec la diète de référence. Cependant, la concentration du cytochrome b_5 et l'activité de la NADH–cytochrome-

ABBREVIATIONS: ANOVA, analysis of variance; EFA, essential fatty acid(s); PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; DBI, double bond index.

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Printed in Canada / Imprimé au Canada

b_5 réductase sont respectivement 33 et 43% plus élevées que celles du groupe témoin au même moment. Lorsque des rats sont nourris avec la diète contenant l'huile de tournesol, la concentration du cytochrome *P*-450 et l'activité de la NADH-cytochrome-b_5 réductase au 120^e jour sont respectivement 11 et 23% inférieures à celles du groupe témoin, mais la concentration du cytochrome b_5 est 10% plus élevée que celle du groupe témoin. Chez les rats nourris avec la diète contenant l'huile de poisson, la concentration du cytochrome *P*-450 et les activités de la NADPH-cytochrome-*c* réductase, de l'aniline hydroxylase et de l'aminopyrine *N*-déméthylase au 120^e jour sont respectivement 30, 48, 41 et 31% plus élevées que celles des rats nourris avec la diète de référence. La corrélation entre l'activité de ces enzymes et la quantité totale d'acides gras polyinsaturés ($n - 3$) dans la diète est très bonne ($0{,}84 < r < 0{,}93$), $P < 0{,}05$. L'étude de la composition en acides gras des microsomes de foie de rats nourris avec la diète sans graisse indique des taux élevés d'acides gras 16:1($n - 7$), 18:1($n - 9$) et 20:3($n - 9$). Une diminution prononcée des acides gras 18:2($n - 6$) et 20:4($n - 6$) est notée. Les animaux nourris avec la diète contenant l'huile de tournesol ont des taux élevés d'acides gras 22:4($n - 6$) et 22:5($n - 6$) et une faible concentration d'acides gras monoinsaturés. Cependant, lorsque des rats sont nourris avec la diète contenant de l'huile de poisson, les microsomes de foie sont très enrichis en acides gras 20:5($n - 3$) et 22:6($n - 3$) et, en même temps, leur contenu en acide arachidonique est diminué. D'après ces résultats, nous concluons que les lipides alimentaires induisent des changements non seulement dans la composition en acides gras des lipides des microsomes du foie, mais également dans les activités des enzymes du système d'oxydase à fonction mixte. De plus, ces résultats suggèrent que les acides gras essentiels pourraient jouer un rôle dans la régulation des activités des protéines membranaires intrinsèques.

Mots clés : cytochrome *P*-450, cytochrome b_5, NADH-cytochrome-b_5 réductase, NADPH-cytochrome-*c* réductase, aminopyrine *N*-déméthylase, aniline hydroxylase, microsomes, foie de rat.

[Traduit par la rédaction]

Introduction

During recent years much interest has been focused on the function of microsomal mixed-function oxidase system of mammalian liver owing to its important function in the metabolism of various xenobiotics and endogenous substrates and also to its key role in the biotransformation of physiologically important compounds such as fatty acids, prostaglandins, leukotrienes, and steroids (Estabrook et al. 1972; White and Coon 1980). The mixed-function oxidases are membrane bound and phosphatidylcholine, as one of the structural components of microsomal membranes, is necessary for proper function of the system (Lu 1976). It is well known that rats require not only dietary protein, but also lipid substances for normal synthesis of microsomal cytochrome *P*-450 and hydroxylating enzymes (Marshall and McLean 1971). The physiological role of the hepatic microsomal mixed-function oxidase system has been shown to be modified by nutritional factors and lipids are one of the important factors (Campbell 1977; Miller 1976; Antal et al. 1982). Variations in dietary lipids with different fatty acids produce corresponding variations in the fatty acid composition of phospholipids of the microsomal membranes (Laitinen et al. 1975) and, thus, alter the membrane conformation and fluidity, resulting in changes in the activity of mixed-function oxidase (Wade and Norred 1976). Several reports suggest that deprivation of dietary lipid results in changes in relative content of microsomal phospholipid fatty acids and associated with these changes are decreased metabolism of various xenobiotics, decreased content of hepatic microsomal cytochrome *P*-450, and decreased binding of substrates to cytochrome *P*-450 (Baker et al. 1983; Wills 1980). Although dietary fat is known to alter the structural lipids of various cell membranes and, in turn, to induce changes in membrane function, the exact mechanisms by which these effects are brought about are not clear. However, membrane fatty acid composition, cholesterol-to-phospholipid ratio, and optimal bilayer thickness (McMurchie and Raison 1979; Sinensky et al. 1979) are considered important factors in bringing about these effects. Purposeful manipulation of these enzymes on other hand may have far-reaching consequences in enhancing or preventing chemical carcinogenesis, in modifying chronic medication, or in treating toxicities. If, indeed, as recently postulated, 80–90% of human cancer is due to environmental chemicals and chemical carcinogens require metabolic activation, the question is posed whether nutritional manipulation may modify carcinogenic susceptibility. Similarly, nutritional status could also significantly affect drug pharmacologic response and environmental chemical insult. But the significance of these changes in terms of the time course of alteration has not received much attention. For this reason, the present study was initiated to determine the rate of alteration of oxidative enzyme activities and changes of fatty acid composition in liver microsomes of rats fed various dietary fats at different ages.

Materials and methods

Animals and diets

Experiments were performed on male weanling rats of the Sprague–Dawley strain weighing 40 ± 5 g purchased from Iffa Credo, l'Arbresle, France. Rats were divided in five groups of 30 animals each and maintained under standardized conditions of temperature (22 ± 1°C) and relative humidity (70%) with a 12-h-light:12-h-dark cycle. The animals were fed from day 21 to 120 with a commercial rat diet or a semisynthetic diet containing no fat or 10% fat consisting of peanut–rapeseed oil, sunflower oil, or salmon oil. Fish oil was supplemented with 100 mg α-tocopherol/100 g oil. Diets were stored in the dark at 4°C. The composition of diets and the fatty acid composition of commercial dietary fats are presented in Tables 1 and 2.

Preparation of microsomes

At various ages (21, 40, 60, 90, and 120 days), animals were killed by decapitation without anaesthesia. Liver was quickly excised and washed with buffer solution (0.1 M Tris–HCl, 0.32 M sucrose, 0.9% NaCl, pH 7.4), suspended in the same buffer solution at 25% (w/v), and homogenized in a Potter homogenizer. The homogenates were centrifuged for 5 min at 8000 × *g* and the supernatants were centrifuged for 30 min at 16 000 × *g*. The resulting supernatants were centrifuged for 60 min at 105 000 × *g* and microsomal pellets were resuspended in the same buffer to obtain 2 mg protein/mL. Protein content was estimated by the procedure of Lowry et al. (1951), with bovine serum albumin as a standard.

Enzyme assays

Microsomal cytochromes *P*-450 and b_5 concentrations were measured by the method of Omura and Sato (1964). The concentration of cytochrome *P*-450 was calculated from the CO difference spectrum of dithionite-reduced samples using an extinction coeffi-

TABLE 1. Composition (g/100 g) of the experimental diets

Ingredients	Diets				
	Control	Fat free	Peanut–rapeseed oil	Sunflower oil	Fish oil
Casein	23	22.5	22.5	22.5	22.5
Glucose	58	63.5	53.5	53.5	53.5
Lipids	5	<0.1	—	—	—
Cellulose	6	6	6	6	6
Mineral mixture[a]	7	7	7	7	7
Vitamin mixture[b]	1	1	1	1	1
Peanut oil	—	—	4.8	—	—
Rapeseed oil	—	—	5.2	—	—
Sunflower oil	—	—	—	10	—
Salmon oil	—	—	—	—	10

[a]Mineral composition of the mixture (mg/100 g): K_2HPO_4, 20.0; $CaCO_3$, 34.6; $CaHPO_4 \cdot 2H_2O$, 26.55; NaCl, 13.70; $MgSO_4 \cdot 7H_2O$, 3.42; $CuSO_4 \cdot 5H_2O$, 0.042; $MnSO_4 \cdot H_2O$, 0.27; $FeSO_4 \cdot 7H_2O$, 1.02; $ZnSO_4 \cdot H_2O$, 0.15; $CoCO_3$, 0.0008; KI, 0.0016.

[b]Composition of vitamin supplement per kilogram (triturated in dextrose) (United States Biochemical Corp., Cleveland, Ohio): thiamin–HCl, 10 mg; inositol, 170 mg; cyanocobalamin, 0.05 mg; menadione, 40 mg; α-tocopherol, 170 IU; choline chloride, 1360 IU (ergocalciferol).

TABLE 2. Fatty acid composition (%) of the experimental diet

Fatty acids	Diets			
	Control	Peanut–rapeseed oil	Sunflower oil	Fish oil
14:0	1.08	1.06	0.22	7.48
16:0	20.70	7.32	7.21	15.90
17:0	—	0.06	—	0.88
18:0	12.33	3.32	7.42	2.76
20:0	0.49	0.84	0.44	—
22:0	—	1.51	1.00	3.32
Σ SFA	34.80	14.11	16.93	29.80
16:1($n-9$)	0.27	0.21	—	11.33
16:1($n-7$)	1.34	—	0.10	1.18
18:1($n-9$)	42.36	52.22	26.14	15.90
18:1($n-7$)	2.29	—	—	4.43
20:1($n-9$)	0.92	3.12	0.22	8.80
Σ MUFA	47.18	55.18	26.40	41.46
18:2($n-6$)	16.52	27.10	55.75	3.15
18:3($n-6$)	0.15	0.02	0.20	—
20:4($n-6$)	—	—	—	0.65
Σ PUFA($n-6$)	16.67	27.12	56.55	3.79
18:3($n-3$)	1.29	3.00	0.10	0.65
18:4($n-3$)	—	—	—	1.91
20:5($n-3$)	—	—	—	10.16
22:5($n-3$)	—	—	—	2.41
22:6($n-3$)	—	0.55	—	9.63
Σ PUFA($n-3$)	1.29	3.55	0.10	24.76
($n-6$)/($n-3$)	12.92	7.63	565.50	0.13

NOTE: Values represent the average obtained from at least three separate extractions and analyses of the respective diets and are relative amounts, expressed as a percentage of the total identified fatty acids.

cient 91 $mM^{-1} \cdot cm^{-1}$ for the absorption difference between 450 and 490 nm.

Cytochrome b_5 concentration was estimated from the difference spectrum between dithionite-reduced and oxidized samples. An extinction coefficient between 424 and 409 nm of 185 $mM^{-1} \cdot cm^{-1}$ was used to calculate the concentration of this molecule. NADPH–cytochrome-*c* reductase activity was assayed by the method of Omura and Takesu (1970), by monitoring the rate of reduction of cytochrome *c* (50 μM) at 550 nm in a 1-mL volume containing 120 mM potassium phosphate, 0.8 mM potassium cyanide buffer, a suitable amount of microsomal protein (about 0.2 mg), and 0.1 mM NADPH.

NADH–cytochrome-b_5 reductase activity was measured by the method of Hrycay and Prough (1974), with some modifications, employing potassium ferricyanide (1 mM) as the artificial electron acceptor and measuring its rate of reduction at 420 nm in 1 mL of reaction medium containing 1.2 mM potassium buffer, 0.1 mM NADH, and about 10 μg enzyme. Assays were performed at 30°C.

Aminopyrine *N*-demethylase activity was determined by measuring the production of formaldehyde produced using the Nash reagent as described by Matsubara et al. (1977).

Aniline hydroxylase activity was determined by following the formation of *p*-aminophenol from aniline, according to the method of Imai et al. (1966), as modified by Matsubara and Tochino (1975).

TABLE 3. Fatty acid composition (%) of liver microsomal lipids of rats fed various dietary lipids for a 3-month period

Fatty acids	Diets				
	Control	Fat free	Peanut–rapeseed oil	Sunflower oil	Fish oil
14:0	0.50 ± 0.01	0.44 ± 0.09	0.32 ± 0.02	0.60 ± 0.05	0.55 ± 0.07
16:0	23.10 ± 0.66	20.85 ± 0.11	21.37 ± 0.30	18.00 ± 0.20	24.93 ± 0.15
16:1($n-9$)	0.40 ± 0.06^a	2.33 ± 0.28^b	0.64 ± 0.08^a	0.90 ± 0.03^c	0.31 ± 0.01^a
16:1($n-7$)	3.12 ± 0.43^a	5.68 ± 0.55^b	1.62 ± 0.11^c	1.94 ± 0.10^d	2.41 ± 0.07^a
17:0	0.32 ± 0.05^a	0.32 ± 0.09^a	0.70 ± 0.10^b	0.62 ± 0.02^c	0.50 ± 0.04^a
18:0	18.60 ± 0.17	16.10 ± 0.10	18.94 ± 0.25	20.55 ± 0.17	19.44 ± 0.70
18:1($n-9$)	13.20 ± 0.24^a	20.50 ± 0.91^b	13.10 ± 0.20^a	4.87 ± 0.12^c	10.54 ± 0.40^d
18:1($n-7$)	4.77 ± 0.18^a	8.70 ± 0.34^b	3.51 ± 0.14^a	4.60 ± 0.08^a	2.47 ± 0.11^c
18:2($n-6$)	8.30 ± 0.50^a	1.04 ± 0.06^b	11.30 ± 0.80^c	10.02 ± 0.50^{ac}	2.90 ± 0.05^{d,b}
18:3($n-6$)	—	—	0.30 ± 0.01	0.44 ± 0.04	0.15 ± 0.01
20:0	0.23 ± 0.01	0.37 ± 0.02	tr	0.50 ± 0.05	tr
20:1($n-9$)	0.30 ± 0.05	0.33 ± 0.07	0.45 ± 0.02	0.21 ± 0.03	0.34 ± 0.05
20:1($n-7$)	0.10 ± 0.02^a	0.40 ± 0.01^b	0.22 ± 0.01^c	0.10 ± 0.01^a	0.32 ± 0.01^d
20:3($n-9$)	—	14.50 ± 0.20	—	0.40 ± 0.02	—
20:3($n-6$)	1.00 ± 0.02	0.30 ± 0.02	1.20 ± 0.04	1.77 ± 0.05	0.90 ± 0.01
20:4($n-6$)	19.40 ± 0.13^a	5.94 ± 0.10^b	20.15 ± 0.50^a	28.65 ± 1.02^c	12.91 ± 0.12^d
20:5($n-3$)	—	—	0.90 ± 0.01	—	6.90 ± 0.02
22:0	0.40 ± 0.01	0.28 ± 0.02	0.33 ± 0.10	0.20 ± 0.02	0.18 ± 0.01
22:1($n-9$)	0.10 ± 0.02	0.21 ± 0.05	0.10 ± 0.02	0.25 ± 0.04	0.20 ± 0.02
22:4($n-6$)	0.32 ± 0.02	—	0.34 ± 0.03	0.60 ± 0.05	0.30 ± 0.02
22:5($n-6$)	0.80 ± 0.01^a	1.10 ± 0.01^b	0.40 ± 0.02^c	0.95 ± 0.01^a	0.35 ± 0.01^{c,d}
22:5($n-3$)	0.53 ± 0.01	—	0.62 ± 0.01	0.44 ± 0.02	1.90 ± 0.02
22:6($n-3$)	5.00 ± 0.01^a	1.08 ± 0.03^b	5.11 ± 0.03^a	3.12 ± 0.25^c	12.02 ± 0.01^d
24:0	0.76 ± 0.02	0.60 ± 0.01	—	0.45 ± 0.04	1.30 ± 0.10
24:1($n-9$)	0.38 ± 0.05	0.50 ± 0.11	0.20 ± 0.02	tr	0.12 ± 0.03

NOTE: Results are expressed as the percentage of total fatty acids. Values (means ± SD, $n = 6$) not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript letter appears, values are not significantly different. tr, trace.

Lipid analysis

Lipids were extracted from aliquots of microsomes from each group according to Folch et al. (1975). Lipids were transesterified by treatment with methanol – cyclohexane – acetyl chloride for 60 min at 100°C according to the method of Lepage and Roy (1986). The methyl esters were analyzed on a Delsi gas chromatograph equipped with a flame ionization detector and a silica capillary column (length, 30 m; internal diameter, 0.32 mm; stationary phase, Omegawax Supelco, France). Helium was used as the carrier gas. The oven, injector, and detector temperatures were maintained, respectively, at 200, 230, and 250°C. Identification of fatty acids was performed by comparison with authentic commercial standards and with mixtures of known fatty acids composition. Areas were calculated with Merck-Hitachi 2 500 integrator and fatty acid concentrations were reported as a percentage of total fatty acid content. The double bond index was calculated from the sum of the percentages of each unsaturated fatty acid times the number of double bonds in that acid.

Statistical analysis

All data are presented as means ± SD. The effects of different dietary fats were examined by ANOVA and the effect of individual diets was compared for statistical significance ($P < 0.05$) using the unpaired Student's t-test.

Results

Lipid composition of microsomal membranes

The fatty acid compositions of the various dietary fats used in the experiment are shown in Table 2. The major unsaturated fatty acids in the control diet and peanut–rapeseed oil diet were linoleic 18:2($n-6$), oleic 18:1($n-9$), and α-linolenic 18:3($n-3$) acids. The sunflower oil diet was particularly rich in linoleic acid and very low in ($n-3$) fatty acids. In the fish oil diet, the major unsaturated fatty acids were eicosapentaenoic 20:5($n-3$), palmitoleic 16:1($n-7$), oleic 18:1($n-9$), and docosahexaenoic 22:6($n-3$) acids. The fatty acid composition of liver microsomes was quite different among the various groups, reflecting the type of dietary fat given (Tables 3 and 4). The fat-free diet included in the liver microsomal membranes an increase in oleic 18:1($n-9$), palmitoleic 16:1($n-7$), and 5,8,11-eicosatrienoic 20:3($n-9$) acids. A concomitant decrease in linoleic 18:2($n-6$), arachidonic 20:4($n-6$), and docosahexaenoic 22:6($n-3$) acids was observed, as compared with the control group. Higher ratios of 20:3($n-9$)/20:4($n-6$), 18:1($n-9$)/(18:0) (which are commonly used as an index of EFA deficiency), and 20:3($n-6$)/18:2($n-6$) indicate a Δ9- and Δ6-desaturase stimulation (Holman 1968). In animals fed the peanut–rapeseed oil diet, the main result was a marked increase in arachidonic acid in liver microsomal membranes. The double bond/saturated fatty acid ratio was not significantly different from the control group. When the animals were fed the sunflower oil diet, the proportion of ($n-6$) series fatty acids was increased, particularly 22:4($n-6$) and 22:5($n-6$), and that of 22:6($n-3$) was decreased, resulting in an increase of ($n-6$)/($n-3$) molar ratio (11.91 versus 5.39 in microsomes of rats fed standard diet). The total amounts of the saturated fatty acids and of the double bond index/saturated fatty acids ratios were not altered. In animals fed the fish oil diet, there was a general increase of the content of ($n-3$) fatty acids in liver microsomes compared with the control group. Linoleic and arachidonic

TABLE 4. Indices of lipid differences (%) in liver microsomal membranes from rats fed different dietary lipids

Indices	Diets				
	Control	Fat free	Peanut–rapeseed oil	Sunflower oil	Fish oil
Σ SFA	42.75 ± 0.90	38.08 ± 0.41	41.33 ± 0.67	40.72 ± 0.53	45.42 ± 0.96
Σ MUFA	21.89 ± 0.98^{a}	37.94 ± 2.16^{b}	19.54 ± 0.56^{a}	12.87 ± 0.40^{c}	16.39 ± 0.65^{c,d}
Σ ($n-6$)	29.82 ± 0.23^{a}	8.38 ± 0.19^{b}	33.39 ± 0.67^{c}	42.43 ± 1.67^{d}	17.36 ± 0.21^{e}
Σ ($n-3$)	5.53 ± 0.02^{a}	1.08 ± 0.03^{b}	5.73 ± 0.04^{c}	3.56 ± 0.27^{c,d}	20.82 ± 0.05^{e}
Σ PUFA	35.35 ± 0.25^{a}	23.96 ± 0.24^{b}	39.12 ± 0.71^{a}	46.39 ± 0.96^{c}	38.18 ± 0.26^{a}
($n-6$)/($n-3$)	5.39 ± 0.02^{a}	7.75 ± 0.02^{a}	5.82 ± 0.01^{a}	11.91 ± 1.52^{b}	0.83 ± 0.03^{c}
DBI/SFA	3.71 ± 0.01	3.20 ± 0.10	4.02 ± 0.03	4.49 ± 0.18	4.30 ± 0.02
18:1/18:0	0.70 ± 0.03^{a}	1.27 ± 0.01^{b}	0.69 ± 0.03^{a}	0.23 ± 0.05^{c}	0.54 ± 0.01^{a}
20:3($n-9$)/20:4($n-6$)	—	2.44 ± 0.02	—	—	—
20:3($n-6$)/18:2($n-6$)	0.12 ± 0.02^{a}	0.32 ± 0.01^{b}	0.11 ± 0.04^{a,c}	0.17 ± 0.02^{c}	0.31 ± 0.05^{d,b}
20:4($n-6$)/20:3($n-6$)	19.40 ± 0.52	19.66 ± 1.12	16.75 ± 1.35	16.18 ± 1.02	14.33 ± 2.30

NOTE: See legend to Table 3. Values calculated from Table 3.

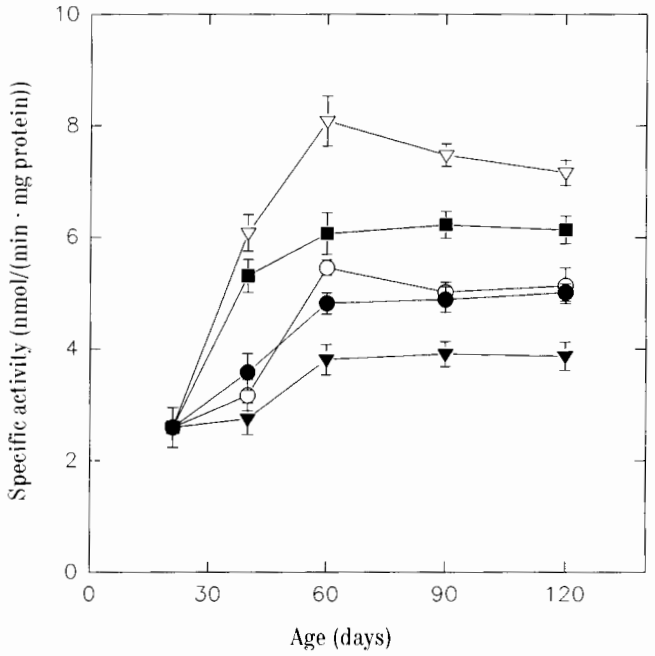

FIG. 1. Specific activity of NADH–cytochrome-b_5 reductase in liver microsomes of rats fed various dietary fats at different ages. ●, control diet; ▽, fat-free diet; ○, peanut–rapeseed oil; ▼, sunflower oil diet; ■, fish oil diet.

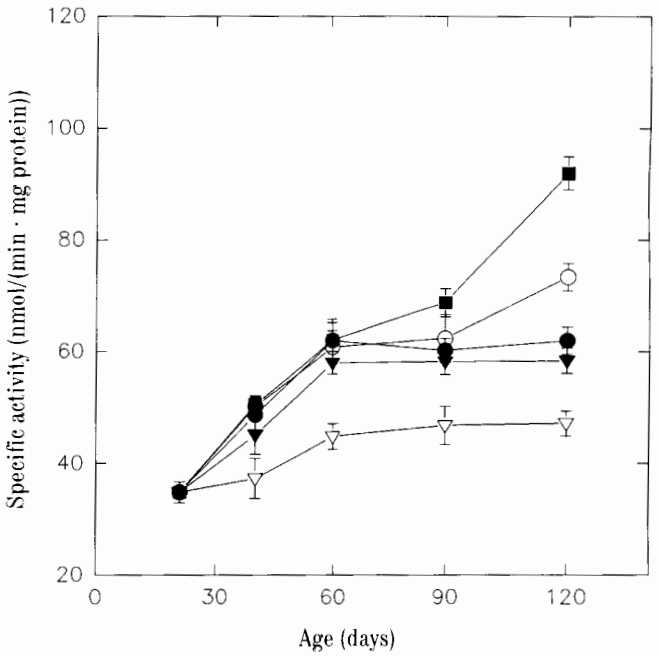

FIG. 2. Specific activity of NADPH–cytochrome-*c* reductase in liver microsomes of rats fed various dietary fats at different ages. Symbols are the same as those in Fig. 1.

acids, on other hand, decreased, markedly. The high levels of docosahexaenoic and eicosapentaenoic acids evidently depress the elongation of the ($n-6$) series of fatty acids (Brenner 1984). Lower ratios of 20:3($n-6$)/18:2($n-6$) and 20:4($n-6$)/20:3($n-6$) could indicate that Δ6- and Δ5-desaturase enzymes were inhibited. The double bond index/saturated fatty acid ratio was similar to that of the control animals, but the ($n-6$)/($n-3$) ratio naturally decreased profoundly.

Oxidative enzyme activities

The components and microsomal activities of the mixed-function oxidase system in rats at different ages are shown in Figs. 1–6. It was noticed that in rats fed a fat-free diet, the mixed-function oxidase activities increased throughout development from 21 to 60 days of age and then decreased slightly to a plateau. The cytochrome *P*-450 concentration and aniline hydroxylase, aminopyrine *N*-demethylase and NADPH–cytochrome-*c* reductase activities of liver microsomes at 120 days were, respectively, 26, 16, 10, and 24% lesser than those of rats fed the control diet. However, cytochrome b_5 concentration and NADH–cytochrome-b_5 reductase activity were significantly increased, respectively 33 and 43% higher than those of the control group at the same time. When rats were fed the sunflower oil diet, the cytochrome *P*-450 concentration and NADH–cytochrome-b_5 reductase activity at 120 days were, respectively, 11 and 23% lesser than those of the control group. The aminopyrine *N*-demethylase and aniline hydroxylase activities varied in a similar manner and also tended to be lesser than those of the control group, but the differences were not significant. The cytochrome b_5 concentration was 10% higher than that of the control group. In rats fed a peanut–rapeseed oil diet, slight differences were found compared with control group in the mixed-function oxidase. In rats fed a salmon oil diet,

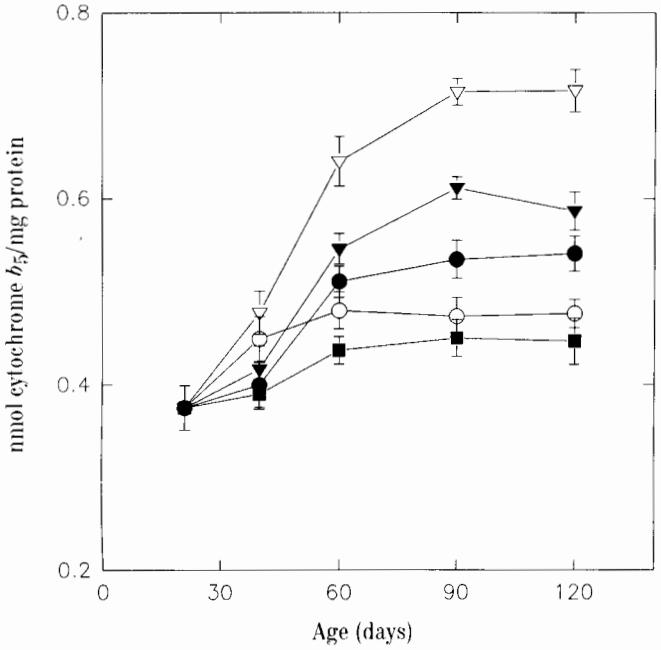

FIG. 3. Cytochrome b_5 concentration in liver microsomes of rats fed various dietary fats at different ages. Symbols are the same as those in Fig. 1.

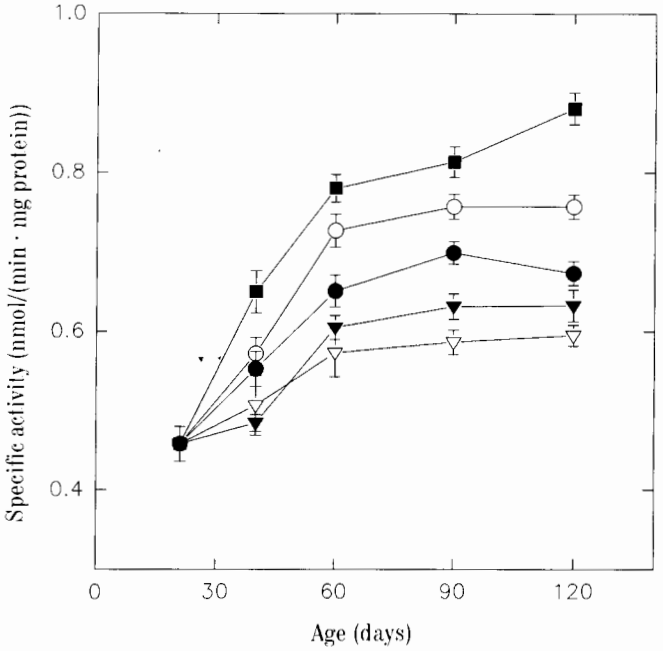

FIG. 5. Specific activity of aminopyrine *N*-demethylase in liver microsomes of rats fed various dietary fats at different ages. Symbols are the same as those in Fig. 1.

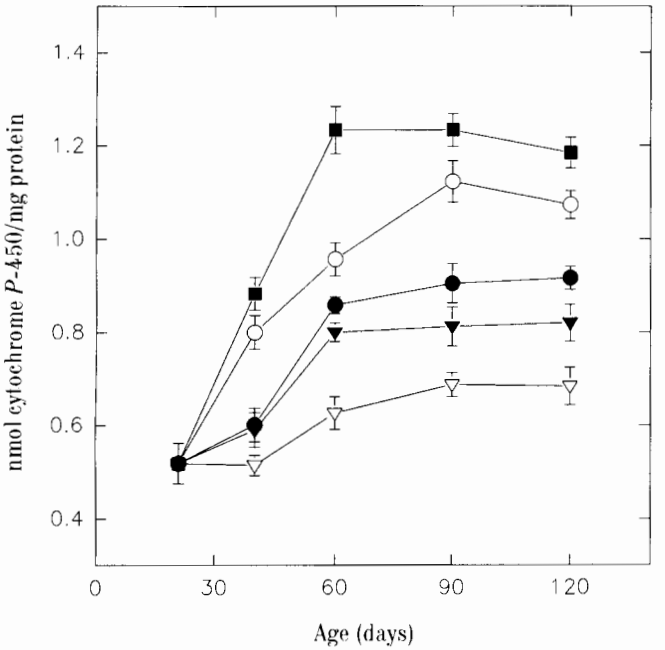

FIG. 4. Cytochrome *P*-450 concentration in liver microsomes of rats fed various dietary fats at different ages. Symbols are the same as those in Fig. 1.

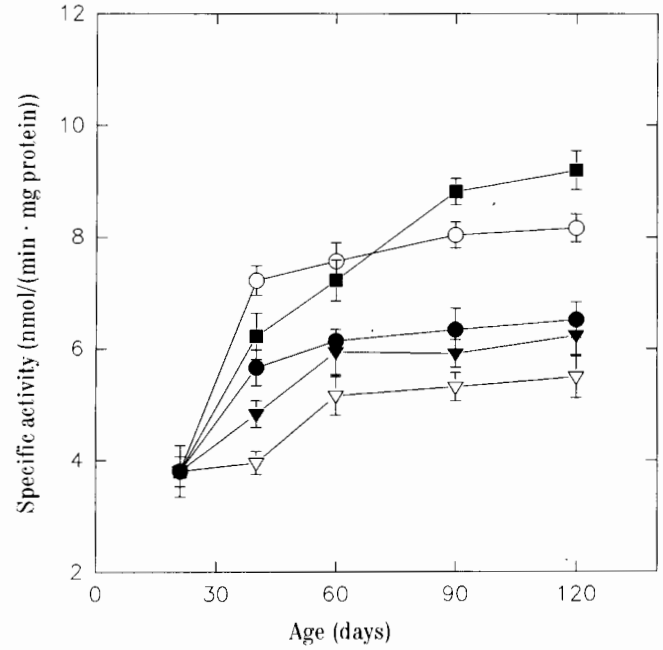

FIG. 6. Specific activity of aniline hydroxylase in liver microsomes of rats fed various dietary fats at different ages. Symbols are the same as those in Fig. 1.

the cytochrome *P*-450 concentration and NADPH–cytochrome-*c* reductase, aniline hydroxylase, and aminopyrine *N*-demethylase activities at 120 days were, respectively, 30, 48, 41, and 31% higher than those of rats fed the control diet. The results (Table 5) indicate a close correlation ($0.87 < r < 0.93$), $P < 0.05$ between the Σ PUFA($n-3$) and these enzymes. However, the cytochrome b_5 concentration was decreased significantly compared with control group ($P < 0.05$).

Discussion

The present study is the first, to our knowledge, to examine the rate of alteration of hepatic mixed-function oxidase system of developing rats fed different dietary fats. Although the enzymes are localized in the same membrane, it appears clearly from our results that they are not similarly influenced by different dietary fats. The cytochrome b_5 content and NADH–cytochrome-b_5 reductase activity had a similar pattern of variation and increased during the

TABLE 5. Effects of dietary fats on the components of the liver microsomal mixed-function oxidase system of rats at 120 days, and correlation with dietary Σ PUFA($n-3$) and Σ PUFA($n-6$)

	NADH–cytochrome-b_5 reductase	NADPH–cytochrome-c reductase	Cytochrome b_5	Cytochrome P-450	Aminopyrine demethylase	Aniline hydroxylase
Diets						
Control	5.01 ± 0.16^{a}	62.02 ± 2.45^{a}	0.54 ± 0.02^{a}	0.92 ± 0.03^{a}	0.67 ± 0.02^{a}	6.52 ± 0.32^{a}
Fat free	7.16 ± 0.23^{b}	47.16 ± 2.18^{b}	0.72 ± 0.02^{b}	0.68 ± 0.04^{b}	0.60 ± 0.01^{a}	5.49 ± 0.37^{a}
Peanut–rapeseed oil	5.13 ± 0.32^{a}	73.34 ± 2.47^{c}	$0.48 \pm 0.02^{a,c}$	1.07 ± 0.03^{c}	0.76 ± 0.02^{b}	$8.16 \pm 0.25^{b,c}$
Sunflower oil	3.87 ± 0.25^{c}	$58.39 \pm 2.27^{a,b}$	0.59 ± 0.02^{a}	$0.82 \pm 0.04^{a,b}$	0.63 ± 0.02^{a}	6.24 ± 0.35^{a}
Fish oil	$6.13 \pm 0.25^{a,b}$	91.99 ± 2.94^{d}	0.45 ± 0.03^{c}	$1.19 \pm 0.03^{d,c}$	0.88 ± 0.02^{c}	9.20 ± 0.35^{c}
Correlation coefficient						
Σ ($n-6$)	−0.98	0.02	−0.35	0.12	−0.10	0.02
Σ ($n-3$)	0.14	0.93	−0.73	0.84	0.92	0.87

NOTE: Values of enzyme activities are expressed as the means ± SD for six animals (for units, see Figs. 1–6). Those not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript letter appears, values are not significantly different.

period of lipid deprivation. The increase may be related to the probable increase in desaturase activities (see Table 4). The first desaturase that responded positively to this change was Δ9-desaturase, which enhances the activity and production of monoenoic acids. These are key amphipathic enzymes assembled by the NADH-cytochrome-b_5 electron-transferring system (Farias et al. 1975) located in the microsomal membrane where they control polyunsaturated fatty acid biosynthesis (Century 1973). However, aminopyrine *N*-demethylase, aniline hydroxylase, and NADPH-cytochrome-*c* reductase activities varied in a manner similar to cytochrome *P*-450 level. Their activities were significantly lower than that of the control group at all ages. These results are consistent with data previously presented by Row and Wills (1976). These authors showed that the cytochrome *P*-450 and rate of oxidative demethylation were lowest when a fat-free diet was fed. This decrease could be closely related to a paucity of fatty acids needed for synthesis of the phospholipid matrix of the endoplasmic reticulum. In addition, there was no significant change in NADPH-cytochrome-*c* reductase activity, suggesting that this enzyme is not influenced by dietary 18:2($n-6$) and 18:3($n-3$). The results obtained here further support the findings reported by other investigators (Norred and Wade 1972; Wade and Norred 1976; Christon et al. 1988). The increase in cytochrome *P*-450 level and NADPH-cytochrome-*c* reductase, aminopyrine *N*-demethylase, and aniline hydroxylase activities in the fish oil group would suggest that 20:5($n-3$) and 22:6($n-3$) might be responsible for the increased activities. These enzymes were correlated very well ($0.84 < r < 0.93$), $P < 0.05$, with dietary Σ PUFA($n-3$) (Table 5). On other hand, this increase could be due to changes in the microsomal membrane fluidity or to the specific effect of ($n-3$) fatty acids on the enzymes itself. Specific interactions between ($n-3$) PUFA and particular domains of proteins may affect catalytic properties of the enzymes, or localized changes in membranes may affect enzyme–substrate interactions (Johannsson et al. 1981). In this view, Davison and Wills (1974) suggested that a species of phosphatidylcholine or phosphatidylethanolamine containing 18:2 in the β-position is essential in the membrane for maximum oxidative demethylation activity in the endoplasmic reticulum. A high concentration of ($n-3$) unsaturated acids, particularly 20:5($n-3$), in the diet enhances the rate of oxidative demethylation. 18:2($n-6$) or 20:5($n-3$) in the liver microsomes as components of phospholipids thus appear to be important in holding the cytochrome *P*-450 enzyme complex in an active conformation in the membrane.

In conclusion, these experiments have demonstrated the ability of the dietary lipid to regulate the mixed-function oxidase system in the liver microsomes and the importance of the fatty acid composition of the membrane phospholipids. Also, biological functions of membranes, such as membrane-bound enzyme functions and transport systems, are influenced by the membrane physical properties, which are determined by fatty acid composition of polar lipids, polar head group composition, and membrane cholesterol content. Polar and nonpolar regions of the phospholipid molecule may interact, with changes in the conformation of a membrane-associated protein altering either its catalytic activity or the protein's interaction with other membrane proteins (Coleman 1973). These data, as well as the results reported by other authors (Trewhell and Collins 1973; Chignell and Chignell 1975), imply that the membrane protein–lipid interactions depends on the nature of the physiochemical makeup of the membrane lipid, which depends in part on dietary intake of fat.

Acknowledgments

This work was supported by the Institut national de la santé et de la recherche médicale. The authors are grateful to Alan Strickland for his English assistance.

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