Dose-Response Effect of Dietary Docosahexaenoic Acid on Fatty Acid Profiles of Serum and Tissue Lipids in Rats

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The dose-response effects of dietary docosahexaenoic acid (22:6*n*−3, DHA) on the fatty acid profiles of total lipids of rat serum and tissues were investigated. Rats were fed diets containing graded levels of purified DHA at 0, 1.0, 3.4, and 8.7% of total energy in the diets for 2 weeks. It was found that each tissue had its own peculiar composition of fatty acids which differed markedly from that of circulating serum lipid. This composition was basically influenced dose-dependently by the dietary lipids with graded levels of DHA, but to different degrees in different tissues. Those of brain were most resistant and of heart most susceptible to dietary DHA.

Keywords: *Dose-response; docosahexaenoic acid; n − 3 polyunsaturated fatty acid; tissue; fatty acid profile*

INTRODUCTION

n − 3 polyunsaturated fatty acids (PUFA's) such as eicosapentaenoic acid (20:5*n*−3, EPA) and docosahexaenoic acid (22:6*n*−3, DHA) which are abundant in fish oils have attracted attention. This is due to the epidemiological and experimental findings that these fatty acids have a hypolipidemic effect on circulating blood and also show a possible prophylactic effect against development of thrombosis and atherosclerosis, resulting in a lower incidence of cardiovascular diseases (Dyerberg, 1986; Herold and Kinsella, 1986; Harris, 1989; Nestel, 1990; Simopoulos, 1991). In recent years, studies with nonhuman primates and human newborns have shown that DHA is essential for the normal functional development of the retina and brain, particularly in premature infants (Simopoulos, 1991; Neuringer et al., 1988). Furthermore, differential effects of EPA and DHA on serum lipids (Kobatake et al., 1984; Ikeda et al., 1994; Frøyland et al., 1996), fatty acid *β*-oxidation in mitochondria and peroxisomes (Aarsland et al., 1990; Willumsen et al., 1993a,b; Frøyland et al., 1996), and prostanoid production (Von Schacky and Weber, 1985; Ikeda et al., 1994) have been demonstrated using purified EPA and DHA. The effects of EPA and DHA on prostanoid production could partly be achieved through the reduction of arachidonic acid (20:4*n*−6, AA) content in tissue phospholipids. Lands (1991) reported that the content of AA in platelet phospholipids directly reflects their synthesis of thromboxane A_2. Ikeda et al. (1994) reported similar results in the aortic PGI_2 production in DHA-administered rats. These results suggest that the observations obtained for *n* − 3 PUFA-rich fish oil feeding result primarily from the combined effects of major component fatty acids, EPA and DHA, and also that the differences in the proportion and/or amount of these fatty acids given to animals may explain the inconsistent observations noticed occasionally in fish oil feeding. Accordingly, it is important to elucidate the dose-response effect of EPA or DHA separately on tissue fatty acid profiles.

n − 3 fatty acids, particularly DHA, have structural roles in glycerolipids of all cell membranes in the tissue, influencing membrane viscosity and permeability, and thereby possibly affecting the enzyme activity of membrane proteins, receptors, and ion channels (Spector and Yorek, 1985; Murphy, 1990). DHA is present in unusually high concentrations in phospholipids from retina (Aveldaño deCaldironi and Bazan, 1980; Fliesler and Anderson, 1983), brain (Sun and Sun, 1972), and mammalian spermatozoa (Poulos et al., 1973). Studies using labeled DHA suggested that membrane DHA of developing brain and retina is supplied through circulating blood lipid by the liver and thus the metabolism of DHA is developmentally regulated by and under the control of the liver (Martin et al., 1994), implying an importance of the metabolism of DHA in the liver. Most studies have used fish oil and/or fish oil concentrate to examine the effect of dietary DHA on tissue fatty acid composition. However, these oils usually contain a considerable amount of EPA and other *n* − 3 and *n* − 6 PUFA's. Only a few works have examined the effects of dietary purified DHA (Croset and Kinsella, 1989; Taniguchi et al., 1993; Ikeda et al., 1994; Frøyland et al., 1996). Even in these studies, the fatty acid profiles of various tissues and the dose-response effect were not investigated.

In this study we report a dose-response effect of dietary purified DHA on the fatty acid profiles of serum and various tissue lipids in rats. The diets were designed to provide a constant amount of LA and graded

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Table 1. Composition of Experimental Diets (g/100 g of Diet) and Fatty Acid Composition of Dietary Lipids (Percent) for Rats

		group				
		1	2	3	4	5
DHA level (energy %)[a]		0	0	1.0	3.4	8.7
LA level (energy %)[a]		1.9	9.0	2.0	2.0	2.1
basic ingredients[b]		90.0	90.0	90.0	90.0	90.0
test lipids[c]		10.0	10.0	10.0	10.0	10.0
olive oil		9.54	5.0	8.98	7.32	4.00
safflower oil		0.46	5.0	0.50	0.63	0.88
DHA concentrate[d]		0.00	0.0	0.52	2.05	5.12
fatty acid	name					
16:0		10.1[e]	8.8	9.5	8.2	5.2
16:1($n-7$)		1.0	0.6	1.1	0.8	0.5
18:0		3.2	2.8	3.0	2.7	1.6
18:1($n-9$)	OA	75.9	45.9	72.2	60.4	35.7
18:2($n-6$)	LA	9.0	41.4	9.2	9.1	9.7
18:3($n-3$)		0.7	0.5	0.6	0.4	0.4
20:5($n-3$)	EPA	0.0	0.0	0.0	0.4	1.0
22:1($n-11$)		0.0	0.0	0.0	0.6	1.4
22:3($n-3$)		0.0	0.0	0.0	0.9	2.2
22:5($n-3$)	DPA	0.0	0.0	0.0	0.9	2.0
22:6($n-3$)	DHA	0.0	0.0	4.4	15.6	40.2
$\sum n-6$		9.0	41.4	9.2	9.1	9.7
$\sum n-3$		0.7	0.5	5.0	18.2	45.8
$n-6/n-3$		12.9	82.8	1.8	0.5	0.2
DBI[f]		1.0	1.3	1.2	1.8	3.2

[a] The Atwater energy factors were used for the energy percent calculation. [b] The basic ingredients of the diets in all the groups were casein, 20.0 g, DL-methionine, 0.3 g, cornstarch, 15.0 g, sucrose, 22.5 g, glucose, 22.5 g, cellulose powder, 5.0 g, AIN-76 mineral mixture, 3.5 g, AIN-76 vitamin mixture, 1.0 g, and choline bitartrate, 0.2 g. [c] Fat energy percent is 21.6%. [d] The purity of DHA concentrate (ethyl ester form) is 83%. [e] Values of fatty acids less than 0.3% are not shown. [f] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. OA, oleic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; and DHA, docosahexaenoic acid.

amount of DHA. The control DHA-free lipid contained LA comparable to the top amount of dietary DHA.

MATERIALS AND METHODS

Animals and Diets. Male Sprague-Dawley rats, 4 weeks of age, weighing 75−85 g at the beginning of the experiment, were housed individually in stainless steel wire-bottomed cages at a constant temperature of 22 ± 1 °C and humidity of 50−60% with a 12-h light−dark cycle. The compositions of experimental diets based on AIN-76 purified diet for rats (American Institute of Nutrition, 1980) are shown in Table 1. The energy content of all the diets was 416 kcal/100 g (1.74 MJ/100 g), where the Atwater energy factors (4, 9, and 4 kcal/g for protein, fat, and carbohydrate, respectively) were used. Vitamin E (VE) content of all the diets was adjusted to 13.4 mg/100 g diet as (*R*,*R*,*R*)-α-tocopherol equivalent by analyzing the content of tocopherol analogues in dietary lipids (Saito et al., 1992) and then by adding *all-rac*-α-tocopheryl acetate to the diets. The relative biological activities for (*R*,*R*,*R*)-α-, (*R*,*R*,*R*)-*β*-, (*R*,*R*,*R*)-*γ*-, and (*R*,*R*,*R*)-*δ*-tocopherols were taken as 100:25:5:0.1 in the calculation (Mino et al., 1988). The lipid content of the diets was 10 wt % and the fat energy 21.6%. DHA levels of the diets for the five test groups were adjusted to 0, 0, 1.0, 3.4, and 8.7% of total energy, respectively, by combining olive oil, safflower oil, and DHA concentrate. The DHA concentrate was prepared from fatty acid ethyl esters of sardine oil and the composition of its main fatty acids was as follows (%): 20:5$n-3$, 2.3; 22:1$n-11$, 2.1; 22:3$n-3$, 4.6; 22:6$n-3$, 83.1. The fatty acid composition of the dietary lipids was analyzed by using gas−liquid chromatography and is indicated in Table 1. The DHA-free control lipid (group 2) contained about 41% of linoleic acid, which was comparable to the top amount of DHA (group 5). Also, each test lipid was prepared to supply linoleic acid at at least 2% of total energy as an essential fatty acid of $n-6$ type, where the proportion of linoleic acid was approximately 9%.

Feeding Trial. After being fed the basal diet containing 5% (wt %) olive oil for 3 days, six to seven rats of each group were maintained on the experimental diets for 14 days. Food and water were available *ad libitum*. To prevent autoxidation of DHA concentrate in the diets, each diet was prepared beforehand without adding the concentrate and stored at −20 °C. DHA concentrate stored at −80 °C under nitrogen was mixed with the diet every day immediately before feeding. Furthermore, the diets were given to rats in the evening and removed the next morning. After fasting overnight, the rats were killed by cardiac puncture and the tissues were promptly excised, washed with isotonic saline, and weighed. The tissues were stored at −80 °C until analysis. Serum was separated by centrifugation at 2700*g* for 15 min at 4 °C.

Lipid Analyses. Liver microsomes were prepared as described (Saito and Yamaguchi, 1988). Total lipid was extracted from the liver, liver microsomes, kidney, brain, heart, testis, and serum according to the method of Folch et al. (1957). Chloroform/methanol (2:1, v/v) solution containing 0.45 mM 2,6-di-*tert*-butyl-*p*-cresol was used for the extraction. Total lipids from liver and brain were separated into non-phosphorus lipids and phospholipids with a silica cartridge (Juaneda and Rocquelin, 1985). Fatty acid methyl esters of dietary, serum, and tissue lipids were prepared as follows: lipids were saponified with 0.5 M sodium hydroxide−methanol solution and the resultant free fatty acids were converted into methyl esters by using boron trifluoride−methanol solution (140 g/L). The methyl esters were extracted with *n*-hexane and analyzed by gas−liquid chromatography. The fatty acid composition of the DHA concentrate was analyzed by gas−liquid chromatography with a flame ionization detector (5890, Series II, Hewlett-Packard Co., Wilmington, DE) by using a Quadrex CPS-1 capillary column (25 m × 0.25 mm i.d.). The temperature of the oven was programmed from 150 to 200 °C at a rate of 2 °C/min. Injector and detector temperatures were 250 °C. Helium was employed as the carrier gas. Fatty acid methyl esters of dietary lipids, total lipids from tissues and serum, and phospholipids from liver and brain total lipids were separated by gas−liquid chromatography with dual-flame ionization detectors (Model 103, Ohkura gas−liquid chromatograph, Tokyo, Japan) by using a 2 m × 2.5 mm i.d. glass column containing diethylene glycol succinate on 80/100 mesh Uniport B (150 g/kg) (Saito et al., 1990). Injector and column temperatures were 240 and 195 °C, respectively. Nitrogen gas was employed as the carrier gas. Standard mixtures of fatty acid methyl esters (Nihon Chromato Works Ltd., Tokyo, Japan) were used for identification of peaks.

Statistical Analysis. Statistical analyses of multiple treatment effects were conducted by using one way analysis of variance with comparison of means made by Duncan's multiple-range test at the 1% level of significance (Duncan, 1957).

RESULTS

Rats consumed 14.7−15.7 g of diet a day and gained 6.0−7.0 g of body weight a day for 14 days. Food intake and body weight gain of all the groups were not significantly different. Tissue weights also did not significantly differ among the groups.

The fatty acid composition of serum total lipid is shown in Table 2. The compositions were affected by those of the dietary lipids. Among the saturated fatty acids (SFAs), the proportion of palmitic acid tended to be greater in groups 1 and 3 than in group 5 and was generally higher than the percent stearic acid in all the groups. The proportion of oleic acid (18:1$n-9$, OA) reflected well dietary levels of OA and was increased

Table 2. Fatty Acid Composition (Percent) of Serum Total Lipid in Rats Fed Diets Containing Graded Levels of DHA[a]

		group				
		1	2	3	4	5
DHA level (energy %)		0	0	1.0	3.4	8.7
LA level (energy %)		1.9	9.0	2.0	2.0	2.1
fatty acid	name					
14:0		0.6 ± 0.1a	0.5 ± 0.1ab	0.3 ± 0.2bc	0.2 ± 0.1c	0.2 ± 0.2bc
14:1(*n*−7)		0.0 ± 0.1a	0.2 ± 0.1a	0.1 ± 0.1a	0.1 ± 0.1a	0.2 ± 0.1a
16:0		19.4 ± 1.4a	17.7 ± 1.3a	19.9 ± 3.3a	18.1 ± 0.7a	16.8 ± 1.1a
16:1(*n*−7)		3.6 ± 0.4a	2.3 ± 0.5b	2.6 ± 0.3b	2.6 ± 0.6b	2.0 ± 0.3b
18:0		9.4 ± 1.6a	11.4 ± 1.7a	10.2 ± 1.1a	9.4 ± 1.5a	9.2 ± 0.8a
18:1(*n*−9)	OA	40.7 ± 4.7a	21.0 ± 2.9b	32.8 ± 2.8c	26.9 ± 2.0d	18.9 ± 1.2b
18:2(*n*−6)	LA	8.6 ± 0.7a	19.6 ± 1.2b	11.8 ± 0.8c	10.4 ± 0.6d	9.2 ± 0.8ad
20:0		0.2 ± 0.1a	0.3 ± 0.2a	0.1 ± 0.2a	0.0 ± 0.1a	0.2 ± 0.2a
20:1(*n*−9)		0.6 ± 0.1a	0.5 ± 0.1a	0.6 ± 0.1ab	0.5 ± 0.0a	0.7 ± 0.1b
20:2(*n*−6)		0a	0.2 ± 0.1b	0a	0a	0a
20:3(*n*−9)		0.9 ± 0.2a	0b	0.4 ± 0.1c	0b	0.1 ± 0.1b
20:3(*n*−6)		0.5 ± 0.2a	0.6 ± 0.2ab	0.8 ± 0.1b	0.3 ± 0.1c	0.4 ± 0.1c
20:4(*n*−6)	AA	11.6 ± 2.3a	21.2 ± 3.4b	9.8 ± 1.7ac	7.6 ± 0.8c	8.8 ± 2.0ac
20:5(*n*−3)	EPA	0.2 ± 0.3a	0.2 ± 0.2a	2.3 ± 0.4b	6.6 ± 0.6c	8.1 ± 0.9d
22:4(*n*−6) + 24:1(*n*−9)	DTA	0.1 ± 0.2a	0.7 ± 0.1b	0a	0a	0.1 ± 0.2a
22:5(*n*−6)	DPA	0.5 ± 0.1a	1.7 ± 0.8b	0.1 ± 0.1a	0.4 ± 0.2ac	0.9 ± 0.2c
22:5(*n*−3)	DPA	0a	0.1 ± 0.2ab	0.5 ± 0.3b	1.5 ± 0.3c	2.0 ± 0.4d
22:6(*n*−3)	DHA	2.6 ± 0.5a	1.8 ± 0.5a	8.0 ± 1.1b	14.4 ± 1.3c	21.2 ± 2.7d
others		0.2 ± 0.4a	0a	0.3 ± 0.4ab	0.9 ± 0.3b	0.9 ± 0.5b
∑PUFA[b]		25.0 ± 3.4a	46.2 ± 2.9b	33.6 ± 2.9c	41.2 ± 1.7d	50.7 ± 1.7e
∑MUFA[c]		45.0 ± 4.7a	23.9 ± 3.2b	36.1 ± 3.0c	30.1 ± 2.4d	21.7 ± 1.4b
∑SFA[d]		29.6 ± 2.0ab	30.1 ± 1.1a	29.7 ± 1.8a	27.8 ± 2.1ab	26.5 ± 2.0b
P/S[e]		0.9 ± 0.1a	1.5 ± 0.1b	1.1 ± 0.1c	1.5 ± 0.1b	1.9 ± 0.2d
∑*n* − 6		21.4 ± 3.1a	44.0 ± 3.4b	22.4 ± 2.3a	18.7 ± 1.2a	19.3 ± 2.4a
∑*n* − 3		2.7 ± 0.6a	2.3 ± 0.8a	10.8 ± 1.1b	22.5 ± 2.0c	31.3 ± 2.6d
n − 6/*n* − 3		8.1 ± 2.0a	21.2 ± 7.4b	2.1 ± 0.3c	0.8 ± 0.1c	0.6 ± 0.1c
DBI[f]		1.3 ± 0.1a	1.7 ± 0.1b	1.6 ± 0.1b	2.1 ± 0.1c	2.6 ± 0.1d
DI[g]		1.41	1.12	0.90	0.76	1.00

[a] Data are presented as the mean ± SD ($n = 6$ for groups 1 and 2, and $n = 7$ for groups 3, 4, and 5). Means within the same row not followed by a common letter differ significantly ($P < 0.01$). [b] PUFA, polyunsaturated fatty acid. [c] MUFA, monounsaturated fatty acid. [d] SFA, saturated fatty acid. [e] P/S, ∑PUFA/∑SFA. [f] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. [g] Desaturation index = [20:3(*n*−6) + 20:4(*n*−6)]/18:2(*n*−6). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

in groups 1 and 3 and decreased in group 5 compared to group 2 (control group). This was also the case for the total proportion of monounsaturated fatty acids (MUFAs) in groups 1, 3, and 5. The value for LA was higher in group 2, reflecting the dietary supply of LA, as was that for AA, converted from LA. So, the total proportion of *n* − 6 PUFA's was significantly higher in group 2 than in the other groups. The proportion of DHA progressively increased in response to dietary supply, as did those of EPA and *n* − 3 docosapentaenoic acid (22:5*n*−3, DPA), resulting in a similar change in the total proportion of *n* − 3 PUFA's. This was also the case for total proportion of PUFA, *P*/*S* ratio, and double bond index (DBI). The desaturation index (DI) was highest in group 1, the group supplied the lowest amount of dietary PUFA's.

The fatty acid profile of total lipid of the liver is given in Table 3. The compositions were affected by those of the dietary lipids and changed generally like those of serum, particularly the *n* − 3 PUFA's and group 5. However, more palmitic acid and less *n* − 6 PUFA's such as LA and AA were present in the liver as compared with serum. But the differences between liver and serum lipids in each group decreased as the dietary supply of DHA increased. This may partly be related to the nature of the liver, which stores palmitic acid as a primary component of depot fat. The P/S ratio and DBI increased in liver in response to the dietary supply of DHA as in serum. The DI was highest in group 1, which received the lowest dose of PUFA's.

The fatty acid composition of total lipid of liver microsomes is shown in Table 4. Compared with liver, the degree of unsaturation was higher on the whole as assessed by the total proportion of PUFA and DBI, that of monounsaturation inversely lower, and that of saturation nearly the same. There was almost no difference in stearic acid content among the groups. The proportion of LA was significantly high and that of AA very high in group 2 compared to the other groups. AA levels were generally higher in the liver microsomes than in the liver in all groups. However, the percent LA and AA decreased or tended to decrease with increase in the dietary supply of DHA.

The proportion of DHA in liver microsomes increased progressively in a dose-dependent manner (Table 4). When the values for DHA in the liver microsomes and liver are compared, those of the former were particularly high in the group given a low dose of DHA (group 3) but differed little from the latter in the group supplied the highest dose of DHA (group 5). This was also the case in the DBI. The EPA and *n* − 3 DPA changed similarly to DHA in the liver microsomes, too. The DIs of the liver microsomes were higher in general than those of the liver, particularly in group 2.

The fatty acid composition of phospholipids separated from total lipid of the liver was similar to that of liver microsomal lipid in all the groups (data not shown).

The fatty acid composition of total lipid of the kidney is given in Table 5. The composition did not necessarily reflect the dietary fatty acid composition. The propor-

Table 3. Fatty Acid Composition (Percent) of Liver Total Lipid in Rats Fed Diets Containing Graded Levels of DHA[a]

		group				
		1	2	3	4	5
DHA level (energy %)		0	0	1.0	3.4	8.7
LA level (energy %)		1.9	9.0	2.0	2.0	2.1
fatty acid	name					
14:0		1.0 ± 0.2a	0.7 ± 0.2b	0.6 ± 0.1b	0.4 ± 0.0c	0.3 ± 0.1c
14:1(*n*−7)		0.1 ± 0.1a	0.2 ± 0.1b	0.2 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.1b
16:0		29.5 ± 2.7a	24.7 ± 2.7bc	26.2 ± 1.6b	21.9 ± 1.5cd	20.6 ± 0.8d
16:1(*n*−7)		5.2 ± 0.7a	3.3 ± 1.1b	3.4 ± 0.4b	2.5 ± 0.2bc	1.8 ± 0.3c
18:0		9.3 ± 1.8a	12.3 ± 1.6b	10.3 ± 0.9a	12.8 ± 0.9b	13.7 ± 0.6b
18:1(*n*−9)	OA	39.9 ± 2.7a	27.4 ± 2.3b	39.6 ± 2.0a	28.4 ± 2.0b	18.4 ± 1.3c
18:2(*n*−6)	LA	3.6 ± 0.6a	12.7 ± 1.9b	5.2 ± 0.7c	6.0 ± 0.5c	6.0 ± 0.4c
20:0		0a	0.3 ± 0.1b	0a	0a	0a
20:1(*n*−9)		0.3 ± 0.1a	0.4 ± 0.1a	0.3 ± 0.1a	0.5 ± 0.1b	0.5 ± 0.1b
20:2(*n*−6)		0a	0.3 ± 0.2b	0a	0a	0a
20:3(*n*−9)		0.5 ± 0.2a	0b	0.1 ± 0.1b	0b	0b
20:3(*n*−6)		0.4 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.1a
20:4(*n*−6)	AA	6.9 ± 2.0a	12.7 ± 1.9b	4.8 ± 0.6c	5.3 ± 0.6c	6.4 ± 0.5ac
20:5(*n*−3)	EPA	0a	0a	1.7 ± 0.5b	5.6 ± 0.7c	7.2 ± 0.3d
22:4(*n*−6) + 24:1(*n*−9)	DTA	0a	0.6 ± 0.1b	0a	0a	0a
22:5(*n*−6)	DPA	0.6 ± 0.2a	2.2 ± 0.9b	0a	0.3 ± 0.1a	0.6 ± 0.1a
22:5(*n*−3)	DPA	0a	0a	0.3 ± 0.2a	1.7 ± 0.4b	2.7 ± 0.2c
22:6(*n*−3)	DHA	2.6 ± 0.7a	2.2 ± 0.3a	6.8 ± 1.1b	14.2 ± 1.1c	21.2 ± 1.4d
∑PUFA[b]		14.7 ± 3.6a	31.0 ± 4.3b	19.4 ± 2.7c	33.4 ± 2.1b	44.5 ± 1.8d
∑MUFA[c]		45.5 ± 3.1a	31.1 ± 3.0b	43.6 ± 2.3a	31.6 ± 2.1b	20.9 ± 1.5c
∑SFA[d]		39.8 ± 1.6a	37.9 ± 2.6a	37.1 ± 1.7ab	35.0 ± 1.9b	34.6 ± 1.1b
P/S[e]		0.4 ± 0.1a	0.8 ± 0.2b	0.5 ± 0.1a	1.0 ± 0.1b	1.3 ± 0.1c
∑*n* − 6		11.5 ± 2.8a	28.8 ± 4.1b	10.4 ± 1.3a	12.0 ± 0.5a	13.3 ± 0.8b
∑*n* − 3		2.6 ± 0.7a	2.2 ± 0.3a	8.8 ± 1.5b	21.4 ± 1.8c	31.1 ± 1.6d
n − 6/*n* − 3		4.4 ± 0.4a	13.1 ± 1.6b	1.2 ± 0.2c	0.6 ± 0.1c	0.4 ± 0.1c
DBI[f]		1.0 ± 0.1a	1.4 ± 0.2b	1.3 ± 0.1b	1.9 ± 0.1c	2.4 ± 0.1d
DI[g]		2.01	1.03	1.01	0.94	1.12

[a] Data are presented as the mean ± SD ($n = 6$ for groups 1 and 2, and $n = 7$ for groups 3, 4, and 5). Means within the same row not followed by a common letter differ significantly ($P < 0.01$). [b] PUFA, polyunsaturated fatty acid. [c] MUFA, monounsaturated fatty acid. [d] SFA, saturated fatty acid. [e] P/S, ∑PUFA/∑SFA. [f] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. [g] Desaturation index = [20:3(*n*−6) + 20:4(*n*−6)]/18:2(*n*−6). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

tions of SFA's did not differ practically among the groups and those of MUFA's were highest in group 1, but there was no substantial difference among the other groups, unlike in serum and liver. The proportion of LA was a slightly higher in group 2, which was supplied the greatest amount of dietary LA. However, values for AA tended to be higher in kidney than in liver in all groups. The values for DHA in groups 1 and 2 were very low but increased gradually in groups 3, 4, and 5 in a dose-related manner, although the extent of the increase was rather small in kidney compared to liver lipid. Characteristically, the EPA content in groups 3, 4, and 5 was comparable to the DHA content, with higher values for kidney than liver, whereas $n - 3$ DPA content was decreased in the kidney. The changes in the percent total PUFA, MUFA, and SFA were generally small in the kidney lipids as judged by the close *P*/*S* ratios and DBIs among all the groups. Accordingly, the kidney seems to be less susceptible to alterations in dietary fatty acid compositions. In addition, the DIs were higher in the kidney, even higher than those of the liver microsomes. Hence, the level of activity of $n - 6$ fatty acid metabolism appears to be high in the kidney.

The fatty acid composition of brain total lipid is shown in Table 6. The composition was generally quite resistant to changes in the dietary fatty acids, and thus the compositional differences in the fatty acids among all of the groups were very small. In addition, the percent total SFA was particularly high, about 50%, in all the groups and the percent total PUFA was low. The content of LA in brain lipid was reduced in all the groups. The AA content did not increase a lot even in group 2, which was supplied the most dietary LA. The level of DHA in groups 3−5 changed little regardless of remarkable change in the dietary DHA level. However, the value for $n - 6$ docosatetraenoic acid (DTA) was distinctively higher in brain than in other tissues in all the groups. Moreover, no EPA was detected in brain lipids, although it was contained in circulating blood lipids as shown in Table 2.

The fatty acid composition of phospholipids separated from total lipids in brain was almost the same as that of brain total lipid in all the groups (data not shown). Accordingly, the fatty acid composition of total lipids in brain is thought to reflect the composition of cell membrane phospholipids.

The fatty acid profile of total lipid of the heart is given in Table 7. The heart was the organ most affected by dietary DHA. The proportion of DHA increased in response to dietary supply; the value was higher for heart than for any other tissues in DHA-administered groups 3−5. Even in group 3, supplied the lowest amount of DHA, the proportion of DHA was increased relative to the other tissues analyzed. However, the value for EPA did not increase in groups 3−5 as compared with that in the liver lipids, even though the circulating blood lipids contained EPA at appreciable levels. On the other hand, values for $n - 6$ LA and AA were highest in group 2, supplied the most LA, and decreased as the dietary level of DHA increased. Values for SFA were similar in all the groups.

Table 4. Fatty Acid Composition (Percent) of Liver Microsomal Total Lipid in Rats Fed Diets Containing Graded Levels of DHA[a]

		group				
		1	2	3	4	5
DHA level (energy %)		0	0	1.0	3.4	8.7
LA level (energy %)		1.9	9.0	2.0	2.0	2.1
fatty acid	name					
14:0		0.5 ± 0.1a	0.4 ± 0.1b	0.3 ± 0.1bc	0.2 ± 0.0c	0.2 ± 0.1c
14:1(*n*−7)		0.1 ± 0.2a	0.1 ± 0.1a	0a	0.0 ± 0.1a	0.1 ± 0.1a
16:0		16.6 ± 0.6a	16.3 ± 0.5a	17.2 ± 0.9a	18.5 ± 0.7b	19.0 ± 0.5b
16:1(*n*−7)		2.7 ± 0.3a	1.6 ± 0.4bc	1.8 ± 0.2b	1.6 ± 0.1bc	1.4 ± 0.1c
18:0		21.3 ± 0.4ab	22.3 ± 0.5a	21.4 ± 1.1ab	21.1 ± 0.8ab	20.8 ± 0.7b
18:1(*n*−9)	OA	20.0 ± 1.3a	12.2 ± 0.6b	17.3 ± 1.0c	14.3 ± 0.4d	11.0 ± 0.4b
18:2(*n*−6)	LA	7.6 ± 0.7ac	11.1 ± 0.9b	8.4 ± 0.6a	6.8 ± 0.3c	5.8 ± 0.2d
20:0		0a	0a	0a	0a	0a
20:1(*n*−9)		0.4 ± 0.2a	0.3 ± 0.1a	0.3 ± 0.1a	0.4 ± 0.1a	0.3 ± 0.1a
20:2(*n*−6)		0a	0.6 ± 0.1b	0.0 ± 0.1a	0a	0.1 ± 0.2a
20:3(*n*−9)		1.5 ± 0.3a	0b	0.6 ± 0.2c	0b	0b
20:3(*n*−6)		1.3 ± 0.3a	0.9 ± 0.1b	1.4 ± 0.2a	0.6 ± 0.1bc	0.5 ± 0.0c
20:4(*n*−6)	AA	19.6 ± 1.2a	25.1 ± 1.0b	12.1 ± 0.5c	9.4 ± 0.6d	9.9 ± 0.5d
20:5(*n*−3)	EPA	0.2 ± 0.2a	0a	3.3 ± 0.8b	7.3 ± 0.6c	8.1 ± 0.5c
22:4(*n*−6) + 24:1(*n*−9)	DTA	0.1 ± 0.2a	0.8 ± 0.1b	0c	0c	0c
22:5(*n*−6)	DPA	1.5 ± 0.3a	4.0 ± 1.4b	0.2 ± 0.2c	0.5 ± 0.1a	0.6 ± 0.0a
22:5(*n*−3)	DPA	0.2 ± 0.2a	0.1 ± 0.2a	0.9 ± 0.2b	1.9 ± 0.3c	2.1 ± 0.2c
22:6(*n*−3)	DHA	6.4 ± 0.5a	4.1 ± 0.3b	14.7 ± 0.9c	17.3 ± 0.6d	20.1 ± 0.6e
∑PUFA[b]		38.2 ± 0.9a	46.7 ± 1.1b	41.6 ± 1.3c	43.8 ± 0.7d	47.2 ± 0.6b
∑MUFA[c]		23.2 ± 1.3a	14.2 ± 0.8b	19.3 ± 1.2c	16.2 ± 0.5d	12.8 ± 0.5b
∑SFA[d]		38.5 ± 0.7a	39.0 ± 0.4ab	38.9 ± 0.6ab	39.8 ± 0.6bc	40.0 ± 0.7c
P/S[e]		1.0 ± 0.0a	1.2 ± 0.0b	1.1 ± 0.1c	1.1 ± 0.0c	1.2 ± 0.0b
∑*n* − 6		30.1 ± 0.4a	42.6 ± 1.2b	22.2 ± 0.4c	17.3 ± 0.6d	16.8 ± 0.6d
∑*n* − 3		6.7 ± 0.7a	4.1 ± 0.3b	18.9 ± 1.2c	26.6 ± 0.7d	30.3 ± 0.4e
n − 6/*n* − 3		4.5 ± 0.5a	10.4 ± 0.8b	1.2 ± 0.1c	0.7 ± 0.1cd	0.6 ± 0.1d
DBI[f]		1.7 ± 0.0a	1.9 ± 0.1b	2.0 ± 0.1c	2.2 ± 0.1d	2.4 ± 0.0e
DI[g]		2.73	2.34	1.61	1.47	1.80

[a] Data are presented as the mean ± SD ($n = 6$ for groups 1 and 2, and $n = 7$ for groups 3, 4, and 5). Means within the same row not followed by a common letter differ significantly ($P < 0.01$). [b] PUFA, polyunsaturated fatty acid. [c] MUFA, monounsaturated fatty acid. [d] SFA, saturated fatty acid. [e] P/S, ∑PUFA/∑SFA. [f] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. [g] Desaturation index = [20:3(*n*−6) + 20:4(*n*−6)]/18:2(*n*−6). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

The fatty acid composition of total lipid of the testis from groups 2, 4, and 5 is shown in Table 8. The testis was resistant to increase in the proportions of *n* − 3 PUFA's as estimated by the low ∑*n*−3 compared to other tissues analyzed. And inversely, the value for *n* − 6 PUFA's was high as evidenced by the relatively high ratios of *n* − 6/*n* − 3 and DIs. The high *n* − 6/*n* − 3 ratio is primarily due to the *n* − 6 DPA content; higher than in any other tissue studied. This contrasts with the high proportion of *n* − 6 DTA in the brain. The proportion of DHA in testis increased with increasing intake of DHA, but the respective values for DHA in groups 2, 4, and 5 were the lowest among any tissue analyzed. Consequently, values for EPA and *n* − 3 DPA were also low. However, summed values of *n* − 6 DPA and DHA, the major 22-carbon PUFA's in the testes of most animal species, were nearly the same in all the groups. The sum of the values for all 22-carbon PUFA's, *n* − 6 DTA, *n* − 6 DPA, *n* − 3 DPA, and *n* − 3 DHA, in each group was also nearly the same. The proportions of SFAs were almost constant on the whole in all the groups.

DISCUSSION

Dose levels as well as the compositional diversity of fatty acids affect tissue fatty acid profiles, which influence membrane viscosity and permeability, resulting in variations in the enzyme activity of membrane proteins, receptors, and ion channels (Stubbs and Smith, 1984; Spector and Yorek, 1985; Lee, 1986; Murphy, 1990; Saito, 1994). The fatty acid composition of total lipids of serum was dose-dependently influenced, particularly by the dietary level of DHA (Table 2). The change in the proportion of DHA in serum lipid was accompanied by a similar change in the proportion of EPA and *n* − 3 DPA. Resultantly, the fatty acid composition of serum lipids differed markedly in each group, particularly in terms of *n* − 3 PUFA level, thereby influencing the fatty acid profile of each tissue through circulating blood.

In liver total lipid (Table 3), proportions of LA and AA in *n* − 6 PUFA's reflected dietary LA levels, where AA was thought to be converted metabolically from ingested LA because the dietary lipids did not contain a detectable amount of AA. At the same time, however, the effect of dietary DHA levels on the level of LA and AA was practically negligible. In *n* − 3 PUFA's of liver total lipid, the percent DHA increased in a dose-related manner, which conformed with increases in EPA and *n* − 3 DPA. The dietary supply of EPA and *n* − 3 DPA was very small compared with that of DHA but the proportions of these fatty acids in liver total lipid were rather high, e.g., approximately 33% and 12% of DHA, respectively, in groups 4 and 5. Hence, it is conceivable that retroconversion of DHA to EPA and *n* − 3 DPA occurred. Retroconversion of DHA to EPA has been reported in rat hepatocytes (Grønn et al., 1991), rats (Ikeda et al., 1994; Frøyland et al., 1996), and humans (Von Schacky and Weber, 1985). However, no increase

Table 5. Fatty Acid Composition (Percent) of Kidney Total Lipid in Rats Fed Diets Containing Graded Levels of DHA[a]

		group				
		1	2	3	4	5
DHA level (energy %)		0	0	1.0	3.4	8.7
LA level (energy %)		1.9	9.0	2.0	2.0	2.1
fatty acid	name					
14:0		0.6	0.5	0.5	0.5	0.5
14:1($n-7$)		1.8	1.9	2.1	2.1	2.1
16:0		23.6	23.1	23.9	22.6	23.6
16:1($n-7$)		2.2	1.6	1.6	1.6	1.5
16:2($n-7$)		0.6	0.7	0.6	0.6	0.6
18:0		14.7	15.2	14.7	15.2	15.7
18:1($n-9$)	OA	23.3	15.5	18.3	17.1	14.6
18:2($n-6$)	LA	6.3	10.8	9.3	8.4	7.4
20:0		0	0	0	0	0
20:1($n-9$)		0.4	0.3	0.3	0.3	0.3
20:2($n-6$)		0	0.3	0	0	0
20:3($n-9$)		0.4	0	0	0	0
20:3($n-6$)		0.7	0.9	0.6	0.4	0.3
20:4($n-6$)	AA	22.9	26.2	18.5	15.1	14.2
20:5($n-3$)	EPA	0	0	3.7	7.5	9.0
22:4($n-6$) + 24:1($n-9$)	DTA	0.4	0.7	0	0	0
22:5($n-6$)	DPA	0.3	0.8	0	0	0
22:5($n-3$)	DPA	0	0	0.4	0.5	0.6
22:6($n-3$)	DHA	1.8	1.5	5.3	7.8	9.5
∑PUFA[b]		33.4	41.9	38.4	40.5	41.6
∑MUFA[c]		27.7	19.3	22.3	21.1	18.5
∑SFA[d]		38.9	38.8	39.1	38.3	39.8
P/S[e]		0.9	1.1	1.0	1.1	1.0
$\sum n-6$		30.6	39.7	28.4	24.1	21.9
$\sum n-3$		1.8	1.5	9.4	15.8	19.1
$n-6/n-3$		17.0	26.5	3.0	1.5	1.1
DBI[f]		1.6	1.7	1.7	1.9	2.0
DI[g]		3.75	2.51	2.05	1.85	1.96

[a] Poole samples from six animals for groups 1 and 2, and from seven animals for groups 3, 4, and 5 were analyzed. [b] PUFA, polyunsaturated fatty acid. [c] MUFA, monounsaturated fatty acid. [d] SFA, saturated fatty acid. [e] P/S, ∑PUFA/∑SFA. [f] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. [g] Desaturation index = [20:3($n-6$) + 20:4($n-6$)]/18:2($n-6$). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

in the $n-3$ DPA level was observed after purified DHA was administered to rats (Ikeda et al., 1994; Frøyland et al., 1996). We observed an increase in the $n-3$ DPA level with increases in the dietary DHA supply although the elevation was not as high as for EPA (Table 3). This discrepancy could be ascribed to the amount of DHA administered to rats. The dietary amount of DHA in the current experiment is higher than in other studies (Ikeda et al., 1994; Frøyland et al., 1996), presuming that DHA supplied in a large amount is directly retroconverted efficiently to EPA, which is then converted to $n-3$ DPA by the elongase. This metabolic pathway has also been suggested in isolated rat liver cells by Grønn et al. (1991). The relative levels of DHA, EPA, and $n-3$ DPA in kidney lipid support this theory (Table 5). Previously (Saito et al., 1996) we reported a plasma triacylglycerol-lowering effect of DHA in rats and this effect may also be attributable to the action of EPA retroconverted from DHA. On the other hand, Kobatake et al. (1994), Ikeda et al. (1994), and Frøyland et al. (1996) observed no plasma triacylglycerol-lowering effect when purified DHA was administered in a lesser amount to rats. Anyhow, the precise metabolic pathways of retroconversion of DHA remain to be elucidated.

In the fatty acid composition of liver microsomal lipid (Table 4), higher proportions of PUFA's, lower proportions of MUFAs, and nearly the same and a constant proportion of total SFA on the whole were observed. This is ascribed to the fact that microsomal lipid is mostly composed of membrane phospholipids which contain large amounts of $n-6$ and $n-3$ PUFA's but little deposited fatty acids (Hansen, 1994). The similarity in fatty acid profiles between liver microsomal lipid and the liver phospholipids separated from total lipid (data not shown) supports this idea. However, AA is the major component of liver microsomal lipid when LA is the primary source of dietary PUFA's (group 2), whereas DHA and EPA are when DHA is the primary dietary source. In addition, DHA is relatively abundant even at the lowest dose of DHA (group 3) as compared with the value for liver total lipid shown in Table 3. It is indicated that DHA is efficiently incorporated into liver phospholipids even at a lower dose of DHA (Croset and Kinsella, 1994).

When the fatty acid profiles of serum total lipid (Table 2) and liver total lipid (Table 3) are compared, similarities are evident, particularly in the $n-3$ PUFA's such as DHA, EPA, and DPA of the DHA-administered groups. Because the dietary source of $n-3$ PUFA's is exclusively DHA, the fatty acid composition of lipids in serum appeared to reflect lipid metabolism in the liver, since lipoproteins are transported from the liver, the major organ of lipid metabolism, to serum.

In kidney lipid (Table 5), as compared with liver lipid (Table 3), the fatty acid composition is rather insensitive to dietary fatty acid changes and $n-6$ PUFA's, particularly AA, are more abundant as a whole. In $n-3$ PUFA's, the DHA content increases dose-dependently; not only was it only about a half of that of liver lipid in each DHA-administered group, but also the degree of its increase was far less than that of liver lipid. Characteristically, the value for EPA was comparable

Table 6. Fatty Acid Composition (Percent) of Brain Total Lipid in Rats Fed Diets Containing Graded Levels of DHA[a]

		group				
		1	2	3	4	5
DHA level (energy %)		0	0	1.0	3.4	8.7
LA level (energy %)		1.9	9.0	2.0	2.0	2.1
fatty acid	name					
14:0		0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
14:1($n-7$)		3.3 ± 0.1a	3.4 ± 0.2a	3.3 ± 0.2a	3.5 ± 0.2a	3.4 ± 0.3a
16:0 + 16:0 DMA[b]		21.7 ± 0.7a	22.0 ± 0.7a	21.6 ± 0.6a	22.1 ± 0.6a	21.7 ± 0.3a
16:1($n-7$)		0.7 ± 0.1a	0.5 ± 0.3a	0.6 ± 0.3a	0.7 ± 0.1a	0.7 ± 0.1a
18:0 DMA[b]		5.0 ± 0.2a	4.9 ± 0.3a	5.0 ± 0.3a	5.1 ± 0.4a	5.1 ± 0.5a
18:0 + 18:1 DMA[b]		20.4 ± 0.7a	20.5 ± 0.3a	20.3 ± 0.7a	20.1 ± 0.4a	20.1 ± 0.5a
18:1($n-9$)	OA	20.3 ± 0.5a	19.4 ± 0.3b	20.3 ± 0.3a	20.0 ± 0.3abc	19.6 ± 0.5c
18:2($n-6$)	LA	0.5 ± 0.1a	0.7 ± 0.1b	0.6 ± 0.1ab	0.5 ± 0.1ab	0.5 ± 0.1ab
20:0		0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.1a
20:1($n-9$)		1.6 ± 0.1a	1.5 ± 0.1ab	1.5 ± 0.1ab	1.5 ± 0.1ab	1.4 ± 0.1b
20:2($n-6$)		0a	0a	0a	0a	0a
20:3($n-9$)		0a	0a	0a	0a	0a
20:3($n-6$)		0.4 ± 0.0a	0.4 ± 0.0a	0.4 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.0a
20:4($n-6$)	AA	9.8 ± 0.4a	10.2 ± 0.2a	9.1 ± 0.3b	9.0 ± 0.3b	8.8 ± 0.3b
20:5($n-3$)	EPA	0a	0a	0a	0a	0a
22:4($n-6$) + 24:1($n-9$)	DTA	3.3 ± 0.1a	3.4 ± 0.1a	3.0 ± 0.1b	2.9 ± 0.1bc	2.7 ± 0.1c
22:5($n-6$)	DPA	0.5 ± 0.1a	0.8 ± 0.1b	0.2 ± 0.2c	0.3 ± 0.2ac	0.3 ± 0.2ac
22:5($n-3$)	DPA	0a	0a	0.1 ± 0.2a	0.1 ± 0.2a	0.5 ± 0.4b
22:6($n-3$)	DHA	12.0 ± 0.3a	11.9 ± 0.4a	13.3 ± 0.4b	13.2 ± 0.5b	14.1 ± 0.7c
∑PUFA[c]		26.5 ± 0.7a	27.4 ± 0.6ab	26.9 ± 0.5ab	26.5 ± 0.7a	27.6 ± 0.7b
∑MUFA[d]		25.9 ± 0.6a	24.9 ± 0.6b	25.7 ± 0.4a	25.7 ± 0.4a	25.1 ± 0.6ab
∑SFA[e]		47.5 ± 0.4a	47.8 ± 0.4a	47.3 ± 0.2a	47.7 ± 0.4a	47.3 ± 0.4a
P/S[f]		0.6 ± 0.1a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.1a	0.6 ± 0.0a
$\sum n-6$		14.5 ± 0.4a	15.5 ± 0.3b	13.5 ± 0.4c	13.1 ± 0.4c	13.0 ± 0.3c
$\sum n-3$		12.0 ± 0.3a	11.9 ± 0.4a	13.4 ± 0.3b	13.4 ± 0.5b	14.8 ± 0.9c
$n-6/n-3$		1.2 ± 0.0a	1.3 ± 0.0b	1.0 ± 0.0c	1.0 ± 0.0c	0.9 ± 0.1d
DBI[g]		1.5 ± 0.1a	1.6 ± 0.0ab	1.6 ± 0.0ab	1.6 ± 0.1a	1.6 ± 0.1b
DI[h]		21.68	16.28	15.97	18.86	17.45

[a] Data are presented as the mean ± SD ($n = 6$ for groups 1 and 2, and $n = 7$ for groups 3, 4, and 5). Means within the same row not followed by a common letter differ significantly ($P < 0.01$). [b] Dimethylacetal. [c] PUFA, polyunsaturated fatty acid. [d] MUFA, monounsaturated fatty acid. [e] SFA, saturated fatty acid. [f] P/S, ∑PUFA/∑SFA. [g] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. [h] Desaturation index = [20:3($n-6$) + 20:4($n-6$)]/18:2($n-6$). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

to that of DHA in each DHA-administered group in kidney lipid. It is conceivable that the level of activity for incorporating DHA from serum lipid is low in the kidney, or the activity of retroconversion of DHA to EPA is high. The precise mechanisms for this change remain to be elucidated.

The fatty acid composition of lipids was less variable in the brain (Table 6) than in the other tissues studied. Similar results have been obtained for α-linolenic acid deficiency (Bourre et al., 1992) and DHA administration (Taniguchi et al., 1993) in rats. However, a tendency in response to the graded supply of DHA is to increase the amounts of DHA. Characteristically, little LA but quite a lot of $n - 6$ DTA is present in brain compared to serum and other tissues even in the DHA-administered groups, suggesting high activity of elongase to elongate AA to $n - 6$ DTA. EPA was not detected and $n - 3$ DPA was present in only a tiny amount. However, the values for DHA among the DHA-free diet groups (groups 1 and 2) were the highest of all the tissues studied. This seems to be associated with the fact that brain tissue takes up DHA selectively from lipids in circulating blood through the blood−brain barrier although the level of DHA in serum lipid was low in the groups not given DHA. The incorporation of dietary PUFA's, particularly AA and DHA, into brain phospholipids of many animal species including rats has been described (Crawford et al., 1976). Anyway, in the brain, the percent SFA in lipids is, on the whole, quite high, approximately 50%, whereas the percent PUFA is somewhat lower.

The fatty acid composition of lipids in the heart (Table 7) varied characteristically compared with that of the dietary (Table 1) and serum (Table 2) lipids. The percent DHA in the DHA-administered groups was higher in heart than in serum and in other tissues. Even in the group given the lowest level of DHA (group 3; Table 7), the proportion of DHA was comparable to that of the serum, liver, and liver microsomal lipids of the groups given the most DHA (group 5; Tables 2, 3, and 4, respectively). Because more than 80% of heart total lipid is known to be composed of phospholipids, the fatty acid composition of heart lipid is almost the same as that of membrane phospholipids (Nalbone et al., 1989). Croset and Kinsella (1989) observed that DHA is strongly taken up by mouse heart phospholipids, even in the presence of high dietary LA, i.e., 50 mol %. However, as compared with serum and liver lipids, little EPA is present in heart lipid. Hence, overall, the percent $n - 3$ PUFA's in heart lipids is almost the same as that in liver microsomal lipids in the DHA-administered groups. In addition, the increased DHA content concomitant with the low value for EPA may indicate that the heart incorporates DHA efficiently from lipids in circulating blood but the activity of retroconversion of DHA to EPA is very low. Alternatively, as suggested by Croset and Kinsella (1989), cardiac tissue may have a requirement for 22-carbon chain fatty acids, which is

Table 7. Fatty Acid Composition (Percent) of Heart Total Lipid in Rats Fed Diets Containing Graded Levels of DHA[a]

		group				
		1	2	3	4	5
DHA level (energy %)		0	0	1.0	3.4	8.7
LA level (energy %)		1.9	9.0	2.0	2.0	2.1
fatty acid	name					
14:0		0.3 ± 0.1a	0.2 ± 0.1a	0.2 ± 0.1a	0.1 ± 0.1b	0.1 ± 0.1b
14:1(*n*−7)		2.0 ± 0.1a	1.9 ± 0.2a	1.7 ± 0.1b	1.6 ± 0.2b	1.5 ± 0.1b
16:0		13.2 ± 1.1ab	12.9 ± 0.5a	14.0 ± 0.5b	13.2 ± 0.4ab	12.8 ± 0.7a
16:1(*n*−7)		0.9 ± 0.3a	0.6 ± 0.2a	0.8 ± 0.2a	0.7 ± 0.3a	0.7 ± 0.2a
18:0 DMA[b]		0.7 ± 0.1a	0.8 ± 0.1a	0.8 ± 0.1a	0.8 ± 0.1s	0.7 ± 0.1a
18:0		21.4 ± 0.7a	21.4 ± 0.6a	20.4 ± 0.6a	20.7 ± 1.0a	20.9 ± 1.0a
18:1(*n*−9)	OA	17.2 ± 2.2a	12.5 ± 1.1b	16.4 ± 1.7a	13.4 ± 1.6b	11.1 ± 1.1b
18:2(*n*−6)	LA	11.3 ± 1.8a	16.5 ± 1.3b	9.3 ± 0.8c	9.0 ± 0.9c	7.0 ± 0.9d
20:0		0a	0.1 ± 0.1b	0a	0a	0a
20:1(*n*−9)		0.3 ± 0.1ab	0.3 ± 0.1ab	0.3 ± 0.0ab	0.2 ± 0.1a	0.4 ± 0.1b
20:2(*n*−6)		0a	0.1 ± 0.1b	0a	0.0 ± 0.1a	0.0 ± 0.1a
20:3(*n*−9)		0.5 ± 0.1a	0b	0b	0b	0b
20:3(*n*−6)		0.5 ± 0.2a	0.3 ± 0.1b	0.4 ± 0.1a	0.3 ± 0.1b	0.2 ± 0.0b
20:4(*n*−6)	AA	19.5 ± 0.8a	19.5 ± 0.6a	12.1 ± 0.4b	10.3 ± 0.5c	9.5 ± 0.7c
20:5(*n*−3)	EPA	0.4 ± 0.4a	0.1 ± 0.2a	0.5 ± 0.2a	1.0 ± 0.2b	1.1 ± 0.3b
22:4(*n*−6) + 24:1(*n*−9)	DTA	1.0 ± 0.1a	2.0 ± 0.2b	0.3 ± 0.0c	0.2 ± 0.1c	0.3 ± 0.1c
22:5(*n*−6)	DPA	1.1 ± 0.1ac	3.3 ± 0.8b	0.6 ± 0.1a	0.9 ± 0.1ac	1.2 ± 0.1c
22:5(*n*−3)	DPA	1.3 ± 0.3ab	1.1 ± 0.6a	1.5 ± 0.4ab	1.7 ± 0.3ab	1.8 ± 0.3b
22:6(*n*−3)	DHA	8.5 ± 1.0a	6.5 ± 0.7a	20.5 ± 1.6b	25.7 ± 1.6c	30.4 ± 1.3d
others		0a	0a	0.1 ± 0.2a	0.2 ± 0.2a	0.2 ± 0.2a
∑PUFA[c]		44.0 ± 3.2a	49.4 ± 1.1b	45.1 ± 1.7a	49.0 ± 1.2b	51.5 ± 0.9b
∑MUFA[d]		20.4 ± 2.5a	15.2 ± 1.2b	19.2 ± 1.8a	16.0 ± 1.7b	13.7 ± 1.2b
∑SFA[e]		35.4 ± 0.9a	35.5 ± 0.8a	35.4 ± 0.3a	34.8 ± 0.9a	34.5 ± 0.6a
P/S[f]		1.2 ± 0.1a	1.4 ± 0.1bc	1.3 ± 0.1ab	1.4 ± 0.0c	1.5 ± 0.0c
∑*n* − 6		33.4 ± 2.0a	41.7 ± 1.5b	22.6 ± 0.9c	20.9 ± 1.0c	18.1 ± 1.3d
∑*n* − 3		10.1 ± 1.6a	7.6 ± 1.1a	22.5 ± 2.1b	28.4 ± 1.8c	33.4 ± 1.2d
n − 6/*n* − 3		3.4 ± 0.5a	5.6 ± 1.1b	1.0 ± 0.1c	0.7 ± 0.1c	0.5 ± 0.1c
DBI[g]		1.9 ± 0.1a	2.0 ± 0.1a	2.3 ± 0.1b	2.5 ± 0.1c	2.7 ± 0.1d
DI[h]		1.76	1.20	1.34	1.18	1.39

[a] Data are presented as the mean ± SD ($n = 6$ for groups 1 and 2, and $n = 7$ for groups 3, 4, and 5). Means within the same row not followed by a common letter differ significantly ($P < 0.01$). [b] Dimethylacetal. [c] PUFA, polyunsaturated fatty acid. [d] MUFA, monounsaturated fatty acid. [e] SFA, saturated fatty acid. [f] P/S, ∑PUFA/∑SFA. [g] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. [h] Desaturation index = [20:3(*n*−6) + 20:4(*n*−6)]/18:2(*n*−6). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

not specific for DHA: Values for *n* − 6 DPA as well as *n* − 3 DPA were somewhat higher in heart even in the DHA-administered groups than in serum or in the other tissues except testis. On the other hand, the values for LA and AA decreased as the dietary level of DHA increased. This suggests that DHA, competing with LA and AA, is preferentially incorporated at the *sn*-2 positions of cardiac membrane phospholipids.

It has been reported that modifying the fatty acid composition of heart mitochondrial cardiolipin by feeding a high fish oil diet diminishes respiratory function (Yamaoka et al., 1988). In the current study as well as that by Croset and Kinsella (1989), DHA was shown to be preferentially incorporated into heart lipids even at the lowest dose of DHA. Accordingly, higher intake of DHA might cause functional disorder of the heart. Further investigation into the relationship between incorporation of DHA in cardiac organelle phospholipids and functional disorder is needed.

As shown in Table 8, the testis differed characteristically in fatty acid composition from the brain and heart. In the testis, high affinity for *n* − 6 PUFA's was noticed as evidenced by the higher *n* − 6/*n* − 3 ratios and DIs even in the DHA-administered groups. This is primarily due to the large amount of *n* − 6 DPA, comparable to AA. In addition, the proportion of *n* − 6 DPA did not change dose-dependently in the DHA-administered groups. Bourre et al. (1992) also observed no reduction in *n* − 6 DPA in testes of α-linolenic acid deficient adults rats. This suggests that *n* − 6 DPA of testis tissue is resistant to dietary manipulation as was the case in brain tissue. In *n* − 3 PUFA's, the DHA content increased dose-dependently but was still low compared with the other tissues analyzed. This was also the case for EPA and *n* − 3 DPA. However, the summed values for *n* − 6 DPA and DHA, the major 22-carbon PUFA's in the testes of most animal species (Bieri and Prival, 1965), were almost the same in all the groups, as were the values for all 22-carbon PUFA's. A reciprocal relationship may exist between *n* − 6 DPA and DHA in the testis with *n* − 6 DPA being the preferred fatty acid in rats. Bieri and Prival (1965) report that each animal species has a characteristic testis fatty acid composition and *n* − 6 DPA is the major component in rats, whereas it is DHA in humans. Accordingly, although rat testis prevails in *n* − 6 PUFA metabolism, testis lipids are relatively insensitive to exogenous fatty acid changes even after high intake of DHA as also suggested elsewhere (Astorg et al., 1987; Chanmugam et al., 1991).

In conclusion, each tissue had its own peculiar composition of fatty acids differing from that of circulating serum. This composition was basically influenced dose-dependently by the dietary lipids with graded levels of DHA, but to different degrees in different tissues. Those of brain were most resistant and of heart most susceptible to dietary DHA.

Table 8. Fatty Acid Composition (Percent) of Testis Total Lipid in Rats Fed Diets Containing Graded Levels of DHA[a]

		group		
		2	4	5
DHA level (energy %)		0	3.4	8.7
LA level (energy %)		9.0	2.0	2.1
fatty acid	name			
14:0		0.4 ± 0.2a	0.4 ± 0.1a	0.5 ± 0.1a
14:1(*n*−7)		2.4 ± 0.3a	2.5 ± 0.1a	2.6 ± 0.3a
16:0		30.4 ± 1.3a	31.3 ± 0.4a	31.4 ± 0.5a
16:1(*n*−7)		1.9 ± 0.8a	2.3 ± 0.5a	2.6 ± 0.8a
16:2(*n*−7)		0.7 ± 0.2a	0.8 ± 0.2a	0.7 ± 0.1a
18:0		6.1 ± 0.8a	6.0 ± 0.4a	5.8 ± 0.2a
18:1(*n*−9)	OA	16.6 ± 3.1a	19.1 ± 1.9a	18.5 ± 1.2a
18:2(*n*−6)	LA	5.9 ± 2.0a	4.3 ± 0.3ab	3.6 ± 0.2b
20:0		0a	0a	0a
20:1(*n*−9)		0.2 ± 0.1a	0.3 ± 0.1a	0.4 ± 0.1a
20:3(*n*−9)		0.1 ± 0.2a	0.5 ± 0.1b	0.5 ± 0.1b
20:3(*n*−6)		0.6 ± 0.1a	1.0 ± 0.1b	0.9 ± 0.1b
20:4(*n*−6)	AA	14.1 ± 1.6a	11.5 ± 0.9b	11.3 ± 0.5b
20:5(*n*−3)	EPA	0.5 ± 0.6a	0.7 ± 0.2a	1.2 ± 0.5a
22:4(*n*−6) + 24:1(*n*−9)	DTA	2.1 ± 0.3a	1.1 ± 0.1b	1.1 ± 0.3b
22:5(*n*−6)	DPA	14.2 ± 1.8a	11.0 ± 1.0b	9.6 ± 0.9b
22:5(*n*−3)	DPA	0a	0.5 ± 0.0b	0.6 ± 0.1c
22:6(*n*−3)	DHA	1.3 ± 0.3a	4.6 ± 0.5b	6.1 ± 0.8c
others		2.4 ± 0.5a	2.3 ± 0.4a	2.0 ± 0.3a
∑PUFA[b]		39.4 ± 2.3a	35.9 ± 1.9b	35.6 ± 1.0b
∑MUFA[c]		21.1 ± 3.7a	24.1 ± 2.3a	24.1 ± 1.6a
∑SFA[d]		36.9 ± 1.9a	37.7 ± 0.5a	37.7 ± 0.6a
P/S[e]		1.1 ± 0.1a	1.0 ± 0.1b	0.9 ± 0.1b
∑*n* − 6		36.8 ± 2.4a	28.9 ± 1.9b	26.5 ± 1.0b
∑*n* − 3		1.8 ± 0.8a	5.8 ± 0.7b	7.6 ± 0.6c
n − 6/*n* − 3		24.1 ± 10.7a	5.1 ± 0.8b	3.4 ± 0.4b
DBI[f]		1.8 ± 0.1a	1.8 ± 0.1a	1.8 ± 0.0a
DI[g]		2.48	2.92	3.37

[a] Data are presented as the mean ± SD ($n = 6$ for group 2, and $n = 7$ for groups 4 and 5). Means within the same row not followed by a common letter differ significantly ($P < 0.01$). [b] PUFA, polyunsaturated fatty acid. [c] MUFA, monounsaturated fatty acid. [d] SFA, saturated fatty acid. [e] P/S, ∑PUFA/∑SFA. [f] Double bond index expresses mean double bond number and is the sum of the fraction of each fatty acid × the number of double bonds in that acid. [g] Desaturation index = [20:3(*n*−6) + 20:4(*n*−6)]/18:2(*n*−6). OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

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