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Sex Differences in n-3 and n-6 Fatty Acid Metabolism in EFA-Depleted Rats (42547)

YUNG-SHENG HUANG AND DAVID F. HORROBIN

Efamol Research Institute, Kentville, Nova Scotia, Canada B4N 4H8

Abstract. We studied the effect of sex on the distribution of long-chain n-3 and n-6 fatty acids in essential fatty acid-deficient rats fed γ-linolenate (GLA) concentrate and/or eicosapentaenoate and docosahexaenoate-rich fish oil (FO). Male and female weanling rats were rendered essential fatty acid deficient by maintaining them on a fat-free semisynthetic diet for 8 weeks. Thereafter, animals of each sex were separated into three groups (n = 6) and given, for 2 consecutive days by gastric intubation, 4 g/kg body wt per day of GLA concentrate (containing 84% 18:2n-6), n-3 fatty acid-rich FO (containing 18% 20:5n-3 and 52% 22:6n-3), or an equal mixture of the two oil preparations (GLA + FO). The fatty acid distributions in plasma and liver lipids were then examined. GLA treatment increased the levels of C-20 and C-22 n-6 fatty acids in all lipid fractions indicating that GLA was rapidly metabolized. However, the increases in 20:3n-6 were less in females than those in males, while those in 20:4n-6 were greater, suggesting that the conversion of 20:3n-6 to 20:4n-6 was more active in female than in male rats. FO treatment increased the levels of 20:5n-3 and 22:6n-3 and reduced those of 20:4n-6. The increase in n-3 fatty acids was greater in females than that in males and the reduction in 20:4n-6 was smaller. Consequently, the sum of total long-chain EFAs incorporated was greater in females than that in males. The administration of n-3 fatty acids also reduced the ratio of 20:4n-6 to 20:3n-6 in GLA + FO-treated rats indicating that n-3 fatty acids inhibited the activity of δ-5-desaturase. However, this effect was not affected by the sex difference.

Male animals are generally more susceptible than female animals to essential fatty acid (EFA) deficiency (1–8). Female rats retain higher levels of 20:4n-6 (arachidonic acid, AA) in tissues during EFA deficiency (3, 7). Female sex hormones may facilitate the conversion of 18:2n-6 (linoleic acid, LA) to AA (7, 8). This view was supported by our previous studies of animals treated with an antiestrogen drug, tamoxifen, which inhibits the conversion of LA to AA (9). Generally, the formation of AA from LA in mammals requires the function of two desaturases and one elongase. LA is converted to 18:3n-6 (γ-linolenic acid, GLA) by δ-6-desaturase (D6D) (10, 11). GLA is rapidly elongated to 20:3n-6 (dihomo-γ-linolenic acid, DGLA) and subsequently desaturated by δ-5-desaturase (D5D) to AA (10–12). The purpose of the present study was to locate the possible biochemical step which is modulated by sex hormones. We fed to EFA-deficient rats a GLA concentrate which bypasses the regulating step D6D (10, 11) and provides the DGLA substrate for D5D activity. This enabled us to assess the effect of sex on D5D activity without the complication of D6D activity by examining the ratios of 20:4n-6/20:3n-6 in various lipid fractions. Previously, Brenner and Peluffo (13) showed that 22:6n-3 (docosahexaenoic acid, DHA)—a long-chain n-3 metabolite—substantially inhibited the desaturation of 18:2n-6 (13), while we have reported that 20:5n-3 (eicosapentaenoic acid, EPA)—another major long-chain n-3 metabolite—suppressed the conversion of DGLA to AA (14). Since n-3 metabolites exerted effects similar to those found in male animals, we compared the effects of sex on n-6 fatty acid metabolism in the absence or presence of the C-20 and C-22 n-3 fatty acids.

Materials and Methods. *Animals and diets.* Twenty-four male and female weanling Sprague–Dawley rats (Charles River Breeding Laboratories, Montreal, Quebec) were maintained on a fat-free (FF) semisynthetic diet (Teklad Test Diets, Madison, WI) for a period of 8 weeks to render them EFA deficient. The composition of the FF diet, which has been fully described previously (15), was 20% vitamin-free casein, 70.2% sucrose, 5% cellulose, 3.5% mineral mix (AIN-76), 1% vitamin mix, and 0.3% DL-methionine. Prior to the feeding, six animals of each sex (group D, EFA deficient) were killed in the fed state by exsanguination under light ether anesthesia. Blood was collected by heart puncture into test tubes

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containing EDTA (1 mg/ml blood). Plasma was separated by centrifugation and stored at −20°C. Livers were rapidly excised, rinsed, blotted, weighed, and frozen. The remaining animals of each sex were randomly divided into three groups of six rats each. Animals were maintained on the FF diet and were in addition given daily by gastric intubation 4g/kg body wt of (i) GLA concentrate (group G), (ii) EPA and DHA-rich fish oil (FO) (group F), or (iii) an equal mixture of the two oil preparations (group M) for 2 days. GLA concentrate (containing 16% LA and 84% GLA ethyl esters) was prepared from evening primrose oil (Efamol Ltd., Guildford, UK), while EPA and DHA-rich fish oil (containing 18% EPA and 52% DHA ethyl esters) was prepared from fish oil (POLEPA, a product of Efamol Ltd.) by the method of Haagsma *et al.* (16). Animals were then sacrificed and plasma and liver were collected as described above.

Lipid analyses. Plasma and liver lipids were extracted according to the method of Folch *et al.* (17). Lipid fractions, i.e., cholesteryl esters (CE), triglycerides (TG), and total phospholipids (PL), were separated by thin-layer chromatography. Fatty acids of plasma and liver lipids were methylated and analyzed by gas–liquid chromatography (GLC) as described previously (18). Plasma cholesterol and TG levels were measured enzymatically using a Cobas-Bio centrifugal analyzer (Hoffmann–La Roche, Etobicoke, Ontario). The concentrations of liver cholesterol (19) and TG were determined by GLC using 5α-cholestane and triheptodecanoin as internal standards. Plasma and liver PL were quantitated by the method of Bartlett (20).

Statistics. Statistical comparisons between lipid fatty acid compositions in male and female rats and the effects of different fat administrations were made using two-way analysis of variance and Student's *t* test.

Results. In the present study, the male rats grew significantly faster than the female rats during the period of fat deprivation. The growth rates of both male and female rats under a similar condition has been shown previously (21). To adjust for the difference in body sizes between the male and the female rats, the dosage of either GLA concentrate or n-3 fatty acid-rich FO was given according to body weight.

There were no significant differences in total plasma and liver lipid concentrations between the sexes and among different dietary groups. Therefore, the data on fatty acid percentage composition reflect closely the absolute fatty acid levels. Since percentages of n-3 and n-6 fatty acids in PL and CE were significantly higher than those in TG, we presented in this report only the changes observed in plasma and liver PL and CE.

Figures 1–4 show the fatty acid compositions of plasma and liver PL and CE in male and female EFA-deficient rats administered different oil preparations. The changes are summarized as follows:

(i) The levels of 20:3n-9 in all lipid fractions

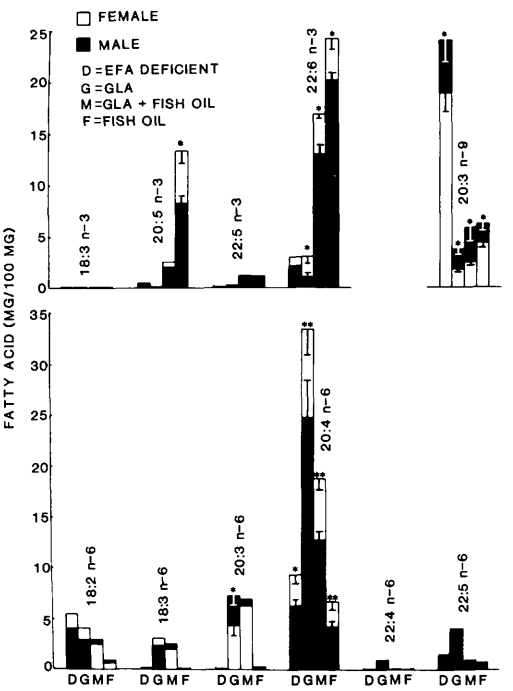

FIG. 1. The distribution of n-3, n-6, and n-9 fatty acids in plasma phospholipids of male (■) and female (□) rats. All data are means of six determinations. The bars represent, in sequence, samples collected from EFA-deficient animals (group D), and EFA-deficient animals receiving daily by gastric intubation 4 g/kg body wt of GLA concentrate alone (group G), an equal mixture of GLA concentrate and EPA and DHA-enriched fish oil (group M), or fish oil alone (group F) for 2 days. *,**: Comparisons between male and female rats showed a significant difference at $P < 0.05$ and 0.01, respectively. Vertical line represents SEM.

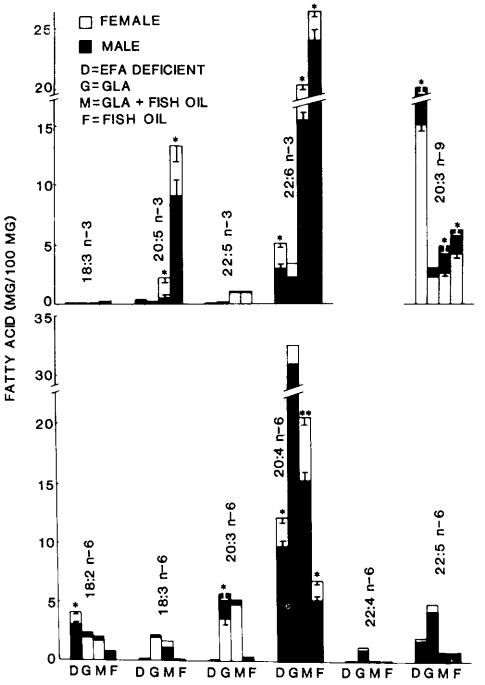

FIG. 2. The distribution of n-3, n-6, and n-9 fatty acids in liver phospholipids of male (■) and female (□) rats. All data are means of six determinations. The bars represent, in sequence, samples collected from EFA-deficient animals (group D), EFA-deficient animals receiving daily by gastric intubation 4 g/kg body wt of GLA concentrate alone (group G), an equal mixture of GLA concentrate and EPA and DHA-enriched fish oil (group M), or fish oil alone (group F) for 2 days. *,**: Comparisons between male and female rats showed a significant difference at $P < 0.05$ and 0.01, respectively. Vertical line represents SEM.

were significantly elevated in animals deprived of fats for 8 weeks, confirming the presence of EFA deficiency. Following 2 days of fat feeding, the levels of 20:3n-9 were significantly reduced in plasma PL and CE and in liver PL. The reduction was greatest in the GLA-treated rats (group G), followed by GLA + FO- (group M) and FO- (group F) treated rats. The levels of 20:3n-9 were consistently lower in female than in male rats, regardless of fat treatment.

(ii) The levels of n-3 fatty acids in all lipid fractions in the nontreated (group D) or GLA-treated (group G) EFA-deficient rats were generally very low. Administration of FO to EFA-deficient rats (groups M and F) rapidly elevated the levels of 20:5n-3 and 22:6n-3 and to a lesser extent the levels of 22:5n-3. The elevations of 20:5n-3 and 22:6n-3 were directly related to the increasing dietary n-3 acid contents. In group F, 22:6n-3 was the major n-3 fatty acid in plasma and liver PL, whereas 20:5n-3 was the major n-3 acid in plasma CE. In liver CE, the levels of 20:5n-3 and 22:6n-3 were equally important. In all lipid fractions, the levels of n-3 fatty acids were significantly higher in female than in male rats.

(iii) Prior to the feeding, the levels of 18:2n-6 and 20:4n-6 were low in EFA-deficient rats (group D). GLA treatment (group G) significantly elevated the levels of 18:3n-6, 20:3n-6, 22:4n-6, and 22:5n-6, and particularly the levels of 20:4n-6. With the exception of 20:3n-6, the levels of n-6 fatty acids were generally

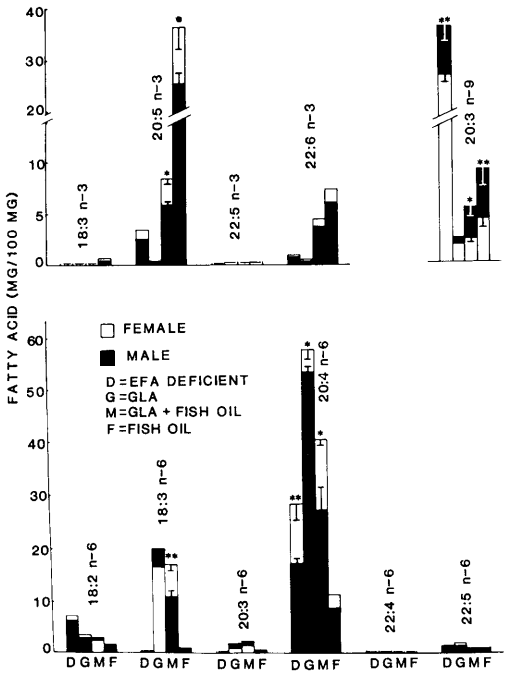

FIG. 3. The distribution of n-3, n-6, and n-9 fatty acids in plasma cholesteryl esters of male (■) and female (□) rats. All data are means of six determinations. The bars represent, in sequence, samples collected from EFA-deficient animals (group D), EFA-deficient animals receiving daily by gastric intubation 4 g/kg body wt of GLA concentrate alone (group G), an equal mixture of GLA concentrate and EPA and DHA-enriched fish oil (group M), or fish oil alone (group F) for 2 days. *,**: Comparisons between male and female rats showed a significant difference at $P < 0.05$ and 0.01, respectively. Vertical line represents SEM.

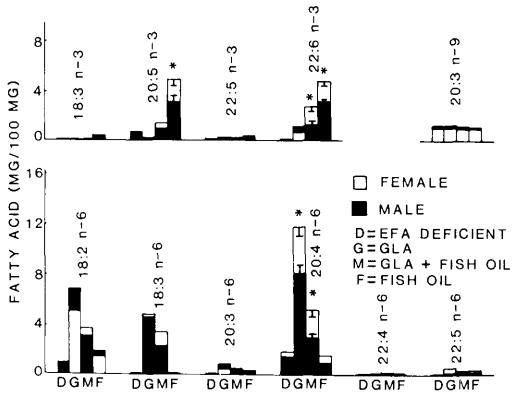

FIG. 4. The distribution of n-3, n-6, and n-9 fatty acids in liver cholesteryl esters of male (■) and female (□) rats. All data are means of six determinations. The bars represent, in sequence, samples collected from EFA-deficient animals (group D), EFA-deficient animals receiving daily by gastric intubation 4 g/kg body wt of GLA concentrate alone (group G), an equal mixture of GLA concentrate and EPA and DHA-enriched fish oil (group M), or fish oil alone (group F) for 2 days. *,**: Comparisons between male and female rats showed a significant difference at $P < 0.05$ and 0.01, respectively. Vertical line represents SEM.

higher in female than in male rats. When dietary GLA was replaced partly (50%) by fish oil, the levels of 20:4n-6, 22:4n-6, and 22:5n-6 fatty acids in all lipid fractions were all significantly reduced, whereas those of 20:3n-6 were not affected (in male rats) or were increased (in female rats). When dietary GLA was replaced wholly with fish oil, significant amounts of 20:4n-6 could be observed only in plasma and liver PL and in plasma CE.

Discussion. In this study, both male and female rats deprived of fat for 8 weeks showed a significant elevation in 20:3n-9 and decreases in n-3 and n-6 fatty acid levels which clearly demonstrated the presence of EFA deficiency. Administration of n-3 and/or n-6 fatty acids for a period of only 2 days rapidly reduced the levels of 20:3n-9, while it elevated the total levels of n-3 and n-6 fatty acids. These changes indicated that the biochemical features of EFA deficiency were rapidly reversed by either n-3 or n-6 fatty acids, although the effectiveness of n-3 and n-6 fatty acids was not exactly equal. Our results also showed a consistently higher level of 20:3n-9 and lower incorporation of n-3 and n-6 fatty acids in male than in female rats (Table I), confirming the reports that the female rat is less susceptible to EFA deficiency (1–8). The differences between the sexes in EFA incorporation were considerably greater in the animals given fish oil. Total EFA incorporation in both sexes was substantially less in the fish oil groups. However, if EFAs are expressed in relation to the number of double bonds, incorporation in fish oil group was not different from (in CE) or even greater than those in GLA groups (Table II).

GLA treatment (group G) elevated the levels of 20:4n-6 indicating that conversion of 18:3n-6 to 20:4n-6 was rapid. However, the increase in the intermediate 20:3n-6 was greater, whereas that of 20:4n-6 was smaller in male than in female rats. This resulted in a lower 20:4n-6/20:3n-6 ratio in male rats and suggests a lower rate of conversion of 20:3n-6 to 20:4n-6 in males (Table III). Partial replacement of GLA with FO (group M) significantly reduced the levels of 20:4n-6, whereas it had no effect on or even increased the levels of 20:3n-6, suggesting that FO reduced the ratios of 20:

TABLE I. THE SUM OF THE PERCENTAGE OF n-3 AND n-6 FATTY ACIDS PRESENT IN VARIOUS LIPID FRACTIONS IN MALE AND FEMALE EFA-DEFICIENT RATS TREATED DAILY BY GASTRIC INTUBATION WITH 4 g/kg BODY WEIGHT OF GLA CONCENTRATE (GROUP G), AN EQUAL MIXTURE OF GLA AND EPA AND DHA-ENRICHED FISH OIL (GROUP M), OR FISH OIL ALONE (GROUP F) FOR 2 DAYS[a]

	Group G		Group M		Group F	
	Male	Female	Male	Female	Male	Female
Plasma CE	80.7 ± 2.1	82.8 ± 0.3	55.0 ± 6.4	77.1 ± 3.7**	45.4 ± 7.9	61.2 ± 10.2*
PL	43.5 ± 15.2	53.8 ± 4.4	42.0 ± 5.1	51.5 ± 2.9**	35.9 ± 1.1	46.8 ± 3.1**
Liver CE	23.0 ± 2.3	25.0 ± 3.0	12.2 ± 4.2	18.9 ± 4.1*	10.2 ± 3.6	14.0 ± 2.3
PL	50.8 ± 1.4	48.2 ± 1.7	42.7 ± 3.6	53.0 ± 2.3**	42.2 ± 4.1	50.0 ± 4.9*

[a] Means ± SD ($n = 6$).
* $P < 0.02$; ** $P < 0.001$.

TABLE II. EFFECT OF SEX ON THE DEGREE OF UNSATURATION[a] OF PLASMA AND LIVER LIPIDS IN EFA-DEFICIENT RATS TREATED DAILY BY GASTRIC INTUBATION WITH 4 g/kg BODY WEIGHT PER DAY OF GLA CONCENTRATE (GROUP G), AN EQUAL MIXTURE OF GLA CONCENTRATE AND EPA AND DHA-ENRICHED FISH OIL (GROUP M), OR FISH OIL ALONE (GROUP F) FOR 2 CONSECUTIVE DAYS

	Group		Group M		Group F	
	Male	Female	Male	Female	Male	Female
Plasma CE	306.0	313.4	230.5	269.9	242.5	297.1
PL	184.6	204.3	214.6	246.1	226.3	272.9
Liver CE	79.3	88.4	46.9	74.6	52.1	71.9
PL	209.1	202.6	210.2	255.7	244.3	267.7

[a] Degree of unsaturation = sum of (percentage of polyunsaturated fatty acid × number of double bonds).

4n-6/20:3n-6 (Table III). This confirms our previous report that fish oil can exert an inhibitory effect on δ-5-desaturation (14). This effect was not affected by the sex difference.

Administration of FO to EFA-deficient rats (group M) also elevated the levels of 20:5n-3 and 22:6n-3 in plasma and liver lipids. The ratios of 22:6n-3/20:5n-3 in plasma PL were 6.5 and 6.6 and in liver PL were 9.6 and 9.1 respectively for male and female rats (Table IV) and were significantly higher than that (2.9) in the dietary fish oil. On the other hand, the ratios of 22:6n-3/20:5n-3 in plasma CE (0.6 and 0.5) and liver CE (1.4 and 2.0) were lower than the 22:6n-3/20:5n-3 ratio in the diet, indicating that the incorporations of 20:5n-3 and 22:6n-3 into lipid fractions might be specific. The high levels of 20:4n-6 in plasma and liver PL of GLA + FO rats (group M) might have competed with the incorporation of 20:5n-3 into PL fractions (22, 23). Indeed in rats given no n-6 fatty acids (group F), where the levels of 20:4n-6 were reduced, the incorporation of 20:5n-3 was significantly elevated as shown by the low 22:6n-3/20:5n-3 ratios in this group (Table IV). The levels of 20:4n-6 in plasma and liver CE in GLA-treated rats (group G) were high, whereas those of C-22 n-6 fatty acids were very low. Similarly, the levels of 20:5n-3 in FO-treated rats were very high, whereas those of 22:6n-3, particularly in plasma CE, were less significant. These observations suggest that the esterification of cholesterol was more specific to C-20 than to C-22 acids. In plasma, lecithin–cholesterol acyltransferase (LCAT) is responsible for the esterification of cholesterol (24). Sgoutas (25) has demonstrated that the specificity of LCAT in rat plasma favors 20:4n-6 as a substrate. Our results showed that 20:5n-3 might become the alternate favorite in the absence or at low levels of 20:4n-6.

TABLE III. EFFECT OF SEX ON 20:4n-6/20:3n-6 RATIOS IN PLASMA AND LIVER LIPIDS OF EFA-DEFICIENT RATS TREATED DAILY BY GASTRIC INTUBATION WITH 4 g/kg BODY WEIGHT PER DAY OF GLA CONCENTRATE OR AN EQUAL MIXTURE OF GLA CONCENTRATE AND EPA AND DHA-ENRICHED FISH OIL FOR 2 DAYS

	Phospholipids		Cholesteryl esters	
	Male	Female	Male	Female
Group G				
Plasma	3.4	7.7	28.4	62.1
Liver	5.6	8.4	9.7	16.5
Group M				
Plasma	1.8	2.9	11.1	20.6
Liver	2.9	4.2	4.1	7.2

TABLE IV. EFFECT OF SEX ON 22:6n-3/20:5n-3 RATIOS IN PLASMA AND LIVER LIPIDS OF EFA-DEFICIENT RATS GIVEN 4 g/kg BODY WEIGHT PER DAY BY GASTRIC INTUBATION OF AN EQUAL MIXTURE OF GLA CONCENTRATE, EPA AND DHA-ENRICHED FISH OIL, OR FISH OIL ALONE FOR 2 DAYS

	Phospholipids		Cholesteryl esters	
	Male	Female	Male	Female
Group G				
Plasma	6.5	6.6	0.6	0.5
Liver	9.6	9.1	1.4	2.0
Group M				
Plasma	2.4	1.9	0.3	0.2
Liver	2.6	2.0	1.0	1.0

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