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Differences in long chain polyunsaturates composition and metabolism in male and female rats ☆

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ABSTRACT

Human studies and some animal work have shown more docosahexaenoic acid (DHA) and arachidonic acid (ARA) was accumulated or converted from precursors in females compared to males. This study explored in-depth the effect of gender on fatty acid composition and polyunsaturated fatty acid metabolism in rats fed one of two well-defined diets containing 10% total fat. One diet contained 15% of linoleic acid (LA) and 3% of α-linolenic acid (ALA) of the total fatty acids (LA+ALA diet), while the other diet contained 15% LA and 0.05% ALA (LA diet). At the age of 20 weeks, all animals were orally administered a single dose of a mixture of deuterium-labeled LA and ALA. Caudal venous blood was then drawn at 0, 2, 4, 8, 12, 24, 48, 96 and 168 h. The concentrations of the deuterated precursors and their metabolites in plasma total lipids were quantified by GC/MS negative chemical ionization. Endogenous fatty acids were quantified by GC/FID analysis. When expressed as the percentage of oral dosage, female rats accumulated more precursors and more products, deuterated DHA and deuterated n-6 docosapentaenoic acid ($^2H_5$-DPAn-6), in plasma than did male rats in both the LA+ALA diet and the LA diet. For the endogenous non-labeled PUFA, greater concentrations of DHA and DPAn-6 were similarly observed in female rats compared to males within each diet. A lower concentration of non-labeled ARA was observed only in female rats fed the LA+ALA diet. In summary, greater endogenous and exogenous DHA and DPAn-6 was observed in female rat plasma and this was independent of dietary ALA status.

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1. Introduction

In 1929, Burr and Burr [1] first discovered that n-6 polyunsaturated fatty acids (PUFA) are necessary for proper growth and development of mammals and thus termed them essential fatty acids (EFAs). Ever since, much research has been conducted on all aspects of EFAs including possible gender differences. Since the first study by Loeb and Burr [2], which reported a gender difference in EFA requirements, a series of studies in the 1960s by Lyman and Ostwald [3,4] observed that female rats utilized EFAs more efficiently, especially arachidonic acid (ARA), an n-6 PUFA. This series of studies suggested that the gender difference in EFA metabolism was strongly related to sex and gonadal hormones, mainly estrogen and testosterone [3–6]. It was found that increased hormone levels were correlated with changes in liver microsomal phospholipid fatty acid composition, and possibly involving the underlying mechanism of calcium uptake activity [7,8]. But these differences were not observed in an *in vitro* rat microsomal system using a radio isotopic technique [9].

It was not until the late 1970s that the n-3 PUFAs were considered to be essential nutrients in mammals [10–13]. During the past decade, the gender differences in n-3 PUFAs conversion and fatty acid compositions in human subjects has been investigated following the report by Burdge et al. [14–17]. Several reviews [18–20] indicate that docosahexaenoic acid (DHA) levels were significantly higher for both endogenous fatty acid composition and conversion from the isotopic precursor α-linolenic acid (ALA). This increase in DHA in females has been reported to be closely related to hormone levels [21–23] probably through an up-regulation of the mRNA expression of FADS2, the gene that encodes the Δ6-

Abbreviations: ALA, 18:3n-3, α-linolenic acid; ARA, 20:4n-6, arachidonic acid; AUC, area under the time-course curve; BF_3, boron trifluoride; BHT, butylated hydroxytoluene; DPAn-3, 22:5n-3, n-3 docosapentaenoic acid; DPAn-6, 22:5n-6, n-6 docosapentaenoic acid; DHA, 22:6n-3, docosahexaenoic acid; EFA, essential fatty acid; EPA, 20:5n-3, eicosapentaenoic acid; FID, flame ionization detector; GC, gas liquid chromatography; HUFA, highly unsaturated fatty acid; LA, 18:2n-6, linoleic acid; LC PUFA, long chain polyunsaturated fatty acid; MS, mass spectrometry; MUFA, monounsaturated fatty acids; NCI, negative chemical ionization

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desaturase in human primary hepatocytes [24] and in rat liver [25,26].

However, gender differences reported in many previous studies were observed under dietary circumstances where 18:3n-3, DHA and other highly unsaturated fatty acids (HUFA) were consumed. The 20- and 22-carbon fatty acids with three or more double bonds are categorized as HUFA. Thus, it was unclear whether the higher DHA observed in females was due to direct dietary intake or *de novo* synthesis from dietary 18:3n-3, or both. Furthermore, studies of gender during the last decade have focused upon n-3 PUFA without attention to n-6 PUFAs. This study investigated the possible gender effect on both the n-3 and n-6 fatty acid compositions as well as *de novo* synthesized PUFAs in rats using two well-defined diets containing only linoleic acid (LA) and ALA for dietary PUFA content. One diet contained a high level (3 wt% of total fatty acids) of 18:3n-3 (LA+ALA diet), while the other contained an extremely low level, with only 0.04 wt% of 18:3n-3 (LA diet); both diets included 15 wt% of 18:2n-6 but no long chain PUFA (LC PUFA). Stable isotope tracer techniques coupled with mass spectrometry negative-chemical ionization analysis (GC/MS NCI) were employed to quantify the *de novo* synthesis of deuterated LC PUFA metabolized from deuterated C18 PUFAs.

2. Materials and methods

2.1. Animal and diet

All animal procedures were carried out in accordance with the NIH Animal Care and Welfare guidelines under the animal study proposal #LMBB-YL-11, which was approved by the Animal Care and Use Committee, National Institute on Alcohol Abuse and Alcoholism, NIH. The animal facility was under conventional conditions with controlled temperature 22 °C and illumination (12 h light-dark cycles); food and water were provided ad libitum. The animals in this study came from the second generation of in-house breeding, as described in a previous report [27]. Briefly, the time-pregnant female, Long-Evans hooded rats on the third day of gestation were obtained from Charles River Laboratory (Portage, MI, USA), and they were immediately placed on one of two pelleted custom diets. One diet was high in n-3 PUFA and contained 15% of 18:2n-6, 3.1% of 18:3n-3 and 1.3% 22:6n-3, while the other was extremely low in n-3 PUFA and contained 15% of 18:2n-6, 0.04% of 18:3n-3 without 22:6n-3 (LA diet). The offspring were weaned onto the same diet as the mother at three weeks of age. At 16 weeks of age, animals on the high n-3 PUFA diet were switched to the LA+ALA diet, which included 15% of 18:2n-6 and 3.1% of 18:3n-3 but with no DHA nor other HUFA. These animals consumed the LA+ALA diet for 4 weeks and then groups were separated according to gender and designated as group HM (male) and group HF (female), n=6 for each group. The animals on the LA diet continued to consume the same LA diet and were designated as group LM (male) and group LF (female), n=6 for each group. All diets contained 10 g of fat per 100 g diet and were modified from the AIN-93G formulation [28]. The compositions of the two experimental diets and the corresponding study groups are presented in Table 1.

2.2. Isotope and chemicals

Deuterium-labeled ethyl linolenate ($^2H_5$-17, 17, 18, 18, 18-18:3n-3) and deuterium-labeled ethyl linoleate ($^2H_5$-17, 17, 18, 18, 18-18:2n-6) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA); isotopic purity was greater than 95% for the former and 98% for the latter. A dual labeled isotope, deuterated and carbon-13 labeled gamma-linolenate ethyl ester (7,8-$^{13}C_2$, 99%; 2,2,3,3,4,4,5,5,6-$^2H_9$, 98%-18:3 n-6) was used as an internal standard for the quantification of the labeled fatty acids and their *in vivo* metabolites. Its chemical purities were greater than 95%. Olive oil and boron trifluoride-methanol solution (BF_3 14% in methanol) were acquired from Sigma-Aldrich Chemical (St. Louis, MO); methanol was obtained from Burdick & Jackson (Muskegon, MI); hexane and chloroform were from EMD Chemicals Inc. (Gibbstown, NJ); butylated hydroxytoluene (BHT) was from Acros (Geel, Belgium). Standard docosatetraenoic ethyl ester (22:3n-3) and reference standards GLC–462 containing 28 fatty acid methyl esters (FAME) were purchased from Nu-Chek Prep (Elysian, MN). All chemicals were commercially purchased and used without further purification.

Table 1
Nutrient composition of experimental diets and study groups.

Ingredient[a]	**Amount** (g/kg of diet)	
Protein (20%)		
Casein (vitamin free)	200	
Carbohydrate (60%)		
Cornstarch	150	
Dextrose	199.5	
Maltose-dextrin	150	
Sucrose	100	
Others (10%)		
Cellulose	50	
Mineral & salt mix	35	
Vitamin mix	10	
L-Cystine	3	
Choline bitartrate	2.5	
tert-butylhydroquinone	0.02	
Fat (10%)	100	
	LA+ALA diet	**LA diet**[b]
HCO	77.5	81
Safflower oil	17.7	19
Flaxseed oil	4.8	–
Fatty acid composition[c] (wt% of total fatty acids)		
Saturates	77.2	80.85
Monounsaturates	4.3	3.92
18:2n-6	15.3	15.13
18:3n-3	3.12	0.04
Study groups		
HM (male)	+	
HF (female)	+	
LM (male)		+
LF (female)		+

[a] HCO, hydrogenated coconut oil.
[b] The experimental diets [45,46] were modifications of the AIN-93G [28]. (LA+ALA diet=Control diet, LA diet=n-3 Def in previous studies).
[c] C20 and C22 PUFA were undetectable.

2.3. Administration of isotopes and sampling

At the age of 20 week, fasting animals were administrated by gavage a single oral dose of an oil mixture of $^2H_5$-18:3n-3 ethyl ester (12.5 mg/kg BW) and $^2H_5$-18:2n-6 ethyl ester (62.5 mg/kg BW) in olive oil as the vehicle. Caudal vein blood (0.2–0.3 mL) was drawn from each animal at 2, 4, 8, 12, 24, 48, 96 and 168 h after administration. Control samples, at 0 h, were taken two days prior to the initiation of the study. The coefficient of variance for the sampling time was less than 5% for all animals during the entire experimental period. Plasma was separated shortly after collection at 1750 $g \times 5$ min in Eppendorf microcentrifuge, then was frozen and stored at −80 °C until analysis.

2.4. Total lipid extraction and fatty acid analysis

Plasma total lipids were extracted with chloroform: methanol (2:1) according to Folch [29]. Briefly, 100 μl of rat plasma was