Effect of essential fatty acid deficiency on membrane fatty acid content and growth hormone stimulation of rat pituitaries during postnatal development

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Abstract Fatty acid composition of anterior pituitary cell membranes of rats deprived of essential fatty acids (EFA) and of rats receiving a standard diet was determined during postnatal development and in adults. Pregnant rats were fed an EFA-deficient diet and the offspring were fed the same diet after weaning. In parallel, effects of the diet on growth and on growth hormone (GH) responsiveness to GHRH stimulation were determined in control animals. Membrane content of arachidonic acid (20:4n-6) and of its elongation product adrenic acid (22:4n-6) increased regularly from day 2 to day 12 after birth. EFA-deficiency resulted on day 2 in increased oleic acid and in substitution of arachidonic and adrenic acids by corresponding elongation-desaturation products of oleic acid: eicosatrienoic (20:3n-9) and docosatrienoic (22:3n-9) acids. At the age of 24 days, n-9 series fatty acid reached the same level as in adult animals. Two-day-old EFA-deficient rats paradoxically exhibited a higher level of 20:4n-6 as compared to control rats. EFA-deficiency also decreased growth rate and GH pituitary responses to GHRH during the prepubertal period. These results suggest that changes in the lipid structure and in pituitary secretion properties elicited by EFA-deficiency depend upon the stage of development.—**Soares, M. C., M. L. Aléssio, C. L. Léger, M. T. Bluet-Pajot, H. Clauser, A. Enjalbert, C. Kordon, and D. E. Wandscheer.** Effect of essential fatty acid deficiency on membrane fatty acid content and growth hormone stimulation of rat pituitaries during postnatal development. *J. Lipid Res.* 1995. **36:** 1401–1406.

Supplementary key words arachidonic acid • essential fatty acids • phospholipids

The relationship between changes in lipid composition, physical properties, and biological functions of membranes has been extensively studied. Membrane functions depend upon cholesterol concentrations (1) as well as upon polar (head group) and hydrophobic (fatty acid) parts of phospholipids (2). Essential fatty acids (EFA) represent 20–50% of total membrane fatty acids (2, 3). Membrane lipid composition can be modified by dietary lipids in animals (4) as well as in humans (5). As EFA are exclusively of dietary origin, their shortage induces EFA deficiency. Cells compensate for such deficiency by increasing oleic acid bioconversion through the desaturation/elongation system (6). The subsequent shift in the double bond position of fatty acid chains has marked consequences on membrane physical properties (7).

EFA deficiency causes numerous dysfunctions in animals (8, 9) and humans (10, 11), e.g., cardiovascular alterations (12), dermal/allergic disturbances (13, 14), and retarded growth (9, 15).

In addition, changes in membrane lipid composition can alter membrane functions, in particular those controlling hormone/receptor interactions (16), or receptor coupling with enzymes as adenylate cyclase (17) or phospholipase A_2 (18, 19). On the other hand, EFA are precursors of eicosanoids, a large family of signaling molecules including prostaglandins, thromboxanes, prostacyclins, and leukotrienes as well as linear oxygenated derivatives of 20-carbon fatty acids (20).

Another important role of phospholipids is represented by phosphoinositide hydrolysis by phospholipase C, which results in inositol phosphates (IPs) and diacylglycerol (DAG) release. In turn, these moieties trigger intracellular Ca^{2+} mobilization and protein kinase C (PKC) activation, respectively (21, 22). Finally, phopholipase A_2 leads to the release of polyunsaturated fatty acids from the *sn*-2 position of the glycerol backbone. Arachidonic acid, the major fatty acid released, and 2-lysophospholipids produced by PLA_2 action are both considered as important intracellular signals (23–25).

Abbreviations: EFA, essential fatty acids; STD, standard (control) diet; DED, EFA-deficient diet; GH, growth hormone; GHRH, growth hormone releasing factor; PL, phospholipid.

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GH secretion by adenohypophyseal cells is predominantly controlled by two neuropeptides, GHRH and SRIF, which, respectively, stimulate and inhibit its secretion (26–28). The corresponding receptors are coupled to adenylate cyclase (29), intracellular Ca^{2+} (30, 31), and presumably PLA_2 (32–34).

For these reasons EFA deficiency represents an interesting model for studying the role of polyunsaturated fatty acids and their functional relevance to membrane functions. In a previous study, we demonstrated specific, EFA deficiency-induced changes in subcellular fractions of anterior pituitary membranes; some fractions were more able than others to preserve their arachidonic acid content (35). In the present work, consequences of EFA deficiency have been determined on fatty acid composition of membrane phospholipids of rat anterior pituitaries and correlated with growth hormone responses to GHRH in vitro at different stages of postnatal development.

MATERIAL AND METHODS

Animals

Adult female Wistar rats weighing 200–250 g were maintained during pregnancy on a standard (STD) or EFA-deficient diet (DED) under controlled temperature (22–25°C) and light (12-h light/12-h dark). Diets differed only by the lipid composition: 5% of corn oil for STD and 5% of hydrogenated copra oil for DED (**Table 1**). The day of birth was considered as day 0 of life. Ten pups at most were kept with each mother in either diet group. Male pups before weaning were killed by decapitation on day 2, 12, or 24. Male adult rats were killed 6 weeks after weaning. They were kept on the same diet as their mother throughout the experiment. Pituitaries (anterior pituitary for 12-, 24-day-old pups and adult rats or whole pituitary for 2-day-old pups) were dissected on ice and used for biochemical analyses or incubation.

TABLE 1. Composition of standard (STD) and EFA-deficient (DED) diets

Diet	Wt %	% W/W STD[a]	% W/W DED[b]
Casein	20.7		
DL-Methionine	0.1		
Cellulose	1.8		
Corn starch	46.8		
Sucrose	21.0		
Oil	5.0		
Vitamin mix[c]	0.9		
Mineral mix[d]	3.7		
Fatty acids[e]			
10:0			3.8
12:0			60.5
14:0		0.55	21.0
16:0		12.8	8.7
18:0		2.0	8.5
18:1n-9		27.5	0.6
18:2n-6		56.0	0.2
18:3n-3		0.8	
22:3n-9		1.2	

[a]Diet containing corn oil.
[b]Diet containing hydrogenated copra oil.
[c]INRA.
[d]Composition (wt %): $CaHPO_4$ (38); K_2HPO_4 (24); $CaCO_3$ (18.1); NaF (0.1); NaCl (7.0); MgO (2.0); $MgSO_4 \cdot 7H_2O$ (9.0); $FeSO_4 \cdot 7H_2O$ (0.7); $ZnSO_4 \cdot H_2O$ (0.5); $MnSO_4 \cdot H_2O$ (0.5); $CuSO_4 \cdot 5H_2O$ (0.1); $A1_2(SO_4)_3K_2SO_4 \cdot 24H_2O$ (0.02); $Na_2SeO_3 \cdot 5H_2O$ (0.001); KCl (0.008).
[e]Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl (n) end of the molecule is also indicated.

Membrane lipid composition

After pituitary homogenization and separation of the total membrane fraction, lipids were extracted (36) in the presence of 0.005% BHT to preserve polyunsaturated fatty acids from oxidative damage. The SepPak procedure (37) was used for lipid separation into total phospholipid (PL), neutral lipid, and glycolipid fractions. The PL fraction was evaporated under nitrogen and dissolved in 1 ml chloroform. After assessing phosphorus contents according to Bartlett (38), transmethylations were carried out according to Berry, Cevallos, and Wade (39). Fatty acid methyl ester analyses were performed using a capillary column gas chromatography technique. Briefly, a Stang apparatus (model DANI 6500-HR) fitted with the programmed temperature vaporizer (PTV) injection system (in the total sample injection mode) and a flame ionization detector was used with a Durabond-Wax glass capillary column (60 m × 0.25 mm, 0.25-μm thickness of the bonded phase). The treatment of chromatographic information was achieved by means of the 3000 Series Chromatography Data System supplied by Nelson-Analytical in combination with a Tandon TM 7002 IBM PC-AT computer. Chromatographic peaks were identified by comparison of their retention times with those of authentic fatty acid methyl esters.

Pituitary incubation

Incubation of paired hemipituitaries was performed at 37°C in medium 199 (Gibco, Glasgow, Scotland), containing 20 mM HEPES (pH 7.2) in a metabolic shaker with 95% O_2, 5% CO_2, according to the technique of Enjalbert et al. (40) modified by Wandscheer et al. (34). After a 30-min preincubation, hemipituitaries were incubated for two periods of 60 and 45 min. Aliquots of 500 μl were successively removed from the medium after 1 h for basal (non-stimulated) GH assay. GHRH (10^{-6} M) was added at that time and two further aliquots were sampled for stimulated GH assay. After incubation, hemipituitaries were washed, homogenized, and frozen at −20°C for GH-content determination.

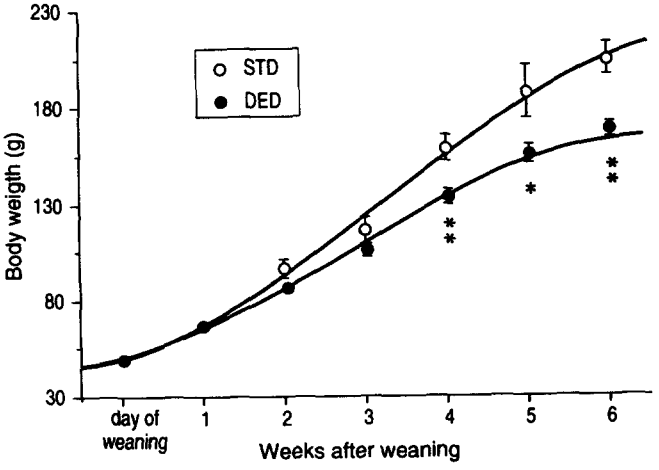

Fig. 1. Average body weights of rats born of EFA-deficient or control mothers and receiving (after weaning) an EFA-deficient (DED, ●) or control (STD, ○) diet. Weights with * and ** are significantly different, $P < 0.05$ and $P < 0.01$, respectively, from control weights.

GH radioimmunoassay

The double antibody RIA method (41) was used for determining GH plasma concentrations. Materials were supplied by National Institute of Diabetes and Digestive and Kidney Diseases for anti-GH antibody, and by Antibodies Inc. (Davis, California) for goat anti-rabbit gamma globulins.

Statistical analysis

Analysis of variance ANOVA for repeated measurements was used for statistical comparison between experimental groups; $P < 0.05$ was considered significant.

RESULTS

Body weights and EFA-deficiency

Animals receiving EFA-deficient diet presented a significantly decreased body weight from the 5th week after weaning. An 18% decrease as compared to STD animals can be observed at the 6th week (**Fig. 1**).

Fatty acid composition of membrane phospholipids

Palmitic (16:0), stearic (18:0), oleic (18:1n-9), and arachidonic (20:4n-6) acids represent major fatty acids in the membrane phospholipids of rat anterior pituitaries in STD as well as in DED animals (**Fig. 2A-2D**). The effect

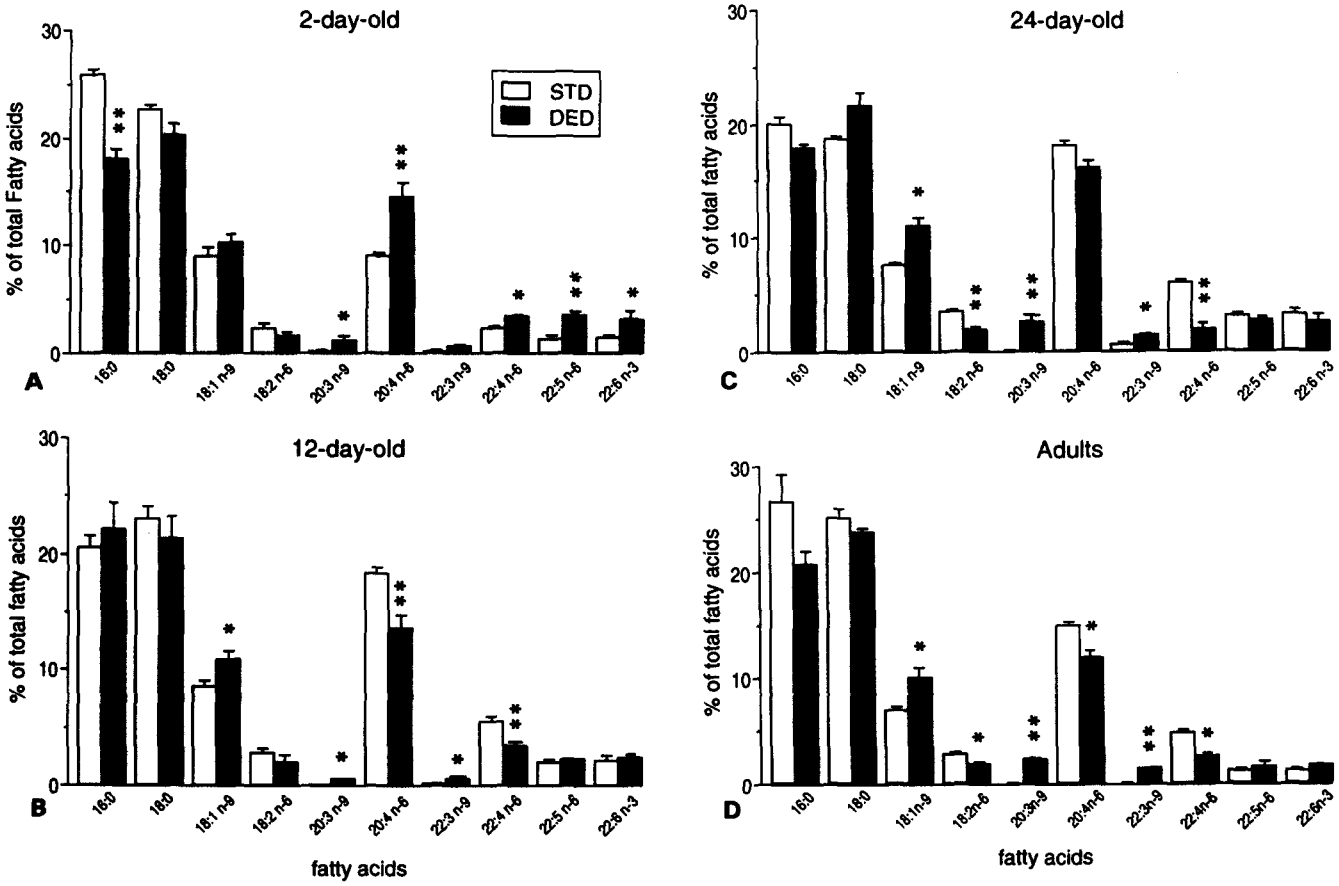

Fig. 2. Effect of the EFA-deficient diet (DED) on phospholipid fatty acid composition of anterior pituitary membranes at different ages. A: 2 days old; B: 12 days old; C: 24 days old; D: adults. Results are expressed in weight percentage. Values are mean ± SE of 5-9 separate determinations on 10 animals. Results with * and ** are significantly different, $P < 0.05$ and $P < 0.01$, respectively, from control values.

of deficiency was already visible on the 2nd day of life, as judged by increased concentrations of the eicosatrienoic acid 20:3n-9 and docosatrienoic acid 22:3n-9, two EFA-deficiency markers (1.2 ± 0.6% and 0.6 ± 0.2%, respectively, versus 0.1 ± 0.01% for both in STD rats). At 24 days, the markers reached levels observed in EFA-deficient adults. Oleic acid increased progressively in DED rats. It was not significantly different at 2 days, but was 27%, 46%, and 56% higher at 12 days, 24 days, and in the adults, respectively.

Arachidonic (20:4n-6) and adrenic (22:4n-6) acids were markedly affected by EFA deprivation, but in an age-dependent manner. For the STD rats, they increased until day 24 and tended to diminish thereafter (Fig. 2 and **Fig. 3**). Interestingly, both arachidonic and adrenic acids were paradoxically higher on day 2 in DED than in STD animals (14.4 ± 1.3% versus 8.9 ± 0.2%), whereas from the 12th day on they were significantly lower.

Linoleic acid was consistently lower in EFA-deficient animals, but the difference was only significant after day 24 (1.9 ± 0.1% versus 3.4 ± 0.2%). It is of interest to note that low levels of linoleic acid were concomitant with high levels of arachidonic acid in deficient 2-day-old animals.

Effect of EFA-deficiency on pituitary responsiveness to GHRH

Basal secretion rates from hemipituitaries of EFA-deficient rats were slightly, but not significantly, lower than those of corresponding controls of the same age (**Fig. 4**). Responsiveness to GHRH in controls appeared as soon as 2 days after birth, but the amplitude of the GH response observed at that time was quite low. It increased steadily thereafter until adulthood. In contrast, pituitaries from EFA-deficient animals did not respond to GHRH until 24 days of age. In adults, a slightly, but not significantly, decreased responsiveness to GHRH stimulation was still present after EFA-deprivation. Pituitary

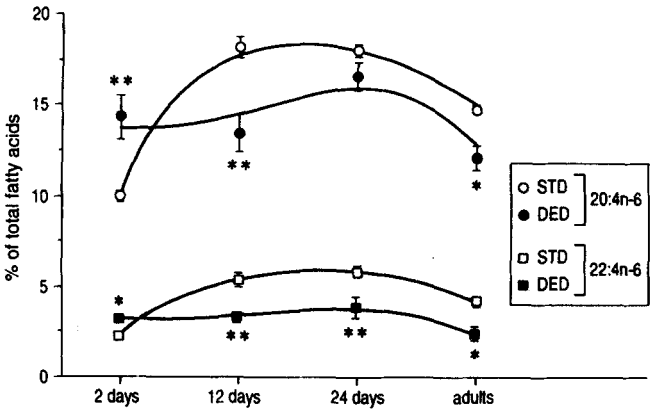

Fig. 3. Effect of EFA-deficient diet (DED) on phospholipid fatty acid composition in relation to age: percent of arachidonic acid (20:4n-6) and adrenic acid (22:4n-6). Results with * and ** are significantly different, $P < 0.05$ and $P < 0.01$, respectively, from control values.

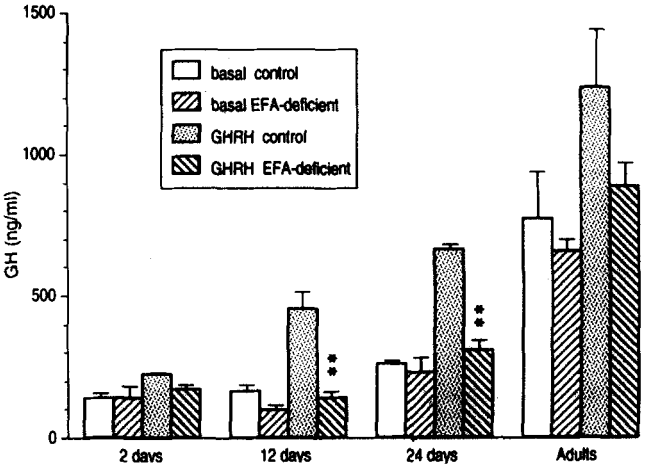

Fig. 4. Effect of EFA deficiency on basal and GHRH-stimulated growth hormone secretion from incubated anterior pituitaries in relation to age. Pituitary fragments were incubated for 45 min. Data reported are the mean ± SE of 6-10 separate determinations; **, significant difference ($P < 0.01$) with respect to control values.

GH contents of STD and DED rats were not significantly different at any stage of development (**Table 2**).

DISCUSSION

The purpose of the present study was to assess changes occurring during development in membrane phospholipid fatty acid composition and pituitary growth hormone responsiveness in rats born from normal or EFA-deficient mothers and receiving normal or EFA-deficient diet after weaning.

In pituitary membrane phospholipids, arachidonic and 20-/22-carbon n-6 and n-3 polyunsaturated fatty acid concentrations were found to be paradoxically higher 2 days after birth in EFA-deficient animals than in con-

TABLE 2. Pituitary growth hormone (GH) content in control and EFA-deficient rats

Age of Rats	Diet	GH
days		*ng/mg protein*
2	STD	20.5 ± 5.8
	DED	27.1 ± 3.0
12	STD	37.0 ± 3.2
	DED	30.3 ± 7.6
24	STD	58.0 ± 10.0
	DED	51.1 ± 10.9
Adult	STD	77.3 ± 14.0
	DED	70.4 ± 1.3

Anterior pituitary GH content from the early postnatal development stages to the adult stage in animals fed standard (STD) or EFA-deficient (DED) diets. For details, see the Animals section in Materials and Methods.

trols. This was no longer the case for older animals (from 12 days to adults) essentially because at those ages fatty acids increased dramatically in animals on STD, but they remained almost unchanged in animals on DED (see Fig. 3 for data on 20:4n-6 and 22:4n-6).

EFA are preferentially incorporated into developing tissues (42). Higher levels of polyunsaturated fatty acids in early development stages in pups born from EFA-depleted mothers could result from a process analogous to the arachidonic acid magnification reported in the placenta during EFA deficiency (43, 44). There is also evidence for regulation by the liver of extrahepatic lipid composition in EFA-deprived animals (45). Stimulation of hepatic Δ6- and Δ5-desaturases has been observed in EFA-deficient pregnant rats (46, 47). Alternatively, hepatic-PLA_1-generated 2-acyl-lysophosphatidylcholine could also supply EFA to extrahepatic tissues (48). Taken altogether, those arguments suggest that increased mobilization of arachidonic acid from the mother is able to compensate for consequences of EFA-deprivation in the fetus.

Fetal metabolism may also partly account for the high levels of 20-22-carbon polyunsaturated fatty acids found in animals born from EFA-deficient mothers. A limited Δ5-desaturase activity develops during fetal life and overtakes that of Δ6 desaturase by the end of pregnancy (46, 49). This could explain the burst of 20-22-carbon tetraenoic acid production in the first days after birth.

Interestingly, EFA deficiency correlated with a marked decrease in pituitary GH reponses to GHRH. Under our conditions, sensitivity to GHRH did not strictly correlate with membrane 20:4n-6 concentrations, as it remained low at all stages of development tested, in spite of changes in the fatty acid concentrations during that period.

Although in vitro GH responses to GHRH after EFA deprivation do not reflect exclusively GH regulation in vivo, which also involves participation of other peptides such as somatostatin, VIP, or galanine (50), it is considered a fair indication of GH regulation. The fact that GH responsiveness recovers spontaneously in young adults agrees with results of a previous study that indicated that GH responses to GHRH from cultured adult pituitary cells were not affected by EFA-deficiency (51).

Taken altogether, the present data suggest that changes in fatty acid composition of membrane lipids resulting from exposure to EFA-deficient diets correlate with a temporary dysfunction of growth hormone regulation. That dysfunction may account for the growth retardation observed in EFA-deficient subjects, and is thus likely to be of pathophysiological relevance.

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