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Regulation of Desaturase Gene Expression by Dietary Fatty Acids: Effects on Liver, Brain and Kidney

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Introduction
Among factors regulating polyunsaturated fatty acid (PUFA) biosynthesis, it has been shown that dietary fatty acids play a key role. Their influence is dependent on their family (n-6 or n-3), their chain length, and their number of double bonds (1–3). Their effects on desaturase activities remain controversial. For example, Hoy et al. (4) showed in rats that low dietary supplementations with oils rich in n-3 PUFA had no effect on desaturation, whereas Blond et al. (5) demonstrated that dietary 18:3n-3 (ALA) inhibited both Δ6 and Δ5 desaturations. Linseed oil has been shown to inhibit Δ6 (1), but increase Δ5 desaturation (2). On the contrary, Christiansen et al. (6) observed an increase of Δ6 and Δ5 desaturase activities with such a diet. More recent studies have shown that dietary n-6 or n-3 PUFA decreased hepatic mRNA of Δ6 desaturase by ~80% and that of Δ5 desaturase by ~60% (7). Nevertheless, the control diet used in those investigations was a high-glucose/low-fat diet, which stimulates desaturation. In sum, discrepancies exist among the data on regulation of PUFA biosynthesis by dietary fatty acids; these discrepancies result from differences in control diets (fat-free, high-glucose, high-fat), the origin or doses of administered fats (oils, fatty acid mixtures, purified fatty acids), the age of the animals, or the duration of the treatments.

The aim of the present work was to clarify these data, by studying the effects of purified fatty acids on both Δ6-desaturase activity and gene expression. Because the choice of the control diet is of primary importance for such a nutritional study; the control regimen contained 4% lipids, qualitatively equilibrated in fatty acids; this corresponded to the level of fats generally found in diets administered to rodents.

Materials and Methods
Animals and Diets. Adult Wistar rats (n = 16) were provided by Dépré (Centre d’Élevage Dépré, Saint Doulchard, France) and maintained at 22 ± 2°C, 60 ± 5% humidity, with a 12-h light:dark cycle. The rats had free access to food and tap water. On arrival, rats were fed commercial standard pellets (Souffratar, U.A.R A04, Villemoisson Sur Orge, France) for 1 wk. Then, they were divided into four groups. The control group was fed a standard semisynthetic diet containing (g/100 g): 56.4 cornstarch, 19.0 casein, 10.0 cellulose powder, 4.5 saccharose, 3.8 minerals, 2.0 vitamins (U.A.R.), 0.3 methionine (Sigma Chemical, St. Louis, MO) and 4% lipids (ISIO 4 oil, Lesieur, Boulogne Billancourt, France, containing 40% 18:1(n-7 + n-9), 45.9% of 18:2n-6, 1.6% of 18:3n-3). The other three groups received the standard diet in which the 4% lipids was replaced by 4% oleic acid (18:1 n-9, group OA), linoleic acid (18:2n-6, group LA), or γ-linolenic acid (18:3n-3, group ALA) (Nu-Chek-Prep, Elysian, MN) as the only source of lipids. These fatty acids were esterified according to Berdeaux et al. (8) and added to the diet just before feeding. The stability of the ethyl esters, under conditions including exposure to air, was confirmed by gas-liquid chromatography (GLC). Food intake was measured daily and body weight weekly. After 15 d of this regimen, the rats were killed, and liver, kidney, and brain preserved.

Fatty Acid Composition Analysis. Lipid extraction followed the procedures described by Bellenger et al. (9). Fatty acid methyl esters were prepared by transmethylation using 14% boron trifluoride in methanol (10), and analyzed by GLC (Hewlett-Packard model 417 GLC; Hewlett-Packard, Downers Grove, IL) equipped with a flame ionization detector and a 30-m capillary fused silica column coated with Carbowax 20M (Applied Science Labs, State College, PA). Fatty acids were identified by their relative retention times in comparison with a fatty acid standard (Nu-Chek-Prep) (11).

Desaturase Activities and Gene Expression. The Δ6 n-6 and Δ6 n-3 desaturase activities were measured according to Narce et al. (12) using liver microsomal membrane preparations (13). The expression of Δ6-desaturase (Δ6D) was determined by Northern blotting, after tissue mRNA extraction. Total RNA was isolated from frozen liver according to Chomczynski and Sacchi (14), and quantified by ultraviolet (UV) light absorption at 260 nm. The integrity of the RNA used for the RNase protection analysis was verified by fractionating 5 μg of total hepatocyte RNA on a 1% agarose gel containing 6.7% formaldehyde and visualizing RNA by ethidium bromide staining. Electrophoresis and Northern blotting were performed as follows: 50 μg of total RNA from each liver was denatured in 50% formamide, 17% formaldehyde, 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (20 mM MOPS, 0.5M sodium acetate, pH 7.0, 1mM EDTA) for 10 min at 65°C, and run on a denaturing agarose gel in MOPS buffer. Transfer onto nylon membranes (HybondN, Amersham, Les Ulis, France) was achieved by capillary blotting using 10 X SSC buffer (1.5 M NaCl, 0.15 M trisodium citrate, pH 7.4). Membranes were then air-dried and irradiated with UV light at 254 nm in a spectrolinker XL-1000 UV crosslinker (Spectronics) with an energy of 1.2 × 10⁻³ J/cm². The mRNAs were then hybridized with human DNA probes. Full-length cDNA clones were used for Δ6D and 18S. Inserts from these different clones were purified using Qiaquick Spin Columns (Qiagen, Courtaboeuf, France) and then 32p-radiolabeled using a random priming labeling kit (multiprime DNA labeling kit, RPN 1601 Y,
Amersham, Orsay, France). RNA blots were prehybridized for 3 h in 50% formamide with 5X Denhardt, 200 mM Tris HCl, 26 mM pyrophosphate tetrasodium salt, 1% sodium dodecyl sulfate, 10% sul fate dextran, 1% sodium chloride and 100 μg/ml denatured herring sperm DNA and hybridi zed for 18 h in the same medium, except for sodium chloride, with 250 μg/ml denatured herring sperm DNA in the presence of 2 x 10^6 cpm of labeled cDNA Δ6D probe or 2 x 10^5 cpm of labeled cDNA 18S probe. The prehybridization and hybridization temperature was 42°C. Membranes were then washed successively once in 3X SSC, 0.1% SDS, at room temperature, once in 3X SSC, 0.1% SDS, at 50°C for 30 min, and twice in 1X SSC, 0.1% SDS, at 50°C for 30 min. Membranes were then air-dried and hybridization was revealed by autoradiography at −80°C using Kodak X-Omat AR film and intensifying screens. Hybridization signals were quantified on a Biorad image analyzer (Biorad, Ivry sur Seine, France).

Statistical Analyses. Results were expressed as the arithmetic mean for each group ± SEM. For each diet, after ANOVA, the means from groups OA, LA, and ALA were classified using Duncan’s multiple range t-test. P < 0.05 was considered significant.

Results

Weight Gain and Food Intake. Weight gain and food intake of OA and ALA groups did not differ from controls at the end of the experiment; in the LA group, however, they were significantly lower compared with controls (Fig. 1).

Fatty Acid Composition of Liver Total Lipids. The total amount of saturated fatty acids was significantly lower in the ALA group than in controls (Fig. 2). A smaller amount of monounsaturated fatty acids was observed in groups LA and ALA, due to a decrease of oleic acid (−44%). On the contr ary, the content of this fatty acid was higher in the OA group (+24%). The total amount of n-6 fatty acids was significa ntly lower in groups OA and ALA (−21 and −38%, respectively) than in controls, reflecting a marked decrease of linoleic e in group OA, and of linoleic and arachidonic acids in group ALA. On the contrary, the LA group had an increase of fatty acids, due to 20:4n-6 (+14%) and 22:4n-6 (+100%), to 18:2n-6 (+24%) and 22:5n-6 (+200%) compared with controls. The amount of n-3 fatty acids was 6.6-fold higher in ALA group than in the control group, indicating a tremendous increase of 18:3n-3, 20:5n-3, and 22:5n-3. The n-6/n-3 rati o was significantly lower by 41% and 90% in the OA and ALA groups, respectively, compared with controls.

n-6 and n-3 Desaturase Activities. Liver microsomal Δ6-desaturase activities are presented in Figure 3. Diets containing OA or LA as the only source of lipids induced a significant increase of the n-6 Δ6-desaturase activity (+46%, +45%, respectively) compared with controls; however, significant change was observed in the ALA group. Concerning n-3 Δ6-desaturase activities (Fig. 3), the resin showed that diets containing LA or ALA, as the only source of lipids, induced a significant increase of n-3 Δ6-desatur activity (+75% and +102%, respectively) compared with controls, whereas OA had no effect on this desaturase activity.

Δ6-Desaturase Gene Expression. In liver, Δ6D gene expression (Fig. 4) increased, irrespective of the source lipids. Oleic acid provided in the diet as the only source of lipids yielded the highest induction of Δ6D gene expression; LA had a greater effect than ALA on this gene. However, brain (Fig. 5) and kidney (Fig. 6), Δ6-desaturase expression did not change, irrespective of the fatty acid provided.

Fig. 1. Weight gain and food intake in rats fed a semisynthetic diet (Control) or that diet with lipids replaced by oleic acid (Group OA), linoleic acid (Group LA), or α-linolenic acid (Group ALA). Values are means ± SEM; means with different superscripts are significantly different, P < 0.05.
Discussion

Together, these results point out that purified oleic acid, linoleic acid, and α-linolenic acid, provided as the only source of lipids, modulate Δ6-desaturase activities and expression. Moreover, these fatty acids induce changes in fatty acid composition of liver total lipids, in relation to the composition of the diet. Changes in liver fatty acid composition under the influence of dietary fatty acids have been described extensively in the literature (1,2,6,15–18). In the

Fig. 2. Fatty acid composition of liver total lipids in rats fed diets as described in the legend to Figure 1. Values are means ± SEM; means with different superscripts are significantly different, P < 0.05.

Fig. 3. Liver microsomal n-6 (upper panel) and n-3 (lower panel) Δ6-desaturase activities in rats fed diets as described in the legend to Figure 1. Values are means ± SEM; means with different superscripts are significantly different, P < 0.05.
Fig. 4. Liver Δ6 gene expression in rats fed diets as described in the legend to Figure 1. Upper panel: blots are shown for each control and experimental animal. Lower panel: values are means ± SEM, using 18S as standard; means with different superscripts are significantly different, \( P < 0.05 \), according to Duncan’s multiple range test.

group fed ALA, the level of 20:5n-3 increased tremendously, reflecting the increase in the amount of its substrate (18:3n-3) in the dietary regimen, whereas the quantity of docosahexaenoic acid (DHA; 22:6n-3) did not change. Christiansen et al. (6) noted similar results in rats supplemented with linseed oil. On the contrary, when fish oil replaced linseed oil, the level of DHA increased markedly (6). These data seem to indicate that when ALA is administered as the only source of fatty acids, its final derivate, DHA, is not synthesized. A metabolic cascade is blocked at the eicosapentaenoic : (EPA) level. Recently, de Antuerno et al. (19) and d’Andre et al. (20) demonstrated that a single Δ6-desaturase was

Fig. 5. Brain Δ6 gene expression in rats fed diets as described in the legend to Figure 1. Upper panel: blots are shown for each control and experimental animal. Lower panel: values are means ± SEM, using 18S as standard; means with different superscripts are significantly different, \( P < 0.05 \), according to Duncan’s multiple range test.
Fig. 6. Liver Δ6 gene expression in rats fed diets as described in the legend to Figure 1. Upper panel: blots are shown for each control and experimental animal. Lower panel: values are means ± SEM, using 18S as standard; means with different superscripts are significantly different, *P* < 0.05, according to Duncan's multiple range test.

olved in the conversion of the 18C and 24C fatty acids. Thus, the n-3 metabolic cascade observed in our experiment could be due to a competition between the desaturation of LA and of EPA because of the high amount of C-18 substrate in group ALA. Such a result could also be reinforced by a lower availability of endogenous EPA compared with that provided by the diet.

The most important outcome concerning Δ6-desaturase is its activity increased under the effects of both LA and LA. The activity of n-3 Δ6-desaturase was more important than that of n-6 Δ6-desaturase as described previously (21). In the group fed ALA, the n-3 Δ6-desaturase activity was higher than in the group fed LA. On the other hand, the n-6 6-desaturase activity was higher in the group fed LA than the group fed ALA. Our results were similar in part to those reported by Christiansen et al. (6), using linseed or sunflower oil. However, the data reported by Choi et al. (22) showed higher n-6 Δ6-desaturase activity under the effect of dietary linseed oil than with sunflower. The reasons for these discrepancies remain unclear, but may be due to the differences between the diets and/or the feeding protocols, i.e., Christiansen et al. (6) used 20% fat in the regimen, whereas Choi et al. (22) used 10%. These data demonstrate clearly at n-6 and n-3 Δ6-desaturase activities were regulated differently by dietary PUFA. These differences may be related to the substrate affinities for the enzyme, or to different regulations at the gene level. This hypothesis was reinforced by our results with the oleic acid diet because n-6 Δ6-desaturation is increased by 46% in group OA, when n-3 Δ6-desaturase did not change. Considering this result, the hypothesis that the existence of one or two isoforms for 18C Δ6-desaturase should be reconsidered. The higher levels of Δ6-desaturase transcripts observed in all experimental groups were not consistent with a previous report showing a 60% decrease in n-6 Δ6-desaturase activity and gene expression under the effect of sunflower oil or fish oil (23,24). Nevertheless, in these reports, the animals were fed a high-glucose, fat-free diet for 10 d, and then the same diet enriched with triolein as a control; such a diet could be considered to be highly lipogenic. Thus, major differences in the protocols, mainly in the choice of control diets, could explain such contradictory results. The results for liver n-6 Δ6-desaturase activities very closely reflected the results obtained for Δ6-desaturase transcripts. On the contrary, those for n-3 Δ6-desaturase did not. The reasons for such differences remain unidentified; however, these observations require further investigation of the 18C Δ6 desaturation step in the two PUFA families, in spite of the data from several authors (19,20,25) evoking the existence of only one desaturase for both 18C and 24C fatty acids. In agreement with Cho et al. (23), we found a high level of Δ6-desaturase expression in brain, even though a low level of Δ6-desaturase activity has been reported in this tissue (26,27). However, none of our experimental diets seemed to influence Δ6-desaturase expression in cerebral or kidney tissues, suggesting that the regulation of this enzyme by dietary fatty acids is tissue specific.

In conclusion, this work demonstrates the following: (i) PUFA are able to induce the Δ6-desaturase gene, depending on the choice of the control diet; and (ii) there are unexpected differences in the effects of these diets on the first desaturation step of the two PUFA families (n-6 and n-3), supporting the hypothesis that the desaturation of 18:2n-6 and 18:3n-3 fatty acids might be regulated differently. The intracellular or nuclear phenomena involved, such as the influence of the sterol regulatory element binding protein-1 (28–30), are yet to be elucidated.
References


