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Accelerated Myelinogenesis by Dietary Lipids in Rat Brain

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Abstract: Our previous work showed an early development of behavioral reflexes in rats whose mothers had been fed, during pregnancy and lactation, a lipid fraction extracted from yeast grown on *n*-alkanes (which contain 50% odd-chain fatty acids) in comparison with controls fed a margarine diet. To clarify whether the observed changes might be linked to an early myelination, we have investigated mRNAs involved in myelin synthesis in the brains of offspring at 5 days of age by northern blot and in situ hybridization. Northern blot analysis showed that proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) mRNAs were higher in animals on the lipid diet compared with controls. In situ hybridization with probes specific for PLP, myelin basic protein, and MOG mRNA showed significantly higher numbers of positive cells in test animals compared with controls in all brain regions. This study shows an acceleration of myelinogenesis induced by dietary lipids. These data can give a new insight in the therapeutical approaches involved to promote repair in demyelinating diseases. **Key Words:** Dietary lipids—Myelin gene expression—Development—Rat brain.
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Myelination is one of the major biological events occurring during the development of the brain. Myelin formation in CNS occurs as the result of a coordinate expression of a specific genetic program of the oligodendrocytes. The program can also be affected by environmental factors; consequently, only the correct interaction of such factors makes the formation and maintenance of the myelin sheath possible. Among environmental factors, the important role of diet and, in particular, of dietary lipids is becoming more evident (Yeh, 1988). Neonatal fat intake has been shown to influence and regulate myelin-specific mRNA levels. In particular, a postnatal deficiency of essential fatty acids (EFAs) reduces myelin basic protein (MBP) and proteolipid protein (PLP) mRNA levels in the brain (DeWille and Farmer, 1992).

Our previous studies have shown that when synthetic diets containing a lipid fraction derived from *n*-alkane–grown yeast (which contains 50% odd-chain fatty acids) are administered during pregnancy and throughout lactation, behavioral development is accelerated (Gozzo et al., 1978, 1981) and brain biochemistry and morphology are modified in the offspring (Salvati et al., 1984). These data indicate that it is possible that dietary lipids interfere with brain development and accelerate some patterns of its maturation.

To clarify if the observed behavioral modification in newborn rats is related to the accelerated myelinogenesis, we investigated the effects of this diet on mRNAs involved in myelin synthesis in the early postnatal period. Knowledge of the factors that stimulate the synthesis of myelin components is important both to provide new insight into the myelinogenesis process and to be the basis for therapeutical approaches to promote repair in demyelinating disorders.

EXPERIMENTAL PROCEDURES

Animals and diets

During pregnancy and lactation, female Sprague–Dawley rats from Charles River Laboratories (Como, Italy) were fed synthethic diets containing casein 21%, rice starch 53%, sucrose 10%, salt mixture (AIN-76) 4%, vitamin mixture (AIN-76) 1%, cod liver oil 1%, margarine 9%, and corn oil 1%. In the test diet, the margarine and corn oil were replaced by the lipid fraction obtained from yeast (*Candida lypolitica*) grown on *n*-alkanes.

The composition of the lipid fraction extracted from hydrocarbon-grown yeast and the fatty acid contents of the two diets are shown in Tables 1 and 2. The litter size in each group was culled to eight pups at birth. Three littermates

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Abbreviations used: EFA, essential fatty acid; IGF-I, insulin-like growth factor I; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; PUFA, polyunsaturated fatty acid; SDS, sodium dodecyl sulfate.

TABLE 1. *Composition of lipid fraction of microbial lipids*

Fraction	% of total
Steroids	2.7
Nonpolar lipids	14.0
Phospholipids	76.1
Other polar lipids	7.2

were killed by decapitation at 5 days of age. From every litter, three brains were quickly removed, fixed in 4% paraformaldehyde in phospate-buffered saline (pH 7.4) for 3 h, and routinely embedded in paraffin. Brains from the remaining five pups were removed and dissected in the medulla oblongata, the cerebellum, and the cerebral cortex, and the isolated regions, pooled for each experiment, were quickly processed for total RNA extraction. For the northern blot analysis, three independent experiments were performed.

DNA probes

The following probes were used: cDNA encoding for rat PLP in pGEM3 vector (Milner et al., 1985) and rat myelin oligodendrocyte glycoprotein (MOG) in pBSII vector (Gardinier et al., 1992).

Bacteria strains containing plasmids were propagated under standard culture conditions in LB medium. Supercoiled plasmid DNA was isolated by standard alkaline-lysis procedures and processed with the appropriate restriction endonucleases (Sambrook et al., 1989).

The GAPDH probe was a full-length cDNA clone encoding rat glyceraldehyde-3-phosphate dehydrogenase (Fort et al., 1985).

The cDNA probes were labeled using a (Boehringer Mannheim) random-primed DNA-labeling kit, following the manufacturer's instructions. Unincorporated deoxyribonucleoside triphosphates were removed by chromatography through Sephadex G50 columns (Pharmacia).

Northern blot hybridization

Total cellular RNA was extracted from the medulla, the cerebellum, and the cerebral cortex obtained from rats at 5 days of age as described previously (Chomczynski and Sacchi, 1987). Tissues were homogenized in Utraspec RNA (Biotecx Laboratories, Inc., U.S.A.) and RNAs isolated were quantified by OD_{260}. Equal amounts (20 μg/lane) of total RNA were denaturated and fractionated on a 1% agarose/formaldehyde gel (Lehrach et al., 1977) and blotted onto an Amersham Hybond N nylon membrane. The RNAs were cross-linked to membrane by heating for 2 h at 80°C in vacuo.

Prehybridization was performed using 500 m*M* sodium phosphate, pH 7.2, 7% sodium dodecyl sulfate (SDS), 1 m*M* EDTA, pH 8, for 1 h at 65°C and then hybridized in the same solution for 24 h at 65°C to random-primed ^{32}P-labeled cDNA using 1.5 or 2 $\times$ 10^6 cpm of probe/ml of hybridization buffer. After hybridization, the membranes were washed three times briefly in 50 m*M* sodium phosphate, pH 7.2, 1% SDS. Washed filters were exposed to Kronex Du Pont films with intensifying screens at −80°C. Autoradiographs were scanned with a 2202 Ultrascan laser densitometer (LKB).

The integrity of the RNA and the equal loading in each lane were controlled with a glyceraldehyde-3-phosphate dehydrogenase probe.

In situ hybridization

In situ hybridization was performed with a nonradioactive system, using digoxigenin-labeled riboprobes. The technique has been described in detail previously (Breitschopf et al., 1992). In brief, the riboprobes used were generated from plasmids containing cDNA inserts specific for PLP (Milner et al., 1985), MBP (Mentaberri et al., 1986), and MOG (Gardinier et al., 1992). cRNA probes were synthesized by transcribing linearized plasmids in both directions using the appropriate RNA polymerases. The sense strand served as a negative control. After in situ hybridization, the sections were also stained with hematoxylin.

Quantitative analysis of in situ hybridization

A semiquantitative analysis of mRNA-containing cells was performed in the upper portions of the cervical spinal cord, the medulla oblongata, the deep cerebellar white matter, the centrum semiovale, and the parietal cerebral cortex. The number of positive cells was determined in multiple high-power fields of 0.0234 mm^2 in each area. The following scores from 0 to 4 were used: 0, no cells positive; 1, one cell positive; 2, one to five cells positive; 3, six to 20 cells positive; 4, >20 cells positive.

More detailed quantitative analysis was performed in the central white matter of the cerebellum (around the dentate nucleus) and in the centrum semiovale. For the latter, standardized serial sections at the level of the midthalamus were used. Ten high-power microscopic fields adjacent to the lateral ventricle between the lateral angle of the ventricle and the corpus callosum were analyzed. The number of in situ hybridization positive cells was counted and recalculated as stained oligodendrocytes per square millimeter.

Statistical analysis was done using the statistical package StatView II for the Macintosh computer. The nonparametric group test (Mann–Whitney *U* test) was used.

RESULTS

There were no significant differences in the body and the brain weights between control and test rats. Data, given as mean ± SD values from 15 animals,

TABLE 2. *Fatty acid composition of the diets (%)*

Fatty acids	Margarine diet	Test diet
14:0	1.2	1.8
15:0	0.5	7.3
16:0	16.7	10.5
16:1 (n-7)	2.3	7.4
17:0	0.7	3.4
17:1 (n-5)	1.5	29.2
17:2 (n-5)	0.3	12.8
18:0	5.3	1.7
18:1 (n-9)	28.1	11.9
18:2 (n-6)	41.4	11.4
18:3 (n-3)	2.0	2.6
Σ odd-chain	3.0	52.7

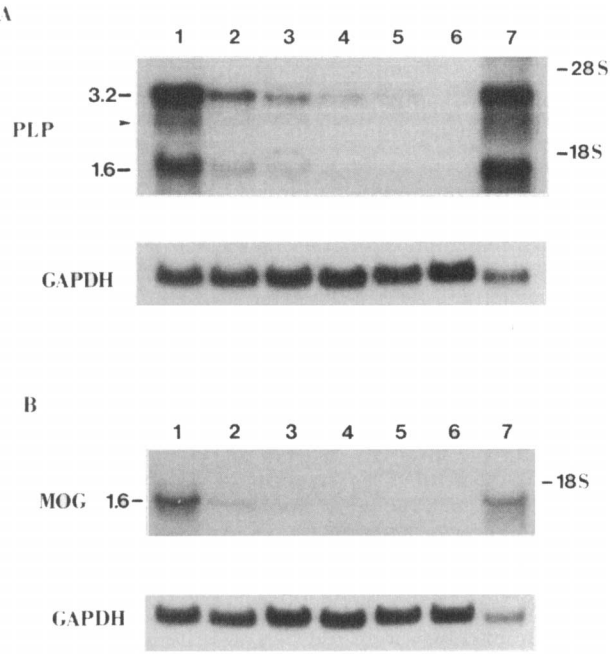

FIG. 1. Expression of PLP (**A**) and MOG (**B**) mRNAs in the medulla (lanes 1 and 2), the cerebellum (lanes 3 and 4), and the cerebral cortex (lanes 5 and 6) of rats 5 days old and in brain of rats 14 days old (lane 7). Lanes 1, 3, and 5 are test samples; lanes 2, 4, and 6 are control samples. Each lane contains 20 μg of total RNA from the indicated tissues. Autoradiography films were exposed for 1 week. Filters were stripped and subsequently rehybridized to a rat glyceraldehyde-3-phosphate dehydrogenase cDNA as a control for equivalent loading of samples. The arrowhead indicates 2.4-kb mRNA PLP.

were the body weight expressed in grams (15.6 ± 1.8 vs. 15.8 ± 2.2) and the brain weight expressed in milligrams (650 ± 0.11 vs. 660 ± 0.13) from control and test rats, respectively.

The expression of PLP and MOG mRNAs in the medulla, the cerebellum, and the cortex was analyzed by northern blot in 5-day-old rats. As shown in Fig. 1, the levels of PLP and MOG transcripts in test and control animals increase in an approximate gradient from hindbrain to forebrain, which is directly related to the gradient of myelination; the strongest hybridization signal per microgram of RNA was observed in the medulla, and the cerebral cortex exhibited the lowest.

Similar to what is reported in the literature (Milner et al., 1985), PLP mRNAs have two transcripts, of 3.2 kb and 1.6 kb. In controls, the major 3.2-kb PLP mRNA band was clearly detected in the medulla oblongata and the cerebellum, whereas the minor 1.6-kb PLP mRNA band was detected only in the medulla oblongata (Fig. 1). By contrast, in test animals, the 3.2-kb PLP transcript was well detected in each region examined and the 1.6-kb PLP mRNA band was clearly visible both in the medulla oblongata and in the cerebellum. Moreover, the 2.4-kb PLP transcript that appears as a minor mRNA band later in brain development (Milner et al., 1985) is already detected in the medulla of the test group.

The temporal order of PLP and MOG expression is different; the MOG expression is delayed compared with PLP (Gardinier et al., 1992). In fact, in controls, the MOG transcript was visible only in the medulla. By contrast, in test animals, the MOG mRNA band was detected in both the medulla and the cerebellum, showing an accelerated gradient of developmental expression compared with controls.

The relative intensity of myelin protein mRNA levels in test animals was always higher than in controls.

In situ hybridization with probes specific for PLP, MBP, and MOG mRNA stained oligodendrocytes in the brain and the spinal cord. In addition, Schwann cells of cranial and spinal roots were detected by specific probes. MBP mRNA was found in the perinuclear oligodendrocyte cytoplasm as well as in peripheral oligodendrocyte processes, whereas PLP and MOG mRNA was restricted to perinuclear areas (Fig. 2a–c). No reaction was found in control sections, stained with the respective sense probes.

In the developing rat brain, a gradient of mRNA expression for the different myelin proteins was found from caudal to cranial regions. The highest density of reactive cells was found in the white matter of the spinal cord, followed by the medulla oblongata and the central areas of the cerebellar white matter. Only few cells with mRNAs were present in the subcortical regions of the cerebellum and the centrum semiovale. Whereas the staining intensity and the number of reactive cells were similar for PLP and MBP mRNAs, the expression of MOG mRNA was significantly lower (Fig. 2d–q). In all brain regions analyzed by semiquantitative techniques, rats fed the test lipid diet showed more positive oligodendrocytes visualized by in situ hybridization and a more pronounced expression of myelin protein mRNAs compared with rats fed the control diet (Fig. 3).

Detailed quantitative analyses of myelin protein mRNA-expressing cells was performed in both the cerebellum and the centrum semiovale. The results revealed a significantly increased number of oligodendrocytes that were stained for either PLP or MOG mRNA, in rats fed the test lipid diet compared with those fed the control diet (Fig. 4).

DISCUSSION

Myelinogenesis is a scheduled process that proceeds in a caudocranial direction involving different tracts at different times. On the one hand, the time course for myelin development is well known, and on the other hand, relatively little is known about the factors that regulate this process. Nevertheless, several lines of evidence suggest that environmental factors including diet play an important role in myelination. Severe protein-calorie and protein malnutrition (Fuller et al., 1984)

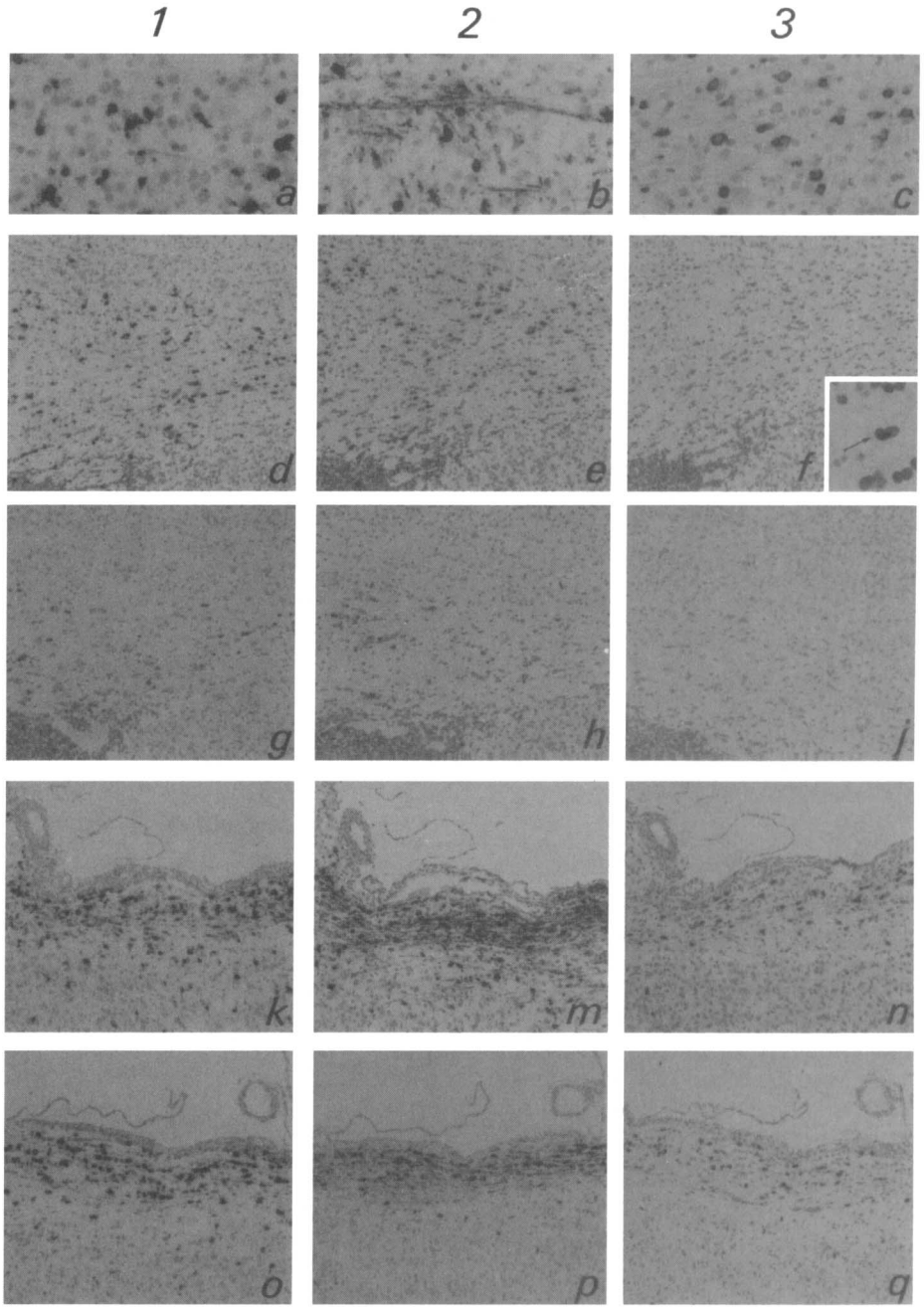

FIG. 2. In situ hybridization with riboprobes specific for PLP mRNA (lane **1**), MBP mRNA (lane **2**), and MOG mRNA (lane **3**) visualizing oligodendrocytes (purple) in the spinal cord (k-q) and in the centrum semiovale (d–j) on serial tissue sections that have been counterstained for cell nuclei with hematoxylin (blue). **a-c:** The different staining patterns of oligodendrocytes, perinuclear with PLP- and MOG-specific riboprobes (a, c), perinuclear and cell processes with MBP-specific riboprobe (b) (×680). **d-f:** Centrum semiovale of rats fed lipid diet, showing various numbers of positively stained oligodendrocytes with the respective riboprobes (×210). **Insert** in f: Positively stained cell (arrow) (×680). **g–j:** Centrum semiovale of control animals showing in all three examples the reduced numbers of stained oligodendrocytes compared with animals fed lipid diet (×210). **k–n:** Spinal cord of rats fed lipid diet showing enhanced reactivity in the white matter compared with control animals (**o–q**) with all three riboprobes (×210).

and an EFA-deficient diet (McKenna and Campagnoni, 1979; Berkow and Campagnoni, 1981, 1983) during development reduce mRNA levels of myelin proteins (DeWille and Farmer, 1992; Royland et al., 1992, 1993) and cause hypomyelination in rat brain. Results of this study show, instead, changes in the opposite direction in progeny of rats fed during pregnancy and lactation phospholipids containing odd-chain fatty acids extracted from hydrocarbon-grown yeast. Both in situ hybridization studies and northern blot analysis have shown that the expression of myelin proteins is higher in tests than in the control animals in each region examined of the brain. It is worth noting that the patterns of brain development of control rats fed margarine were similar to those of other control animals fed standard laboratory diets in previous and in this experiment (Gozzo et al., 1978, 1982). Dietary microbial lipids are positively involved in the control of the CNS myelinogenesis process without affecting the brain and body weight. To our knowledge, an in vivo positive effect on the myelinogenesis process is observed in the transgenic mouse line that overex-

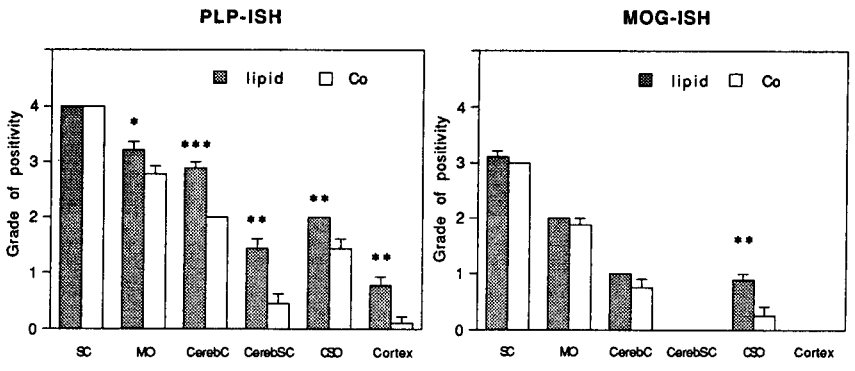

FIG. 3. Distribution of oligodendrocytes in various rat brain regions. The density of positive cells for PLP mRNA and MOG mRNA was estimated in a semiquantitative way using in situ hybridization (ISH). Values represent mean and SEM of nine animals (PLP-ISH: lipid, Co; MOG-ISH: lipid) and eight animals (MOG-ISH: Co), respectively. Statistically significant differences between rats fed test lipid diet (lipid) and rats fed control diet (Co) are shown; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. SC, cervical spinal cord; MO, medulla oblongata; CerebC, central cerebellum; CerebSC, subcortical cerebellum; CSO, centrum semiovale.

presses insulin-like growth factor I (IGF-I); however, an increase in brain and body weight was also observed (Carson et al., 1993).

The increase in myelin protein mRNAs is reflected in an increased number of differentiated oligodendrocytes and an enhanced synthesis of myelin-specific proteins. Our previous results (Salvati et al., 1984) have shown that the levels of myelin proteins were higher in the light myelin fraction isolated from rats treated in similar experimental conditions at 7 and 14 postnatal days. By 21 days, the levels of myelin proteins were present in the test group at control levels. Further results (Confaloni et al., 1993) have shown that at 7 postnatal days in rats fed microbial lipid, MBP- and PLP-positive fibers are already present in a more rostral level such as the corpus callosum. In controls, the staining fibers were detected later in brain development. Taken together these findings indicate that microbial lipids play a positive role in the control of the early phases of the process and do not alter the mechanisms involved in arresting its synthesis and formation. Such mechanisms appear to be difficult to keep under control in transgenic mice for IGF-I, because in this condition the CNS myelin content is greater than that in the brain of the controls (Carson et al., 1993).

Although microbial lipids are constituted by different lipidic fractions, our previous results lead us to hypothesize that such effects are due to their high content of odd-chain fatty acids (Gozzo et al., 1982). We performed studies to establish whether early behavioral development (Gozzo et al., 1978) observed in pups whose mothers were fed diets containing microbial lipids during pregnancy and lactation could be ascribed to a specific fraction of the yeast lipid. Our results have shown a similar behavioral development only in pups fed fatty acids isolated from the yeast lipids. Diets containing commercial soya lecithin also induced similar effects but to a lesser degree, whereas diets containing an unsaponified steroid fraction extracted from *n*-alkane-grown yeast adversely affected behavioral maturation (Gozzo et al., 1982). Changes in myelin development could determine the early development of behavioral parameters. A delay in behavioral development was instead observed in rats fed the EFA-deficient diet in which hypomyelination was present (Galli et al., 1975).

We observed the presence of odd-chain fatty acids

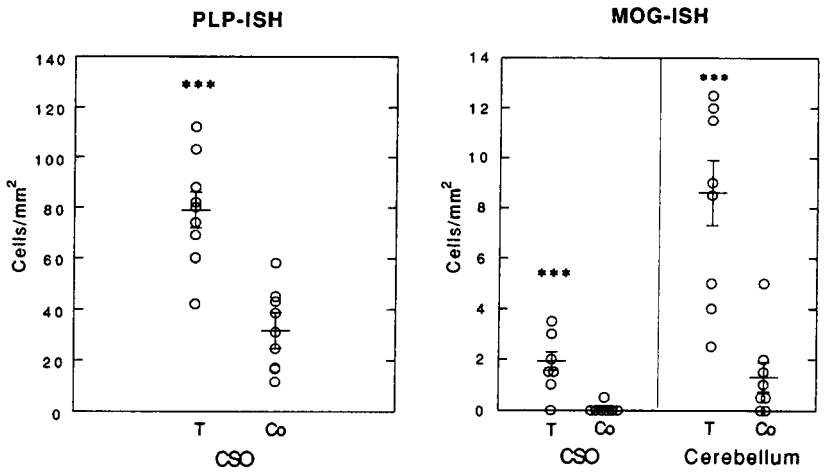

FIG. 4. Number of oligodendrocytes in various brain regions. Oligodendrocytes positively stained for MOG mRNA or PLP mRNA were counted in both the cerebellum and the centrum semiovale (CSO) in rats fed either test lipid diet (T) or rats fed control diet (Co). There was a statistically significant difference between groups; $***p < 0.001$. Number of animals: PLP-ISH (CSO) T: n = 9, Co: n = 9; MOG-ISH (CSO, cerebellum) T: n = 9, Co: n = 8.

in brain lipids of rats both at birth and at weaning (Bernardini et al., 1978). A high percentage of odd-chain fatty acids in the test animals compared with controls at birth, as well as their increase during development, suggest that these acids cross the placenta, are secreted in the maternal milk, and cross the blood–brain barrier. Taken together, these findings suggest that fatty acids, which are known to be important structural membrane components, may also play a role in myelin synthesis.

In recent years, there is growing evidence that fatty acids may regulate the gene expression at the transcriptional level (Distel et al., 1992; McDougald and Lane, 1995; Baillie et al., 1996). Recent studies on primary hepatocytes have shown that polyunsaturated fatty acids (PUFAs) reduce mRNA levels of various hepatic genes (Clarke and Jump, 1993; Jump et al., 1994; Liimatta et al., 1994). In preadipose cells, fatty acids (>12 C atoms) are potent inducers of various genes that encode proteins directly involved in fatty acid metabolism (Amri et al., 1995). Because PUFA modulation of gene expression is very rapid, a direct effect on gene transcription has been suggested. However, the molecular mechanism by which PUFA may regulate gene transcription still remains unclear.

Myelin synthesis is a process involving coordinate gene expression of myelin-specific proteins. In our experiments, we observed a coordinated accelerated expression in MBP, PLP, and MOG mRNAs in test rats. The analysis of these gene promoters has revealed the presence of common regulatory elements (Gardinier et al., 1992; Daubas et al., 1994; Roth et al., 1995). Moreover, Brendt et al. (1992) have shown that some of these sequences bind unidentified transcriptional factors. On the basis of this information, we can speculate that odd-chain fatty acids can modulate in a coordinated way the expression of myelin-specific genes activating directly or indirectly common transcriptional factors.

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REFERENCES

Amri E. Z., Bonino F., Ailhaud G., Abumrad N. A., and Grimaldi P. A. (1995) Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. *J. Biol. Chem.* **270,** 2367–2371.

Baillie R. A., Jump D. B., and Clarke S. D. (1996) Specific effects of polyunsaturated fatty acids on gene expression. *Curr. Opin. Lipidol.* **7,** 53–55.

Berkow S. E. and Campagnoni A. T. (1981) Essential fatty acid deficiency: effect of cross-fostering mice at birth on brain growth and myelination. *J. Nutr.* **111,** 886–894.

Berkow S. E. and Campagnoni A. T. (1983) Essential fatty acid deficiency: effect of cross-fostering mice at birth on myelin levels and composition. *J. Nutr.* **113,** 582–591.

Bernardini M. P., Salvati S., Serlupi Crescenzi G., Tagliamonte B., and Tomassi G. (1978) Nutritional studies on the lipid fraction of n-alkane grown yeast. II. Effect of different dietary levels on odd-chain fatty acid composition of rat brain. *Nutr. Rep. Int.* **17,** 137–145.

Berndt J. A., Kim J. G., and Hudson L. D. (1992) Identification of *cis*-regulatory elements in the myelin proteolipid protein (PLP) gene. *J. Biol. Chem.* **267,** 14730–14737.

Breistschopf H., Suchanek G., Gould R. M., Colman D. R., and Lassmann H. (1992) *In situ* hybridization with digoxigenin-labeled probes: sensitive and reliable detection method applied to myelinating rat brain. *Acta Neuropathol. (Berl.)* **84,** 581–587.

Carson M. J., Behringer R. R., Brinster R. L., and McMorris F. A. (1993) Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice. *Neuron* **10,** 729–740.

Chomczynski P. and Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159.

Clarke S. D. and Jump D. B. (1993) Regulation of gene transcription by polyunsaturated fatty acids. *Prog. Lipid Res.* **32,** 139–149.

Confaloni A., Avellino C., Malvezzi Campeggi L., and Salvati S. (1993) Accelerated myelinogenesis induced by dietary lipids in rat. *Dev. Neurosci.* **15,** 94–99.

Daubas P., Pham-Dinh D., and Dautigny A. (1994) Structure and polymorphism of the mouse myelin/oligodendrocyte glycoprotein gene. *Genomics* **23,** 36–41.

DeWille J. W. and Farmer S. J. (1992) Postnatal dietary fat influences mRNAs involved in myelination. *Dev. Neurosci.* **14,** 61–68.

Distel R. J., Robinson G. S., and Spiegelman B. M. (1992) Fatty acid regulation of gene expression. Transcriptional and post-transcriptional mechanism. *J. Biol. Chem.* **267,** 5937–5941.

Fort P., Marty L., Piechaczyk M., Sabrouty S. E., Dani C., Jeanteur P., and Blanchard J. M. (1985) Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucleic Acids Res.* **13,** 1431–1442.

Fuller G. N., Johnston D. A., and Wiggins R. C. (1984) The relationship between nutritional adequacy and brain myelin accumulation: a comparison of varying degrees of well fed and undernourished rats. *J. Neurosci. Res.* **10,** 289–293.

Galli C., Messeri P., Oliverio A., and Paoletti R. (1975) Deficiency of essential fatty acids during pregnancy and avoidance learning in the progeny. *Pharmacol. Res. Commun.* **7,** 71–77.

Gardinier M. V., Amiguet P., Linington C., and Matthieu J. M. (1992) Myelin/oligodendrocyte glycoprotein is a unique member of the immunoglobulin superfamily. *J. Neurosci. Res.* **33,** 177–187.

Gozzo S., Oliverio A., Salvati S., Serlupi Crescenzi G., Tagliamonte B., and Tomassi G. (1978) Nutritional studies on the lipid fraction of n-alkane grown yeast. IV. Effects on behavioral development. *Nutr. Rep. Int.* **17,** 357–366.

Gozzo S., Di Felice M., and Salvati S. (1981) Ontogenetic alterations of the cerebral cortex in rat caused by a diet containing a lipid fraction extracted from yeast (*Candida lypolitica*) grown on n-alkanes. *Int. J. Neurosci.* **13,** 219–227.

Gozzo S., Oliverio A., Salvati S., Serlupi Crescenzi G., Tagliamonte B., and Tomassi G. (1982) Effects of dietary phospholipids and odd-chain fatty acids on the behavioural maturation of mice. *Food Chem. Toxicol.* **20,** 153–157.

Jump D. B., Clarke S. D., Thelen A., and Liimatta M. (1994) Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J. Lipid Res.* **35,** 1076–1084.

Lehrach H., Diamond D., Wozney J. M., and Boedtker H. (1977) RNA molecular weight determinations by gel electrophoresis

under denaturation conditions, a critical reexamination. *Biochemistry* **16,** 4743–4751.

Liimatta M., Towle H. C., Clarke S. D., and Jump D. B. (1994) Dietary polyunsaturated fatty acids interfere with the insulin/glucose activation of L-type pyruvate kinase gene transcription. *Mol. Endocrinol.* **8,** 1147–1153.

McDougald O. A. and Lane D. M. (1995) Transcriptional regulation of gene expression during adipocyte differentiation. *Annu. Rev. Biochem.* **64,** 345–373.

McKenna M. C. and Campagnoni A. T. (1979) Effect of pre- and post-natal essential fatty acid deficiency on brain development and myelination. *J. Nutr.* **109,** 1195–1204.

Mentaberri A., Adesnik M., Atchinson M., Norgard E. M., Alvarez F., Sabatini D. D., and Colman D. R. (1986) Small basic proteins of myelin from central and peripheral nervous systems are encoded by the same gene. *Proc. Natl. Acad. Sci. USA* **83,** 1111–1114.

Milner R. J., Lai C., Nave K. A., Lenoir D., Ogata J., and Sutcliffe J. G. (1985) Nucleotide sequences of two mRNAs for rat brain myelin proteolipid protein. *Cell* **42,** 931–939.

Roth M. P., Malfroy L., Offer C., Sevin J., Enault G., Borot N., Pontarotti P., and Coppin H. (1995) The human myelin oligodendrocyte glycoprotein (MOG) gene: complete nucleotide sequence and structural characterization. *Genomics* **28,** 241–250.

Royland J. E., Konat G. W., Kanoh M., and Wiggins R. C. (1992) Down-regulation of myelin-specific mRNAs in the mechanism of hypomyelination in the undernourished developing brain. *Dev. Brain Res.* **65,** 223–226.

Royland J. E., Konat G. W., and Wiggins R. C. (1993) Abnormal upregulation of myelin genes underlies the critical period of myelination in undernourished developing rat brain. *Brain Res.* **607,** 113–116.

Salvati S., Conti de Vergiliis L., Di Felice M., de Gier J., Demel R. A., Serlupi Crescenzi G., Tomassi G., and Tagliamonte B. (1984) Morphological and biochemical changes in myelin subfractions of developing rats fed microbial lipids. *J. Neurochem.* **42,** 634–643.

Sambrook J., Fritsch E. F., and Maniatis T. (1989) *Molecular Cloning, 2nd edit.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Yeh Y. Y. (1988) Maternal dietary restriction causes myelin and lipid deficits in the brain of offspring. *J. Neurosci. Res.* **19,** 357–362.