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The administration of pig brain phospholipids versus soybean phospholipids in the diet during the period of brain development in the rat results in greater increments of brain docosahexaenoic acid

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Abstract

Dietary porcine brain phospholipids are much more efficient than soybean phospholipids for ensuring a normal (optimal obtained with lab chow diet) level of docosahexaenoic acid (DHA) in tissues and brain subcellular fractions (brain myelin and nerve endings). Two weeks before mating, rats were divided into two groups (one group was subdivided into subgroups, fed with varying amounts of porcine brain phospholipids; the other group was divided into subgroups fed varying amounts of soybean phospholipids). Pups were killed when 21 days old. DHA (22:6(n-3)) increased up to normal levels in parallel with increasing amounts of (n-3) fatty acids (omega-3 fatty acids) in the diet, up to 60 mg with dietary porcine brain phospholipids and up to 200 mg with soybean phospholipids. Thus a smaller amount of dietary brain phospholipids resulted in the same level of DHA in tissues as a larger amount of dietary soybean phospholipids. In contrast, 22:5(n-6) declined when (n-3) fatty acids in the diet increased. It stabilized at 60 mg of (n-3) fatty acids/100 g diet with brain phospholipids, and approximately 200 mg/100 g diet with soybean phospholipids. As 22:5(n-6) replaced DHA in tissue when (n-3) fatty acids were not sufficient in the diet, this result shows that the recovery of a normal (and minimal) amount of 22:5(n-6) was obtained with lower dietary levels of brain phospholipids compared with soybean phospholipids.

Keywords: Docosahexaenoic acid; Phospholipids; Triglycerides; Brain development; Soybean oil; Omega-3

A number of nutritional studies have clearly demonstrated that alpha-linolenic acid is essential. In animals models, as well as in humans, alpha-linolenic acid deficiency in the diet dramatically alters the fatty acid composition, membrane fluidity and membrane-bound enzyme activities of various organs, including the brain. Moreover, this deficiency alters electrophysiological parameters, such as the electroretinogram or even auditory response [4,5] and learning performance [13]. In fact, membranes contain only very limited amounts of alpha-linolenic acid, but large amounts of long chain polyunsaturated docosahexaenoic acid (DHA) derived from it. During brain development, the requirement for very long chain polyunsaturated fatty acid is very high. Thus it could be of interest to supply the very long chains directly in the diet in the form of phospholipids.

Phospholipids extracted from eggs obtained from hens fed a (n-3)-rich diet have been added to human infant formula milks in order to provide the very long chains found in human milk [2,10,17]. Very long chain polyunsaturated fatty acids are essential nutrients in infancy [12,14,18,22,23,26] to ensure optimal biochemical, electrophysiological and cognitive functions.

Dietary long-chain polyunsaturated fatty acids from different sources affect fat and fatty acid accretion in rats [1]. Nutritional deprivation of alpha-linolenic acid decreases but does not abolish turnover and availability of unacylated docosahexaenoic acid and docosahexaenoyl-CoA in rat brain [11]. (n-3) fatty acid (omega-3 fatty acid) deficiency decreases phosphatidylserine accumulation selectively in neuronal tissues [15]. As in Carrié [9], the intake of DHA-ethylester and the egg-phosphatidylcholine diet effectively enhances maze-learning ability and brain functions in old mice [20]. An open trial of plant-source

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derived phosphatydilserine for treatment of age-related cognitive decline was assayed [24]. Oral administration of soybean lecithin transphosphatidylated phosphatidylserine improves memory impairment in aged rats [25]. However, evidence from randomized trials does not support the use of lecithin in the treatment of patients with dementia. A moderate effect cannot be ruled out, but results from the small trials to date do not indicate priority for a large randomized trial [16].

Lysophosphatidylcholine is a preferred carrier form of docosahexaenoic acid to the brain [19]. On the other hand, there is evidence that entry of polyunsaturated fatty acids into brain microvessels involves phospholipase A2 and lipoprotein-induced methylation of phosphatidylethanolamine [21].

In a previous work, we calculated the dietary (n-3) fatty acid requirement for obtaining and maintaining a physiological level of (n-3) fatty acids in membranes as determined by the docosahexaenoic acid content. We concluded that, during the combined maternal and perinatal period, the (n-3) fatty acid requirement for adequate deposition of (n-3) fatty acids in the nervous tissue (and liver) of pups is 2-fold lower if animals are fed very long chain polyunsaturated fatty acids found in dietary brain phospholipids rather than alpha-linolenic acid from vegetable oil triglycerides [4,6]. Thus this experiment was designed to determine whether soybean phospholipids are as efficient as triglycerides or brain phospholipids in providing n-3 fatty acids. In contrast to brain phospholipids, soybean phospholipids contain nearly no detectable very long polyunsaturated chains, but large amounts of alpha-linolenic acid.

Female Wistar rats were bred in our laboratory and fed a semi-purified diet containing 5% lipids. This diet was deficient in alpha-linolenic acid (African peanut oil) was added to provide the previously defined optimal amount of linoleic acid [7]. Two weeks before mating, animals were divided into two groups (one group was subdivided into subgroups

Table 1
Fatty acid content of the various lipids used in the diets (mg/100 g fatty acids)[a]

Fatty acids	Porcine PL	Soybean PL
14:0	0.30	
15:0		
16:0	15.00	17.36
17:0	0.25	0.06
18:0	21.18	3.90
20:0	0.36	0.10
22:0	0.36	1.13
24:0	0.36	
∑ SFA	37.51	21.55
16:1n-9	0.51	
16:1n-7	0.64	0.06
18:1n-9	25.16	10.86
18:1n-7	6.00	0.33
20:1n-9	2.00	
20:1n-7	0.88	
22:1n-11	0.15	
22:1n-9	0.43	
22:1n-7	0.23	
24:1n-9	1.06	
24:1n-7	0.58	
∑ MUFA	37.64	11.25
18:2n-6	0.80	59.73
18:3n-6		0.06
20:2n-6		
20:3n-6	0.60	
20:4n-6	8.60	
22:4n-6	4.03	
22:5n-6	0.88	
∑ PUFA n-6	14.91	59.79
18:3n-3		7.36
18:4n-3		
20:5n-3		
22:5n-3	0.30	
22:6n-3	9.29	
∑ PUFA n-3	9.60	7.36
n-6/n-3	1.55	8.12

[a] Values are the mean of six determinations on at least three different batches. Porcine brain phospholipids and soybean phospholipids were used in this study. Bovine brain phospholipids and soybean oil triglycerides were used in previous studies [3,5] (respectively) and are included for comparison. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; and PUFA: polyunsaturated fatty acids.

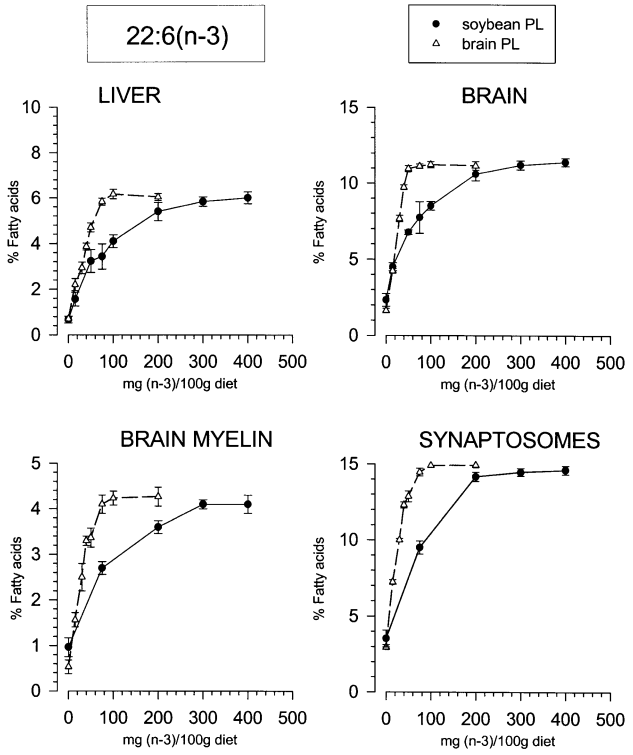

Fig. 1. 22:6(n-3) concentration in brain, liver, myelin and synaptosomes as a function of dietary (n-3) fatty acids. Open triangles: porcine brain phospholipids. Full circle: soybean phospholipids. % of total fatty acids. Mean values ± SD.

fed varying amounts of brain porcine phospholipids (total phospholipids, glycero ans sphingo); the other group was divided into subgroups fed varying amounts of soybean phospholipids). Subgroups were fed different amounts of (n-3) fatty acids, as follows for porcine brain phospholipids: 0, 15, 30, 40, 50, 75, and 100 mg of (n-3) fatty acids/100 g of diet; and for soybean phospholipids: 0, 15, 50, 75, 100, 200, 300, 400 mg of (n-3) fatty acids/100 g of diet. Due to the limited number of rats, less points were obtained for brain myelin and synaptosomes preparations: 0, 75, 200, 300, 400 mg of (n-3) fatty acids/100 g of diet. The (n-3) fatty acids given were esterified to phospholipids obtained from either an extract of porcine brain or soybean phospholipid prepared by the Institut Ponroy and absorbed on Aerosyl (50:50).

Pups were killed at weaning (when 21 days old). The overall composition of the diet has been previously published using bovine brain phospholipids [3] or vegetable oil triglycerides [5]. Table 1 gives the fatty acid profiles of the various lipids used in the diets. Subcellular fractionation (myelin and nerve endings), lipid extraction, transmethylation, and fatty acid analysis by gas-liquid chromatography were performed as previously described [3,5,6].

Each data point in the figures represents the mean value of at least five different brains and livers; at least three organs originated from animals from different litters. For brain myelin and nerve endings, each data point represents the mean value of at least four different preparations; each density gradient preparation required at least four animals. Thus, each individual data point represents at least 16 animals (from at least three different litters). Statistical analyzes were performed using Student's *t*-test and analysis of variance (two-way, $\alpha = 0.05$). Experimental protocols were approved and comply with government directives (Ministry of Agriculture authorization No. 03007, of 4 June 1991).

In the tissues and subcellular fractions examined, saturated and monounsaturated fatty acids were not significantly altered. Fig. 1 shows that in order for DHA (22:6 (n-3)) to reach normal (optimal obtained with lab chow diet) levels with increasing amounts of (n-3) fatty acids in the diet, 60 mg were required with dietary brain phospholipids and 200 mg with soybean phospholipids. Thus the same levels of DHA in tissues were reached with smaller amount of brain phospholipids, rich in DHA, in diet than with larger amounts of soybean phospholipids, rich in alpha-linolenic

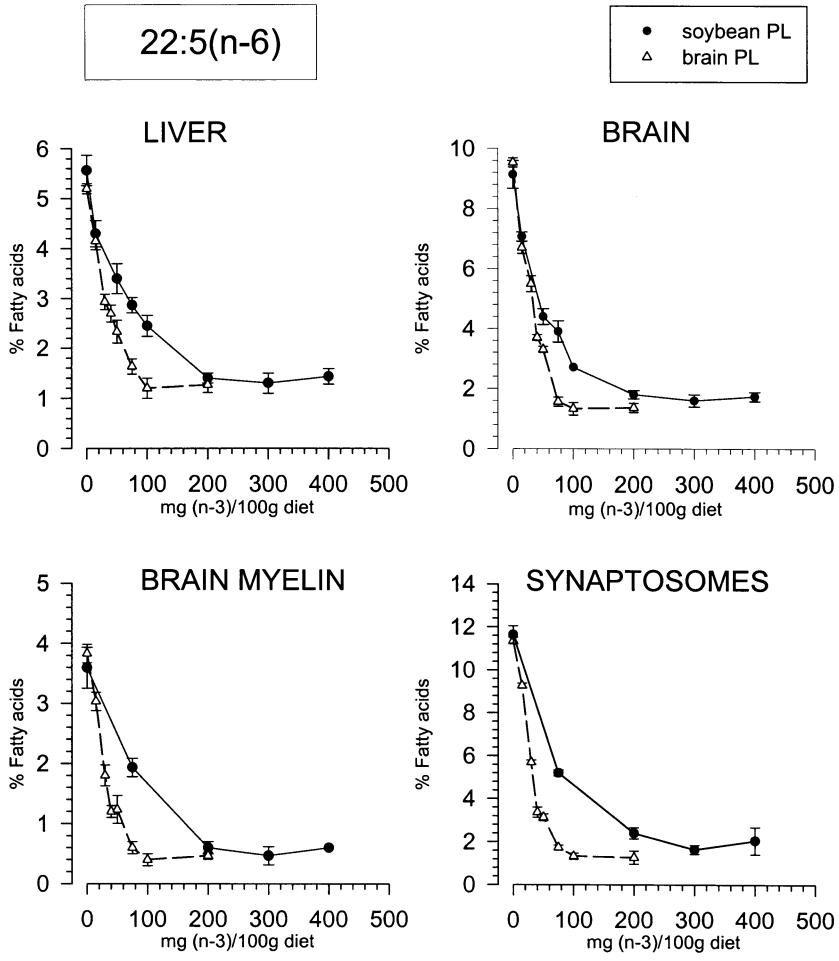

Fig. 2. 22:5(n-6) concentration in brain, liver, myelin and synaptosomes as a function of dietary (n-3) fatty acids. Same remark as in Fig. 1.

acid. Conversely, the same amount of (n-3) fatty acids in the diet resulted in a lower level of DHA in tissues when soybean phospholipids were used compared with dietary brain phospholipids.

In whole forebrain, arachidonic acid (20:4(n-6)) was also not affected regardless of the diet. In contrast, 22:5(n-6) (Fig. 2) declined when (n-3) fatty acids in the diet increased. It stabilized at 60 mg of (n-3) fatty acids/100 g diet with brain phospholipids, and approximately 200 mg/100 g diet with soybean phospholipids. As 22:5(n-6) replaced DHA in tissue when (n-3) fatty acids were not sufficient in the diet, this result shows that the recovery of a normal (and minimal) amount of 22:5(n-6) was obtained with lower dietary levels of brain phospholipids compared with soybean phospholipids.

With soybean phospholipids, as previously found with vegetable soybean oil triglycerides [5], when alpha-linolenic acid was not sufficient in the diet, DHA was replaced in tissues and subcellular fractions by 22:5(n-6). Thus the sum of 22:6(n-3) + 22:5(n-6) was constant, regardless of the diet. The results obtained with soybean phospholipids in this study were essentially the same as those previously obtained with soybean triglycerides [5], although the fatty acid profiles are not exactly the same (15:0, 0, 12.1; 16:0, 17.3, 0; 18:(n-9), 10.9, 22.1; 18:2(n-6), 59.7, 53.2; 18:3(n-3), 7.4, 7.6; for soybean phospholipids and soybean oil, respectively).

The logical explanation is that phospholipids provide the preformed very long chain fatty acids and these are used directly for membrane synthesis in various tissues. But it can not be excluded that there is some kind of preservation of the (n-3) fatty acids that prevents them from entering the various pools (e.g. tissue triglycerides, cholesterols esters and other lipids) when given as phospholipids rather than triglycerides. The results obtained in this study are in agreement with those of Wijendran [27] showing the efficacy of dietary aracidonic acid provided as triglycerides or phospholipids as substrates for brain arachidonic accretion in baboon neonates: phospholipids are about 2.1-fold more effective than triglycerides as substrates for brain arachidonic acid accretion.

In conclusion, dietary porcine brain phospholipids are much more efficient than soybean phospholipids for attaining normal levels of DHA in tissues and subcellular fractions. This should be taken into account when providing essential fatty acids for animal models (for neurochemical research) and for humans, and more particularly for infants (formula milks) and during development and ageing. If using lipids from animal brains could raise problems, it must be kept in mind that, very probably, phospholipids from other source such as eggs, if they have the same fatty acid profile, are also efficient. We have recently demonstrated that specific phospholipid fatty acid composition in mice is affected by (n-3) dietary polyunsaturated fatty acid deficiency, and reversed without significant difference with phospholipid supplementation from either pig brain or egg yolk [8]. Moreover, these two kind of phospholipids reverse behavioral and biochemical alteration in deficient mice [9].

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