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Developmental and neurobehavioral effects of perinatal exposure to diets with different ω-6:ω-3 ratios in mice

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Abstract

Objective: To investigate in mice the effect of diets enriched with soy or sunflower oil with different ω-6:ω-3 ratios on gestation, reproductive success, physical maturation, and the neurobiological development of the pups.

Methods: Dams were assigned, throughout gestation and lactation, to different groups: a commercial diet (CD), a soy oil–enriched diet (SOD), or a sunflower oil–enriched diet (SFOD). Measurements during gestation were dams' body weights and daily food intakes. Measurements in the offspring were physical parameters (body weight, body length, body mass index, fur appearance, pinna detachment, incisor eruption, eye opening, and puberty onset) and behavioral preweaning tests (surface righting reflex, negative geotaxis, and cliff avoidance).

Results: The SOD and SFOD dams became significantly heavier than the CD dams from gestational days 14 and 19, respectively, to parturition. There were no significant differences in gestational length or food consumption during pregnancy or lactation or in maternal weight during lactation. Diets did not modify litter size, sex ratio, survival index at weaning, or body weight. The SFOD and SOD offspring were significantly shorter than the CD offspring at weaning. The mean offspring physical scores of SOD and SFOD offspring were higher than CD offspring and simple reflexes were earlier in the SOD and SFOD groups. In SFOD offspring, puberty onset was significantly delayed, at postnatal days 26 and 27 in male and female offspring, respectively.

Conclusion: This study suggests that the maintenance of an adequate ω-6:ω-3 ratio is necessary for the optimal growth and development of murine offspring. In populations that do not have sufficient provision of polyunsaturated fatty acids in the diet, their consumption would be advisable during gestation and lactation because these improve most neurodevelopmental outcomes included in this study.

Keywords: Soy oil; Sunflower oil; Perinatal development; Polyunsaturated fatty acid; Neurobehavior

Introduction

Unlike cellular protein composition, which is genetically determined, cell membrane polyunsaturated fatty acid (PUFA) quality and proportion are largely influenced by diet [1]. Membrane bilayers are more polyunsaturated in metabolically active tissues than in less active ones. It has been postulated that this feature results in an increased molecular activity of membrane proteins. The amount of membrane PUFAs and their composition can act as signals for nervous system development and maturation [2]. Supplementing diets with PUFAs may be beneficial during pregnancy, parturition, lactation, and fetal development. In contrast, during gestation, maternal overnutrition, obesity, and high saturated fat intake may be as harmful to the developing baby as undernutrition [1,3–5].

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Throughout pregnancy, PUFAs are transferred from the mother to the fetus across the placenta. Recent studies have suggested that ω-3 fatty acid intake has a significant impact on growth, vision, brain, and vital organ development in breast-fed infants [3,6–8]. Therefore, maternal PUFA deficiency could adversely affect fetal development. Conversely, an increased maternal intake of these compounds before, during, and after pregnancy could minimize such risks by ensuring an adequate supply through the perinatal period, childhood, and the entire lifespan [4].

Through the last trimester of pregnancy and the first postnatal months, arachidonic acid (an ω-6 FA) and docosahexaenoic acid (DHA; an ω-3 FA) accumulate in membrane phospholipids of the nervous system in humans and mice [5,9]. The ω-3 PUFA content of the neonatal brain has been associated with improved cognitive capability [1,10,11] and supplementing infant formulas with DHA has, therefore, been recommended to improve neurodevelopmental outcomes. At present, there is considerable information about the impact of supplementing a pregnant mother's food with DHA on the development of her infants [3,12–15].

Currently, the richest natural sources of dietary long-chain ω-3 PUFAs are triacylglycerols extracted from marine resources. Large populations, however, especially those of low economic status, do not regularly consume fish because it is not naturally available or due to cost limitations. This is also the limiting reason for consuming infant formulas enriched with ω-3 PUFAs. One of the main alternative sources of ω-3 PUFAs is soy oil. Tofail et al. [16] provided diets supplemented with fish oil or soy oil as an ω-3 source to pregnant women during the last trimester of pregnancy. They did not find significant differences between the two diet groups in developmental or behavioral outcomes of the children at 10 mo of age. Further studies are necessary to elucidate whether results obtained when administering soy oil are similar to those obtained when DHA is given to pregnant mothers or added to infant formulas.

The hypothesis of this study was that essential FA supplementation during pregnancy and lactation would modify the physical and/or behavioral development of the pups. The maintenance of an adequate ω-6:ω-3 ratio might benefit offspring development.

The objective of this study was to investigate in mice the effects of diets enriched with soy or sunflower oil with their different ω-6:ω-3 ratios on gestation, reproductive success, physical maturation, and the neurobiological development of the pups.

Materials and methods

All procedures performed in the present work were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication 85-23, revised 1996).

Breeding was conducted by placing two female Albino swiss mice in a male's cage. Females were monitored for vaginal sperm plugs on a daily basis and once the plug was detected (considered gestational day 0 [GD0]), the female was removed from the male's cage and individually housed with bedding made from wood shavings. Animals were maintained under a standard 14-h light/10-h dark photoperiod and controlled temperature (22 ± 2 °C) and food and water were provided ad libitum.

Table 1
FA composition (grams per 100 g of total FA), kilocalories per gram, and percentage of kilocalories as fat of total dietary energy of the diets

FA	CD	SOD	SFOD
16:0	16.79	13.48	11.5
18:0	6.37	2.11	2.02
20:0	0.3	traces	traces
22:0	traces	traces	0.5
24:0	traces	traces	traces
16:1	2.46	3.05	2.51
18:1 (ω-9)	30.1	30.21	38.2
20:1	0.56	0.34	0.37
22:1	traces	traces	traces
18:2 (ω-6)	40.87	45.12	42.87
18:3 (ω-3)	2.14	4.94	1.4
ω-6:ω-3	19	9	31
kcal/g	2.79	3.10	3.10
% kcal as fat	12.58	25.23	23.23

CD, commercial diet; FA, fatty acid; SFOD, sunflower oil–enriched diet; SOD, soy oil–enriched diet.

Diets

Dams were randomly assigned to different groups, according to the diet they would receive throughout gestation and lactation: a commercial diet (CD; $n = 5$), a soy oil–enriched diet (SOD; $n = 4$), or a sunflower oil–enriched diet (SFOD; $n = 5$). The CD contained 3.9% fat, 43% carbohydrates, and 18% proteins.

The SOD and SFOD were prepared by adding to 95 g of a pelleted CD (Gepsa Grupo Pilar, Argentina) 5 g of commercial soy oil (Sojola, 100% pure soy oil, Aceitera General Deheza, Córdoba, Argentina) or sunflower oil (Natura, 100% pure sunflower oil, Aceitera General Deheza). To prevent oxidation, oils were added with butyl hydroxytoluene (2 g/L) [17] and diets were prepared once a week and stored at refrigeration temperatures (4 °C). A fresh amount of diet was provided daily to further protect against oxidation. Table 1 lists diet FA composition, calories (kilocalories per gram), and the percentage of kilocalories as fat of total dietary energy in each diet.

Diets were fed to the dams from the first GD and throughout lactation. The offspring continued receiving their respective diets after weaning (postnatal day 21 [PND21]) until adulthood. This report includes the parameters evaluated until the onset of puberty.

Measurements during gestation included mothers' body weights (on GD1, GD7, GD14, and GD19) and daily food intake.

Dams were allowed to deliver spontaneously and delivery day was recorded as PND0. The number of pups was counted on PND0 but they were not weighed or handled. Pups were