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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

sexed on PND1 and eight pups were randomly selected from each litter (four males and four females). Gender was confirmed on PND2, PND3, and PND4. During the testing protocol, whole litters were separated from the dams and maintained no more than 30 min in a warmed environment. Every offspring was tested with one trial each day.

Physical parameters

Litter body weight (grams) and pup body length (centimeters, from the middle of the head to the base of the tail) were measured on PND7, PND14, and PND21. Body mass index was calculated by dividing mean body weight by the square of mean body length of each litter.

Puberty onset was determined as the descent of both testes (PND21 to PND27) or a vaginal opening (PND21 to PND28).

Other physical parameters monitored were fur appearance, emergence of immature fuzz; bilateral pinna detachment, unfolding of an external ear; lower incisor eruption; eye opening, both eyes completely open. To determine the appropriate day to measure these parameters and to avoid excessive animal handling, an initial study was made of the appearance of each parameter in six litters of animals without any treatment and established the optimal day as the one before at least 95% of animals acquired each parameter (Table 2). In agreement with Kihara et al. [18], a schedule was devised indicating the age of testing.

Behavioral preweaning tests

Surface righting reflex is a standard test for labyrinth (inner ear) function and body-righting mechanisms [19,20]. Each animal was placed on its back on a flat surface for 4 s and then released. The time required to regain all four paws in contact with the surface was recorded with a stopwatch. The number of animals with successful responses under 2 s was recorded. Negative geotaxis reflects the function of the vestibular system [21]. The time taken to complete a 180-degree turn when placed in a head-down position on a 45-degree inclined cardboard surface and the number of animals with successful responses under 30 s were recorded. Cliff avoidance is an index of behavioral teratology in rodents, which can be impaired by motor, arousal, or cognitive dysfunction. It is a marker of the maturity of sensory and motor functions associated with development [22]. Each animal was placed on a table with the forepaws and nose over the edge (height 20 cm). The time required to complete backing and turning away from the edge and the number of animals with successful responses within 30 s were recorded.

The day for performing behavioral tests in experimental groups was determined in the same way that was previously described for some physical parameters, and results are presented in Table 2. Because the variables in these animals were not sex-dependent, the experimental unit considered was the litter.

Table 2
Schedule of physical parameters and behavioral tests battery in mice pups*

Physical parameters	Age of testing (PND)
Fur appearance	3
Pinna detachment	3
Incisor eruption	10
Eye opening	13
Behavioral tests	
Cliff avoidance (<30 s)	8
Negative geotaxis (<30 s)	9
Surface righting reflex (<2 s)	10

PND, postnatal day

* The day to devise the schedule was selected from the performance of each pup in six litters without any treatment. We established the optimal PND as the one before at least 95% of the animals acquired each physical parameter or was able to perform each test.

Physical parameters and results from behavioral tests were measured in each pup and percentages of animals that had acquired the parameter in each litter were recorded. Results are presented as the mean of the litters' means (between litters).

In accord with Wainwright et al. [23], scores were calculated for physical and behavioral performances. The percentage of appearance of each parameter in the litter on the scheduled day was divided by 100 to obtain values ranging from 0 to 1. The physical score averaged four physical parameters: fur appearance, pinna detachment, lower incisor eruption, and eye opening. The behavioral score represents the performance at the behavioral tests (surface righting reflex, negative geotaxis, and cliff avoidance) between litters. Thus, each score represents the percentage of maturity of those physical parameters or behavioral tests independently.

Statistical analysis

Values are expressed as mean ± standard error of the mean. Results were analyzed by two-way repeated measures analysis of variance or one-way analysis of variance, as appropriate. Post hoc testing was performed with Tukey's test and statistical significance was set at $P < 0.05$. Statistical procedures were performed by using Infostat 1.1 (Group Infostat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina).

Results

There were no significant differences between dams' body weights during the first 2 wk of gestation. Dams fed with SOD became significantly heavier than those on CD from GD14 to parturition and than the SFOD dams from GD19 to parturition. There was a clear diet effect ($F = 12.6$, $P < 0.01$) with weight increase over time ($F = 180.7$, $P < 0.01$) and an interaction between treatment and time ($F = 2.7$, $P = 0.03$). The evolution of body weight was thus different in gestation with the different treatments, with a greater rate of weight increase in the SOD and SFOD groups than in the CD group. SOD dams were also

significantly heavier than CD and SFOD days on lactation day 1 ($F = 5.5$, $P = 0.02$). After lactation day 1 until lactation day 21, there were no significant differences among groups in maternal body weight. There is a weight decrease over time ($F = 18.7$, $P < 0.01$) and an interaction between treatment and time ($F = 2.5$, $P = 0.04$). The evolution of body weight was also different in lactation with the different treatments (Fig. 1). There were no significant group differences in gestational length or in food intake during pregnancy or lactation (Table 3).

Table 4 presents the results of physical parameters and behavioral test measurements in pups from dams fed the CD, SOD, or SFOD. Diets did not modify litter size, sex ratio, or survival index at weaning, although there was a tendency toward increased postnatal mortality from birth to weaning in the SOD group. The different ω-6:ω-3 ratios did not affect body weight at birth or during lactation. There was growth over time ($F = 664.79$, $P < 0.01$), but no interaction was detected between time and diet. SOD mice, however, tended to have greater body weight. SFOD mice were significantly shorter than CD mice at PND7 and they were also shorter than SOD mice at PND14. At weaning, SOD and SFOD mice were significantly shorter than CD mice. Statistical analysis showed a diet effect ($F = 16.10$, $P < 0.01$), a time effect ($F = 1492.17$, $P < 0.01$), and time-by-treatment interaction ($F = 7.96$, $P < 0.01$). Matching the differences in length, there were significant differences in body mass index between treatments at PND7, PND14, and PND21 ($F = 472955.1$, $P < 0.01$), but there was no time effect or time-by-diet interaction. In the other physical parameters, there was a trend to earlier development with the SOD and SFOD. Fur appearance and pinna detachment occurred earlier in SFOD mice ($F = 108.18$, $P < 0.01$; $F = 2.83$, $P < 0.05$), and eye opening was earlier in SOD mice compared with the other two groups ($F = 5.3$, $P < 0.05$).

Behavioral test results were also altered by diets. Compared with CD animals, SOD and SFOD mice showed earlier onset of the surface righting reflex ($F = 3.48$, $P < 0.05$). In negative geotaxis, when placed on a 45-degree angle slope, most SOD mice turned around toward the top sooner than CD and SFOD mice ($F = 3.85$, $P < 0.05$). In cliff avoidance, the CD animals tended to explore the edges of the board and showed stereotyped sniffing rotational behavior on the test table sooner than SOD and SFOD mice ($F = 25.04$, $P < 0.01$), some of whom remained frozen until the end of the test. SFOD mice were the most delayed.

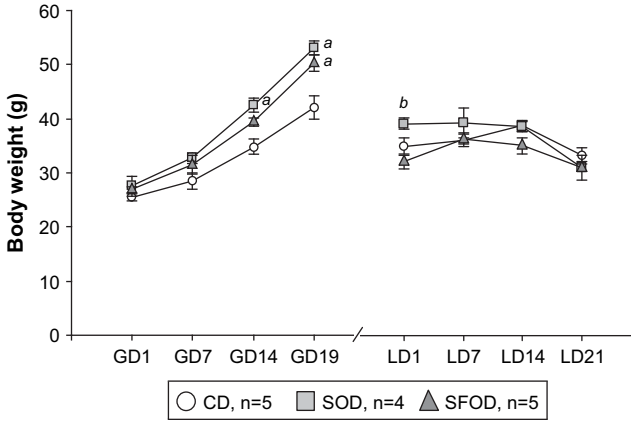

Fig. 1. Body weight during gestation or lactation in murine dams fed with a CD, an SOD, or an SFOD. Results are expressed as mean ± SEM. [a] $P < 0.05$ versus CD, [b] $P < 0.05$ versus CD and SFOD. CD, commercial diet; GD, gestational day; LD, lactation day; n = number of animals; SFOD, sunflower oil–enriched diet; SOD, soy oil–enriched diet.

The mean physical scores of SOD and SFOD mice were higher than in CD mice ($F = 5.15$, $P < 0.05$). The mean behavioral score, however, was lowest in SFOD mice ($F = 15.05$, $P < 0.01$; Fig. 2).

Regarding the onset of puberty, the vaginal opening began on PND21 in CD and SOD female offspring, but on PND23 in SFOD offspring, when almost 50% of pups from the other diets had already reached puberty onset. Although 100% of CD and SOD offspring were mature at PND27, SFOD offspring demonstrated delayed sexual maturity (Fig. 3). There were significant differences among the treatments ($F = 9.39$, $P < 0.01$), between the different times assessed ($F = 39.86$, $P < 0.01$), and time-by-treatment interaction ($F = 4.36$, $P < 0.01$), which implied that speed of maturation was different among treatments. Although descent of both testes began on PND21 with the three diets, on PND26 it was still delayed in SFOD offspring even though 100% of CD and SOD offspring had already reached puberty. There was an interaction between time and treatment ($F = 2.34$, $P < 0.01$), which

Table 3
Maternal and birthing outcomes of mouse dams exposed to CD, SOD, or SFOD*

	CD	SOD	SFOD
No. of dams	5	4	5
Gestational length (d)	19.6 ± 0.24	20.00 ± 0.00	20.40 ± 0.24
Maternal cumulative food consumption GD1–GD18 (g)	78.22 ± 4.96	82.93 ± 3.23	74.58 ± 4.15
Maternal cumulative food consumption PND1–PND21 (g)	302.11 ± 4.68	293.31 ± 7.71	288.81 ± 3.53
No. of pups/litter	10.14 ± 1.50	10.5 ± 0.29	11.2 ± 0.20
Survival index at weaning (%)	95.00 ± 1.67	90.63 ± 6.14	100 ± 0.00
Male/female ratio at birth	1.22 ± 0.08	0.82 ± 0.10	1.82 ± 0.45

CD, commercial diet; GD, gestational day; PND, postnatal day; SOD, soy oil–enriched diet; SOD, sunflower oil–enriched diet

* The survival index at weaning was calculated as the percentage of pups alive at weaning with respect to the number of pups at birth. Results are expressed as mean ± SEM.

Table 4
Physical parameters and behavioral tests in offspring from mouse dams exposed to CD, SOD, or SFOD*

	CD	SOD	SFOD
Physical parameters			
Body weight (g)			
PND7	5.26 ± 0.26 ($n = 5$)	6.05 ± 0.64 ($n = 4$)	5.17 ± 0.69 ($n = 5$)
PND14	9.60 ± 0.23 ($n = 5$)	10.39 ± 0.56 ($n = 4$)	9.04 ± 0.92 ($n = 5$)
PND21	13.15 ± 0.38 ($n = 5$)	14.07 ± 1.74 ($n = 4$)	12.94 ± 1.03 ($n = 5$)
Body length (cm)			
PND7	3.46 ± 0.02 ($n = 40$)	3.30 ± 0.02 ($n = 32$)	3.23 ± 0.06 ($n = 40$)†
PND14	4.57 ± 0.07 ($n = 38$)	4.52 ± 0.02 ($n = 29$)	4.05 ± 0.10 ($n = 40$)‡
PND21	5.45 ± 0.02 ($n = 38$)	5.13 ± 0.06 ($n = 29$)†	5.02 ± 0.07 ($n = 40$)†
Body mass index (g/cm^2)			
PND7	0.44 ± 0.02 ($n = 5$)	0.55 ± 0.05 ($n = 4$)§	0.50 ± 0.06 ($n = 5$)‡
PND14	0.47 ± 0.01 ($n = 5$)	0.50 ± 0.02 ($n = 4$)†	0.57 ± 0.06 ($n = 5$)†
PND21	0.40 ± 0.04 ($n = 5$)	0.52 ± 0.05 ($n = 4$)†	0.50 ± 0.03 ($n = 5$)†
Fur appearance (%)	67.50 ± 3.06	68.75 ± 3.61	100 ± 0.00‡
Pinna detachment (%)	67.50 ± 10.16	84.25 ± 5.18	90.00 ± 4.08†
Incisor eruption (%)	67.50 ± 7.50	90.63 ± 5.98	67.50 ± 7.50
Eye opening (%)	33.21 ± 11.50	76.25 ± 8.93†	65.00 ± 10.00
Behavioral tests (%)			
Cliff avoidance	95.00 ± 3.06	75.00 ± 8.84§	57.50 ± 3.06‡
Negative geotaxis	87.50 ± 3.95	96.88 ± 3.13§	87.50 ± 3.95
Surface righting reflex	90.00 ± 7.29	100.00 ± 0.00†	100.00 ± 0.00†

CD, commercial diet; GD, gestational day; PND, postnatal day; SOD, soy oil–enriched diet; SOD, sunflower oil–enriched diet

* Values are means ± SEMs. Body weight was measured in the litter (n = number of litters); body length was individually measured (n = number of animals). Body mass index was calculated by dividing the mean body weight by the square of the mean body length (n = number of litters). Percentages of fur appearance, pinna detachment, incisor eruption, eye opening, and behavioral tests were measured in each litter (CD, $n = 5$; SOD, $n = 4$; SFOD, $n = 5$; 8 pups each).

† $P < 0.05$ versus CD.

‡ $P < 0.05$ versus CD and SOD.

§ $P < 0.05$ versus CD and SFOD.

implied that the speed of maturing was different among the treatments (Fig. 4).

Discussion

Several sources of information have suggested that humans evolved on a diet with a ω-6:ω-3 FA ratio of 1:1 [1], whereas this ratio in Western diets is currently 10:1 to 20-25:1, indicating that they are deficient in ω-3 FAs compared with that on which humans evolved and their genetic patterns were established [1,11,24]. It has been demonstrated that the total amounts of PUFAs in wild animals are higher than those in farmed animals [25–28]. Research is ongoing for the production of ω-3 FA-enriched products. It is essential in the process of returning the ω-3 FAs into the food supply that the balance of ω-6:ω-3 FAs in the diet that existed during evolution is maintained [29,30].

The aim of this study was to assess the effect of diet supplementation with vegetable oils during gestation and lactation on reproductive success in pups' physical growth and behavioral development.

The two experimental diets led to the pregnant females being heavier during late pregnancy. Their offspring showed some adverse effects in physical and neurobehavioral outcomes, including delayed puberty onset and poor cliff avoidance behavior among SFOD offspring and shortened body length with the SOD and SFOD. Nevertheless, all the offspring reached maturity, despite the slight delays detected.

There were some beneficial effects from the diets: fur appearance, pinna detachment, incisor eruption, and eye opening were accelerated, and the surface righting and negative geotaxis reflexes were also premature. The early acquisition of some physical parameters and simple reflexes as described in our report could be advantageous in the wild or natural setting.

It is well known that pregnancy may be a high-risk period for developing obesity, and support for this is provided in the present study by the data from SOD and SFOD dams during pregnancy. In the groups fed with hypercaloric oil–supplemented diets, the dams showed significant increases in body weight. Despite more calories from fat in the oil-supplemented diets, no differences were found in daily food intake. Other results have suggested that pregnant rats on a fat-enriched diet reduce their ingestion to maintain similar caloric intake compared with controls [31,32]. Keesey and Hirvonen [33] proposed the existence of a ''body weight set-point'' in rodents and humans whereby body weight decrease or increase is corrected by altering food intake and energy expenditure to maintain the target body weight. This weight-control mechanism was not found in dams in this study and this may explain the body weight increase. During early and middle gestation, fat storage is normally facilitated

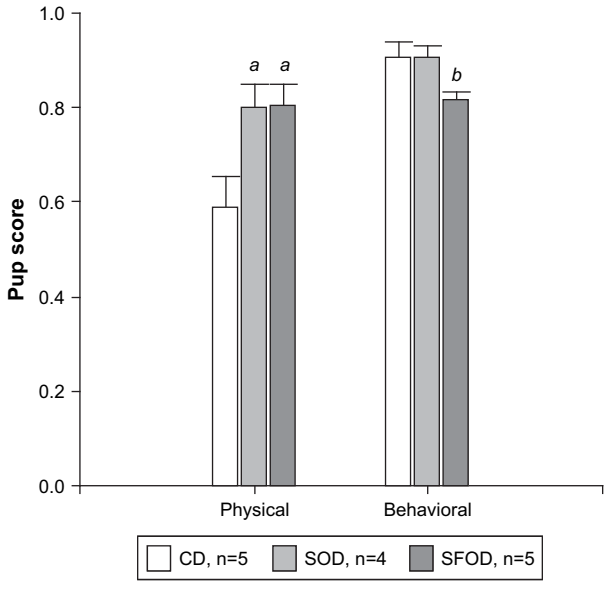

Fig. 2. Physical (fur appearance, pinna detachment, lower incisor eruption, and eye opening) and behavioral scores in mouse pups exposed from gestation to a CD, an SOD, or an SFOD. Results are expressed as mean ± SEM. [a] $P < 0.05$ versus CD, [b] $P < 0.05$ versus CD and SOD. CD, commercial diet; n = number of litters; SFOD, sunflower oil–enriched diet; SOD, soy oil–enriched diet.

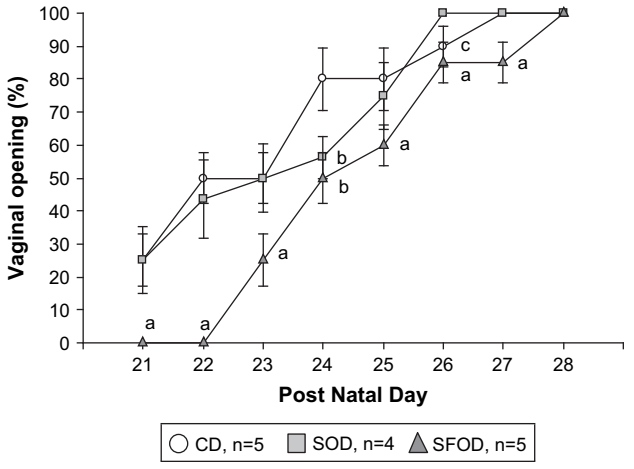

Fig. 3. Vaginal opening in mouse pups exposed from gestation to a CD, an SOD, or an SFOD. Results are expressed as mean ± SEM of litter means. [a] $P < 0.05$ versus CD and SOD, [b] $P < 0.05$ versus CD, [c] $P < 0.05$ versus CD and SFOD. CD, commercial diet; n = number of litters; SFOD, sunflower oil–enriched diet; SOD, soy oil–enriched diet.

through increased FA synthesis within adipose tissue [34] and through elevated adipose tissue lipoprotein lipase activity [35,36], an enzyme believed to regulate exogenous triacylglycerol uptake by this tissue [37].

In this study, the different ω-6:ω-3 ratios did not have significant effects on gestational length, litter size, and birth weight. Similar results were reported when feeding with balanced diets [24] or with an excess of ω-3 FAs [16,38,39]. These results agree with those of Church et al. [15] and Lands et al. [40], that rat dams fed with PUFA-excessive or -deficient diets used compensatory mechanisms during pregnancy to maintain some variables related to gestational and pup development.

Mammals usually produce approximately equal numbers of sons and daughters, but there are circumstances in which ruminants and other mammalian species, especially rodents and primates, appear able to skew the sex ratio of their offspring. Rosenfeld and Roberts [41] from a study performed in mice suggested that the age of the mother and maternal diet, rather than the maternal body condition per se, play directive roles in controlling the sex ratio. Diets supplemented with excessive amounts of long-chain PUFAs favored male offspring in opossums [42]. Conversely, in mice, a corn oil–enriched diet had a preponderance of female-biased litters compared with a fish oil–enriched one [43]. In this study, the offspring sex ratio did not differ in any group, possibly because the ω-6:ω-3 ratio of the diets were not as high as those employed in previous reports.

No significant group differences were detected in pup weights during lactation or at weaning. However, some studies in rats have shown that the balance between ω-6:ω-3 PUFAs in the maternal diet, rather than the amount of ω-6 or ω-3 PUFAs per se, could be important for adipose tissue growth and for maintaining adequate serum concentrations of leptin in the offspring [44]. Excessive maternal intake of ω-3 PUFAs led to reduced adipose tissue mass and lower serum leptin levels in suckling pups compared with the pups of dams fed with balanced ω-6:ω-3 diets or with excess ω-6 [15,44,45]. Diets in this study were balanced (SOD) or with a moderate excess of ω-6 (SFOD). This could explain the maintenance of offsprings' body weight during lactation.

The body length of pups from dams fed with SFOD was shorter from PND7 until weaning, whereas that of SOD pups was significantly shorter at PND21. Similar results

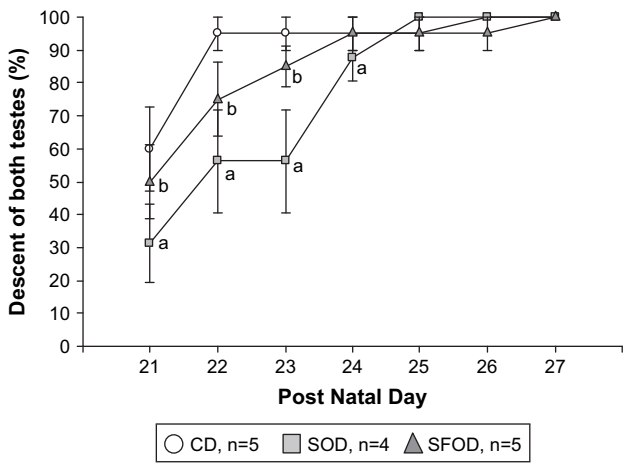

Fig. 4. Descent of both testes in mouse pups exposed from gestation to a CD, an SOD, or an SFOD. Results are expressed as mean ± SEM of litter means. [a] $P < 0.05$ versus CD and SFOD, [b] $P < 0.05$ versus CD and SOD. CD, commercial diet; n = number of litters; SFOD, sunflower oil–enriched diet; SOD, soy oil–enriched diet.

have been obtained in rats by other groups administering excessive fish oil [15,45] or high ω-6 diets [44] to the mothers. Recently, Komlos and Breitfelder [46] and Hundley [47] found that black women are shorter than white women and they considered that a poor diet or fast food could be the main culprit. The moderate excess of ω-6 in the SOD and SFOD could be responsible for this finding. However, further studies are necessary to elucidate these results. The body mass index increase in both groups could be the consequence of the above-mentioned shortening.

The results in the physical parameters of fur appearance, pinna detachment, lower incisor eruption, and eye opening were seen sooner in SOD or SFOD pups than in CD pups, although in some cases these did not reach statistical significance. The earlier eye opening in SOD offspring may reinforce the importance of ω-3 in the diet for eye maturation [7].

The score of those parameters showed a clear improvement in early development when enriched diets were administered, which could be attributed to the moderate excess of ω-6 in these diets. This supports the idea that, although the ω-6:ω-3 ratio is important, the amount of each essential FA administered in the diet must be also taken into account [48]. It should also be noted that the percentage of kilocalories as fat given by the two enriched diets is also higher than that in the CD.

The delayed vaginal opening observed in the group fed with the SFOD contrasts with the findings of Hilakivi-Clarke et al. [49,50] describing precocious puberty in female mouse offspring when their mothers were fed with a high ω-6 diet. This may be due to the fact that those diets were higher in ω-6 (43% or 46% of calories from corn oil) than that employed in the present study (25% of calories from sunflower oil). Any consideration of the effects of the SOD and SFOD on sexual maturation in females must take into account the fact that linoleic acid is the precursor for arachidonic acid biosynthesis, because, in excess, this inhibits prostaglandin synthesis, which is involved in the physiologic control of sexual development [51]. However, these effects were not as evident in the descent of testes in males. Further studies are necessary to elucidate if these differences are associated with the ω-6:ω-3 ratio, the net amount of each FA, or some other compounds incorporated into the diet.

When analyzing the behavioral test results, it is important to consider that negative geotaxis and surface righting reflex evaluate aspects related to vestibular or labyrinth (inner ear) functions, whereas cliff avoidance evaluates more complex neural functions reflecting the maturity of sensory–motor functions associated with nervous development.

The negative geotaxis and surface righting reflex results show that the SOD improved both reflexes, whereas the SFOD did not modify the former and improved the latter. However, the cliff avoidance test results are clearly different, indicating that both supplemented diets exerted a harmful effect and that this was worse with the SFOD, and, because DHA increases motor skill development in preterm infants [52], it can be hypothesized that the ω-3 deficiency in the SFOD was responsible for this impairment. This is not true, however, for the SOD because, despite having an adequate ω-6:ω-3 balance, it impaired the response to the test. The moderate excess of ω-6 may explain this result. These factors do not seem to affect the other two reflexes, which, as already outlined, evaluate less complex aspects of nervous system physiology. Results obtained with the behavioral score in this study are not useful because they include a harmful effect that is somewhat misleading. It may be concluded that the SOD and SFOD benefit the acquisition of simple reflexes but do not improve cliff avoidance, where a more complex development is needed.

The present results show that maternal nutrition during pregnancy and lactation has significant effects on the physical and neurobiological development of offspring. It is worth noting that, biologically, the control of neural, sexual, and/or physical growth and development in general are subject to control mechanisms mediated by different chemical messengers and feedback mechanisms. Some of these may therefore be modified differentially.

In this study, the SOD and SFOD showed mostly accelerated physical and behavioral development during the preweaning period. In contrast, there was delayed development during the postweaning period (vaginal opening, testes descent) with the SFOD. An age-related effect of this diet should be taken into account, considering that a multitude of factors including lipids and diet may lead to puberty-timing alterations and pubertal progression [46,53]. Further research will be needed to clarify this topic.

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 PUFAs in the diet, their consumption would be advisable during gestation and lactation because these improve most neurodevelopmental outcomes included in this study.

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