A Postnatal Diet Containing Phospholipids, Processed to Yield Large, Phospholipid-Coated Lipid Droplets, Affects Specific Cognitive Behaviors in Healthy Male Mice¹–³

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Abstract

Background: Infant cognitive development can be positively influenced by breastfeeding rather than formula feeding. The composition of breast milk, especially lipid quality, and the duration of breastfeeding have been linked to this effect.

Objective: We investigated whether the physical properties and composition of lipid droplets in milk may contribute to cognitive development.

Methods: From postnatal day (P) 16 to P44, healthy male C57BL/6JOlaHsd mice were fed either a control or a concept rodent diet, in which the dietary lipid droplets were large and coated with milk phospholipids, resembling more closely the physical properties and composition of breast milk lipids. Thereafter, all mice were fed an AIN-93M semisynthetic rodent diet. The mice were subjected to various cognitive tests during adolescence (P35–P44) and adulthood (P70–P101). On P102, mice were killed and brain phospholipids were analyzed.

Results: The concept diet improved performance in short-term memory tasks that rely on novelty exploration during adolescence (T-maze; spontaneous alternation 87% in concept-fed mice compared with 74% in mice fed control diet; \( P < 0.05 \)) and adulthood (novel object recognition; preference index 0.48 in concept-fed mice compared with 0.05 in control-fed mice; \( P < 0.05 \)). Cognitive performance in long-term memory tasks, however, was unaffected by diet. Brain phospholipid composition at P102 was not different between diet groups.

Conclusions: Exposure to a diet with lipids mimicking more closely the structure and composition of lipids in breast milk improved specific cognitive behaviors in mice. These data suggest that lipid structure should be considered as a relevant target to improve dietary lipid quality in infant milk formulas. J Nutr doi: 10.3945/jn.115.224998.

Keywords: postnatal diet, breast milk, dietary lipid droplets, brain development, cognitive behavior

Introduction

Infancy is a period of rapid growth and development during which the brain is particularly sensitive to environmental influences, including nutrition. Cognitive development in infants can be positively influenced by duration and exclusivity of breastfeeding in comparison with infant milk formula (IMF)⁷ as the sole source of nutrition during the first months of life (1–4).

Differences in nutritional quality between breast milk and IMF may contribute to this effect. An important part of the daily energy intake of infants is derived from dietary fat. Milk fat not only provides energy for growth, but also supplies lipids and their essential precursors, which are used by and accumulate in the developing brain. Of particular relevance to brain and cognitive development is the FA composition of milk. Long-chain PUFAs (LCPUFAs), such as DHA, rapidly accumulate in the brain during pre- and postnatal life in an age-specific pattern (5, 6) and support various essential neurodevelopmental processes (7–10). Breastfed infants were shown to have a higher brain DHA concentration than formula-fed infants (11, 12), which could be explained by different (13) and more variable concentrations (14, 15) of DHA and other PUFAs in breast milk than in IMF. Although a direct relation between milk FA composition and cognitive development is difficult to prove in...
humans, numerous rodent studies have shown that dietary LCPUFAs can affect brain FA composition and cognitive performance (16).

Besides having a unique FA composition, human milk contains biologically active lipids, including polar lipids such as phosphatidylcholine and sphingomyelins (17), that contain important nutrients for brain development, such as choline (18). Although the total amount of these polar lipids in human milk is <1% of total fat (19, 20), IMF is usually void of polar lipids.

We investigated an aspect of lipid quality beyond composition, namely, the way in which lipid components are structured in milk. The mammary gland secretory cells release breast milk containing TGs and other nonpolar lipids as lipid globules, which are enveloped by a triple-layer membrane—the so-called milk fat globule membrane (MFGM)—which consists of phospholipids and other polar lipids, cholesterol, (glyco-)proteins, and enzymes (21). These membrane-enveloped lipid globules have a diameter of ~4 μm (21, 22). In contrast, commercially available IMFs contain TGs as a lipid source; because of processing such as homogenization, lipid droplets have a far smaller diameter of 0.3–1.0 μm, with milk proteins adhering to the surface (23). The physical properties of dietary lipids, including lipid droplet size and surface, are relevant to digestion and absorption kinetics, affecting lipid bioavailability and metabolic fate (24–28). Altered lipid bioavailability during critical periods of development could alter organ development, with possible long-term consequences for function and capacity (responsiveness). Indeed, we showed in a mouse model that a diet provided between postnatal day (P) 16 and P42 containing lipid droplets with physical properties more closely resembling those found in breast milk, i.e., larger in size, and coated with phospholipids (Naturis) improved later-life metabolic health after challenging with a mild Western-style diet (29, 30). These results are in line with reported protective effects of breastfeeding on childhood obesity and metabolic health (31) and suggest that, besides LCPUFA composition, the physical structure and composition of lipid droplets may be a relevant aspect of lipid quality in milk. Given the important role of dietary lipids in brain development, we investigated whether a diet with large, phospholipid-coated lipid droplets in early life may impact brain development and cognitive function during the dietary intervention as well as later in life.

Methods

Mice and experimental design. All experimental procedures were approved by the Dutch Animal Experimental Committee in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), and complied with principles of laboratory animal care. Experimental groups were obtained from C57BL/6J Ola Hsd dams and housed in a controlled environment (12-h/12-h light/dark cycle, humidity, and temperature) with ad libitum access to food and water unless specified otherwise. Breeding pairs were fed semisynthetic rodent diets (AIN-93G; Research Diet Services). On P2, litters were randomly assigned and culled to 6 pups/dam (male-to-female ratio 4:2 or 5:1). On P16, dams with litters were randomly assigned to 1 of 2 experimental diets (3 dams with litters/diet) consisting of either control IMF (control diet) or Nuturis IMF (concept diet). The experimental diets were provided to the dams and litters in the form of dough on the cage floor to allow easy and early access to the diet by the pups. Between P16 and P21, pups consumed experimental diets but were also allowed to drink milk from the dam. Although pups can rely on solid foods from P16 onward, early weaning can have a profound influence on neurodevelopment and behavior (32) and was therefore avoided. Male pups were weaned on P21 onto their respective diets and single-housed on P34. On P44, mice were switched to a maintenance semisynthetic rodent diet (AIN-93M) until dissection on P102. Mice were weighed on a weekly basis throughout the experiment. A total of 27 male offspring (control diet, n = 13; concept diet, n = 14) were used for this study.

Experimental diets. The experimental diets (Research Diet Services; Supplemental Table 1) were semisynthetic, with a macronutrient and micronutrient composition that was based on the AIN formulation of AIN-93G purified diets for laboratory rodents as described (33), and were produced as previously reported (29, 30). The diets consisted of 28.3% wt:wt control or concept IMF powder and were complemented with additional protein, carbohydrates, and micronutrients to mimic AIN-93G composition. The fat portions of the diets were entirely derived from the IMFs, which were similar in total lipid content and FA composition (Supplemental Table 2), but differed in physical properties and composition of dietary lipid droplets because of the addition of milk phospholipid and processing adjustments. IMF powders were clinical study products for infants aged 0–6 mo. The control IMF was produced according to the standard stage I IMF recipe and processing procedures (Danone Nutricia Research). The concept IMF was generated by adding 4 g phospholipid sourced from bovine milk/kg IMF (Cream Serum; Royal Friesland Campina), and processing was adjusted to obtain phospholipid-coated lipid droplets that were larger than those in standard IMF (patent EP2825062A1) (34). Lipid droplet size distribution (Supplemental Table 3) was measured as previously described (29, 35). The structural characteristics and composition of the lipid droplets in the concept IMF are described elsewhere in detail (35).

Behavioral testing. Mice were subjected to various cognitive tests during adolescence (P35–P43) and adulthood (P70–P101) to assess possible short- and long-term effects of early exposure to the dietary intervention. All behavioral assessments were performed during the light phase.

Open field test. Effects of the postnatal dietary intervention on locomotor activity and exploratory behavior at juvenile (P35) and adult (P78) age were measured by using the open field test, with the use of the same settings and equipment as previously described (36). Briefly, mice were introduced into a square open field and exploration was tracked for 5 min. Total distance moved and time spent in the center square of 9 equally sized squares were measured. The open field test was used as a control assay to monitor basal levels of locomotor activity and exploration that could affect outcomes in other behavioral tests.

Novel object recognition test. To determine the effects of the postnatal diet on the ability of the mice to recognize a change in a previously explored environment, mice were subjected to the novel object recognition (NOR) test on P35 and P78. NOR testing was conducted subsequent to the open field test with the use of similar settings and video tracking software and according to a protocol described before (37). The test was divided into three 5-min trials in the same arena: object familiarization, novel place recognition (NPR), and NOR. Mice were returned to their home cage between trials. Mice were introduced in the west corner of the arena in each trial. During the familiarization trial, the arena contained 2 identical objects (plastic LEGO brick stacks of identical color but different shape) placed in the north and south corners of the arena. For NPR testing 2 h later, mice were introduced in the same arena with identical objects, one of which was placed in the east instead of the south corner. For NOR testing 2 h after NPR testing, mice were introduced in the same arena with a familiar object in the north corner and a novel object in the east corner. The basic measure was the time taken by the mice to explore the objects in the NPR and NOR trial. Performance was evaluated by calculating a discrimination index (N = F/N + F), where N = time spent exploring the object in the new location (NPR) or the novel object (NOR) and F = time spent exploring the object in the familiar location (NPR) or the familiar object (NOR). The discrimination index was calculated for the first 2 min after introduction into the arena. A higher index reflects better memory performance.
**T-maze.** The effect of postnatal diet on short-term spatial memory was assessed with the use of the T-maze spontaneous alternation test at juvenile (P36 and P37) and adult (P79 and P80) ages, as previously described (38). In short, the mouse was placed into the base of a T-shaped box and was allowed to choose freely the left or right arm to explore, which was closed up by a sliding door immediately after entry. After 30 s, the mouse was transferred back to the starting position and was again allowed to choose. A successful alternation was scored if mice chose to enter the previously unvisited goal arm. This procedure was repeated 6 times distributed across 2 d with ≥1 h in between. The percentage of test trials with a successful alternation was used as a measure of short-term spatial memory.

**Barnes maze.** The effects of the postnatal dietary intervention on spatial learning and memory during adolescence were assessed with the use of the Barnes maze (BM; between P38 and P41), as previously described in detail (36). Mice were introduced in the center of a circular platform with 24 holes equally spaced at 5 cm from the perimeter. One of the holes was designated as the target hole, providing access to an escape box mounted underneath the maze. During acquisition trials (P38, 2 trials; P39, 3 trials; P40, 3 trials; and P41, 1 trial in the morning), mice learned to associate extra maze visual cues with the location of the target hole. Trials ended after 5 min or when the target hole was found. Latency to reach the target hole was recorded for each session. During the probe trial (P41, in the afternoon) the escape hole was identical to all 23 other holes and a target zone was defined that comprised the previous escape hole and the holes next to the left and right of the escape hole. The proportion of hole visits in the target zone (probability) was calculated as follows: total number of visits to a hole in the target zone divided by the total number of hole visits.

Subsequently, the BM was used to determine possible effects of postnatal diet on behavioral flexibility. During the reversal-acquisition trials (P42, 3 trials; P43, 1 trial in the morning) of maximum 5 min, the target hole was located at the exact opposite position to its former location and the acquisition of the new escape location was measured as an indicator for behavioral flexibility. Reversal acquisition trials were followed by a reversal probe trial (P43, afternoon) as described above.

**Spontaneous behavior.** The spontaneous behavior of the mice was video recorded in a home cage environment between P73 and P79 with the use of a PhenoTyper (model 3000; Noldus Information Technology) and software as described in detail elsewhere (39). After 2 d of habituation, the X–Y coordinates of mice were acquired at 15 coordinates/s over 3 consecutive days. Data were processed to generate 20 key behavioral variables describing kinematic markers of move and arrest segments, characteristics of sheltering behavior, activity bout characteristics, habituation effects across days, the effect of the light/dark phase, and activity patterns during light/dark phase transitions.

**Radial-arm maze.** Effects of postnatal dietary intervention on spatial learning and memory during adulthood were assessed with the use of the 8-arm radial-arm maze (RAM; between P90 and P101). The apparatus consisted of a maze with 8 identical and equally spaced arms radiating from the center that could be baited with sweetened rewards (14 mg Dextrose Precision Pellets F05684; Bio-Serve) at the far end of the arm. The baits were located behind a horizontal obstacle on the floor of the arm, preventing mice from seeing the reward from the center. Inaccessible food rewards were present at the ends of all 8 arms, behind a fence, preventing mice from using the odor of the baits as a cue. Extramaze visual cues with different shapes were attached to the walls surrounding the apparatus. The mouse was placed in the center and was allowed to explore the arms. During acquisition, the same 3 arms were baited with food pellets over 20 consecutive trials (10 d; 2 trials/d). The trials ended either when all food pellets were consumed or after a maximum of 10 min. Reference memory was calculated as visits to baited arms divided by total visits and working memory performance was assessed by calculating the number of re-entries to previously visited arms divided by total visits in the same trial (working memory errors). The acquisition trials were followed by a probe trial on day 10 during which no arm was baited, and the ratio of entries into previously baited and unbaited arms was recorded for 10 min. To increase motivation for seeking food pellets, mice were provided with daily food below ad libitum intake to reach 90% of initial body weight starting 10 d before testing. Mice were weighed on a daily basis.

**Brain phospholipid analysis.** On P102, mice were killed by decapitation and brains were weighed. Brain material was lyophilized and stored at –80°C until further analysis. Brain phospholipids were quantified by an external laboratory (Biocrates Life Sciences) with the use of an MS-based metabolomics approach. The biologically most abundant members of (lyso-) glycerophospholipids, i.e., phosphatidyl-cholines, -ethanolamines, -serines, and -glycerols, as well as sphingolipids, i.e., sphingomyelins, ceramides, dihydroceramides, and 2-hydroxycyl ceramides, were analyzed by a high-throughput flow injection electrospray ionization tandem-MS screening method. Five internal standards were used to compensate for matrix effects, and 43 external standards were used for a multipoint calibration. Comparisons of diet groups were calculated on log2-transformed measurements for all metabolites yielding ≥75% of values above detection limit. Total brain phospholipid content and phospholipid class distribution (percentage of phosphorylated-cholines, -ethanolamines, -serines, -glycerols, sphingomyelins, and ceramides) was calculated.

**Statistical analysis.** The effects of the experimental diet on behavior, brain weight, phospholipid content, and phospholipid class distribution were tested with the use of SPSS 15.0.1 software. A Student’s t test was used to compare between the diet groups the behavioral variables in the T-maze; object recognition test; BM and RAM probe trials; spontaneous behaviors in the home cage; and the brain weight, brain phospholipid content, and phospholipid class distribution. The acquisition of learning over various trials in the BM and RAM and the possible influence of dietary exposure on acquisition were analyzed by repeated-measures ANOVA with the fixed factors time (trial day) and diet. The effects of the experimental diet on individual brain metabolites were analyzed by using in-house MetIDQ software with the use of the statistical computing environment R, with the R package Limma being applied for moderated statistical tests. A t test was used to compare between the diet groups and false discovery rate was calculated to correct for multiple comparisons. All data are presented as means ± SEMs unless indicated otherwise. Differences were considered to be significant at P < 0.05.

**Results**

**General health and home cage behavior.** The body weight gain of mice throughout the experiment was similar between diet groups (on P102: control, 27.4 ± 0.6 g; concept, 26.8 ± 0.6 g). In addition, there were no differences in spontaneous behavior of mice in the home cage during adulthood (Supplemental Table 4).

**Open field test.** The total distance moved (meters) and time spent in the center (seconds) were comparable between diet groups at adolescence (control, 42.5 ± 23.8 m and 126 ± 16 s; concept, 44.5 ± 25.1 m and 112 ± 11 s, respectively) and in adulthood (control, 56.8 ± 37.6 m and 29 ± 6 s; concept, 55.1 ± 31.9 m and 28 ± 4 s, respectively).

**NOR.** At adult age, the discrimination index during the novel object trial was significantly increased by early-life exposure to the concept diet (P = 0.038; Figure 1B), but the discrimination index was not different between groups during adolescence (Figure 1A). The discrimination index during the object location trial was similar in the diet groups at adolescent (control, 0.2 ± 0.1; concept, −0.1 ± 0.2) and adult (control, 0.2 ± 0.2; concept, 0.1 ± 0.2) age.

**T-maze test.** In adolescent mice, alternation performance in the T-maze was significantly greater in those fed the concept diet.
diet ($P = 0.037$; Figure 2A), but there was no difference between diet groups in alternation performance in adulthood (Figure 2B).

**BM test during adolescence.** During acquisition, mice showed a significant decrease in the latency to reach the target hole over training days ($P < 0.001$; Supplemental Figure 1A), but the decrease was not different between diet groups, indicating similar learning rates. The overall probability of visiting a hole in the target octant during the probe trial was not affected by diet (Supplemental Figure 1B), indicating similar spatial reference memory performance in the diet groups. Subsequently, during reversal, the latency to reach the target hole was reduced over training days ($P = 0.001$; Supplemental Figure 1C), but, again, not differently affected by diet. The probability of visiting a hole in the new target octant during the reversal probe trial was not affected by diet either (Supplemental Figure 1D).

**RAM during adulthood.** During acquisition, the number of correct visits to baited arms (reference memory performance) of the mice increased over trials ($P < 0.001$; Supplemental Figure 2A), whereas working memory errors decreased ($P < 0.001$; Supplemental Figure 2C). These changes were comparable between diet groups. Also during the probe trial, reference memory performance and working memory errors were similar between diet groups (Supplemental Figure 2B and D).

**Brain phospholipid profile.** On P102, brain weight was comparable between groups (control, 385 ± 7 mg wet weight; concept, 379 ± 2 mg wet weight). The phospholipid profile was analyzed in lyophilized brain tissue. A total of 275 metabolites were used for statistical analysis of individual lipid species. Of these, 20 metabolites were different between diet groups (9 ceramides, 4 phosphatidylcholines, 3 (lyso)phosphatidylethanolamines, and 4 sphingomyelins) (Supplemental Table 5), but these differences were not significant after false discovery rate correction. Total ceramide plus phospholipid (glycerophospholipid + phosphosphingolipid) concentration was similar between diet groups (control, 79.4 ± 5.2 nmol/mg tissue; concept, 71.9 ± 4.4 nmol/mg tissue). Although the relative phosphatidyl-glycerol concentration was significantly higher in the brains of mice fed the concept diet (0.211% ± 0.008% compared with 0.185% ± 0.006% in control; $P = 0.038$), the relative concentrations of ceramides and other phospholipid classes (phosphatidyl-cholines, -ethanolamines, -serines, sphingo-

**Discussion**

Our study showed that exposure to a diet containing lipids with composition and physical properties closer to human milk lipids (i.e., large, phospholipid-coated lipid droplets) between P16 and P44 improved T-maze performance and NOR in healthy male C57BL/6J Ola Hsd mice. The improved NOR performance was sustained into adulthood. These effects were demonstrated under feeding conditions that met all nutritional requirements for optimal growth and development of rodents. The experimental diets were introduced from P16 onward (directly after normal lactation), and groups showed normal body weight trajectories. Under these conditions, mice are expected to show optimal cognitive performance, and further improvements in cognitive function may seem difficult to achieve. Nevertheless, this study showed that certain aspects of cognitive performance in healthy rodents can be improved by an early-life diet with lipids that mimic the unique structure and composition of MFGM in raw unprocessed milk. To our knowledge, this is the first report that proposes a possible role for the physical structure of dietary lipids in cognitive development and function in healthy subjects.

Although the concept diet improved T-maze and NOR test performances, there were no signs of improved performance in the BM test during adolescence and in the RAM during adulthood. Furthermore, locomotor and general exploratory activity in the open field, and also spontaneous behavior in the home cage, were comparable between diet groups. This collectively suggests that the effects underlying improved performance of concept-fed mice in the T-maze test and NOR test are rather domain specific. NOR and T-maze tests are short-term memory tasks that rely on a rodent’s innate drive to explore novelty (38, 40), whereas the BM and RAM tests rely on different motivations (i.e., escape from an aversive environment and food-motivated behavior, respectively) and involve multiple training sessions to build a (long-term) reference memory of spatial cues around the maze. In rodents, novelty exposure induces a moderate state of arousal. It is well known that cognitive performance has a nonlinear relation with acute stress and arousal, with low to moderate levels of arousal facilitate learning and memory, as opposed to stressful conditions, which can negatively affect

**FIGURE 1** Novel object recognition test. Discrimination index on postnatal day 35 (A) and postnatal day 78 (B) in mice fed a control or concept diet during adolescence. Values are means ± SEMs, $n = 13$ (control) and $n = 14$ (concept). *Different from control, $P < 0.05$. F, time spent exploring the familiar object; N, time spent exploring the novel object.
learning and memory (41). Our data suggests that the concept diet improved novelty-induced arousal and associated learning and memory, but not the form of memory that is associated with more stressful conditions.

Several mechanisms may be postulated to contribute to these effects. First, the entire lipid fraction of the concept diet consisted of lipid droplets with an altered physical structure (i.e., large, phospholipid-coated lipid droplets) from the control diet. Size and surface properties of dietary lipid droplets directly affect digestion and/or absorption kinetics in the digestive tract, which results in different postprandial plasma lipid concentrations (25, 26, 42, 43). Although bioavailability of lipids may affect brain lipid accretion and utilization, which are essential processes for brain growth and development early in life, we do not believe that this mechanism contributed to the long-term cognitive effects observed in the current study, because we did not detect any differences between diet groups in brain weight, phospholipid content, or individual glycerophospholipid or phosphosphingolipid species on P102. There was a small but significant percentage increase in total brain phosphatidylglycerol content in the concept-fed mice, but brain phosphatidylglycerol content was very low (−0.2% in the current study). The more complex dietary lipid structure of the concept diet and the putatively altered postprandial lipid responses may have altered the release of gut-derived satiety hormones such as cholecystokinin (44). Increased cholecystokinin release augments arousal and can facilitate learning and memory processes (45). Moreover, the satiety factor oleoylethanolamide, the release of which is selectively triggered by dietary lipids, can facilitate learning processes by increasing noradrenergic activity in the brain (46). The point here is that, during critical periods of brain development, altered concentrations of these and other satiety factors may modulate circuit formation by prolonged or increased postprandial stimulation of, e.g., the noradrenergic system. This possibility seems all the more likely, because noradrenergic projections in the brain that contribute to arousal continue to develop during postnatal life in rodents (47), and this development may have been affected by the concept diet. Alternatively or in addition, the brief exposure of the lactating dams to the experimental diet (between P16 and P21) could have influenced maternal care and behavior in this period. This could also contribute to the development and long-term functioning of noradrenergic systems (48). Maternal care and behaviors were, however, not systematically recorded in the current study, and possible influences of experimental diets on this remain to be investigated.

Important for consideration of our results is that the altered structure of lipid droplets in the IMF used in the concept diet was generated by the addition of phospholipid sourced from bovine milk containing MFGM, followed by unique technological processing of the IMF (34). The concept diet in the current study therefore did not simply contain phospholipid from bovine MFGM extract as an added ingredient, but, rather, the entire lipid fraction of the concept diet consisted of lipids with an altered structure (large, phospholipid-coated lipid droplets). Previous studies have shown that early-life exposure to concept diet improves long-term metabolic health outcome and protects mice from diet-induced obesity (29, 30), which was caused by the altered lipid structure, but not the mere presence, of the phospholipid ingredients in the concept diet (49). As an added ingredient to the diet, and in higher concentrations than the 0.1% that was used in the current study, MFGM lipid extracts have been reported to improve cognitive performance assessed by a variety of tests in young rats (50), piglets (51), and human infants at 6 mo of age (52), and, more long term, at 12 mo of age (53). However, the experimental formula in the latter study also contained a reduced energy and protein density compared with the control formula, making it difficult to rule out the possible influences of this additional dietary manipulation (53). It needs to be mentioned that the use of bovine milk-derived phospholipid in the concept diet in the present study also increased the content of cholesterol- and MFGM-derived bioactive components, including gangliosides and sialic acid. Cholesterol, gangliosides, and sialic acid are present in human milk and in neuronal and myelin membranes in the brain. A perinatal (maternal) cholesterol deficiency impairs offspring brain weight, brain cholesterol content, and myelination (54, 55), but the relevance of relatively small differences in the cholesterol content of the postweaning diet, after normal lactation, to brain development is most likely negligible. Whereas dietary supplementation with gangliosides and sialic acid early in life may be of biological significance to neuronal development and cognition (56, 57), postnatal supplementation of these ingredients in a low concentration did not improve cognitive performance (50) and did not significantly increase the brain sialic acid content (55) in healthy rats. Together, these results suggest that phospholipid- and other MFGM-derived components, added as an ingredient in a low dose to the postweaning diet, may be of little relevance.
dietary lipids could be a crucial aspect of dietary lipid quality in breast milk. Nonetheless, our study suggests that the overall structure of lipids beyond lipid composition in breast milk contributes to cognitive development in infants remains to be investigated. Nonetheless, our study suggests that the overall structure of dietary lipids could be a crucial aspect of dietary lipid quality in early life.

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LS designed the research and wrote the paper; LS and ML conducted the research and performed the statistical analyses; GvD, LMB, NB, and AJWS reviewed the manuscript; and EMvDh had primary responsibility for the final content. All authors read and approved the final manuscript.

References
3. Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, P44 (directly after lactation) improved the cognitive function of healthy mice during juvenile life while on the diet, and in adult life after previous exposure to the diet. The improvements were specific for age and cognitive domain, and were not associated with changes in the adult brain phospholipid profile. We hypothesize that differences in lipid absorption, digestion kinetics, postprandial hormone responses, and/or alterations in brain circuitry involved in arousal may contribute to the observed effects on behavior. In the current experiment, the effects of the concept diet were observed in healthy mice; therefore, experiments to investigate the effects of the concept diet under conditions that are more challenging to brain development (e.g., early-life stress, nutritional deficiency, or obesogenic environment) are needed. To what extent the physical structure of dietary lipids beyond lipid composition in breast milk contributes to cognitive development in infants remains to be investigated. Nonetheless, our study suggests that the overall structure of dietary lipids could be a crucial aspect of dietary lipid quality in early life.


