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Enriched dairy fat matrix diet prevents early life lipopolysaccharide-induced spatial memory impairment at adulthood

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ABSTRACT

Polyunsaturated fatty acids (PUFAs) are essential fatty acids, which are critical for brain development and later life cognitive functions. The main brain PUFAs are docosahexaenoic acid (DHA) for the n-3 family and arachidonic acid (ARA) for the n-6 family, which are provided to the post-natal brain by breast milk or infant formula. Recently, the use of dairy lipids (DL) in replacement of vegetable lipids (VL) was revealed to potently promote the accretion of DHA in the developing brain. Brain DHA, in addition to be a key component of brain development, display potent anti-inflammatory activities, which protect the brain from adverse inflammatory events. In this work, we evaluated the protective effect of partial replacement of VL by DL, supplemented or not with DHA and ARA, on post-natal inflammation and its consequence on memory. Mice were fed with diets poor in vegetal n-3 PUFA (Def VL), balanced in vegetal n-3/n-6 PUFA (Bal VL), balanced in dairy lipids (Bal DL) or enriched in DHA and ARA (Supp VL; Supp DL) from the first day of gestation until adulthood. At post-natal day 14 (PND14), pups received a single administration of the endotoxin lipopolysaccharide (LPS) and brain cytokine expression, microglia phenotype and neurogenesis were measured. In a second set of experiments, memory and neurogenesis were measured at adulthood. Overall, our data showed that lipid quality of the diet modulates early life LPS effect on microglia phenotype, brain cytokine expression and neurogenesis at PND14 and memory at adulthood. In particular, Bal DL diet protects from the adverse effect of early life LPS exposure on PND14 neurogenesis and adult spatial memory.

1. Introduction

Long chain polyunsaturated fatty acids (PUFAs), in particular docosahexaenoic acid (DHA, 22:6 n-3), from the n-3 family and arachidonic acid (AA, 20:4 n-6) from the n-6 family, are crucial for the optimal development of the brain (recently reviewed in [1–3]). In humans, DHA accumulates in the intrauterine and neonatal period up to 2 years of age [4–6]. The high need and the limited synthesis of these fatty acids in the developing brain require PUFAs supply by the mother through gestation and lactation [7]. This is one of the reasons why breastfeeding is highly recommended by the World Health Organization (WHO). However, when breastfeeding is not possible, formulas have to be used. In milk formula, n-6 and n-3 PUFAs are provided by vegetable oil containing alpha-linolenic acid (ALA) and linoleic acid (LA), which are the precursors of DHA and ARA respectively. In addition of being far from breast milk lipid composition with little content in cholesterol and short-chain fatty acids, these formula do not optimize DHA accretion in the infant brain [8,9]. Several studies have established that the enrichment of maternal diet with long chain n-3 PUFAs such as DHA and/or eicosapentaenoic acid (EPA, 20:5 n-3) leads to higher DHA accretion in the brain of the offspring not only at embryonic and weaning stage but also later in life when switched to a low DHA diet [10–12]. Recently, we demonstrated that adding up dairy blend to the diet leads to increased DHA level in the brain of pups [8,9,13]. Importantly, the partial replacement of vegetable oil containing ALA by dairy fat in infant formula increases DHA level in the brain both at early post-natal stage and adulthood [9,13]. Dairy lipids consumption therefore represents an interesting perinatal nutritional strategy to improve brain DHA accretion over development.

Accumulation of DHA in the brain is a necessary step for both brain shaping and cognitive functions, not only in infants but also

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later in life, including for aging [14,15]. In humans, some studies investigated the link between breast milk, DHA concentration and cognition [16–19]. In most of the studies performed, DHA status was positively associated with academic performances. A high concentration in n-3 PUFAs, especially DHA, in, breast milk, was associated with better academic results in 12-years old girls [20]. Some studies report a short-term benefit of maternal DHA supplementation on cognitive processes, executive functions and memory in preterm [21,22]. Of note, short term DHA enteral supply to preterm born infants poorly improved memory outcome later in life [Makrides, 2010 #1608], further suggesting the importance of promoting DHA accretion in the brain throughout gestation and lactation for optimal cognitive functions [14].

DHA accretion in the brain is not only crucial for optimal brain development [1,3,9,23], but could be protective toward adverse events. The postnatal period is a critical time-window during which an inflammatory episode can have significant and enduring effects on the development of the central nervous system (CNS) with long-term consequences, in particular on cognitive functions [24–30]. In rodents, the early-life exposure to lipopolysaccharide (LPS), a Gram-negative bacteria endotoxin, increases the risk of developing spatial memory alterations later in life [27,31]. Indeed, LPS administration at postnatal day (PND) 14 induces the expression of proinflammatory cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor alpha (TNFα), both at the periphery and in several brain structures (prefrontal cortex and hippocampus) and sustained spatial memory impairment [27,31]. DHA protects from neuroinflammatory processes at adulthood and aging and from their deleterious effect on memory processes [15,32–35]. Recently, the protective effect of DHA-enriched diets on prenatal LPS administration outcomes in the offspring has been revealed [36]. Several in vitro and in vivo data suggest that the protective effects of DHA toward neuroinflammation is linked to the action of DHA and/or its derivatives (resolvins, neuroprotectins, maresins) on microglia, the brain innate immune cells and producers of proinflammatory cytokines [1,34,37,38].

All together, these data suggest that brain DHA is key in the regulation of early life neuroinflammatory events and their detrimental effects on memory later in life. However, the protective effects of diets promoting brain accretion of DHA on long-term effect of post-natal inflammation have been poorly examined. The aim of this study was to evaluate the protective action of partial replacement of vegetable oil by dairy fat in infant formula, supplemented or not with DHA and ARA, on early-life LPS-induced memory impairment.

2. Material and methods

2.1. Animals, diets and experimental design

All experiments were conducted according to the INRA Quality Reference System, and to relevant French (Directive 87/148, Ministère de l'Agriculture et de la Pêche) and European (Directive 86/609, November 24th, 1986, European Community) legislations. They followed ethic protocols approved by the Région Aquitaine Veterinary Services (Direction Départementale de la Protection des Animaux, approval ID: A33-063-920) and by ethics committee (N° 50120112-A). Every effort was made to minimize suffering and the number of animals used. Adult male and female swiss mice (CD1 mice) were obtained from Janvier (St Berthevin, France)before mating. At birth, litters were harmonized at 12 pups/litter with as many males as possible and a minimum of 3 females [39,40]. At weaning, male pups from the same litter were housed in polypropylene cages and maintained in temperature and humidity controlled pathogen-free facility on a 12 h light-dark cycle with ad libitum access to food and water. All studies were performed in males.

Diets were given to mice from the first day of gestation and offsprings were maintained on the same diet until adulthood [9]. Diets contained 5% of total lipids from different sources: n-3 PUFA balanced vegetable lipids (Bal VL), VL supplemented with DHA (0.2%) and ARA (0.4%) (Supp VL), n-3 PUFA balanced dairy lipids (Bal DL), DL supplemented with DHA (0.2%) and ARA (0.4%) (Supp DL) and n-3 PUFA deficient diet (Def VL). In an additional group, Def VL pups were switched to a Bal DL diet from weaning to adulthood (Def VL+Bal DL). Diet composition is described in Tables 1 and 2.

Table 1
Composition of the diets (g/kg diet).

Ingredient	Amount
Casein	180
Cornstarch	460
Sucrose	230
Cellulose	20
Fat	50
Mineral mix[a]	50
Vitamin mix[b]	10

[a] Composition (g/kg): sucrose, 110.7; $CaCO_3$, 240; K_2HPO_4, 215; $CaHPO_4$, 215; $MgSO_4 \cdot 7H_2O$, 100; NaCl, 60; MgO, 40; $FeSO_4 \cdot 7H_2O$, 8; $ZnSO_4 \cdot 7H_2O$, 7; $MnSO_4 \cdot H_2O$, 2; $CuSO_4 \cdot 5H_2O$, 1; $Na_2SiO_7 \cdot 3H_2O$, 0.5; $AlK(SO_4)_2 \cdot 12H_2O$, 0.2; K_2CrO_4, 0.15; NaF, 0.1; $NiSO_4 \cdot 6H_2O$, 0.1; H_2BO_3, 0.1; $CoSO_4 \cdot 7H_2O$, 0.05; KIO_3, 0.04; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.02; LiCl, 0.015; Na_2SeO_3, 0.015; NH_4VO_3, 0.01.

[b] Composition (g/kg): sucrose, 549.45; retinyl acetate, 1; cholecalciferol, 0.25; DL-α-tocopheryl acetate, 20; phylloquinone, 0.1; thiamin HCl, 1; riboflavin, 1; nicotinic acid, 5; calcium pantothenate, 2.5; pyridoxine HCl, 1; biotin, 1; folic acid, 0.2; cyanocobalamin, 2.5; choline HCl, 200; DL-methionine, 200; p-aminobenzoic acid, 5; inositol, 10.

Table 2
Dietary fatty acid composition.

Fatty acids	Def VL	Bal VL	Supp VL	Bal DL	Supp DL
8:0		–	–	0.7	0.7
10:0		–	–	1.6	1.6
12:0	0,22	0.2	0.2	1.9	1.9
14:0	1,24	0.8	0.9	6.2	6.1
15:0	0,07	0.1	0.1	0.6	0.6
16:0	44,11	28.8	28.8	18.4	18.5
17:0	0,11	0.1	0.1	0.3	0.3
18:0	4,58	3.9	4.0	7.0	7.1
20:0	0,36	0.4	0.4	0.3	0.3
22:0	0,08	0.3	0.3	0.4	0.4
24:0	0,10	0.2	0.2	0.2	0.2
Saturated	**50,86**	**34.7**	**34.8**	**37.5**	**37.6**
16:1 n-9	0,03	–	–	0.1	–
16:1 n-7	0,18	0.2	0.2	0.9	0.9
17:1	0,03	–	–	0.1	0.1
18:1 trans	0,12	0.1	0.1	1.3	1.2
18:1 n-9	37,88	47.1	46.2	45.4	44.8
18:1 n-7	0,68	1.1	1.1	1.3	1.3
20:1 n-9	0,14	0.3	0.3	0.4	0.4
Monounsaturated	**39,05**	**48.8**	**48.2**	**49.4**	**48.7**
18:2 n-6	9,58	15.1	15.0	10.8	10.8
20:4 n-6	–	–	0.4	–	0.4
n-6	**9,93**	**15.1**	**15.4**	**10.8**	**11.2**
18:3 n-3	0,16	1.3	1.3	2.2	2.2
22:6 n-3	–	–	0.2	–	0.2
n-3	**0,16**	**1.3**	**1.5**	**2.2**	**2.4**
Polyunsaturated	**10,09**	**16.5**	**17.0**	**13.1**	**13.7**
n-6/n-3	**61,54**	**11.3**	**10.0**	**4.8**	**4.6**