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Research paper

Maternal suppplementation with conjugated linoleic acid reduce anxiety and lipid peroxidation in the offspring brain

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ABSTRACT

Background: Maternal consumption of fatty acids can alter neuronal membrane function, synaptic connections, and protect the brain from alterations caused by disturbances such as lipid peroxidation and anxiety in the offspring. We aimed to investigate how the maternal consumption of conjugated linoleic acid (CLA) interferes in anxiety behavior of the offspring and cerebral lipid peroxidation.
Methods: Three groups were formed: control (CG) - diet without CLA; CLA1 - diet containing 1% of CLA; and CLA3 - diet containing 3% of CLA. These diets were offered to the mothers from the 7th day of gestation until the end of lactation. The following behavioral tests were used: Elevated plus maze (EPM), Open Field (OF) and Light-dark Box (LDB). Levels of malondialdehyde (MDA) and glutathione were measured in the offspring's brains. Data were analyzed by ANOVA followed by the Holm–Sidak post-test or the Kruskal–Wallis test ($p < 0.05$).
Results: CLA1 and CLA3 showed higher number of entries in the open arms and time spent in the central area in EPM, they translocated and ambulated more in the clear area of the LDB and presented more rearing in the OF compared to CG ($p < 0.05$); moreover, they presented higher concentration of glutathione and lower MDA in brain tissue ($p < 0.05$).
Limitations: We evaluated the effect of maternal consumption of CLA on anxiety and lipid peroxidation in rats' offspring, but a similar study should be performed in humans.
Conclusions: Maternal intake of CLA induced a decrease in the parameters of anxiety and cerebral lipid peroxidation in the offspring.

1. Introduction

The maternal diet during pregnancy alters the development of the offspring's brain and their subsequent behavior. The formation of the nervous system occurs during gestation and lactation. This phase is known as the "critical period of development". During this period, the processes of neurogenesis, gliogenesis, cell migration and differentiation, myelinogenesis, formation of synapses, as well as the synthesis and the release of neurotransmitters occur (Madore et al., 2014).

Maternal consumption of lipids in the diet may determinate the type of fatty acid that will accumulate in the offspring's brain tissue (Yetimler et al., 2012; Innis, 2014). This occurs because the lipids from the maternal diet are driven to the maternal liver and are easily transferred to the placenta, involving fatty acid transport and binding proteins. During pregnancy, the fetus can desaturate and elongate essential fatty acids. However, for this to occur, it is necessary that the fatty acids must be supplied in the diet. (Herrera, 2002; Hanebutt et al., 2008).

A lipid composition of neuronal membranes influences a subjective perception. Emotional behavior is substantially regulated by the membrane barrier and synaptic connection yield, and it may cause anxiety disorder (Müller et al., 2015). Neural maturation during the critical period is a more sensitive phase for the development of anxiety-triggering circuits (Leonardo and Hen, 2008).

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Uncontrolled anxiety can occur due to lipid oxidation of neurons. This lack of control can lead to impairment in neural plasticity, membrane fluidity, and cell death (Montuschi et al., 2004; Bakunina et al., 2015). Fatty acids can have an antioxidant effect, reducing lipid peroxidation (Basiricò et al., 2015; Basiricò et al., 2017). Increase in lipid peroxidation may induce changes in brain tissue; thus, causing anxiety disorders (Steenkamp et al., 2017). When offered in the early stages of life, some fatty acids may alter the anxiety parameters of the offspring (Pudell et al., 2014).

One source of polyunsaturated fatty acids (PUFA) is the conjugated linoleic acid (CLA). CLA is characterized by a mixture of positional and geometric isomers of linoleic fatty acid with conjugated unsaturated double bonds. It is formed as an intermediate during the biohydrogenation of linoleic acid to stearic acid in the rumen of animals, but it can also be synthetically produced (Khanal and Dhiman, 2004; Jelińska et al., 2014).

CLA from goat's milk fat during gestation and/or lactation deposited in brain tissue induced changes in the propagation of cortical electrical activity (Soares et al., 2012) and accelerated reflex maturation (Soares et al., 2014). CLA can also reduce angiogenesis in the mammalian brain (Sikorski et al., 2008) and protect cortical cells against neurotoxic elements in vitro (Hunt et al., 2010).

Therefore, we conducted this study with the objective to investigate whether a commercially isolated source of CLA added in different proportions in the maternal diet interferes in offspring's anxiety behavior and cerebral oxidative stress.

2. Materials and methods

2.1. Animals and diet

Primiparous Wistar rats (n = 9, three female by each group) from the Federal University of Pernambuco (UFPE) aged 90–120 days and weight of 250 ± 50 g were used to obtain newborns (n = 36). One female was maintained for each male during the mating period.

After confirmation of pregnancy by vaginal smear, the pregnant rats were housed in individual polypropylene maternity cages under standard conditions: temperature of 22 ± 1 °C, with light-dark cycle (12:00 h, beginning of the light phase at 6:00 h), humidity of ± 65%, receiving feed and water ad libitum. The animals were kept in the Laboratory of Experimental Nutrition of the Federal University of Campina Grande (UFCG).

In the first week of gestation, the rats received commercial feed (Presence - Purina®), and the experimental diet was offered from the 7th day of gestation and throughout lactation. Three groups were formed: Control Group (CG) (n = 11) receiving the experimental diet containing soybean oil; CLA1 group (n = 13) received an experimental diet containing 1% CLA; and CLA3 (n = 12) containing 3% CLA. The blend of CLA used was from the brand Clarinol® Powder (Loders Croklland). The control group received as lipid source only soybean oil, source of essential fatty acids, as recommended by the American Institute of Nutrition for rodents (Reeves et al., 1993) and used in others researches (Melo et al., 2017; Soares et al., 2012). The diet chosen was for animals during gestational and lactating (AIN-93G) (Reeves et al., 1993). All groups received 7% of soybean oil, as shown in Table 1.

After birth, the pups were housed in the cages with their mothers. Postnatal weaning was performed after 21 days, and the mothers were euthanized by cervical dislocation. The litters were standardized into 6 pups, housed in a polypropylene cage (two animals per cage), fed a commercial diet throughout the experiment (Presence - Purina ®). Only the males were used to obtain the data. The research followed the experimental protocol according to the ethical recommendations of the National Institute of Health (Bethesda, USA) regarding animal care. The present study was approved for the use of animals by the Ethics Committee of the Federal University of Paraiba No: 0407/13 (Fig. 1).

Table 1
Composition of control and experimental diets and profile of CLA fatty acid.

INGREDIENT (G/KG)	DIETS		
	CONTROL	CLA1	CLA3
Cornstarch	530	520	500
Casein	199.5	199.5	199.5
Sucrose	100	100	100
Soybean oil	70	70	70
CLA mix isomers	–	10	30
Fiber	50	50	50
Mineral mix	35	35	35
Vitamin mix	10	10	10
L-Cystine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5
Total calories (Kcal)	3.960	4.010	4.110
FATTY ACIDS OF THE SUPPLEMENT CONTAINING CLA			(%)
C14:0 Myristic acid			0.1 ± 0.01
C16:0 Palmitic acid			4.3 ± 0.04
C18:0 Stearic acid			1.4 ± 0.02
C18:1n9c Oleic acid			10.7 ± 0.14
C18:1c11 Cis-vacenic acid			0.6 ± 0.02
C18:2n-6 Linoleic acid			1.0 ± 0.03
C20:0 Arachidic acid			0.2 ± 0.01
C:22 Behenic acid			0.2 ± 0.01
CLA-c9t11 Conjugated linoleic acid			39.2 ± 0.10
CLA-t10c12 Conjugated linoleic acid			38.3 ± 0.07
Other types of CLA			2.5 ± 0.13
CLA-trans,trans			1.5 ± 0.04

*Fatty acid values expressed in % ± SD.

2.2. Maternal and offspring body weight and food consumption

Maternal body weight and food consumption were measured in the beginning and the end of pregnancy and lactation.

The body weight and food consumption of offspring were measured when the animals reached ten weeks of life.

2.3. Determination of the fatty acid profile of CLA

Samples of CLA were sent to Faculty of Veterinary Medicine of University of Lisbon where the FA analysis was conducted. Fatty acid methyl esters (FAME) from samples were prepared by direct transesterification using potassium hydroxide (2 M) in methanol according to Rego et al. (2009). Fatty acid methyl esters were analyzed by gas chromatography with flame ionization detection using a Shimadzu GC 2010-Plus (Shimadzu, Kyoto, Japan) equipped with a SP-2560 (100 m × 0.25 mm, 0.20 μm film thickness, Supelco, Bellefonte, PA, USA) capillary column. The chromatographic conditions were as follow: injector and detector temperatures were set at 250 °C and 280 °C, respectively; helium was used as the carrier gas at 1 mL/min constant flow; the initial oven temperature of 50 °C was held for 1 min, increased at 50 °C/min to 150 °C and held for 20 min, increased at 1 °C/min to 190 °C and then increased at 2 °C/min to 220 °C and held for 40 min. Identification of FAME were achieved by electron impact mass spectrometry using a Shimadzu GC–MS QP2010 Plus (Shimadzu) and according to published chromatograms (Alves et al., 2013). The chromatographic column and the GC conditions were similar to the GC-FID analysis. Additional mass spectrometer conditions were as follow: ion source temperature, 200 °C; interface temperature, 240 °C; emission voltage, 70 eV. Amounts of fatty acids in incubation tubes were expressed as milligrams per flask and determined using the internal standard, assuming direct proportionality between GC-FID peak area and FA weight.