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Effects of maternal protein malnutrition on oxidative markers in the young rat cortex and cerebellum

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Abstract

Malnutrition affects a large number of children worldwide. Inadequate nutrition during pre- and postnatal period may alter brain development resulting in biochemical, physiological and anatomical changes which in turn could cause behavioral abnormalities. The impairment of the central nervous system following protein deficit have been extensively studied and this deprivation produces deleterious effects upon cerebral structures. The aim of this study was to identify oxidative parameters present in the developing brain as consequence of maternal protein malnutrition. Female Wistar rats were fed a normal protein diet (25% casein) or low protein diet (8% casein) from the time of conception up to 21 days after the parturition. In addition, the diets were supplemented or not with L-methionine. Cortex and cerebellum were removed from offspring to determine the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and the levels of lipoperoxidation (TBARS). Our findings demonstrated heterogeneity in response to protein restriction. The levels of lipoperoxidation were increased in the cerebellum of malnourished offspring. Methionine supplementation caused an increase in lipoperoxidation in both brain structures. CAT activity was decreased in the cerebellum of the offspring supplemented with methionine whereas the cerebellum of malnourished pups with or not methionine supplementation showed a decrease in SOD activity. The activity of SOD in the cortex did not differ among groups. CAT activity, however, was increased in the cortex of malnourished pups supplemented or not with methionine. Thus, these results provide clues to the knowledge of malnutrition effects upon the brain.

Keywords: Malnutrition; Oxidative stress; Cerebellum; Cortex

Given that malnutrition is a worldwide concern, it has been widely studied. In particular, a number of studies have focused on the effects of nutrition on brain development demonstrating that changes in dietary nutrients can alter brain morphology as well as its biochemical functions [3,28] thus leading to behavioral changes [20]. Protein deprivation can cause many direct deleterious effects on the brain such as loss of brain weight [10,31,33], altered hippocampal formation [27], impairment of neurotransmitter systems [13,29,33,37], changes in protein phosphorylation [34], and deficit in cognitive functions [15]. In addition, protein deficiency can cause indirect effects on the brain by impairing major organs systems including liver, pancreas, kidney and spleen [10]. In our previous work we reported the relation between protein malnutrition and oxidative stress on hipocamppus [6].

Dietary protein is an important source of essentials amino acids (e.g. cysteine and methionine), which can be used as intracellular antioxidants. Therefore, the decrease in dietary protein content could potentially lead to oxidative stress [39]. Free radicals are physiologically generated by cells and the main enzymatic defenses against free radicals are superoxide dismutase (SOD) and catalase (CAT) [16]. This coupled enzymatic activity is able to reduce the ion superoxide to H_2O [16].

Methionine is an essential amino acid and a necessary precursor for the synthesis of coenzyme A, taurine and glutathione, as well as for many biological processes as methyl group donator to methylation required to DNA expression and epinephrine, phosphatidyl choline, creatine, melatonin

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synthesis [26]. Moreover, methionine is the first amino acid in all eukariotic proteins, since it is encoded by mRNA start code (AUG). In this way, a dietary deficit in methionine could impair protein synthesis with further deficits in organ function.

In this study, we have assessed the effects of maternal prenatal plus postnatal protein malnutrition and methionine supplementation upon the levels of lipid oxidative damage and antioxidant enzymes activities in the cortex and the cerebellum from 21 days old pups. These structures were chosen based on the differences on their vulnerability during distinct stages of development. Cortical development occurs during the fetal period while cerebellar development occurs mainly postnatally [38].

Albino Wistar rats were obtained from our colony, and were maintained at 22 °C on a 12 h light-dark cycle. The protocol was performed in accordance to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil. The nutritional model used in this work is one of the most commonly used for inducing malnutrition. In this method, during the prenatal period the pregnant female is fed with deficient diet [14]. Malnutrition can be sustained postnatally by maintaining the chows throughout the suckling period which was reported to result in less milk production as well as alterations in milk composition, therefore affecting protein content ingested by the pups [14]. Pregnant female Wistar rats fed diets with 25% or 8% protein from conception up to the end of lactation (21 days). It has been reported that maternal protein malnutrition generates underweight pups with cerebral metabolism impairment [9]. Pregnant females were divided into four groups that were given different diets: (1) control, fed with 25% casein - a high quality protein, which avoids additional effects of low quality protein - or normal protein content; (2) fed with 25% casein diet with 0,15% L-methionine supplementation; (3) malnourished fed with 8% casein, (4) malnourished fed with 8% casein with 0,15% L-methionine supplementation. All diets were given *ad libitum* and were isocaloric, contained equal amounts of fat (15%), non-nutritive fiber (1%), mineral (4%) and vitamin mixture (1%) as recommended by the Association of Analytical Chemists [17]. Offspring were sacrificed by decapitation 21 days after birth (weaning date). The cerebellum and the cortex were immediately removed for biochemical analyses as described below.

Lipoperoxidation was assessed by measuring the levels of thiobarbituric acid reactive substances (TBARS), as previously described [11]. Briefly, equal volumes of trichloroacetic acid 10% (TCA) and brain homogenates were incubated with thiobarbituric acid 0.67% (TBA) at 100 °C for 15 min TBARS levels were determined by using a spectrophotometer at 535 nm.

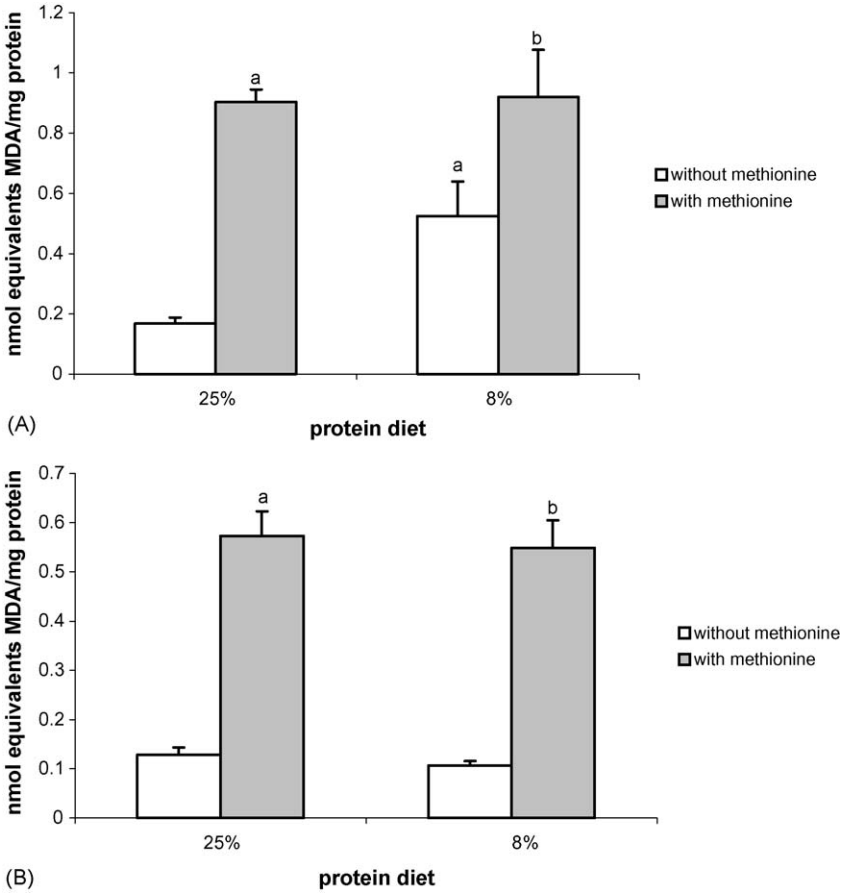

Fig. 1. Thiobarbituric acid reactive species in rat brain. Alterations in lipoperoxidation in cerebellum and cortex with different diets. (A) cerebellum of 21 day-old rats (B) cortex of 21 day-old rats. Animals were killed by decapitation and structures were surgically isolated, washed with saline buffer (pH 7.4) and TBARS content were determinated and expressed as nmol of MDA equivalents/mg protein. Data represent the means ± S.E.M. (a) Statistically different from the control group (25% protein); (b) statistically different from 8% without methionine; $p < 0.05$ ($n = 4$ for each group).