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# Effects of maternal low-protein diet on parameters of locomotor activity in a rat model of cerebral palsy

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ABSTRACT

Children with cerebral palsy have feeding difficulties that can contribute to undernutrition. The aim of this study was to investigate the effect of early undernutrition on locomotor activity and the expression of the myofibrillar protein MuRF-1 in an experimental model of cerebral palsy (CP). In order to achieve this aim, pregnant rats were divided into two groups according to the diet provided: Normal Protein (NP, n = 9) and Low Protein (LP, n = 12) groups. After birth, the pups were divided into four groups: Normal Protein Sham (NPS, n = 16), Normal Protein Cerebral Palsy (NPCP, n = 21), Low Protein Sham (LPS, n = 20) and Low Protein Cerebral Palsy (LPCP, n = 18) groups. The experimental cerebral palsy protocol consisted of two episodes of anoxia at birth and during the first days of life. Each day, nitrogen flow was used (9l/min during 12 min). After nitrogen exposure, sensorimotor restriction was performed 16 h per day, from the 2nd to the 28th postnatal day (PND). Locomotor activity was evaluated at 8th, 14th, 17th, 21th and 28th PND. At PND 29, soleus muscles were collected to analyse myofibrillar protein MuRF-1. Our results show that CP animals decreased body weight ($p < 0.001$), which were associated with alterations of various parameters of locomotor activity ($p < 0.05$), compared to their control. Undernourished animals also showed a decrease ($p < 0.05$) in body weight and locomotor activity parameters. Moreover, CP decreased MuRF-1 levels in nourished rats ($p = 0.015$) but not in undernourished rats. In summary, perinatal undernutrition exacerbated the negative effects of cerebral palsy on locomotor activity and muscle atrophy, but it appears not be mediated by changes in MuRF-1 levels.



## 1. Introduction

Cerebral Palsy (CP) is defined as a complex syndrome which includes a set of movement and posture disorders leading to limitation of activity. It is a static, non- progressive and permanent lesion, caused by insults to the developing nervous system (Bax et al., 2005; Chan and Miller, 2014; Krigger, 2006). The overall prevalence of CP has stabilized in the last 40 years, affecting 2–3.5 children per 1000 live births (Blair, 2010; Colver et al., 2014). However, in underdeveloped or developing countries, this incidence may increase due to favourable conditions for the occurrence of chronic problems like CP (Himmelmann, 2013).

Undernutrition associated with CP presents a high incidence in childhood (46–90%) (Reilly et al., 1996; Sullivan et al., 2000; Troughton and Hill, 2001). Eating disorders are also found in children with CP due to the interaction of several factors such as dysfunction of oral motor control, abnormal neurological matura-

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tion and poor posture when sitting due to instability of the trunk (Fung et al., 2002). Difficulty of swallowing is found in 99% of children with severe CP (Calis et al., 2008). Furthermore, vomiting and contractures on the temporomandibular joint are common in these patients, which makes the eating process slow and unpleasant, contributing even more to a poor nutritional state (Pelegano et al., 1994; Sullivan et al., 2000).

Brain development takes place until 5 years of age in children. Therefore, brain injuries may occur in prenatal, perinatal or postnatal stages (Chan and Miller, 2014). There are several risk factors that can trigger CP, such as malnutrition, incomplete pregnancy (<32 weeks), low birth weight (<2500 g), malformations, multiple pregnancy, trauma, intracranial haemorrhage, periventricular leukomalacia, infections, maternal hypertension, asphyxia, breathing difficulties, bacterial meningitis, hyperbilirubinemia and falls (Bax et al., 2005; Himmelmann et al., 2011; Jones et al., 2007; Koman et al., 2004). The pathological effects of CP may change over time (Bell et al., 2010; Blair, 2010), but the key features are spasticity, increased pathologic reflexes, muscle atrophy and weakness, movement disorders, ataxia, rigidity and characteristic scissor gait (Koman et al., 2004; Krigger, 2006).

Clinical and experimental studies have established that undernutrition damages the neurodevelopment, physical growth parameters and brain structure (Akitake et al., 2015; Naik et al., 2015). Both CP and undernutrition may affect the maintenance of muscle mass (Marcuzzo et al., 2008; Toscano et al., 2008). The reduction of muscle mass found in individuals with CP and/or malnutrition can occur by increasing protein degradation or reducing protein synthesis (Mccarthy and Esser, 2010). The ubiquitin-proteasome system is the major pathway of muscle breakdown mainly under protein or energy restriction conditions (Carbone et al., 2012). This system involves enzymes responsible for marking the protein substrate with multiple ubiquitin molecules. Thus, the protein substrate is recognized by S26 proteasome, which degrades ubiquitinated protein into many peptides (Teixeira et al., 2012). Three enzymatic components are required to mark the protein: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3). These are responsible for connecting ubiquitin with the target protein. MURF-1 (Muscle Ring Finger-1) is known as E3 ligase, which is increased in models of disuse, immobilization, denervation and suspension of the limb (Bajotto et al., 2011; Delfino et al., 2013; Teixeira et al., 2012).

Undernutrition is frequently associated with CP due to eating disorders and postural disabilities (Calis et al., 2007) that are related to this syndrome. Thus, the current study used a model of CP in rats (Coq et al., 2008; Marcuzzo et al., 2008, 2010; Strata et al., 2004) associated with perinatal undernutrition to investigate the effects on the offsprings' phenotypic expression of body weight, locomotor activity, soleus muscle mass and the MuRF-1 myofibrillar protein expression. We hypothesized that undernourished rats who were submitted to experimental CP would show locomotor activity damage, lower soleus muscle mass and increased MuRF-1 myofibrillar protein expression compared to nourished rats with or without CP.

## 2. Experimental procedures

### 2.1. Animals and experimental groups

All experimental procedures followed the guidelines from the National Institute of Health Guide for Care and Use of Laboratory Animals and National Council of Control and Animal Experimentation (Conselho Nacional de Controle e Experimentação Animal—CONCEA) and were approved by the Committee of Ethics in Animal Experimentation of the Federal University of Pernambuco—Brazil (protocol number:23076.014726/2013-74).

Twenty one virgin female albino Wistar rats were obtained from Nutrition Department, Federal University of Pernambuco, Brazil. The animals were maintained in a controlled light–dark cycle (dark 18.00–06.00 h) at room temperature (23 ± 2 °C). A standard laboratory chow diet (52% carbohydrate, 21% protein and 4% lipids – Labina; Purina Agriband) and water were given ad libitum. Females were mated at a ratio of 2:1. The litters were not obtained by consanguineous mating. 21 litters were used, and for all experiments we used at least two male offspring from each litter. After mating confirmation by visualisation of spermatozoa in a vaginal smear, the rats were housed individually and were divided into two groups according to dietary manipulation: Normoproteic Group, fed a control diet containing 17% of protein (NP, 170 g of protein/kg, n = 9), and Low Protein Group, fed an isocaloric low-protein diet containing 8% of protein (LP, 80 g of protein/kg, n = 12; AIN-93) (Reeves et al., 1993) (Table 1). Dams were fed *ad libitum* during pregnancy and lactation and at birth litter size was adjusted to eight pups per dam. Male offspring were used in each litter and females were used only to standardize the size of each litter to eight pups. At least two male pups from each litter were used to form the subgroups according to dam diet and brain palsy induction: Normal Protein Sham Group (NPS, n = 16); Normal Protein CP Group (NPCP, n = 21); Low Protein Sham Group (LPS, n = 20) and Low Protein CP Group (LPCP, n = 18). At weaning, all male pups from experimental subgroups were fed with the previous diet (17% or 8% of protein) until PND 29. The body weight (BW) of dams was recorded every three days during pregnancy and lactation. The BW of pups was recorded at birth and on PND 8, 14, 17, 21 and 28.

### 2.2. Experimental model of cerebral palsy

The experimental model of CP was induced as initially proposed by Strata et al. (2004) and Coq et al. (2008) and reproduced by Marcuzzo et al. (2008, 2010) . This model associates perinatal anoxia with a sensorimotor restriction model of hind limbs with the objective of reproducing the lack of active movement observed in CP.

### 2.3. Analysis of locomotor activity

The study of locomotor activity was performed in an unlighted room attached to the animal facilities. The analysis occurred at PND 8, 14, 17, 21 and 28, according to Aragão et al. (2011). Briefly, the animals were placed in the central area of an open field, where a monitoring apparatus recorded free movement during 5 min. The apparatus consisted of a circular open field (⊘ 1 m), bounded by 30 cm high walls with inner surfaces painted in black with EVA

**Table 1**
Diet composition.

| Nutrients | Amount for 100 g of diet | |
|---|---|---|
| | Normal protein (17% of protein) | Low protein (8% of protein) |
| Casein (85%)[a] | 20 g | 9.41 g |
| Vitamin Mix (AIN-93G) | 1 g | 1 g |
| Mineral Mixture (AIN-93G) | 3.5 g | 3.5 g |
| Cellulose | 5 g | 5 g |
| Choline | 0.25 g | 0.25 g |
| α-Methionine | 0.3 g | 0.3 g |
| Soyabean oil | 7 ml | 7 ml |
| Maize starch | 39.74 g | 50.34 g |
| Dextrinized starch | 13.2 g | 13.2 g |
| Sucrose | 10 g | 10 g |
| Energy density | 16.26 kJ/g | 16.26 kJ/g |

[a] Casein showed 85% purity (85 g protein for each 100 g casein).