ORIGINAL ARTICLE:

Fifty percent of food restriction during gestation reduced the dendritic density of cerebrum and cerebellum of *Rattus norvegicus'* newborn

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ABSTRACT

Objectives: To analyze the influence of 50 percent food restriction during pregnancy to the dendritic density of cerebellum and cerebellum of newborn' *Rattus norvegicus*.
Materials and Methods: Laboratory experimental study with single blind randomized post-test only control group design using animal model; pregnant *Rattus norvegicus* as treatment models. Subjects were divided into two groups: control group and treatment group which was exposed to 50% food restriction (FR 50%). At day 21, both group sacrificed and the cerebrum and cerebelum of the offsprings were prepared and stained with silver impregnation. We used parametric independent t-test in analyzing dendritic density.
Results: In the cerebrum there was a significant difference in dendritic density between control (4.98±2.17) and treatment (2.69±0.76) groups with p=0.001 (p<0.05). In the cerebellum there was ALSO a significant difference in dendritic density between control (7.37±2.23) and treatment groups (3.01±0.64) with p=0.000 (p<0.05).
Conclusions: The dendritic density of cerebrum and cerebellum of newborn *Rattus norvegicus* exposed to 50 percent of food restriction during pregnancy were lower than control.

Keywords: 50 percent of food restriction; pregnancy; dendrite density; *Rattus norvegicus* newborn cerebrum and cerebellum

ABSTRAK

Tujuan: Menganalisis perbedaan kelompok kekurangan gizi dengan kelompok kontrol selama kebuntingan terhadap kepadatan dendrite pada cerebrum dan cerebellum otak *Rattus norvegicus* baru lahir.
Bahan dan Metode: Penelitian analitik eksperimental dengan desain single blind randomized post test only control group, menggunakan hewan coba *Rattus norvegicus* sebagai model perlakuan. Kelompok hewan coba dibagi dua kelompok yaitu kelompok kontrol tanpa diberi perlakuan dan kelompok perlakuan yang diperlakukan *undernutriTION* dengan *food retriction* 50% (FR 50%). Kami menggunakan uji parametric *independen t-test* untuk analisis kepadatan dendrite.
Hasil: Pada cerebrum, terdapat perbedaan bermakna kepadatan dendrite kelompok kontrol (4,98±2,17) dan perlakuan (2,69±0,76) dengan p=0.001 (p<0,05). Pada cerebellum, terdapat perbedaan bermakna kepadatan dendrite kelompok kontrol (7,37±2,23) dan perlakuan (3,01±0,64) dengan p= 0.000 (p<0,05).
Simpulan: Kepadatan dendrite di cerebrum dan cerebellum otak *Rattus norvegicus* baru lahir yang terpapar *food restriction* 50% lebih rendah dibanding kontrol.

Kata Kunci: Nutrisi; food restriction; Maternal Perinatal Undernutrition; dendrit; *Rattus norvegicus*

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INTRODUCTION

The brain is the most complex human organ either from its structural form or its biological function. The brain starts to grow and develop since the baby is still in the womb. The brain has almost half the size of the fetus, However, when grows into adulthood, the brain only weighs 2-4% of the total human body weight.[1]

A 90% brain development process occurs in the first 5 years of life. This growth and development process will be influenced by genetics, daily experience, received responses, nutritional intake, activity and ALSO the stimulation given from the moment in the womb until after the baby is born.[1]

In the womb, brain cells undergo proliferation, migration, differentiation, myelination, synaptogenesis and apoptosis. The number of cells dO not increase since the pregnancy around 26 weeks and the ability of 32-week-old neurobehaviour infants did not differ from term infant. The brain structure is also composed by external stimuli known as the premise: "Stimulation induced morphological changes".[2]

Efforts that can be done during pregnancy to optimize brain growth and development are providing adequate nutrition and stimulation, in addition to the characteristics of the parents. Whereas, the most easily accepted stimulation of the fetus is auditory stimulation, and music is the most harmonious combination of sounds.[2,3]

Maternal food during pregnancy should contain good amounts and nutritional quality. The health condition of infants born is strongly influenced by the mother's nutritional condition during pregnancy. Nutrition and other environment maternal factors affect brain development during the fetal phase and the beginning of life. Nutrition formed the cellular wall in developing fetus including the brain cells. Malnutrition either under or overnutrtion form influence the rapid neural development process, including the formation of synapses and myelenisation and reduce the apoptotic process.[4,5]

Maternal perinatal undernutrition (MPU) may affect central nervous system maturation (CNS), both morphologically and physiologically. Undernutrition causes brain development disorders with specific and different variations in each part of the brain, which can occur both before and after periods of rapid brain development. The longterm effect of the pathophysiological mechanism of the occurrence is also known as "perinatal programming of adult diseases," or "develop-mental origin of health and adult diseases (DOHAD)".[6]

Several studies have shown that early undernutrition is associated with a stress-neuroendocrine system in which the regulation involves areas of the central nervous system. Perinatal development and central nervous system maturation require various factors, such as growth factors like neurotrophin. Neurotropin, including NGF, BDNF, NT-3 and NT-4, are proteins that are known to have important functions in the growth of neuronal cells, especially in controlling the loss of developmental ability of neuron cells.[6,7] Efforts to optimize the fetus brain growth and development are aimed at increasing the number of neuronal and glial cells by reducing apoptosis and increasing the ratio of glia-neurons and also increasing synaptogenesis or dendritic density.[2,3,8]

Dendrites are extensions of neurons that function to receive and process synapses. Dendrites have a variety of forms associated with the type of relationship between neurons in which the shape is strongly influenced by the given stimulus. At the cellular level, dendrites have different characteristics of axons and glia, in which dendrites have their own organelles.[9]

The dendrite complexity describes the number of connections a neuron can accept. The growth and differentiation of synaptic structures that are affected by stimuli (sound, activity, nutrition, stress) are known to contribute to thinking and memory. Dendritic density is a good marker for synaptic conditions in which the amount of dendrite affects how the speed of nerve impulses is delivered.[9]

This study was a continuation of a series of study on combination of musical stimuli and nutrition in the womb to optimize brain growth and aimed to determine the effect of undernutrition during pregnancy to dendritic density in cerebebrum and cerebellum of *Rattus norvegicus'* newborn.

MATERIALS AND METHODS

This study was an experimental analytic study with single blind randomized post-test only control group design. This study used experimental animals *Rattus norvegicus* as a model of undernutrient treatment in place of pregnant women for more invasive research that has been hindered by technique and ethics in its implementation. The subjects were divided into two groups at random. The treatment group was conditioned in malnutrition with 50% food restriction and control group who still got the usual food intake. This study was conducted at Experimental Animal Cage and Pathology Laboratory, Faculty of Veterinary Medicine,

Universitas Airlangga, Surabaya from November 2016 to December 2016.

The inclusion criteria in this study were healthy *Rattus norvegicus* parent weighing 130-150 grams, unmated, ± 3-month-old, and healthy newly born. The exclusion criteria were unhealthy *Rattus norvegicus*' parent during treatment, fetus died in utero, and prematurely born. The number of samples of each group in this study was 13 with a total of 26 samples.

From each *Rattus norvegicus* parent in both groups, we took two fetuses with the heaviest weight. the newborn mice were sacrificed by cervical dislocation, weighed, and their brains were removed and made a silver impregnated preparation using Golgi-Cox method. The rest of the tissues were buried. The density of dendrite neurons in each sample was assessed. The data were presented in tabular form and statistical testing was performed using SPSS 21st edition software. Ethical clearance was obtained from Ethical Commission for Research, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, no. 616-KE. To obtain optimal results, the code was enclosed on the object glass during the calculation of each brain cell and dendrite density. The new code was opened after the inspection so the reviewers and researchers did not know the results of the previous examination.

RESULTS AND DISCUSSION

Characteristics of research subjects

This study used *Rattus norvegicus* parents weighing 130-150 grams which were randomized into 2 groups of 13 parents in each group.

Table 1. Mean weight of *Rattus norvegicus* parents in control and treatment groups

Groups	Body weight of the parents (grams)	
	Mean	SD
Control	143.31	± 4.25
Treatment	144.31	± 3.30

To identify data distribution based on the weight of *Rattus norvegicus* parents body, we performed normality test using Shapiro-Wilk test, which revealed significance of p=0.06 (p> 0.05) in control group and p=0.82 (p>0.05) in treatment group. Then we performed homogeneity test for the samples of *Rattus norvegicus* parents weight, with the result of p=0.11 (p>0.05), showing that the data distribution was normal and homogeneous (p> 0,05).

All *Rattus norvegicus* newborns were weighed, then two with heaviest weights were selected and sacrificed to remove the brain. Two brains of *Rattus norvegicus*'s newborns were made into one dosage and stained with silver impregnation, and then the dendritic spine density was calculated.

Table 2. Mean weight of *Rattus norvegicus* newborns in control and treatment groups

Groups	Body weight of the newborns (grams)	
	Mean	SD
Control	5.57	± 0.50
Treatment	4.78	± 0.76

To find out the distribution of data based on the weight of *Rattus norvegicus* child, we performed the normality test using Shapiro-Wilk test. The result of Saphiro Wilk test showed significance result p=0,13 (p> 0,05) for control group and p=0,07 (p> 0,05) for treatment group. Then we did a homogenity test for samples of *Rattus norvegicus* child weight we got p=0,05 (p> 0,05). The above results show that the distribution of *Rattus norvegicus* newborn weight data in our study was normal and had homogeneous distribution (p> 0.05).

To know the difference between the weight of *Rattus norvegicus* newborn between control group and treatment with normal data distribution, an analysis was performed using Independent T-test. The mean birth weight of the newborn in the control group was heavier than the treatment group (5.57 $\pm$ 0.05 vs 4.78 $\pm$ 0.76). From the result of the analysis with Independent T-Test there was significant difference of weight of *Rattus norvegicus* newborn between control group and treatment with p=0.000 (p<0,05).

Dendritic density analysis in the cerebrum of *Rattus norvegicus* newborns' brain

We performed the normality test with the Shapiro-Wilk test of the dendritic density data in the cerebrum of both groups. From the test we got significant result p=0,63 for control group and p=0.054 in treatment group. This shows the distribution of data on *rattus norvegicus* dendritic density variables that were normally distributed (p> 0.05).

To know the difference of dendritic density on cerebrum control group and treatment with normal data distribution, we analyzed using independent t-test. The mean dendrite density in control group was higher than that in treatment group (4.98 $\pm$ 2.17 vs 2.69 $\pm$ 0.76). From the analysis with Independent t-test there was significant difference in dendritic density between control group and treatment with p=0.001 (p<0,05).

Dendritic density analysis in the cerebellum of *Rattus norvegicus* newborns' brain

We performed the normality test by Shapiro-Wilk test on the dendritic density data in the cerebellum of the two groups. From the test we obtained significant result p=0.84 for control group and p=0.59 in treatment group. This showed that data distribution on dendrite density of the *Rattus norvegicus* were normal (p>0.05). Furthermore, for analyzing dendritic density data with normal distribution, we carried out Independent T-test.

Mean dendritic density in control group was higher than that in treatment group (7.37 $\pm$ 2.23 vs 3.01 $\pm$ 0.64). The Independent T-Test revealed significant difference between control and treatment group with p=0.000 (p<0,05). This study was conducted to compare dendritic density in cerebrum and cerebellum between *Rattus norvegicus* with FR50 model and control to prove that the influence of malnutrition during pregnancy affects dendritic density in brain oF the newborns. This result was in accordance with the initial hypothesis that the dendritic density in the cerebrum and cerebellum *Rattus norvegicus* FR50 newborn model was lower compared with the control group.

Dendrites are extensions of neurons that function to receive and process impulses. Ramon Y Cajal in the book "Histology of the Nervous System" states a "Doctrine Neuron" that is that the dendrite of a neuron receives an impulse derived from the axon of another neuron and passes this impulse to the axon of the neuron. Roughly it can be said that a neuron that has no dendrite will not be able to receive an impulse because the area to receive impulse is limited.[9]

Brain development is partly susceptible during intrauterine development. Nutrition is one of the most important parts affecting the fetus and plays a very important role in the maturity and development of central fetal nervous system function. In humans, maternal deprivation can be shown as intrauterine growth restriction (IUGR) with an increased risk of perinatal death and infant morbidity for a long time. IUGR is also associated with inhibition of development of the nervous system including reduced agility, decreased intelligence and acting ability and poor learning achievement. In addition, malnutrition during the womb will alter the structure of the brain and the transmission linkage of nerve cells.[10]

Nutrition plays an important role in the maturation and functional development of the central nervous system because nutrients provide the energy and substances needed from the development of cell structures and various metabolic functions. Malnutrition can adversely affect many aspects of brain development and function. However, due to ethical relevance, not much research on the influence of prenatal nutrition on human brain development. Economic problems in developing countries are important factors contributing to the incidence of malnutrition.[10,11]

A study by Salas et al. 2015 showed a decrease in the number of dendrite branches in the neuron of pyramid girus cingulatus anterior of rats receiving nutritional restriction by 50% during pregnancy. Cintra et al. in 1996 examined the effects of protein deficiency during pregnancy on the number of dendrites and density of the spine dendrite of the mouse hippocampus area. In protein deficiency group, dendrite levels were found in the apex and basal and the density of spine dendrite decreased in protein deficiency group at the age of 15 days post partum, but the number increased again at age 30, 90, and 220 post partum. A study by Zhang et al. in 2013 showed nutritional deficiency with FR50 (food restriction 50%) during pregnancy led to a decrease in density of spine dendrite in mouse hippocampus. Research on the effect of nutrients on the human brain can only be done by autopsy examination. Bribiesca et al. in 1999 studied 13 infants with severe malnutrition and found that spine dendritic density lower than controls.[12,13,14,15]

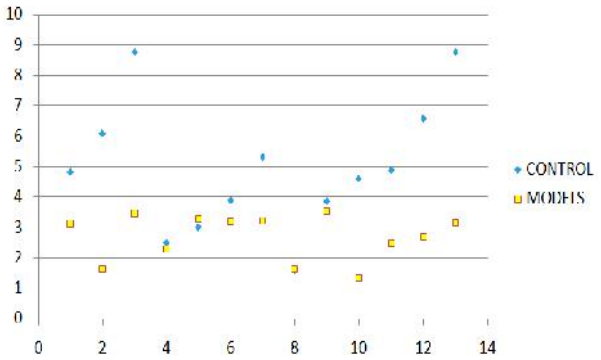

Figure 1. Scatter diagram of dendritic density in the cerebrum *Rattus norvegicus* newborn brain between food restriction group 50% and control group

Several studies were conducted to find out about the effect of nutrition during pregnancy on dendritic density. Salas et al. 2015 examined the effect of nutritional deficiency of FR50 on the 6th day of pregnancy until day 12 (G6 - G12) followed by FR30% in G12 to G19. The results of this study showed that the number of dendrite branches in the anterior pyramid gyrus cinguatus neuron in the group of nutritional deficiencies was significantly lower than the non-malnourished group. Another study by Zhang et al. in 2013 showed similar

results. Zhang et al studied the effect of FR50 during pregnancy from day 7 to lactation period in dendritic density in the mouse brain hippocampus area. In the FR50 group there was less dendritic density than in control. This suggests dendritic density in newborn rats is sensitive to malnutrition during pregnancy. The mechanism of nutritional effects on dendrite density is thought to be due to changes in oxidative stress status, cholinergic system, serotonin system, BDNF, glutamic acid decarboxylase-67 (GAD-67) and nitric oxide synthase.[12,14]

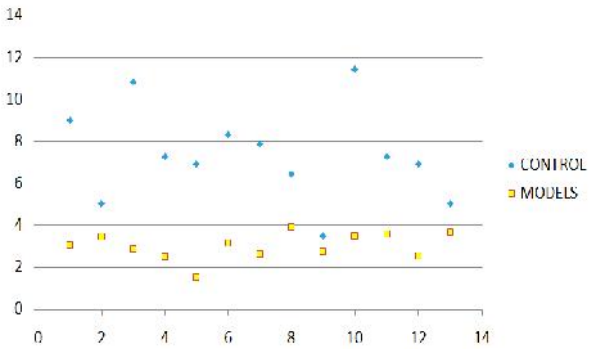

Figure 2. Scatter diagram of dendritic density in the cerebellum *Rattus norvegicus* newborn between food restriction group 50% and control group

Lack of nutrients in this study of 50% food restriction suspected to affect the expression of BDNF, CERB and regulation of neurotransmitter glutamate. The BDNF bond with its receptor, TrkB, will enable Scr Homology 2 domain containing protein adapter (Src). Src will bind to RAS and will phosphorylate ERK ½ through mitogen ERK kinase (MEK) and also phospoinositide-3 kinase (P13K). P13K will also activate ERK ½ and protein kinase B (Akt). ERK with Akt activates mTORCH1 (mammalian Target of Rapamycin C1) and then phosphorylates p70S6K (p70 ribosomal S6 protein kinase) resulting in translational process and protein transcription for dendrite, axon and cell survival.[16,17,18,19,20]

In addition, the neurotransmitter glutamate in the activated cell will bind to AMPA receptors (alpha 3-hydroxy 5-methyl 4-isoxazolepropionic 689 acid) and NMDA (N-methyl D-aspartate) in the post synapse membrane. Activation of AMPA receptors causes membrane depolarization and Ca 2 influx via NMDA and voltage depend Ca2+ channels. Ca2+ binds CaMK (Ca2+/calmodulin-dependent kinase) which is one of the calcium binding proteins. Calcium binding protein activates protein kinase A (PKA) and protein kinase C (PKC). The activated PKA and PKC causes phosphorylation of cAMP Response Element (CRE) to cAMP Response Element Binding Protein (CERB), which will cause transcription of BDNF post synapse.[14,21,22,23]

Several studies have shown that spine dendrite is regulated by glutamanergic transmission and some glutamate receptors on the spine dendrite head. In certain circumstances, the in vitro research shows that N-methyl-D-aspartic acid (NMDA) receptors mediate the instability of filamentous actin (f-actine) associated with dendritic density reduction. This situation will greatly increase in the state of undernutrition during prenatal and perinatal period.[14]

Limitations of the study

This study only proved dendritic density decrease in cerebrum and cerebellum of *Rattus norvegicus* newborn brain during pregnancy and birth because those newborns were directly sacrificed after birth. THIS Study could not follow the post-natal development of the *Rattus norvegicus* newborns with 50% FR during pregnancy.

CONCLUSION

Dendritic density in cerebrum and cerebellum of newborns *Rattus norvegicus* whose exposed to 50% food restriction significantly lower

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