The Effect of Fasting during Pregnancy on Brain-derived Neurotrophic Factor Expression in *Cerebrum* and *Cerebellum*

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Abstract

Background: Fasting for pregnant women is still a debatable issue among society and clinicians. Fasting done by pregnant women causes brain neurons to receive more energy thus increases the connection between the neurons. A brain hormone called Brain-derived Neurotrophic Factor will be affected during fasting. Brain-derived neurotrophic factor (BDNF) plays an important role in neuronal survival and growth, serves as a neurotransmitter modulator, and participates in neuronal plasticity, which is essential for learning and memory. Brain-derived Neurotrophic Factor (BDNF) has an important role in brain development, namely in the formation of new nerve cells and cognitive processes inside the brain which helps to optimize cognitive, learning, and memory functions. Fasting during pregnancy will influence fetal brain cells due to increase in the body's metabolic system which is delivered to *cerebrum* and *cerebellum* neuron cells. The objective of this research is to analyze the effect of fasting during three trimesters pregnancy on Brain-derived Neurotrophic (BDNF) factor expression in *cerebrum* and *cerebellum*.

Method: The design of this research is true experimental laboratory post-test with control group design. The research samples are rats *(Rattus norvegicus)* which consists of 3 groups and 1 control group. First group (X1) was the rats born from mother with 2 days fasting during first trimester pregnancy, second group (X2) was the rats born from mother with 2 days fasting during second trimester pregnancy and third group (X3) was the rats born from mother with 2 days fasting during third trimester pregnancy. The control group (X0) was the rats born from mother without fasting during the whole pregnancy. Brain dissections of new born rats were taken and the brain-derived Neurotrophic Factor expression was studied within munohistochemistry.

Conclusion: Brain-derived Neurotrophic Factor expression in *cerebrum*and *cerebellum*was examined with immunohistochemical method. Data from each sample was assessed using the Remmele Scale Indexmethod (Immunoreactive Score). There was significant *cerebrum* Brain-derived Neurotrophic Factor expression mean found in third trimester (3.90 ± 2.403) with p-value = 0.008 ($p<0.05$). There were no significant *cerebellum* Brain-derived Neurotrophic Factor expression differences found in all trimesters. Fasting during pregnancy is safe for both mother and children. Fasting during pregnancy will increase the Brain-derived Neurotrophic Factor expression so that the brain function will improve as well.

Keywords: Brain-derived, Fasting, Neurotrophic, *Rattus norvegicus*, Pregnancy

Introduction

Fasting is characterized by a series of coordinated metabolic changes designed to save carbohydrates and increase dependence on fat as a substrate for energy supply.[18]Calorie restriction or dietary restrictions are the methods used by limiting the amount of consumed food. Diet retraction is also interpreted as reducing the number of calories that enter the body (about 20-40% of the daily intake that is normally consumed) while maintaining adequate nutrition needed by the body.[20]There are various methods of dietary retention, including alternate-day fasting, that is one day consuming food without restrictions (can be given twice the usual intake) and on a full day the food is reduced.[20]Another method is to try to satisfy animals (not given any food) for several hours with different duration of fasting.

During fasting, the body will signal hunger and stimulate the desire to eat. However, hunger will be halted so that the process of adaptation to the lack of energy sources will occur and energy needs will still be met.[8]

Fasting for pregnant women is still a controversy issue in the community. The public assumes that fasting during pregnancy will pose risks to pregnancy, including intellectual disorders in children, low birth weight, increase *hyperemesis gravidarum*, urinary tract infections, and trigger a decrease in fetal movement in the uterus.[6]

Brain-derived Neurotrophic Factor (BDNF) is one of the neurotrophic factors that support differentiation[7], maturation[1]and survival of neurons in the nervous system[11] and shows a *neuroprotective* effect under adverse conditions, such as glutamatergic timulation, cerebral ischemia, hypoglycemia, and neurotoxicity[12]. Brain-derived Neurotrophic (BDNF) stimulates and controls growth of new neurons from neural stem cells (neurogenesis) [5].

A study showed that fasting could increase Brain-derived Neurotrophic Factor (BDNF) formed in the brain, which helps the body to produce more brain cells, which could increase the fetus brain function[3]. During fasting, the number of mitochondria in each neuron cell of the brain increases. *Mitochondria* causes the brain cells to increase which will affect the brain to be more durable to absorb memories[19]. Duan *et al.* showed that the levels of Brain-derived Neurotrophic (BDNF) was significantly increased in the hippocampus, cerebral cortex, and striatum of rats maintained on a dietary restriction (DR) regimen compared to controls.

Brain-derived Neurotrophic (BDNF) has important role in the formation of new neuron cells and cognitive process in the brain. The brain has few main parts, such as: *cerebrum*, *cerebellum* and brainstem. Functions of the *cerebrum* include: initiation of movement, coordination of movement, temperature, touch, vision, hearing, judgments, reason, problem solving, emotions and learning. While the function of *cerebellum* is to coordinate voluntary muscle movements, maintain postures, balance and equilibrium. The previous studies in this field have been conducted using blood samples of fasted humans or on animals without measuring the level of Brain-derived Neurotrophic (BDNF) expression during pregnancy in the *cerebrum* and *cerebellum*. This study is aimed to analyze the effect of fasting during three trimester pregnancy on Brain-derived Neurotrophic Factor (BDNF) expression in *cerebrum* and *cerebellum*.

Materials and Methods

The study design of this research is true experimental laboratory post-test only with control group design. The subjects in

this research are divided into 4 random groups, consisting of 3 groups which fasted during Trimesters I, II, and III, and 1 control group which did not fast. Fasting during pregnancy is an activity without the consumption of food and drinks with calories for 14 hours (17.00-07.00) with the normal food composition of 70-100/kgbody weight/day. Brain-derived Neurotrophic Factors (BDNF) is defined as a member of the neurotroph in family which has a main function to modulate neuron survival and apoptosis neuron plasticity. Immunohistochemistry method was used to assess the Brain-derived Neurotrophic Factor (BDNF).

The population of this research are mature female *Rattus norvegicus* aged 2-3 months with weights 130-170 grams which were obtained from laboratory and research center at Gadjah Mada University (LPPT UGM). The subjects on this research were young *Rattus Norvegicus* (young rats) with the inclusion criteria: newborn rats from healthy pregnant mother rat (active movements, shiny eyes, soft and thick fur, and has a normal weight), *Rattus norvegicus* mothers which have never been pregnant, and adult *Rattus norvegicus* aged 2-3 months. The total amount of subjects in this research was32, which were divided into 4 groups, each group consisted of8 rats, namely : Control Group X0 which was given standard food and water , Group X1 fasted during trimester I for 2 days (Days 5 and 6, week 1 of pregnancy), Group X2 fasted during trimester II for 2 days (Days 11 and 12, week 2 of pregnancy), and Group X3 which fasted during trimester III for 2 days (Days 17 and 18, week 3 of pregnancy).

Sample collection was done immediately after the *Rattus norvegicus* was born, and then brain samples from the newborn rats were taken for immune on *histochemistry* check to measure the Brain-derived Neurotrophic (BNDF) expression of each sample.

Brain-derived Neurotrophic (BDNF) expression of each sample was graded semi-quantitatively in accordance to Remmele Scale Index (Immuno Reactive Score), which is taking into account the percentage imunnoreactive cell percentage score (A) with the color intensity score (B). Field percentage is seen through a microscope with the magnification of 400X and counted per 100 cells divided by total cells, multiplied by 1000.

Table 1: Semi-quantitative Immunoreactive Score (IRS) in which the final results correspond to the product of two variables (AxB) (Madej et al., 2014)

Point Score	A	B
0	No cells with positive reaction	No color reaction
1	$\leq$ 10% cells with positive reaction	Low intensity of color reaction
2	11%-50% cells with positive reaction	Average intensity of color reaction
3	51%-80% cells with positive reaction	Intense color reaction
4	>80% cells with positive reaction	

To see the difference in Brain-derived Neurotrophic (BDNF) expression in the cerebrum and cerebellum of *Ratus norvegicus* children in the fasting and control groups, normality test will be conducted first. If the data were normally distributed ($p>0.05$) then the Shapiro-Wilk test was used followed by the analysis of variance test (ANOVA). However, if the data is not normally distributed then the Kruskal-Wallis test will be conducted. This study used p-value of 0.05 with 95%

confidence level. All data analysis then statistically counted with statistical package for the social sciences (SPSS) for Windows 23.

Results

Brain-derived *Neurotrophic* (BDNF) expression in cerebrum was analyzed through *immunohistochemical* examination. Data from each sample was assessed using the Remmele Scale Index (*Immunoreactive* Score/IRS). Normality test of Brain-derived Neurotrophic (BDNF) expression in cerebrum of newborn *Rattus norvegicus* was obtained with the result of normal distribution between the control and treatment groups (p<0.05). Then homogeneity test was performed which produced inhomogeneous data with p-value=0.04. Since the data were not homogeneous, the Kruskal-Wallistest was performed.

Table 2: Brain-derived Neurotrophic (BDNF) Result in *cerebrum* of newborn *Rattus norvegicus* with Shapiro-Wilk Test

Group	n	p-value	
		Shapiro-Wilk	Levene's test
X0 (Control)	6	0,19	0,004
X1 (Fasted Trimester I)	6	0,07	
X2 (Fasted Trimester II)	6	0,16	
X3 (Fasted Trimester III)	6	0,20	

Table 3: Brain-derived Neurotrophic (BDNF) Result in *cerebrum* newborn *Rattus norvegicus* with Kruskal-Wallis Test

Group	n	Mean ± SD	p-value
X0 (Control)	6	1,30 ± 0,27	0,008
X1 (Fasted Trimester I)	6	1,77 ± 1,19	
X2 (Fasted Trimester II)	6	2,20 ± 0,35	
X3 (Fasted Trimester III)	6	3,90 ± 2,40	

Based on table 3, there were significant differences in Brain-derived Neurotrophic (BDNF) expression in cerebrum of the four groups (p-value < 0.05), thus further T-test was performed. The result of T2 free sample test showed that X0 group was different than X2 and X3 groups.

Table 4: Brain-derived Neurotrophic (BDNF) Expression in *Cerebrum* of X0 and X1 Groups

Group	n	Mean ± SD	p-value
X0 (Control)	6	1,30 ± 0,276	0,374
X1 (Fasted Trimester I)	6	1,767 ± 1,196	

The result of T2 free sample test showed that there was no significant Brain-derived Neurotrophic (BDNF) expression difference in the *cerebrum* between control group and fasted trimester I group (p-value>0.05).

Table 5: Brain-derived Neurotrophic (BDNF) Expression in *Cerebrum* of X0 and X2 Groups

Group	n	Median (min – max) Mean ± SD	p-value
X0 (Control)	6	1,30 ± 0,276	
X2 (Fasted Trimester II)	6	2,200 ± 0,358	0,001

The result of T2 free sample test showed that there was significant BDNF expression difference in the *cerebrum* between control group and fasted trimester II group (p-value<0.05).

Table 6: Brain-derived Neurotrophic Factor (BDNF) Expression in *Cerebrum* of X0 and X3 Groups

Group	n	Mean ± SD	p-value
X0 (Control)	6	1,30 ± 0,276	
X3 (Fasted Trimester III)	6	3,90 ± 2,403	0,045

The result of T2 free sample test showed that there was significant Brain-derived Neurotrophic (BDNF) expression difference in the *cerebrum*between control group and fasted trimester III group (p-value<0.05).

BDNF expression in *cerebellum* was analyzed through immunohistochemistry examination. Data from each sample was assessed with Remmele Index Scale (Immunoreactive Score/IRS). Normality test of Brain-derived Neurotrophic (BDNF) expression in *Rattus norvegicus* cerebellum was performed using Shapiro-Wilk test.

Table 7: Brain-derived Neurotrophic Factor (BDNF) Result in *cerebellum* of newborn *Rattus norvegicus* with Shapiro-Wilk Test

Group	n	p-value
X0 (Control)	6	0,139
X1 (Fasted Trimester I)	6	0,077
X2 (Fasted Trimester II)	6	0,002
X3 (Fasted Trimester III)	6	0,085

Based on table 7, there was abnormality in distributionof BDNF expression in Group X2 (fasted trimester II) with p-value=0.002 (p-value<0.05). Thus, Kruskal-Wallis test should be done following the previous test.

Table 8: Brain-derived Neurotrophic Factor (BDNF) Result in *cerebellum* of newborn *Rattus norvegicus* with Kruskal-Wallis Test

Group	n	Median (min – max)	p-value
X0 (Control)	6	2,3 (1,0 – 6,4)	
X1 (Fasted Trimester I)	6	2,2 (0,8 – 6,4)	
X2 (Fasted Trimester II)	6	2,8 (2,2 – 6,8)	0,524
X3 (Fasted Trimester III)	6	2,2 (1,2 – 8,4)	

Based on the table 8, there was no significant difference on Brain-derived Neurotrophic (BDNF) expression of the 4 groups in *cerebellum*, with p-value=0.542 (p>0.05).

Discussion

There are reports indicating of a correlation between fasting and Brain-derived Neurotrophic Factor (BDNF) levels during fasting. One of the most comprehensive studies addressing this correlation was carried out by Krisztina Marosi and his colleagues. Their investigations showed that fasting/food deprivation can also induce Brain-derived Neurotrophic (BDNF) expression in neuronal circuits involved in cognition by increasing their activity, and by shifting cellular energy substrate utilization from glucose to ketones. They also added that intermittent fasting and exercise through Brain-derived Neurotrophic (BDNF) induce the *neurogenesis*, by promoting the differentiation of neurons from stem cells, and the survival and synaptic integration of newly generated neurons[17]. There also are several investigations, which indicate that the calorie restriction has beneficial effects on neurotrophic factors such as Brain-derived Neurotrophic (BDNF) in the brain and that the elevation of this factor

can improve the neuro generation in the nervous system[2,10,16].

During fasting, there is a shift in the utilization of brain cell energy substrate from glucose to ketones 3-*hydroxybutyrate* (3OHB). 3OHB can protect neurons against oxidative stress. 3-*hydroxybutyrate* metabolism increases mitochondria which encourage changes in brain expression derived by Brain-derived factors (BDNF) in the cerebral cortex of neurons. The mechanism that induces 3-*hydroxybutyrate* and Brain-derived *Neurotrophic* (BDNF) expression involves ROS (Reactive Oxygen Species) and activation of NF-kB transcription factors. Since Brain-derived Neurotrophic (BDNF) plays an important role in synaptic plasticity and stress resistance, 3OHB (3-*hydroxybutyrate*) can mediate the adaptive response to neurons when fasting.[17]

Brain-Derived Neurotrophic Factor (BDNF) is a member of *neurotropin* which functions to modulate endurance and play a role in the development, maintenance and synaptic plasticity.[17]

Consequently, Brain-derived Neurotrophic Factor (BDNF) is located in the cortex and basal cerebrum in the hippocampus which is a process for thinking, memory and recollection. Brain-derived Neurotrophic Factor (BDNF) is synthesized as pre-proneurothrophin which is divided into pro BDNF and BDNF maturity. Pro Brain-derived Neurotrophic Factor (BDNF) is converted to mature BDNF which is active with the help of prohormone convertase such as purines. Pro Brain-derived Neurotrophic Factor (BDNF) is also produced by neurons that are changed by tissue plasminogen activator (tPA) or plasmin to Brain-derived Neurotrophic Factor (BDNF).[17]

Brain-Derived Neurotrophic Factor (BDNF) is a protein expressed in the brain that covers the areas of the frontal cortex, parietalis, cingulatus, temporal, retrospenial, prirhinal, hippocampus, entorhinal cortex, brain stem and cerebellum.[4] In addition, Brain-derived Neurotrophic Factor (BDNF) concentrations in each area in the brain is different and the highest concentration is in the hippocampus.[7]

According to Manuaba (2010) that, during the first trimester of pregnancy there will be physiological adaptations resulting from hormones in pregnancy and metabolic changes are including a rise in basal metabolism by 15-20% from before pregnancy. During the first trimester of pregnancy the nutrients that enter the body tend to decrease, because the body is still adapting to pregnancy hormones. With poor nutrition during fasting, oxidative stress will be reduced so that the utilization of 3-*hydroxybiturate* will decrease. This might explain the reason there was no significant difference on BDNF expression in trimester I in both *cerebrum* and *cerebellum*in this study.

Cerebellum is the center of the body in controlling the quality of movement. The *cerebellum* also controls many automatic functions of the brain, including: regulating posture or body position, controlling balance, muscle coordination and body movements.[9]In second trimester, body organs have formed, so the metabolic basal requirements will also increase and the need for protein used for fetal growth and development will also rise. In this trimester the body can adapt to changes in pregnancy hormones.[13]During the second trimester, the amount ofnutrients entering the body starts to increase, while the body's metabolic needs also grow in this period. This process will lead to rise in oxidative stress in the body, but the body has a mechanism to deal with this, namely through 3-*hydroxybiturates* that enter the mitochondria and make deacetylation of ROS (reactive oxygen species) molecules such as SOD (superoxide dismutase) enzymes, causing Brain-Derived Neurotrophic Factor (BDNF) to increase in the cerebrum during second trimester pregnancy. In this study, there is no significant BDNF expression difference in

*cerebellum*due to the different function of the *cerebellum*. BDNF is related to cognitive function while *cerebellum* is linked with balance and coordination.

In third trimester pregnancy, the mother's body needs more nutrients for fetal growth and development. Thus, the nutrients that enter the body increases which will elevate the oxidative stress and make the body use more ketones (3-*hydroxybutyrate*). In this study, there was significant difference of Brain-derived Neurotrophic (BDNF) in the *cerebrum* between the control group and fasted third trimester. While in the cerebellum there was no significant Brain-Derived Neurotrophic Factor (BDNF) expression difference between the control group and fasted third trimester group. This condition is due to the fact that the highest Brain-Derived Neurotrophic Factor (BDNF) concentration is located in the hippocampus.

Conclusion

In conclusion, fasting for pregnant women is safe. Fasting during pregnancy will increase Brain-derived Neurotrophic (BDNF) expression and improve brain function. As pregnancy age increases, Brain-derived Neurotrophic (BDNF) will also increase. However, there is still much that needs to be investigated to better understand the effect of fasting since this study was done on *Rattus norvegicus* that could only be fasted for few hours.

Conflict of Interest: There is no conflict of interest in the research.

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Ethical Clearance: All the clinical procedures were carried out following the protocols approved by the Ethics and Review Committee of Wijaya Husada Health Institute.

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