ORIGINAL ARTICLE

Effect of High-Fat Diet on SOD2, GPx, NeuN and BDNF Expression on Frontal Lobe of Obese Rats

Mohammad Ariq Nazar[1,3], Dwi Cahyani Ratna Sari[2], Agung Putra[3,4], Nur Arfian[2]

[1] Master Program of Biomedical Science, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2] Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3] Stem Cell and Cancer Research, Medical Faculty of UNISSULA, Semarang, Indonesia
[4] Department of Pathological Anatomy, Medical Faculty of UNISSULA, Semarang, Indonesia

ABSTRACT

Introduction: Obesity has been demonstrated to induce oxidative stress and inflammation processes that lead to senescence in brain cells. Obesity-induced cellular senescence in the brain is still widely investigated. This study aimed to investigate the expression of antioxidant and neuronal markers in the frontal lobes of obese rats. **Methods:** Eighteen adult rat Sprague Dawley divided into three groups: Control (SO), Obese-2 (DIO2), and Obese-4 (DIO4) were observed. Control rats were fed with a standard diet AIN 76A for two month. In contrast, DIO2 and DIO4 rats were fed with a high-fat diet daily for two and four months, respectively. After being sacrificed, the rats' brains were dissected out then the frontal lobes were used for RNA extraction. Reverse transcriptase PCR of SOD2, GPx, BDNF, NeuN and beta-actin was performed to investigate the relative expression of the antioxidant and neuronal markers. **Results:** DIO2 and DIO4 groups had significantly increased body Weights, blood glucose level and triglyceride level after being fed with a high-fat diet for two and four months, respectively. The DIO4 group had the significantly lowest mRNA expressions of SOD2, GPx, BDNF and NeuN. **Conclusion:** Decreased antioxidant and neuronal markers in the rats frontal lobes were observed as the chronic effect of obesity.

Keywords: Obesity, Frontal lobe, GPx, SOD2, BDNF, NeuN

Corresponding Author:
Dwi Cahyani Ratna Sari, PhD
Email: dwi.cahyani@ugm.ac.id
Tel: +6274 6492521

INTRODUCTION

Obesity is a disproportionation of energy intake and expenditure leading to an increase in body weight caused by an excessive adipose tissue mass accumulation (1). The high level of glucose and lipids may trigger an excessive supply of energy substrates to metabolic pathway, these conditions induce the pathogenesis of several diseases, including neurodegeneration (2). Under an obese condition, regional cerebral blood flow is decreased, especially in prefrontal brain regions that are involved in attention, cognitive, and decision-making functions (3). In addition to, obesity-induced neurodegeneration is promoted by a combination of increased reactive oxygen species (ROS) generation and decreased antioxidant capacity to overcome an oxidative stress (4).

Several studies have reported that high fat diet-induced obese rats had overproduction of mitochondrial superoxide (5,6), and under chronic free radical exposure may lead to a decrease of antioxidant enzyme capacity including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) (7). Excessive superoxide has implicated in the degradation of the nervous system function through blocking neurotrophic factor Janus kinases/signal transducer and activator of transcription proteins (Jak/STAT) (8). Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that has a pivotal role in the nervous system including neuronal survival, differentiation, and synaptic connectivity (9).

BDNF level under obesity shows a decrease of expression in the hippocampus (10), followed by a decrease of NeuN as a mature neuron marker in diet-induced obesity (DIO) (11). However, most of these studies focused on the hippocampus and hypothalamus whereas data on the frontal lobe are scarce (12). Furthermore, another study has reported that DIO in obese rats can increase p16 and p21 senescence marker in the frontal lobe (13). The aim of this study was to investigate the expressions of SOD2, GPx, BDNF, and NeuN in frontal lobe obese rats brains for further potential research developments

in the study of obesity and neurodegenerative diseases.

MATERIALS AND METHODS

Hight-fat diet induce obese animals model

This study was approved by Ethical Committee of Medical Research and Health of Faculty of Medicine Universitas Gadjah Mada with ethical expediency number KE/0627/06/2020. Eighteen Sprague Dawley rats (180-200 grams, young adult of 3 months-old) obtained from Experimental Animal Care Unit of Universitas Gadjah Mada were used for this study. The rats were housed in a cage containing up to three rats each, under the condition of room temperature 25-30°C with 50%-60% humidity, a dark-light cycle of 12:12 hours and were fed water ad libitum. The subject rats were equally divided into three groups, six rats each group, namely: Sham operation (SO), Obese-2 (DIO2), and Obese-4 (DIO4). The SO group received standard pellet diet called AIN76A, while Ob-2 and Ob-4 groups were fed with high fat diet (HFD) 2 months and 4 months, respectively.

The rats were weighed and blood collection at the end of the study. After the due date of high fat diet feeding, DIO2 and DIO4 groups were sacrificed at day-60 and day-120, respectively. The SO group was sacrificed after 2 months of standard pellet diet. The whole brain was harvested and immersed in RNA Later for mRNA assay (Ambion, 7021). The left and right frontal lobes were dissected following the neuroanatomical mapping of the rats brains right before the RNA extraction.

Blood glucose level and triglycerides assay

Blood samples were placed in blood collection tubes (BD Vacutainer®) and incubate for 1 h at room temperature, then centrifuged at 1500 g for 10 min at 4°C. The serum concentrations of blood glucose level and triglycerides were determined using enzymatic colorimetric methods.

RNA Extraction and cDNA synthesis

Frontal lobes tissue was extracted using Genezol RNA Solution (GENEzol™, Cat. No. GZR100) following the manufacturer's instructions. The RNA concentration was quantified using nanodrop. Reverse-transcription for cDNA synthesis was performed using Revertra Ace kit (Toyobo, Cat. No. TRT-101), random primer (TAKARA, Cat. No. 3801), and deoxyribonucleotide triphosphate (dNTP) (Takara, Cat. No. 4030) in a total volume of 20 µl containing 1 µg of total RNA, with PCR condition 30°C for 10 min (annealing), 42°C for 60 min (elongation) and 99°C for 5 min (enzyme deactivation).

Reverse Transcriptase-PCR of antioxidant and neuronal markers

Reverse Transcriptase-PCR of SOD2, GPx, BDNF and NeuN was performed to determine the expression of antioxidant and neuronal marker in the frontal lobes. The RT-PCR was performed using the mixture of cDNA, Taq master mix (Bioron, Germany, Cat. No. S101705) and specific primers (Table I). The PCR products were analyzed on 2% agarose gel along with a 100bp DNA ladder (Bioron, Germany, Cat. No. 306009). The expressions of antioxidant and neuronal marker were quantified using densitometry analysis of the ImageJ software. β-actin expression was used to normalize the expression.

Table I: Primers for PCR analysis

Gene	Primer	*Anneling Temp.*	*Cycles*
β-actin	F : GCAGATGTGGATCAGCAAGC R : GGTGTAAAACGCAGCTCAGTAA	54 °C	35
SOD2	F : ATGTTGTGTCGGGCGGCGTGCAGC R : GCGCCTCGTGGTACTTCTCCTCGGTG	65 °C	35
GPx	F : CTCT CGCGGTGGCACAGT R : CCACCACCGGGTCGGACATAC	62 °C	35
NeuN	R : GCAGATGAAGCAGCACAGAC R : TGAACCGGAAGGGGA TGTTG	58 °C	40
BDNF	R : GCAGATGAAGCAGCACAGA C R : TGAACCGGAAGGGGA TGTTG	60 °C	35

Analysis of statistical

Data are presented as the means ± standard deviation. All calculations were carried out using IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The statistical significance of the differences between the groups was assessed using one way-ANOVA and continued with fisher's least significant difference (LSD) post-hoc analysis. P values: *, $P < 0.05$, **, $P<0.001$, ***, $P<0.000$.

RESULTS

Body weight, glucose level and trigliserida after high-fat diet

After high-fat diet feeding treatment given for each group, the body weight, blood glucose level and triglycerides were measured in each group at the end of study. From Table II, there was a significant increase ($P<0.05$) of body weight, blood glucose level and triglycerides compared to SO.

Expression of antioxidant and neuronal markers in frontal lobes of obese rats

The results obtained from the amplification of the SOD2, GPx, NeuN and BDNF genes showed that there was no significant difference between the SO and DIO2 groups ($p> 0.05$), but there was a significant difference between the SO and DIO4 groups ($p <0.05$) (Table III, Figure 1).

Table II: Body weight, blood glucose and triglyseride level after high-fat diet for two and four months

Levels	SO	DIO2	DIO4
Initial body weight (gram)	187.25 ± 3.7	203.60 ± 19.4	190.6 ± 4.2
Body weight (gram)	218.75 ± 24.2	346.56 ± 34.8*	373.2 ± 19*
Blood glucose level (mg/dL)	80 ± 9.7	123.6 ± 12.7*	125.4 ± 8.2*
Triglyceride (mg/dL)	88.2 ± 9.7	209.8 ± 38.2*	214 ± 50.2*

$p<0.05$ mean values were significantly different from those of the SO group, # $p<0.05$ mean values were significantly different from those of the DIO group.

Table III: The value of relative expression of mRNA of the frontal lobes obese rats.

Levels	SO	DIO2	DIO4
SOD2/ β-actin	3.9 ± 0.4	3.5 ± 0.5	2.8 ± 0.3*#
GPx / β-actin	3.8 ± 0.9	3.0 ± 0.8	2.4 ± 0.2*
NeuN/ β-actin	3.1 ± 0.4	2.5 ± 0.6	2.0 ± 0.2*
BDNF/ β-actin	2.8 ± 0.6	2.7 ± 0.6	2.0 ± 0.2*

*p<0.05 mean values were significantly different from those of the SO group, # p<0.05 mean values were significantly different from those of the DIO group.

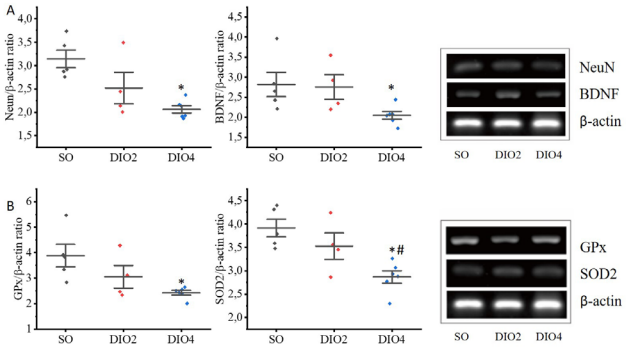

Figure 1: Relative expression of mRNA of the frontal lobes obese rats showed a significant decrease (P<0.05). A. Relative quantification and representative images of gel electrophoresis from RT-PCR products of the BDNF and NeuN expressions. B Relative quantification and representative images of gel electrophoresis from RT-PCR products of the SOD2 and GPx expressions.

DISCUSSION

Obesity is characterized by a pathological accumulation of body adipose tissues that leading to a positive energy balance. To mimic susceptibility to obesity in human, high-fat diets have been used to develop obesity in animals model, DIO animals are believed to mimic better obesity in humans than genetically modified models (14). Small rodents such as rats are the most commonly used preclinical animal model to study metabolic disorders because they have closer physiology to humans (15). Sprague-Dawley exhibit a wide distribution in HFD-induced body weight gain (16). Therefore Sprague-Dawley rats were preferably chosen for this study.

Under obesity conditions, the overload of intracellular lipid storage leading to adipocyte-derived fatty acids leaking into the circulation (17,18). Harmful lipid that circulating in perifer contributes several metabolic disorder including insulin resistance (19,20). Our study demonstrated the body weight, blood glucose level and triglyceride of rats showed a significant increase. The high level of triglyceride in circulation leading to an increase of stress-induced serine-threonine kinases activity subsequently impact of the insulin signaling pathway (21–23). In addition, triglyceride can cross the blood-brain barrier to induce central insulin resistance and also an excessively of ROS formation in brain mitochondria leading to frontal lobe oxidative damage (12,24,25).

The increase of ROS levels might be responded by expressing antioxidant enzymes through the activation of nuclear factor erythroid 2-related factor (Nrf2), Nrf2 activation binding to antioxidant response elements (ARE) as a promoter of antioxidant genes, including SOD2 and GPx (26). We found that there was a significant decrease in the expression of mRNA SOD2 and GPx in DIO4 compare with SO in frontal lobes. These suggest, under the chronic HFD induction may reduce antioxidant enzyme capacity to overcome superoxide. A decrease in antioxidant enzyme levels indicates that there is excessive production of oxidative stress in the frontal lobe, this will trigger a series of pathological events in the brain in the form of decreased synaptic plasticity and neuron cell apoptosis (27).

In line with this finding, we also found there were significant difference expression of mRNA BDNF and NeuN genes in DIO4 compare with SO in frontal lobes. These data indicated that the prolong oxidative stress will interfere with BNDF signaling which implicates decreased expression of NeuN (28). This is supported by other studies that reported the prolong HFD may lead to the decrease of neuronal markers such as BDNF, mitochondrial impairment, and hippocampal neuronal damage in animal models. (29,30). This study has limitations in which we do not analyse of ROS, Nrf2, and ARE pathway which regulates the transcription of antioxidant genes to eliminate stress oxidative. Additional studies are needed to further support the in vivo role of these lipids in the HFD-induced effects in the cortex.

CONCLUSION

In this study, we conclude that the prolong HFD decrease antioxidant and neuronal markers in the rats frontal lobes of obese rats as a result of chronic obesity that might associated with obesity-induced neurodegenerative.

ACKNOWLEDGEMENTS

The authors would like to thank to Mr Mulyana for assisting the animal handling. This study was funded by Dana Masyarakat, Faculty of Medicine, Public Health and Nursing 2021 with grant number 272/UN1/FKKMK/PPKE/PT/2021. The data of this study was used for completting the master program of Biomedical Science for Mohammad Ariq Nazar.

REFERENCES

1. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 2019;15:288–98.
2 Freeman LR, Zhang L, Nair A, et al. Obesity increases cerebrocortical reactive oxygen species and impairs brainfunction. Free Radic Biol Med 2013;56:226–33.

3 Willeumier KC, Taylor D V., Amen DG. Elevated BMI is associated with decreased blood flow in the prefrontal cortex using SPECT imaging in healthy adults. Obesity 2011;19:1095–7.

4 Bruce-Keller AJ, White CL, Gupta S, et al. NOX activity in brain aging: Exacerbation by high fat diet. Free Radic Biol Med 2010;49:22–30.

5 Ma W, Yuan L, Yu H, Xi Y, Xiao R. Mitochondrial dysfunction and oxidative damage in the brain of diet-induced obese rats but not in diet-resistant rats. Life Sci 2014;110:53–60.

6 Aung HH, Tsoukalas A, Rutledge JC, Tagkopoulos I. A systems biology analysis of brain microvascular endothelial cell lipotoxicity. BMC Syst Biol 2014;8:1–15.

7 Bhandari U, Kumar V, Khanna N, Panda BP. The effect of high-fat diet-induced obesity on cardiovascular toxicity in wistar albino rats. Hum Exp Toxicol 2011;30:1313–21.

8 Kaur N, Lu B, Monroe RK, Ward SM, Halvorsen SW. Inducers of oxidative stress block ciliary neurotrophic factor activation of Jak/STAT signaling in neurons. J Neurochem 2005;92:1521–30.

9 Rios M. BDNF and the central control of feeding: Accidental bystander or essential player? Trends Neurosci 2013;36:83–90.

10 Sandrini L, Di Minno A, Amadio P, Ieraci A, Tremoli E, Barbieri SS. Association between obesity and circulating brain-derived neurotrophic factor (BDNF) levels: Systematic review of literature and meta-analysis. Int J Mol Sci 2018;doi: 10.3390/ijms19082281.

11 Hsuchou H, He Y, Kastin AJ, et al. Obesity induces functional astrocytic leptin receptors in hypothalamus. Brain 2009;132:889–902.

12 Batandier C, Poyot T, Marissal-Avry N, et al. Acute emotional stress and high fat/high fructose diet modulate brain oxidative damage through NrF2 and uric acid in rats. Nutr Res 2020;79:23–34.

13 Sari DCR, Rochmah MA, Ananda W, Setyaningsih W, Nugroho DB, Arfian N. Expression of Senescence Markers Increased in the Frontal Lobes of Obese Rats. Malaysian J Med Heal Sci 2020;16:62–6.

14 Lutz TA, Woods SC. Overview of animal models of obesity. Curr Protoc Pharmacol 2012;doi: 10.1002/0471141755.ph0561s58:1–22.

15 Kleinert M, Clemmensen C, Hofmann SM, et al. Animal models of obesity and diabetes mellitus. Nat Rev Endocrinol 2018;14:140–62.

16 Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE. Selective breeding for diet-induced obesity and resistance in Sprague- Dawley rats. Am J Physiol - Regul Integr Comp Physiol 1997;doi: 10.1152/ajpregu.1997.273.2.r725.

17 Oussaada SM, van Galen KA, Cooiman MI, et al. The pathogenesis of obesity. Metabolism 2019;92:26–36.

18 Rekatsina M, Paladini A, Piroli A, Zis P, Pergolizzi J V., Varrassi G. Pathophysiology and Therapeutic Perspectives of Oxidative Stress and Neurodegenerative Diseases: A Narrative Review. Adv Ther 2020;37:113–39.

19 Arnold SE, Arvanitakis Z, Macauley-Rambach SL, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: Concepts and conundrums. Nat Rev Neurol 2018;14:168–81.

20 Guilherme A, Virbasius J V., Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:367–77.

21 ter Horst KW, Gilijamse PW, Versteeg RI, et al. Hepatic Diacylglycerol-Associated Protein Kinase Cε Translocation Links Hepatic Steatosis to Hepatic Insulin Resistance in Humans. Cell Rep 2017;19:1997–2004.

22 Matczuk J, Zalewska A, Łukaszuk B, et al. Insulin Resistance and Obesity Affect Lipid Profile in the Salivary Glands. J Diabetes Res 2016;doi: 10.1155/2016/8163474.

23 Al-Sulaiti H, Diboun I, Banu S, et al. Triglyceride profiling in adipose tissues from obese insulin sensitive, insulin resistant and type 2 diabetes mellitus individuals. J Transl Med 2018;16:1–13.

24 Banks WA, Farr SA, Salameh TS, et al. Triglycerides cross the blood-brain barrier and induce central leptin and insulin receptor resistance. Int J Obes 2018;42:391–7.

25 Song K, Li Y, Zhang H, et al. Oxidative Stress-Mediated Blood-Brain Barrier (BBB) Disruption in Neurological Diseases. Oxid Med Cell Longev 2020;doi: 10.1155/2020/4356386.

26 Tu W, Wang H, Li S, Liu Q, Sha H. The Anti-Inflammatory and Anti-Oxidant Mechanisms of the Keap1/Nrf2/ARE Signaling Pathway in Chronic Diseases. Aging Dis 2019;10:637.

27 Blázquez E, Velázquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: Its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and alzheimer's disease. Front Endocrinol (Lausanne) 2014;5:1–21.

28 Giacobbo BL, Doorduin J, Klein HC, Dierckx RAJO, Bromberg E, de Vries EFJ. Brain-Derived Neurotrophic Factor in Brain Disorders: Focus on Neuroinflammation. Mol Neurobiol 2019;56:3295–312.

29 Cavaliere G, Trinchese G, Penna E, et al. High-Fat Diet Induces Neuroinflammation and Mitochondrial Impairment in Mice Cerebral Cortex and Synaptic Fraction. Front Cell Neurosci 2019;13:1–13.

30 Zhao Z, Yao M, Wei L, Ge S. Obesity caused by a high-fat diet regulates the Sirt1/PGC-1α/FNDC5/BDNF pathway to exacerbate isoflurane-induced postoperative cognitive dysfunction in older mice. Nutr Neurosci 2019;0:1–12.