

SIPS | SURABAYA
2017 | INTERNATIONAL
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SEMINAR

**PROCEEDINGS OF
THE SURABAYA
INTERNATIONAL PHYSIOLOGY
SEMINAR**

Surabaya, October 12-14, 2017

Editors:

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Gestrindo



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FOREWORD

Dean of Faculty of Medicine, Universitas Airlangga

Assalamu'alaikum Wr. Wb.

Distinguished Guests, all the Participants, Ladies and Gentlemen

On behalf of Faculty of Medicine, Universitas Airlangga, it is my great pleasure to welcome all the speakers, moderators, and participants on **Surabaya International Physiology Seminar 2017 (SIPS 2017)**, which will be held from today, October 12th until October 14th, 2017. I would like to express my hearty welcome to all the international speakers, **Prof. Cheng Hwee Ming**, from University of Malaya, Malaysia; **Prof. Daniel John Green**, from University of Western Australia; **Dr. Fadzil Hamzah**, from Sport Center of Changi General Hospital, Singapore and **Dr. Deanne Helena Skelly**, from Griffith University, Australia.

The aim of SIPS 2017 is to provide a platform for academicians, educators, researchers, practitioners, undergraduate and postgraduate students to share and discuss the knowledge of the recent issues, opinions, researchers about the development and innovation of physiology in medical science, dentistry, veterinary, plants and agriculture, sports and sciences.

I believe this event is a great purpose in order to develop knowledge, experiences and best practices that can be applied for the good, especially in the field of healthcare as a whole.

Finally, I would like to express my sincere acknowledgements to those who take part and especially for Department of Medical Physiology, Faculty of Medicine, Universitas Airlangga for their effort in holding this event and wishing all to have success.

Wassalamu'alaikum Wr. Wb.

Prof. Dr. Soetojo, MD.

Faculty of Medicine, Universitas Airlangga

Chair of Committee / Head of Physiology Department, Faculty of Medicine, Universitas Airlangga

Assalamu 'alaikum Wr. Wb

Greetings,

On behalf of SIPS committee and Physiology Department, Universitas Airlangga, we are welcoming to Surabaya, City of Heroes.

This year, the annual meeting of Indonesian Physiology Society (IAIFI) is hosted at Surabaya, entitled "**Surabaya International Physiology Seminar Workshop (SIPS)**". We present some update workshop and lectures in order to bring physiology research from basic to clinical application on humanities, animal welfare and good environment. All participants have opportunities to publish their research in presentation, poster and ISBN proceeding. Selected papers will be submitted to SCOPUS indexed proceeding/ journal and awarded as Best Poster and Best Oral Presentation.

We hope that all participants will get some interesting experiences for next 3 days, 12-14 October 2017. Enjoy our lectures and workshops, taste the culinary and take your time to sightseeing around Surabaya.

Wassalamu 'alaikum wr. wb.

Dr. Bambang Purwanto

Chairman of Committee / Head of Physiology Department
Faculty of Medicine, Universitas Airlangga

Welcome Address - Surabaya International Physiology Seminar Workshop (SIPS)

Dear fellow Physiologists and Participants,

On Behalf of the Indonesian Physiological Society (IAIFI) and the Physiology Department Faculty of Medicine Universitas Airlangga, I would like to welcome you all to Surabaya International Physiology Seminar (SIPS), held on 12-14 of October 2017.

Finally after long-awaited Surabaya gets a turn again to host and organize the International Physiology Seminar. Hence the Steering- and Organizing Committee consisting of young energetic physiologists are determined to make the Seminar a successful one. The theme of the seminar is:

"The Role of Physiology in Translation Research: From Basic to Application"

This annual meeting covers a wide range of topics of Physiology on Medicine, Dentistry, Veterinary, Plants and Agriculture, Sports and Sciences. We sincerely hope that SIPS 2017 enable to provide a platform for academicians, educators, researchers, practitioners and postgraduate students to present and discuss researches, development and innovations in wide range of topics as mentioned above. It will provide all participants to share knowledge, exchange new ideas and their experiences in many research topics, for then it will enhance future collaborations.

With great interest and enthusiasm I look towards the success of this Seminar, and wish all of you every success and a pleasant stay in Surabaya.

May Allah Swt. bestow upon us His Blessings.

On Behalf of the Steering and Organizing Committee Senior Physiologist,
Prof. R. Soedarso Djojonegoro

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High-Calorie Diet Reduces Neuroglia Count

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Abstract: Nowadays, high-calorie food consumption people can unwittingly affect health. Several studies have reported the effects of excess high-calorie food consumption can range from hyperglycaemia to neurodegenerative interference. In the brain, astrocytes function to respond to the modification of molecular brain structure. This study aimed to observe the effect of a high-calorie diet on the brain histology of male mice (*Mus musculus*). In this study, 28 mice were randomly divided into 4 groups: the control group pre (K0); the control group post with a standard diet (K1); the treatment group with 0.15g of glucose diet (K2); and the treatment group with 0.25g of glucose diet (K3). Treatment was given everyday for eight weeks. Brains were then histologically processed and stained with Haematoxylin-Eosin (HE) and observation made of quantitative changes of astrocytes in the cerebral cortex. Results: Data were analyzed with Post-Hoc ANOVA. From the study, significant differences were found between K0 and K2 ($\alpha < 0.05$). In addition, significant differences were obtained in the groups K0 and K3 ($\alpha < 0.05$). Meanwhile, no significant differences were found between groups ($\alpha > 0.05$). In conclusion, a high-calorie diet affects the brain histology of male mice.

1 INTRODUCTION

Nowadays, high-calorie foods are consumed in high quantities by people without them realizing that these types of food may result in bad habits. Eating excessive high-calorie foods can lead to metabolic diseases and cardiovascular disorders, even in the central nervous system (Auer *et al.*, 2015). When the body receives an excessive intake of high-calorie foods long term, this can lead to an increase in blood glucose levels. If this condition continues, it can cause hyperglycemia (Guyton & Hall, 2011). Hyperglycemia can lead to a decrease in the activity of insulin, which serves to increase glucose uptake in most tissue cells (CDC, 2011). In most cell tissues the body needs insulin for glucose uptake, but the insulin-free brain does not. At the time of hyperglycemia, where insulin insufficiency occurs, the brain will still obtain adequate nutrition, but with further consequences, the condition will eventually lead to brain dysfunction (Sherwood, 2014). Other

tissues can produce adenosine triphosphate (ATP) without oxygen; however, ATP cannot occur in the brain because the brain requires oxygen to produce it. Another unique aspect of the brain compared to other tissues is that the brain in normal circumstances can only use glucose as an energy source but cannot store it. Therefore, the brain depends entirely on adequate intake and a constant amount of glucose and oxygen. Brain damage might occur without sufficient intake of oxygen for more than four to five minutes or if its glucose discharge is cut off from 10 to 15 minutes (Sherwood, 2014). Hyperglycemia can lead to excess superoxide production of mitochondria in the endothelial cells of the blood vessels. Increased production of superoxide may lead to increased activation of advanced glycation end-products (AGEs/AGEP). This condition leads to the formation of intracellular reactive oxygen species (ROS) that can: cause angiogenesis defects in response to ischemia; activate a number of pro-inflammatory pathways; and cause epigenetic long-lasting changes that

encourage persistent expression of pro inflammatory genes after hyperglycemic memory (Giacco & Brownlee, 2011). Changes in blood glucose levels affect the brain cells directly or indirectly because glucose is the main source of energy in addition to oxygen. A quarter of the body's oxygen is used for brain cells. This large energy-use brain cell is activated in the mitochondria, in order to maintain brain cell functional activity. In the brain, cells that play a major role in the neurotransmitter process and respond to biochemical changes are called astrocytes. Changes in blood glucose levels in the body can cause the intake of glucose and oxygen to the brain to be disrupted, meaning that the brain cannot run properly. Excessive consumption of a high-calorie diet can affect the body's metabolism, leading to hyperglycemia, which affects the brain tissue directly or indirectly, as described above. This has encouraged researchers to study the impact of a high-calorie diet on the histology of the brains of mice, and to increase public awareness of the impact of a high-calorie diet upon the body, especially brain tissue.

2 METHODS

The protocol used in this study has already been approved by Ethical Committee, Faculty of Veterinary Medicine, Universitas Airlangga.

The standard diet is mouse food obtained from the Faculty of Veterinary Medicine, Universitas Airlangga, and provided with mineral water which can be accessed freely (*ad libitum*) and 0.5cc of mineral water by oral gavage per day. A high-glucose diet is a standard diet, and 0.15g and or 0.25g of glucose in 0.5cc solution by oral administration without considering the weight and *ad libitum* glucose solution with a concentration of 0.05g/ml.

Twenty-eight mice were randomly divided into four groups. The first group (K0) is the pre-treatment control group. The second group (K1) is the standard dietary treatment group. The third group (K2) is a group with a high-glucose dietary treatment of 0.15g. The fourth group (K3) is a group with a high-glucose dietary treatment of 0.25g. The mice were adapted in a cage for 1 week prior to treatment. During the adaptation, they were fed with the standard food and drink *ad libitum*. The first group (K0) was sacrificed after a 1-week adaptation as pre group data. The other group received treatment for 8 weeks. Mice were treated as above, where glucose is administered by oral gavage once

per day for 8 weeks. After being treated for 8 weeks, the mice were all sacrificed. Prior to being sacrificed, the mice were given anesthesia by intraperitoneal injection. The head of the mice was dissected and the brain was taken out and then fixed with formalin buffer. The tissue was then stained using HE (Hematoxylin-Eosin) in the Histotechnic Laboratory of Anatomical Pathology Department of the Faculty of Veterinary Medicine Universitas Airlangga. Microscopic examination of brain tissue was conducted afterward. The astrocytes were then counted from 5 areas, which was at the top, right center, center, left center and bottom, and 400x magnification. Microscope mounted graticulae with 5x5 count chamber. Changes in the amount of astrocyte was counted and the result was analyzed statistically using SPSS.

3 RESULTS

Among 28 mice, there were 9 mice died during the study, 1 mouse in the post control group (K1), 3 mice in the glucose diet treatment group of 0.15g (K2) and 5 mice in the 0.25g glucose diet treatment group (K3). As of this, the amount of mice that performed histology of the brain is from 19 mice.

Table 1: Result of astrocyte count.

Group	N	Astrocytes (%) (Mean ± SD)
K0	7	18.43 ± 5.56
K1	6	16.00 ± 1.89
K2	4	12.75 ± 1.70
K3	2	10.00 ± 1.41

The least amount of astrocytes was obtained in the 0.25 gram (K3) glucose diet treatment group, which was smaller than the other groups. The largest amount of astrocytes was found in the pre control group (K0). The result of normality test shows that the data of astrocytes amount is normal ($\alpha > 0.05$), so the next data is analyzed by parametric statistical test that is one-way ANOVA. One-way ANOVA test results obtained significant results that is 0.040 ($\alpha < 0.05$) which means there are significant differences between groups.

Table 2: Post Hoc anova test result.

GROUP	K0	K1	K2	K3
K0		0.266	0.030*	0.014*
K1	0.266		0.203	0.071

K2	0.030*	0.203		0.414
K3	0.14*	0.071	0.414	

*Significant difference

The result of Post-Hoc Anova test showed that there was a significant difference between the pre-control group (K0) and the 0.15 gram (K2) glucose treatment group and the 0.25 gram (K3) glucose treatment group. No significant difference was found between K1 with K0, K2, and K3. The most significant ratio of astrocytes is between K0 and K3.

4 DISCUSSION

High-calorie diets exaggeratedly cause metabolic abnormalities (Auer *et al.*, 2015). When the body gets a diet which is high in excessive calories for a long term, it can increase blood glucose levels. This can lead to conditions of hyperglycemia (Guyton & Hall, 2011). In addition, blood glucose levels, hyperglycemia also leads to adequate infusion of insulin (CDC, 2011). The condition of hyperglycemia can increase the formation of reactive oxygen species (ROS) in the body and can improve the health of brain tissue (Ding *et al.*, 2004).

The brain consumes 20% of oxygen and 25% of glucose present in the body. Aside of oxygen, glucose is very important for the brain cell, because different from other tissues, in normal conditions the brain can only use glucose as its source of energy (Farooqui, 2015). In conditions of hyperglycemia metabolic acidosis may occur in local brain tissue with increased of brain lactate (Kagansky *et al.*, 2001).

Astrocytes are neuroglia cells in the brain, composed of plastic cells that respond quickly to environmental changes in the brain (Kimelberg & Nedergaard, 2010). An important role of astrocytes in the central nervous system (CNS) is in the process of physiology and pathology occurring in the brain and body (Dong *et al.*, 2001; Biessels *et al.*, 1999). Cohen *et al.* (2016) reported that astrocyte from neonatal mice given low, medium, and high glucose exposure, responded to changes in environmental glucose levels with increased insulin, insulin receptors, and protein levels. Astrocytes secrete insulin but do not respond to stimuli like other insulin-producing cells. This has led to speculation that astrocytic insulin responds to glucose levels, and may be adaptive for cellular homeostasis rather than to affect the environment.

Astrocytes count between the pre-control group and the glucose diet treatment group of 0.15 gram were significantly different. This significant difference indicates a 0.15g glucose diet has an impact on astrocytes. This is due to the high levels of glucose in the blood followed by insulin insufficiency which results in reduced astrocytes as a protective response to neurons from environmental changes (Özdemir *et al.*, 2012; Kelleher *et al.*, 1993). Reduced astrocytes in the 0.15g glucose diet treatment group may also be due to high ROS that elevated blood glucose levels that cause astrocytic damage (Yang *et al.*, 2016; Takahashi *et al.*, 2012; Wang *et al.*, 2012).

High concentrations of glucose in the CNS environment affect astrocytes by increasing the ROS levels that cause oxidative stress (Hsieh *et al.*, 2013) as well as increased production of cytokine inflammation (Shin *et al.*, 2014). Under normal conditions, astrocytes have a major role in the CNS by maintaining extracellular homeostasis of neuroactive substances such as K⁺, H⁺, GABA, and glutamate. The more hyperpolarized membrane potentials compared with neurons can be found in astrocytes that provide the driving forces required for K⁺ spatial buffering and glutamate transport (Kucheryavykh *et al.*, 2007, 2009; Olsen, 2012). When these functions are impaired it will affect brain physiology.

The amount of astrocytes between the pre-control group and the 0.25g glucose diet treatment group was significantly different ($\alpha = 0.014$). The difference was more significant than the amount of astrocytes between the pre-control group and the 0.15g glucose diet treatment group, indicating that astrocytes in the 0.25-gram diet were decreased more. Wang *et al.*, (2012) reported that in experimental animal astrocytes treated with exposure to high glucose levels (15 mM) did not induce apoptosis in astrocytes, while astrocyte experimental animals treated with exposure to extremely high glucose levels (30 mM) experienced apoptosis drastically. This may explain the higher the glucose diet is given, the more astrocytes that apoptosis leads to the less astrocytes that can be calculated.

The researcher found no significant difference for the amount of astrocyte between the pre control and post control groups. No significant difference also found in the astrocyte count between the post control group and the 0.15g glucose diet treatment group. The astrocyte count between the post control group and the 0.25g glucose diet treatment group also have no significant difference. And non-

significant difference was founded in the astrocyte count between the 0.15g glucose diet treatment group and the 0.25g glucose diet treatment group.

The above non-significant differences were thought to be due to study limitations, differences in glucose levels administered between treatment groups were not much different and the majority of researchers looked at the impact of changes in astrocytic glucose levels by evaluation of morphological changes (Ogata & Kosaka, 2012; Wang *et al.*, 2012; Nardin *et al.*, 2007; Ding *et al.*, 2004; Auer *et al.*, 2015). While histological staining is best to be able to see astrocytes and its structure is silver impregnation, gold impregnation and Golgi impregnation (Lopez *et al.*, 2010).

5 CONCLUSION

Based on the study, it can be concluded that long-term high-calorie diets can cause a decrease in the amount of astrocyte in the cerebral cortex of mice and the increased calorie intake is associated with a decrease in the amount of astrocyte in the *cerebral cortex*.

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