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Research Article

The Effect of Physical-Psychological and Psychological Stress on Blood Glucose Levels (*In vivo Study*)

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Abstract

Stress is a condition that burdens a person and affects homeostasis resulting in health problems, including dental and oral health. Stress can be caused by physical, chemical, or psychological stressors. Stressors cause an increase in blood glucose levels due to an increase in the stress hormone cortisol. This study aims to determine the effect of physical-psychological and psychological stress induced by electrical foot shock on blood glucose levels in rats. This research is an experimental laboratory study with a post-test control group design using white male rats as animal stress models. Animal models were divided into six groups, namely physical-psychological stress for 7 days (PPS1), 14 days (PPS2), and 28 days (PPS3); and psychological stress for 7 days (PS1), 14 days (PS2), and 28 days (PS3). The animal models were placed in the experimental box with electrical foot shock. The physical-psychological stress treatment was to flow an electric current of 48 V, 0.5 Hz, 2 mA for 30 minutes daily. In the psychological stress treatment, the animal models were placed without an electric shock, but the animal models were placed together, given an electric shock stressor separated by transparent plastic with a thickness of 5 cm and foam at the bottom of the cage (insulator). The results showed that although there was no effect of electrical foot shock on blood glucose levels in rats experiencing physical-psychological stress for 7, 14, and 28 exposures ($p>0.05$), electrical foot shock affected blood glucose levels in rats with psychological stress ($p<0.05$). It can be concluded that physical-psychological and psychological stress induced by electrical foot shock affected blood glucose levels.

Keywords: blood glucose level; electrical foot shock; physical-psychological stress; psychological stress

INTRODUCTION

Working is a part of human life that provides satisfaction and challenges and causes disruption and threats. These disruptions and threats can trigger both physiological and psychological stress.[1,2] Stress is a physical and psychological condition due to environmental adaptation. Stress represents an unconscious response by a person to avoid or encounter environmental demands, which impacts body homeostasis.[3] Stressors, such as physical, chemical, and psychological aspects, trigger stress. Although these stressors can be of various types, they will impact psychology: fear or anxiety.[4] Several studies described that stressors frequently appeared and affected a person simultaneously so that physicians will determine the difficulty of the source of stressors, especially those related to psychology.[5,6] However, these studies have not explored the impacts of stress.

Based on those impacts, there is the term physical-psychological and psychological stress. Physical-

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psychological stress is caused by harmful stressors to human tissues, such as extreme cold, heat, decreased oxygen concentration, infections, injuries, and strenuous and prolonged physical exercise. Meanwhile, psychological stress represents stress due to changes in life, social relationships, anxiety, anger, and fear.[6]

A stress study is a complex study due to many confounding factors involved. The studies frequently used animal models to explore the impacts of stressors and factors in body life, particularly electrical foot shock as a stressor. It exhibits several advantages, such as:[7] 1) The intensity and duration can control according to the researcher's needs; 2) The electrical foot shock triggers two stresses at once, anxiety (psychological stress) and panic with alteration activity (physical); 3)The stressor is measurable; 4) The stressor provides a new experience of stress compared to sound, light, and temperature stressors.

Electrical foot shock is complex as it triggers acute or chronic physical and psychological stress with varying intensity and duration. This stressor induces behavioral and neurochemical alteration, such as depression, anxiety, tissue damage, and post-traumatic stress disorder. This stressor is expected to decrease the immune system's vulnerability to various diseases.[3]

Exposure to electrical foot shock will over-activate the hypothalamic-pituitary-adrenal axis. This excess stimulation causes energy mobilization (glycogenolysis), but cells and tissues inhibit glucose utilization. This exposure triggers an increase in hyperglycemia.[7] Stress is also one of the risk factors for systemic disorders by neuroendocrine induction. The induction will interfere with metabolism, which enhances glucose dysregulation. The implication of glucose dysregulation presents hyperglycemia and triggers glucose intolerance and diabetes mellitus. Several studies demonstrated that physical and psychological stress triggered hyperglycemia in diabetic and non-diabetic patients.[8,9] Besides, several literature reviews described that hyperglycemia-induced stress increased the risk of oral diseases, such as necrotizing ulcerative gingivitis (NUG), periodontal disease, stomatitis, etc.[10]

Hyperglycemia presents a high level of glucose in circulation. Blood glucose level is one of the screening methods to identify early dysregulation of glucose. It is essential to reduce stress-induced systemic disorders, prevent diabetic complication-induced stress, and reduce deep depression. Physicians recommend that their patients cope with stress by determining the blood glucose level through intake management and glucose regulation.[11] As aforementioned, this study aims to identify the effect of physical-psychological and psychological stress induced by electrical foot shock on blood glucose.

MATERIALS AND METHODS

This research is an experimental laboratory study with a post-test-only control group design. All procedures in this study received approval from the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga No. 275/KE, dated August 2, 2013. This study used forty-two white male rats of the Sprague Dawley strain (*Rattus norvegicus*) as stress animal models. The animal model was adapted to a laboratory environment for a week and fed with ad libitum standard food and drink. The tools and materials used in this study were experimental animal cages, an "Electrical foot shock" box, a stopwatch, disposable syringes, masks, a blood glucose test (Accu check), and chloroform.

Experimental animals used as models must meet the following research criteria: male, 2-3 months old, 250-300 grams, and healthy. These experimental animals were randomly grouped into six groups: physical-psychological stress for 7 days (PPS1), 14 days (PPS2), and 28 days (PPS3); and psychological stress for 7 days (PS1), 14 days (PS2), and 28 days (PS3).

The animal models were placed in treatment cages made from transparent plastic with a thickness of 5 mm, and the dimension was 64x64x16 cm. The treatment was separated into 16 sections: eight for physical-psychological stress and eight for psychological stress. The dimension per section was 16x16x16cm (Figure 1).[12]

The electrical shock was an electrical foot shock with an electric current of about 2-8 mA, 48 volts, and 0.5 Hz for 30 minutes daily. The electrical exposure was at 09.00-10.00 am due to stress hormones being at the highest concentration in the blood, which influences the blood glucose level. Physical-psychological stress used electrical foot shock to induce pain. The stressor was exposed gradually, initiating from 2 mA. If the animal models were unresponsiveness, the electric current was increased by one mA to be three mA. The maximum electric current was eight mA for 30 minutes.[12]

Meanwhile, animal models were not exposed to electrical foot shock in psychological stress groups. The bottom of the cage was free of electrical current. The animal models in these groups experienced stress through visual, auditory, and olfactory contact. Behavioral changes were characterized by the response of experimental animals to psychological stress, such as avoiding the scream sources, hiding, and being silent.[12]

The animal models were euthanized with chloroform overdose on the 7th, 14th, and 28th days. Blood was drawn intracardially. Furthermore, the blood glucose level was measured by enzymatic technique. The results were analyzed by One Way ANOVA, independent T-test, and Least significant differences (LSD) ($p < 0.05$).

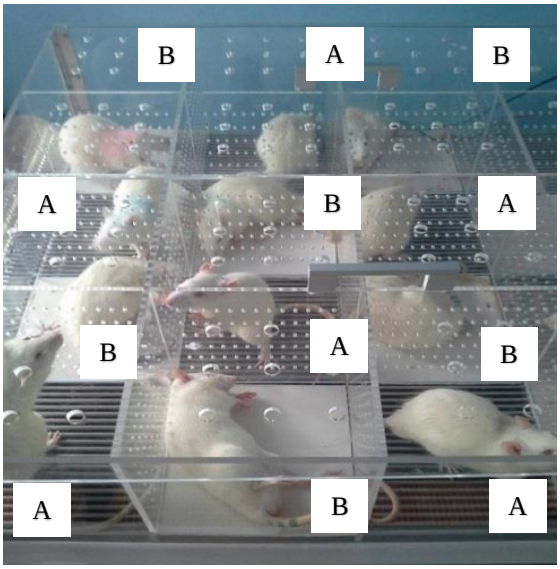

Figure 1. Treatment Cage of Stress Animal Models

A, part of electrical current exposure (physical-psychological stressor); B, part without electrical current (psychological stressor)

RESULT

The results demonstrated that animal models' behavior and blood glucose levels altered after exposure. The behavior of SPF groups showed jumping, running, screaming, and lifting his legs. Therefore, the SP groups avoided screaming sources, hiding, and being silent. Meanwhile, the blood glucose levels showed differences between SPF and SP groups. The blood glucose in SP groups was almost higher than in SPF groups, except SP3. Among the SPF groups, the blood glucose levels were not significantly different ($p>0.05$). Meanwhile, in the blood glucose level of the SP group, there was a significant difference within the groups ($p<0.05$) (Table 1 and Figure 2).

The LSD analyzed the results in the SP groups to determine the difference in blood glucose levels between the groups. The LSD analysis showed a significant difference in blood glucose levels between groups, except between SP1 and SP2 (table 2).

Besides, the data were analyzed by independent T-test to identify the difference in blood glucose levels between SPF and SP

treatments for each exposure time. The analysis displayed no significant difference in blood glucose levels between physical-psychological and psychological stress for 7, 14, and 28 days (table 3).

DISCUSSION

Using rodents, especially rats, as animal models for investigating human physiological and pathological backgrounds requires standards for values and laboratory procedures for comparison and reproducibility of experiments and results. The most important standard for studies related to hyperglycemia is determining glucose levels. Measurement of glucose levels in both humans and animals is more flexible as there is no difference in measuring blood glucose using peripheral blood or whole blood, either using a blood analyzer or a portable glucose check. However, the measurement time primarily determines blood glucose value in both humans and rats, such as in fasting time and randomly or 2 hours post-prandial. Although the blood glucose level in rats could mimic the human condition, the range values of glucose levels in rats and humans were significantly different as the hematocrit value in rats was higher than in humans. Thus, the experiments using animal models must use standard laboratory average values from animal models.[13,14]

Blood glucose levels present a vital role in determining health status. Besides, the blood glucose level is used as a screening tool for diabetes mellitus. The normal range is 70-130 mg/dl (fasting) or less than 140 mg/dl (after eating 2 hours). Therefore, in rats (animal model), it is 50-135 mg/dL.[15] The alteration level indicates metabolism interruptions and causes several disorders. The increase of blood glucose levels and hyperglycemia induces glucose intolerance and diabetes mellitus.[16,17] This condition also impacts the oral environment by enhancing oral bacteria colonization and xerostomia, which triggers the periodontal disease and dental caries.[18]

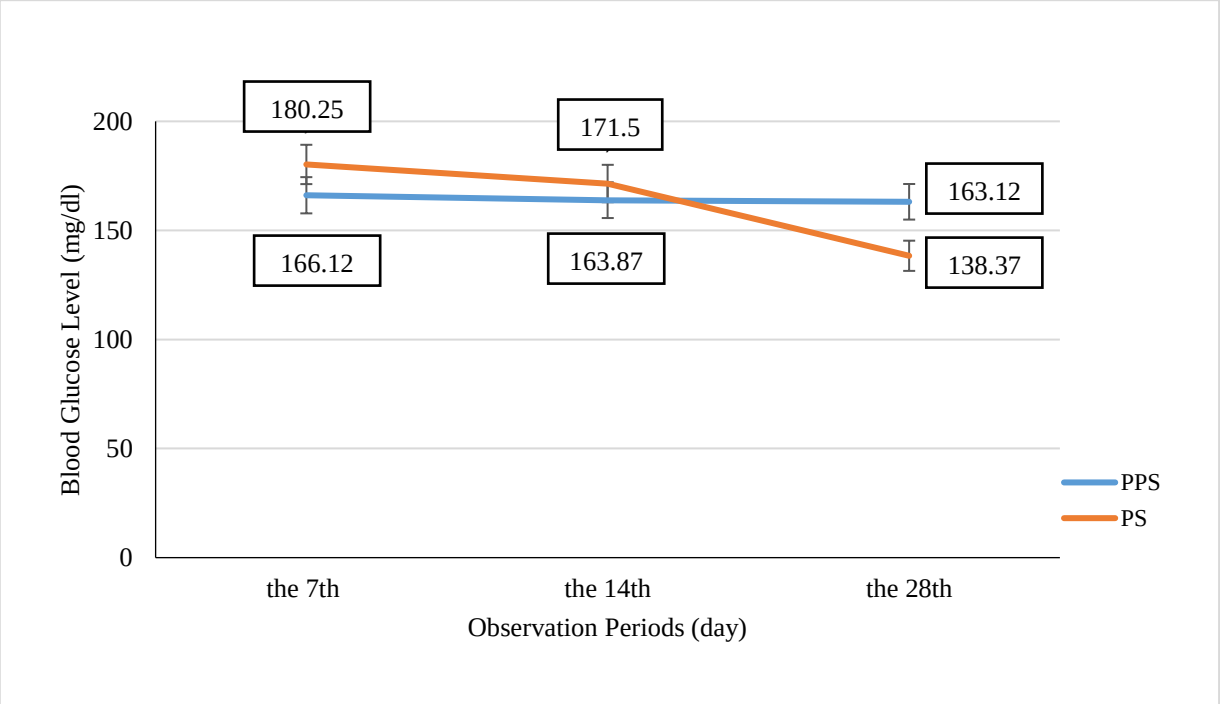

Figure 2. The blood glucose levels of animal models after stress exposure for 7, 14, and 28 days
The data presented the average blood glucose level and standard error.

Table 1. Blood Glucose Level of Animal models (n=42)

Groups	n	Blood glucose level	P value
SPF 1	7	166.12 ± 27.17	
SPF 2	7	163.88 ± 34.37	0.979
SPF 3	7	163.12 ±29.71	
SP 1	7	180.25 ± 39.03	
SP 2	7	171.50 ± 21.19	0.049*
SP 3	7	138.38 ± 17.09	

The data presented the average blood glucose level and standard deviation.
Data were analyzed by one-way analysis of variance ($p<0.05$).
*, significant difference; n, number of samples; SPF 1, physical-psychological stress for 7 days; SPF 2, physical-psychological stress for 14 days; SPF 3, physical-psychological stress for 28 days; SP 1, psychological stress for 7 days; SP 2, psychological stress for 14 days; SP 3, psychological stress for 28 days

Table 2. Summary of Least Significant Difference (LSD) Analysis

Groups	SP1	SP2	SP3
SP1	-	8.750	41.875*
SP2	-8.750	-	33.125*
SP3	-41.875	-33.125*	-

The data presented the p-value of the Least Significant Difference (LSD) Analysis.
*, significant difference; SP 1, psychological stress for 7 days; SP 2, psychological stress for 14 days; SP 3, psychological stress for 28 days

Table 3. Summary of Independent T-test

Groups	df	Mean different	P value
SPF1-SP1	14	14.125	0.098
SPF2-SP2	14	7.625	0.179
SPF3-SP3	14	-24.750	0.059

SPF 1, physical-psychological stress for 7 days; SPF 2, physical-psychological stress for 14 days; SPF 3, physical-psychological stress for 28 days; SP 1, psychological stress for 7 days; SP 2, psychological stress for 14 days; SP 3, psychological stress for 28 days

This study revealed that the glucose levels in both groups were higher than the normal range of blood glucose in rats, although the level tended to decrease each week. It might be that the animal models in the first week were in an acute stress phase. There are three stress phases: the initial (acute) phase, the stress phase, and the final (chronic) phase. In the initial phase, the body will respond by impairing body functions and increasing the cortisol hormone. Increasing the hormone cortisol is to produce energy along with increased metabolism. After that, the body will compensate to maintain homeostasis in the second phase. Then, in the final phase, the body experiences fatigue due to the inability to maintain homeostasis. In this phase, the body will lose the energy and source of energy, decreasing the immune system and blood glucose level.[19]

In the SP groups, blood glucose levels on the seventh day increased significantly. The animal models might be in the early stages of stress (acute phase). In this phase, the body will release the hormone cortisol to increase metabolism, leading to energy production to adapt to stressors. Experimental animals might experience stress compensation during the acute phase by increasing blood glucose levels. When a stressor exceeds a certain threshold, the stress reaction is initiated (fight, flight, or silence). The acute response to stress is time-limited, in which the first week stimulates the peak of release corticosterone. The increasing corticosterone induces gluconeogenesis dan glycolysis that implements hyperglycemia.[16,20–22]

This study showed no significant difference in blood glucose levels in the 7th,

14th, and 28th days of SPF groups. Physical-psychological stress might cause energy use excessively. Energy use might accelerate glucose metabolism, which decreases blood glucose levels. The response of animal models in this study when applied electrical foot shock represented jumping, screaming, frequent movement, and lifting one leg. Previous studies described that being active and more mobilization were practical physical responses to avoid stressors. These responses needed more energy and that glucose is the primary energy source.[23,24]

Furthermore, on the 7th and 14th days, there were no significant differences in blood glucose levels between SP and SPF groups based on the observation periods. It might indicate that the duration of exposure to psychological stress and physical-psychological stress did not affect blood glucose levels.[8,11,25,26] This study contrasts Mustofa's study, in which cortisol and blood glucose levels increased following the stress periods.[27] Early exposure to stress causes a corticosterone surge, which increases excess glucocorticoid secretion. Glucocorticoid secretion mobilizes energy from the liver to the blood circulation, but muscle utilization does not balance the excessive mobilization and triggers hyperglycemia. However, although the intensity was increased, repeated electrical foot shock exposures stimulated activation of the hemostasis pathway and induced adaptation to stress stimuli. The activation of this hemostatic pathway induced normalization of the neuroendocrine system. However, this normalization process depended on exposure to stress; if the animal models are able to maintain, they will be in recovery periods. If they fail, the stressor causes a chronic stress response. These events affected blood glucose levels due to corticosterone levels inducing stress very sensitively.[7,23,27,2823,27]

CONCLUSION

This study demonstrated that psychological and physical-psychological stress affected blood glucose levels in animal models. This study requires further study to investigate the role of cortisol and other parameters to blood glucose metabolism-induced stress, which will be helpful as a specific parameter to identify the risk factors of diabetes mellitus, which can impact oral health.

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