The decrease of histomorphometry and function of pancreas in male albino rats after induced by sleep deprivation

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**Keywords:**
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**Background:** Sleep disturbance accelerates type 2 diabetes mellitus by reducing insulin secretion and by the occurrence of difficulty in controlling sleep behaviour in humans so that it is necessary conduct a study by using experimental animals.

**Objective:** This study aims to determine histophotometric differences of pancreas and differences of glucose tolerance in male albino rats (Rattus norvegicus) after induced by stress paradoxical sleep deprivation (PSD) and total sleep deprivation (TSD).

**Methods:** This study applied a post-test only with control groups consisting of 30 male albino rats (Wistar strain) divided randomly into 3 groups: control group, PSD group and TSD group. The mean number and diameter of islets of Langerhans were calculated per slide and were averaged for each group at a magnification of 400 X. The percentage of beta cells in one islet of Langerhans was (β-p) that was a number of normal beta cells (Bn) divided for the total beta cells (Bt) (normal and damaged) x 100%. The glucose level based on the sampling time was made a curve shape, and the area under the 0-to-120-minute curves (AUC⁰⁻¹²⁰) was calculated by using a trapezoidal formula.

**Results:** The lowest AUC⁰⁻¹²⁰ level of glucose was the control group (14,082 ± 955mg/dL) and the highest was the TSD group (16,293 ± 2,195 mg/dL); the Post-Hoc LSD test showed a significant difference (p<0.05) between the control-PSD group and the TSD-control group. The mean result of pancreatic β cells mass with the lowest number was in the TSD group (76.3 ± 4.8), and the highest result was in the control group (94.3 ± 2.7); Post-Hoc LSD showed a significant difference (p<0.05) in the control-PSD group and the TSD-control group. The lowest mean in cell diameter of islets of Langerhans was in the control group (0.132 ± 0.031 mm), and the highest mean was in the control group (0.213 ± 0.019 mm).

**Conclusion:** Sleep deprivation could reduce pancreatic β cell mass and cell diameter of islets of Langerhans and increase glucose tolerance levels.
glukosa pada tikus putih (Rattus norvegicus) jantan pasca induksi model stres paradoxical sleep deprivation (PSD) dan total sleep deprivation (TSD)

**Metode:** Posttest only with control group dengan 30 ekor tikus putih jantan galur Wistar dibagi menjadi 3 kelompok: kontrol, PSD dan TSD. Rerata jumlah dan diameter pulau Langerhans dihitung tiap slide dan direrata tiap kelompok pada perbesaran 400X. Persentase sel β dalam satu pulau Langerhans adalah (β-p) adalah jumlah sel beta normal (Bn) dibagi total keseluruhan sel beta (Bt) (normal dan rusak) x 100%. Kadar glukosa berdasarkan waktu sampling dibuat bentuk kurva dan dihitung luas daerah di bawah kurva menit 0-120 (LDDK 0-120) menggunakan rumus trapesium.

**Hasil:** Rerata AUC0-120 kadar glukosa dari nilai terendah adalah kelompok kontrol (14.082±955 mg/dL) dan tertinggi pada TSD (16.293±2.195 mg/dL), dilanjutkan uji Post-Hoc LSD menunjukkan perbedaan signifikan (p<0,05) pada kontrol-PSD dan kontrol-TSD. Hasil rerata jumlah sel β pankreas dari terendah TSD (76,3±4,8) dan tertinggi pada kontrol (94,3±2,7) dan dilanjutkan Post-Hoc LSD menunjukkan perbedaan signifikan (p<0,05) pada kontrol-PSD dan kontrol-TSD. Rerata diameter pulau Langerhans terendah TSD (0,132±0,031) mm dan tertinggi pada kontrol (0,213±0,019) mm, uji Post-Hoc LSD menghasilkan perbedaan signifikan (p<0,05) pada kelompok kontrol-PSD dan kontrol-TSD dan PSD-TSD.

**Kesimpulan:** Sleep deprivation menurunkan jumlah sel β pancreas, diameter sel pulau Langerhans dan meningkatkan toleransi glukosa.

**INTRODUCTION**

Circadian rhythm disturbance facilitates the occurrence of type 2 diabetes mellitus, characterized by reduced insulin secretion, and reduced mass of pancreatic β cells, an increase in pancreatic β cell apoptosis and decreased response of pancreatic β cells to glucose contribute for glucose tolerance and reduced insulin secretion.1 Sleep deprivation can increase levels of reactive oxygen species (ROS) in the body, causing oxidative stress affecting the pancreatic and duodenal homeobox factor 1 (PDX-1) which plays a role in differentiation and maintenance of normal function of pancreatic β cells and interferes with DNA binding to PDX-1. This condition changes the expression of insulin genes and disrupts the pancreatic β cells.2

Sleep deprivation interferes glucose metabolism, causes glucose toxicity in islets of Langerhans cells and changes the mass of pancreatic β cells due to the oxidative stress. It happens through changes in enzymatic activity, dysregulation of gene expression and apoptosis so that the arrangement of islets of Langerhans changes either its structure or its function.3,5 This process can be observed by measuring cell diameter of the islets of Langerhans.6

Difficulties in controlling diet and sleep behaviour in humans lead to different research results, so it is still difficult to obtain a clear relationship between glucose metabolism and sleep deprivation as a single cause.7 Experimental studies by using experimental animals need to be conducted to determine the effects of sleep deprivation on glucose metabolism and structures in cells of islets of Langerhans by controlling confounding variables.8

**METHODS**

**Research Design**

This study was a post-test only with control group design conducted from December 2019 to January 2020. This study was conducted at the anatomy laboratory, Faculty of Medicine, Universitas Jenderal Soedirman. This study was approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Jenderal Soedirman Ref No. 8395/ KEPK/ XII/ 2019 on December 13, 2019.

**Experiment Procedure**

The experimental animals were 30 male albino rats (Rattus norvegicus), Wistar strain, 3–4 months old and 100–200 grams in weight. Induction of sleep deprivation used a tank with 123x44x44 cm in measure and with 14 platforms equipped with muscle atonia shock apparatus. Slide preparations were observed with a light microscope equipped with Opti lab. Blood glucose was measured by using a glucometer (Safe AQ Smart®). The other
equipment used were digital scales (Dragon 303®), gastric tube (3 mL), 100 mL measuring cups, beakers and lancets. The materials of this study were drinking water (Aqua®), Neutral Buffered Formalin (NBF) (10%), Haematoxylin Eosin staining, ether, standard laboratory rat feed ingredients, glucose strips (Safe AQ Smart®) and glucose monohydrate (CAS5995-10-1®). Experimental animals were acclimatized for 7 (seven) days. The acclimatization was conducted in a cage with a temperature of 28 ± 2°C, humidity of 75 ± 5% and exposure to natural light for 12 hours of light (07.00-19.00 WIB) and 12 hours of darkness (19.00–07.00 WIB). Food and water were provided ad libitum. The experimental animals were grouped by using completed randomized design (CRD) into three groups: the control group, the PSD group (20 hours/day for 8 days) and the TSD group (24 hours/day for 8 days). Induction of sleep deprivation used a modified multiple platform method (MMPM) equipped with muscle atonia to give a shock effect every 10 minutes. Experimental animals that entered the rapid eye movement (REM) of sleep phase would fall into the water and wake up again. The control group was not treated with sleep deprivation stress model. The PSD group was induced by sleep deprivation for 20 hours (11.00–07.00 WIB) and 4 hours of rest (07.00-11.00 WIB). The TSD group was induced by sleep deprivation for 24 hours (11.00-11.00 WIB) without rest. Induction of sleep deprivation occurred for 8 days, the rats were fasted for 12 hours, and then the initial blood glucose (T0) was measured. The rats were given a glucose load by using a gastric swab at a dose of 2g/KgBW of 2 ml.

Measurement of data
The glucose levels were measured again at 60 (Tg₁) and 120 (Tg₂) minutes by using tail blood samples. The glucose levels based on the sampling time were made a curve shape, and the area under the 0-to-120-minute curves (AUC[0-120]) was calculated by using the trapezoidal formula \( AUC_{0-120} = \frac{(T_0 - T_0)}{2} \times (C_0 + C_1) + \frac{(T_1 - T_1)}{2x} (C_1 + C_2) + \ldots + \frac{(T_n - T_n)}{2} \times (C_n + C_{n-1}). \) The T was time (minute), the C was the concentration of substance in blood (mg/dL), and AUC[0-120] was area under the curve from 0 to nth minutes. The AUC[10-tn] value was analysed to determine whether there was a difference in glucose tolerance between the treatment groups. After the treatment, the rats were terminated, and their pancreas was taken to be made into histological preparations with Haematoxylin Eosin staining. The mean number and diameter of islets of Langerhans were calculated per slide, averaged for each group at a magnification of 400X and analysed by using Image J software. The percentage of beta cells in one islet of Langerhans was (β-p) which was the number of normal beta cells (Bn) divided for the total beta cells (Bt) (normal and damaged cells) x 100%. The percentage of beta cells in one islet of Langerhans is (β-p) which was the number of normal beta cells (Bn) divided for the total beta cells (Bt) (normal and damaged) x 100%. This parameter was used in 4 islets of Langerhans per sample and the results were calculated on average. The damaged cells had following these criteria: pycnotic (nucleus darkens, condensed, and shrinks), karyorrhexis (ruptured and fragmented nucleus), karyolitic (pale nucleus that no longer absorbs colour).

Statistical Analysis
A univariate analysis was used to describe the pancreatic β cell mass and blood glucose levels in terms of mean ± standard deviation. A bivariate analysis applied one way ANOVA test that was continued with Post-hoc Least Significant Difference (LSD) with a confidence level of 95% (α = 0.05).

RESULTS
Figure 1 demonstrates the appearance of islets of Langerhans in each treatment group. Figure 2 shows that the widest diameter of islets of Langerhans Island is the control group (diameter 0.340 mm), the PSD treatment group (diameter 0.258 mm) and that the narrowest is the TSD treatment group (diameter 0.114 mm).
One way ANOVA test results found a significant difference in at least 2 treatment groups, and Post-Hoc LSD test showed significant differences in control group-PSD group (p = 0.000) and PSD-TSD group (p=0.000) in the mean of AUC\(^{0-120}\) of blood glucose and the mean of pancreatic \(\beta\) cells mass. In cell diameter of Langerhans, Post-Hoc LSD test showed significant differences in the control group-PSD group (p = 0.000), the TSD group-control group (p = 0.000) and the PSD group-TSD group (p = 0.012).

![Figure 1](image1.png)

**Figure 1.** Observation of \(\beta\) cells on the islets of Langerhans (HE, 400X). A. (the control group) showed islet cells of Langerhans located peripherally with dark nuclei (alpha cells) (black arrowheads) and centrally located with lighter nuclei (beta cells) (black arrows); B. (the PSD group) showed that the distribution of normal beta cells is less than the control group; C. (the TSD group) showed the distribution of normal beta cells that are less than the control group and islet cells of Langerhans that are damaged in the form of karyorrhexis (yellow arrow).

![Figure 2](image2.png)

**Figure 2.** Cell diameter of islets of Langerhans (A. the Control group; B. the PSD group; C. the TSD group). Cell diameter of islets of Langerhans are smaller than the control, PSD and TSD groups in sequence. Yellow lines show the cell boundaries of islets of Langerhans.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pancreatic (\beta) cell mass (mg/mm(^2))</th>
<th>AUC(^{0-120}) (mg.minutes/dL)</th>
<th>Cell diameter of islets of Langerhans (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.3±2.7</td>
<td>14,082±9,550</td>
<td>0.213±0.019#+</td>
</tr>
<tr>
<td>PSD</td>
<td>76.3±4.8*</td>
<td>15,670±1,352*</td>
<td>0.160±0.011*#</td>
</tr>
<tr>
<td>TSD</td>
<td>76.5±3.5*</td>
<td>16,293±2,195*</td>
<td>0.132±0.031*+</td>
</tr>
</tbody>
</table>

Table 1. Histomorphometric data and glucose tolerance test

Note : Significantly different with the level of significance *(PSD-TSD), # (control-PSD), + (control-TSD)*

**DISCUSSION**

Sleep deprivation induces stress through activating the Hypothamic-Pituitary-Adreal (HPA) axis and increasing plasma cortisol/corticosterone levels in animals which generate oxidative stress characterized by increased reactive oxygen species (ROS).\(^{10}\) The ROS activates casein kinase-2 (CK2) and retromers...
which upregulate the degradation of GLUT-4 in lysosomes. Under normal condition, GLUT-4 function becomes a glucose transporter in peripheral cells, but in oxidative stress condition there is a reduction of the GLUT-4 which increases blood glucose levels and affects the pancreatic β cells to increase their function to produce more insulin through the process of glycolytic flux and TCA cycle. This process escalates production of the ROS by pancreatic β cells, especially by mitochondria, and causes oxidative stress that attacks RNA, proteins, and lipids. Circadian rhythm disturbance reduces melatonin hormone which acts as an endogenous antioxidant by giving electrons to free radicals so that free radicals can no longer attract electrons from cells and DNA, called oxidation chain breakers. An imbalance of antioxidants and ROS causes oxidative stress which disrupts the function of pancreatic β cells, thereby initiating apoptosis through PDX-1 and MafA.

The decrease of pancreatic β cell mass after sleep deprivation between the PSD and TSD groups did not show any significant results. Paradoxical sleep deprivation or rapid eye movement (REM) sleep deprivation are intended to prevent the occurrence of REM sleep phases, while total sleep deprivation is intended to prevent non rapid eye movement (NREM) and REM sleep phases. Both groups did not experienced REM sleep phase; meanwhile, NREM sleep phase was associated with increased blood glucose levels, and REM sleep phase was associated with stable blood glucose levels. In NREM sleep phase, there was a decrease of glucose metabolic activity in the brain by 11% and there was a reduction of peripheral glucose uptake, so in NREM sleep phase there was an increase of glucose levels in the blood. In REM sleep phase, there was a higher use of peripheral glucose than NREM, so blood glucose levels tended to be stable in this phase. The levels of antioxidant enzymes in islets of Langerhans were very low, leading to free radicals easily accumulated in islets of Langerhans. Oxidative stress that occurred in the sleep deprivation was not well compensated due to drastically increased antioxidant levels; a full 48 hours of sleep recovery was required including NREM and REM sleep phases. This proves that when sleep recovery occurs, oxidative stress still proceeds, marked by an increase of antioxidants as compensation. As a consequence, the body needs recovery through sleep recovery in sufficient time.

Increased cortisol due to sleep deprivation inhibits glycogen synthase enzyme and downregulates glycogen formation. Glucose transporter-4 (GLUT-4) and glycogen synthase reduction causes blood glucose levels to remain high although insulin levels are normal. This is known as a peripheral insulin resistance. The sleep deprivation-induced rats in this study experienced increased sympathetic nerve activity decreasing the pancreatic β cells; then, insulin secretion was reduced. Induction of sleep deprivation in rats reduced insulin levels, and this was accompanied by insulin resistance leading to decreased glucose tolerance. Glucose tolerance of the PSD and TSD groups was not statistically significant because sleep cycle disturbance had a greater effect on glucose metabolism than the decreased sleep duration. Epidemiological studies showed that sleep duration of person with obstructive sleep apnoea (OSA) was not decreased, but they still had risks of developing type 2 diabetes mellitus.

One of the targets of oxidative stress in pancreatic β cells is PDX-1 which plays a role in the development and differentiation of the pancreas to maintain β cell function. Mechanism of PDX-1 destruction by oxidative stress is mediated by the c-Jun N-terminal Kinase (JNK) pathway. PDX-1 fails to be translocated from cell nucleus to cytosol making β cells cannot respond to glucose to produce insulin. Furthermore, besides an impact to a decrease of PDX-1 expression, oxidative stress also affects reduction of MafA gene, a transcription factor that is sensitive to glucose levels and plays a role in insulin production. A protein called p38 mitogen-activated protein kinases (MAPKs) is very sensitive to stress. If oxidative stress occurs on islets of Langerhans, p38 will bind
to MafA. This bond makes MafA degraded through Ubiquitin Proteasomal Pathway (UPP). Excessive degradation of PDX-1 and MafA in oxidative stress contributes for damage and dysfunction of pancreatic β cells so that the islets of Langerhans change both in structure and function.

Mechanism of PDX-1 destruction by oxidative stress is mediated by the JNK pathway. PDX-1 fails to be translocated from the cell nucleus to the cytosol; as a result, β cells cannot respond to glucose to produce the hormone insulin. The excessive degradation of PDX-1 and MafA in oxidative stress cause damage and dysfunction of pancreatic β cells so that the islets of Langerhans both in structure and function. The limitations of this study were not measuring the glucose tolerance levels before the treatment. Also, the sampling time of glucose measurement was limited to three times (0, 60 and 120 minutes after treatment) so that the changes of glucose levels were less detailed, and this study did not add sleep recovery procedures. It is suggested to conduct further research to compare blood glucose tolerance levels of pre and post treatment, to measure antioxidant levels, to measure levels of the hormones insulin, leptin, and ghrelin after treatment, and to add sleep recovery procedures to determine whether there is an improvement of glucose tolerance after recovery.

CONCLUSION

The induction of sleep deprivation could reduce the pancreatic β cell mass and cell diameter of islets of Langerhans and improve glucose tolerance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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