Original article:

The RAGE expression in glomerulus and the Malondialdehyde level in the kidney of Diabetes Mellitus rat model after exercise

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Abstract:

Background: The main cause of complications in diabetes mellitus is a condition of oxidative stress due to increased reactive oxygen species (ROS). The increase in ROS can be triggered by the increase in advanced glycation end-products (AGE), which binds to its receptor (RAGE). The result of increased ROS will lead to lipid peroxidation in cell membranes and induce particular increase in malondialdehyde (MDA). ***Objectives:*** The objective of this study was to evaluate the benefits of physical exercise in preventing complications of the kidneys caused by hyperglycemia. ***Method:*** This study was a pure experimental research with post-test only group design. Subjects were 10 male Sprague Dawley rats induced with 35 mg/kgdose of streptozotocin (STZ). The subjects were divided into two groups, i.e. the STZ group and STZ group with physical exercise. Physical exercise was given in 9 weeks using a treadmill, in 5 times/week frequency, moderate intensity, 0 degree of tilt, gradually increased speed and duration. ***Result:*** Regular and measurable physical exercise can decrease the blood glucose level(0,048) and reduce the RAGE expression in glomerulus(0,003) but increase MDA level in kidney (0,767***). Conclusion***: Regular and measurable physical exercise can decrease the blood glucose level and reduce the RAGE expression in glomerulus.

Keywords: Diabetes mellitus; physical exercise; fasting blood glucose; RAGEexpression; kidney MDA level.

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Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia condition. Diabetes mellitus is closely associated with complications.[1] These complications include microvascular complication such as retinopathy, nephropathy and neuropathy, and macrovascular complication such as ischemic heart disease, peripheral heart disease and cerebrovascular disease.[2,3,42] The main cause of complications in diabetes mellitus is the presence of oxidative stress condition.[4,5]

Oxidative stress is formed due to the imbalance between free radicals and the body's defense mechanism, which is caused by the increased production of free radicals, or decreased antioxidant activity, or both.[6,7,8] The main sources of oxidative stress in DM are the auto-oxidation of glucose, the production of reactive oxygen species (ROS) in the mitochondria excess, the nonenzymatic glycation, the activation of polyol sorbitol pathway,[9]the formation of lipid peroxides and the decrease of antioxidant enzymes such as glutathione, superoxide dismutase and ascorbic acid.[6,7]

Nonenzymatic glycation between glucose and amino acids will form the accumulation of advanced glycation end products (AGE). 1% increase of AGE will provide opportunities for 37% microvascular complications.[2] Advanced glycation end products will produce various negative effects through various mechanisms. Firstly, the AGE formation that occurs in the extracellular matrix would lead to the expansion of the matrix and will further narrow the lumen. Secondly, the AGE formation which occurs

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in the intracellular matrix would induce oxidative stress and increase the production of superoxide anions in mitochondria. Thirdly, the interaction between AGE and its receptor (RAGE) may activate proinflammatory, prothrombotic,[2] and profibrotic cascade.[10]

Kidney is the main organ of AGE products disposal. The increased AGE amount in plasma can induce pathologic changes in diabetic rats glomerulus, namely the mesangial matrix expansion, fibrosis and inflammation.[11] Mesangial matrix expansion may cause a decrease in glomerular capillary resulting in a decrease glomerular filtration rate.[12]The increased extracellular matrix may also cause the thickening of gromelural walls and will further induce glomerulosclerosis.[13] Advanced glycation end products that bind to the receptor will lead to the transformation of tubular cells into myofibroblasts, so the tubulus will be hypertrophy.[14] The interaction between RAGE with albumin glycation reduces the expression of nephrin, thereby interfering the normal function of podocytes in the glomerular area which later leads to hyperpermeability and proteinuria.[13] In general, AGE accumulation will accelerate diabetic nephropathy.[3]

A possible source of oxidative stress in DM is the formation of lipid peroxides and the accumulation of their products.[6,7,15] The mechanism that contributes to the formation of lipid peroxides in DM is hyperglycemia which causes the auto-oxidation of glucose, the nonenzymatic glycation between proteins and fats, the increase of sorbitol polyol pathway activity, and the oxidation between AGE and cyclooxygenase, which depends on the formation of prostaglandin H2 (PGH2).[15] Lipid peroxide levels in the body can be measured by the stable end product, namely malondialdehyde (MDA). Elevated levels of MDA would disrupt membranes and cell organelles and would also induce atherosclerosis and microvascular complications in DM.[8,16,17]

A regular and measurable physical exercise is a therapeutic modality for the treatment of DM.[18,19] It may improve glucose homeostasis, reduce the ratio of glucose/insulin, and increase insulin sensitivity.[20] The active muscles do not require insulin for infiltrating glucose into cells. In addition, physical exercise may lead to 7-20 time higher uptake of glucose. The higher demand of energy demand will reduce the occuring glycation reactions, so the number of AGE in plasma and tissues will also decrease.[11] The regular and measurable physical exercise with the proper frequency, intensity, duration and type can increase the capacity and the activities ofbody's antioxidants, such as SOD and glutathione peroxidase (GPx),[22] which results in decreased levels of lipid peroksidase.[23] Furthermore, with a lower number of AGE, the supply of free radicals in the body will also decreases.[18]

The objective of this study was to evaluate the benefits of physical exercise in preventing complications of the kidneys caused by hyperglycemia, with studying blood glucose level, the expression of RAGE in glomerulus and the level of MDA in the kidney.

Materials and Method

This study was a pure experimental research with post-test control group design. The subjects were male white rats with 11-12 week age and 200-250 gram weight. These rats were induced with low-dose streptozotocin (35 mg/kgBW) and had >200 mg/dL plasma glucose or >140 mg/dL fasting glucose. The subjects were divided into two groups. The first group included STZ-induced rats without regular and measurable physical exercise, while the second group consisted of STZ-induced rats with regular and measurable physical exercise.

The regular and measurable physical exercise program was performed 5 days/week for 9 weeks on a treadmill. The treatment was initiated with one week for the adaptation of treadmill exerciseat 5 m/min speed for 10 minutes, followed by 8 weeks for the exercise at gradual speeds ranging from 5m/min to 20 m/min for 1 hour with 0º degrees of tilt.[24]During the first week of treadmill adaptation, the rats ran at 5 m/min speed for 10 minutes. The stage of routine exercise began in the second week at 5 m/min speed for 30 minutes, continued to the third week at 11 m/min speed for 30 minutes, and the fourth week at 14 m/min speed for 45 minutes. From the sixth until ninth week, the exercise was at 2 m/min treadmill speed for 1 hour. The level of fasting blood glucose was measured four times, i.e. in the 3rd day (BG_1), the 17thday (BG_2), the 47th day (BG_3) and 87th day (BG_4) after the induction of STZ.

The RAGE expression in glomerulus was measured by making histological preparations with immunohistochemical staining. The MDA levels in the kidney were measured by generating kidney homogenates and were read by spectophotometry.

Ethical clearance: The study was approved by ethics committee of Faculty of Health Science,'Aisyiyah University, Indonesia

Result

Table 4.1Testing percentage BG_4 difference between the control group (STZ) and treatment group (STZ + exercise)

Group	Mean±SEM	P value	Explanation
Control Group	327,84±70,35	0,048	Significantly different.
Treatment Group	167,94±31,44		

Table 4.2. Testing the RAGE independent t-test difference in glomerulus cells

Group	Mean±SEM	P value	Explanation
Control Group	95,82±0,76	0,003	Significantly different.
Treatment Group	79,12±3,78		

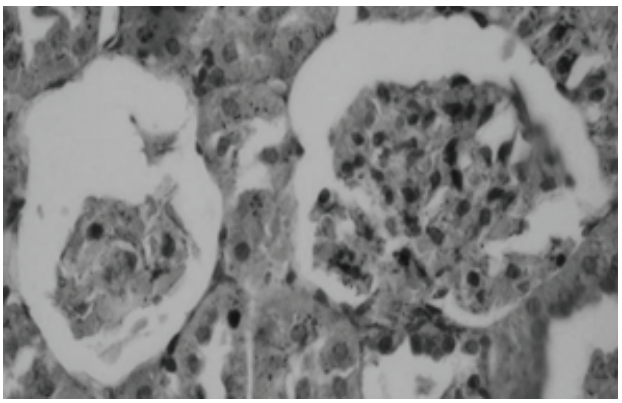

Figure 4.1. AGE receptors inglomerulusof STZ-induced rats

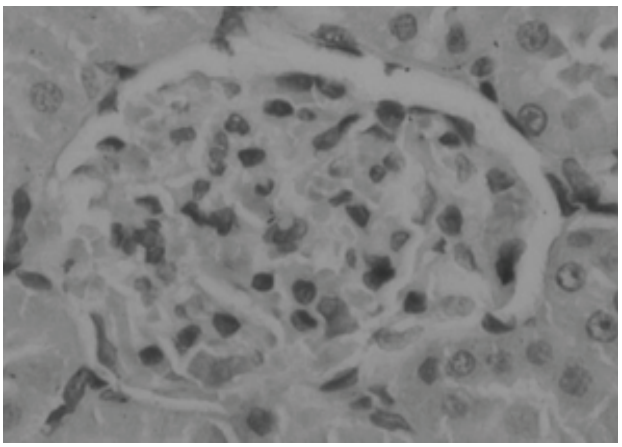

Figure 4.2.AGE receptors inglomerulusof STZ-induced ratswith regular and measurable physical exercise

Table 4.3. Testing the independent t-test differences of MDA level in kidney

Group	Mean±SEM	P *value*	Explanation
Control group	9,03±1,01	0,767	No significant differences
Treatment Group	9,40±0,64		

Discussion

Statistical test results (table 4.1) obtained using independent t-test show a significant difference in rate of blood glucose after treatment in both groups ($p=0.048$; $p\leq0.05$) with the mean difference between the two treatment groups achieves value of 159.90 mg/dL. The BG_4 value of the treatment group is almost close to the normal range, i.e. 167.94 mg/dL, whereas the BG_4 value of the control groupis still high and shows deviation from the normal blood glucose level (327.84 mg/dL) although the levels are still within the range of mild hyperglycemia. The results which show a significant difference between the two groups are probably due to the value of blood glucose of the control group which do not significantly rise because of the pancreas mechanism in compensating hyperglicemia[25] as the result of reversible degeneration of pancreatic β cells by low dose STZ induction.[26]Blood glucose mean decrease of the treatment group after the end of treatment is quite high because the treatment of regular and measurable physical exercise would induce a higher energy demand[11] so the glucose uptake would also increase 30-40 times.[22] The increased energy demand of the skeletal muscles is due to the increase of glucose uptake through glucose transporters, especially GLUT 4.[27] Regular and measurable physical exercise could also protect the damage of pancreatic beta cells in STZ-induced rats in order to prevent degradation of endogenous antioxidant, such as SOD, GSHPx and catalase.[28]

The results of this study (table 4.2) show a significant difference in the expression of RAGE on glomerulus between the control group and the treatment group ($p=0.003$, $p\geq 0.05$). The control group expresses RAGE more (95.82%) than the treatment group (79.12%). The result of this study supports the previous research, which states that high energy demand on the active muscles during a regular and measurable physical exercisecan reduce the amount of AGE in plasma and tissues by reducing reactive glycation products on polyol pathway.[11]

Glomerulus microscopic picture of the control rats shows the thickening of the capsule space of

glomerulus compared with the treated rats (figure 4.1), suggesting the existence of glomerulosclerosis in the control rats (figure 4.2). The result of this study supportsprevious research that figures out that the AGE-RAGE bond would lead to an increase of the basement membrane (type IV collagen),[13] eexcessive production of matrix proteins, expression alteration of matrix metalloproteinase and proteinase inhibitor, oxidative stress induction of mesangial and activation of protein kinase C.[29]Furthermore, these conditions would increase the production of proinflammatory cytokines (TGF-β) and would result in glomerulosclerosis, which is characterized by the capillary wall thickening of glomerulus and renal arteries.[30, 31] In a more advanced state, excessive expression of RAGE will improve albuminuria, serum creatinine and renal hypertrophy.[29]It has been proven that low dose STZ-induced rats experience glomerulosclerosis after 12 weeks with ≥ 250 mg/dL blood glucose.[13] The regular and measurable physical exercise is found to be capable of minimizing glomerular mesangial matrix expansion and tubulointerstitial fibrosis by reducing the amount of AGE, N (epsilon)-carboxy-methyllysine and advanced oxidation protein products.[32,42]

The results of MDA evaluation (table 4.3) on rats' kidneys of the control group (9.03 nmol/g) and those of the treatment group (9.40 nmol/g) show almost similar average, so the test of significance between the two groups results in no significant differences ($p=0.767$; $p \geq 0.05$). It means that physical exercise cannot significantly decrease the MDA in the kidney of diabetic rats. There are some assumptions that may be able to answer why the kidney MDA of STZ-induced ratswith exercise is higher than that of STZ-induced rats without exercise.

The first assumption of why the physical exercise does not significantly affect the MDA level is that the rats have suffered from chronic diabetes so the MDA level in the kidney has been quite high. In this study the rats were induced with STZ (35 mg/kgBW) and left to be chronic for 14 days. The lipid peroxides in the kidney and liver had increased after a week of low-dose STZ induction without kidney histopathologic changes.[33,34] Induction of low dose STZ was able to create the diabetes conditions with minimal damage both for kidney and for liver.[35]

The second assumption is the lack of diet control for the research subjects.A previous study showed a decrease in plasma MDA levels of diabetic subjects after 12 week physical exercise and diet control.[9]Meanwhile, observation on the subjects which were only treated by physical training did not show decreased levels of MDA after 12 weeks and only showed a decrease in MDA after 6 months (24 weeks). Another previous study also showed 1.8% decrease in plasma MDA levels of the subjects after being given physical exercise for 6 months (24 weeks), and did not show any significant decrease when the exercise was conducted for 3 months (12 weeks).[15]Malondialdehyde is the end product of PUFA[36]of which the level is increased due to the improved activity of acyl-CoA.[37]

The third assumption is that the treatment of physical exercise performed in this study is considered insufficient in time. The exercise was only provided for 9 weeks gradually-increased speed and duration. A previous study proved that physical exercise for rats which was aimed at endurance would increase antioxidants and antioxidant enzymes in the skeletal and cardiac muscles after 10 weeks.[38] The research proved that glutathione level in active muscle increased up to 33%, glutathione peroxidase activity increased by 62% and superoxide dismutase level increased by 27% .[38] Increased antioxidant activity would be able to reduce the level of malondialdehyde in the body. Another previous study found that decreased plasma MDA began to occur in week 14 with the provision of regular and measurable physical exercise for the DM subjects,[9] and MDA decreased both in erythrocytes and tissues of rats after 12 week physical exercise (swimming) in various intensity.[28] Physical exercise which is chronically conducted for 8 weeks will evoke side effects, i.e.oxidant change and oxidative stress, which consequently cause the induction of antioxidant enzymes and the synthesis of antioxidants to minimize the effects of those oxidants.[39]

The fourth assumption is the stress experienced by the rats during treatment. Exercise in experimental animals is very susceptible to stress.[40]Researchers can reduce activation of the stress response by maximizing the animal's perceived behavioral control and minimizing the novelty of the exercise situation. For instance, perceived behavioral control can be maximized by, for example, training the animal during the natural active phase of its circadian cycle. To minimize the novelty of the exercise, investigators should expose the animals to the exercise apparatus repeatedly before beginning actual. For example, for treadmill training, repeated exposure to handling and the treadmill apparatus at the same time of day and by the same personnel who will conduct the actual training sessions will greatly reduce the stress response triggered by a novel environment and activity.[40]

Another stress affecting factor was the fact that the treated physical exercise was quite tiring some rats of the treated groups. Some states indicating the fatigue condition of the rats are: poor performance such as being unwiling to run; nudging the electroshock device 4 times in 1 minute; rised temperatures, increased lactic acid and faster pulse. If the rats nudge the electroshock device on the treadmill, it indicates that they have been tired .[40] The exhausting physical exercise would be associated with increased formation of free radicals, primarily due to increased O2 consumption in active tissues.[41]

The fifth assumption is the high MDA as a biomarker of oxidative stress in diabetes is not only caused by hyperglycemia and RAGE. The main sources of oxidative stress in DM are: (1) auto-oxidation of glucose, (2) excessive ROS production in mitochondria, (3) non-enzymatic glycation and, (4) polyol pathway.[9]

In DM condition there is increased consumption of NADPH by the aldose reductase enzyme in the polyol pathway. NADPH is needed to establish the endogenous antioxidant glutathione (GSH). Decreased NADPH would result in reduced GSH and increase oxidative stress. The increase in mitochondrial ROS may be caused by several reasons, namely: (1) mitochondrial components, such as DNA, proteins and lipid membranes, (2) the opening of the mitochondrial permiability transition pore (MPTP), (3) the release of release proapoptosis proteins from mitochondria, such as cytochrome C which can stimulate cell death. The formation of ROS in the mitochondrial respiratory chain as a second messengers for the activation of NF-κB through TNF-α and IL-1. The increased production of superoxide is catalyzed by NADPH oxidase, insulin and XO.[32]

Lipoxygenase is also a producer of free radicals during the enzymatic reaction. The lipoxygenase products especiallly 12 (S)-HETE and 15 (S)-HETE will give proatherogenic effect and can mediate the action of growth factors and proinflammatory cytokines. Sources of ROS which are not derived from the mitochondria also include cyclooxygenase enzyme (COX), which catalyzes the synthesis of various prostaglandins.. Proinflammatory cytokines are able to induce the expression of COX_2 by stimulating NADPH oxidase and production of ROS. Another source of ROS is monooxygenase cytochrome P-450, which will increase CYP2E1 expression - within unusual circumstances CYP2E1 will produce free radicals. Reactive oxygen species (ROS) would activate a stress-sensitive kinase and able to mediate insulin resistance. Activation of various kinases will increase and activate NFκB and activator protein-1 (AP-1) which will eventually: (1) activate c-Jun N-terminal kinase (JNK) and inhibit NFκB kinase-β (IKK), (2) increase gene transcription of proinflammatory cytokines and, (3) increase the synthesis of critical phase reaction.[32]

Conclusion

Regular and measurable physical exercise can decrease the blood glucose level and reduce the RAGE expression in glomerulus but increase MDA level in kidney.

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Conflict of interest: None declared

Author's contribution:

Data gathering and idea owner of this study: Diyah Candra Anita, Sri Lestari Sulistyorini, Sri Kadarsih

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Editing and approval of final draft: Diyah Candra Anita, Sri Lestari Sulistyorini, Sri Kadarsih

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