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Diabetes & its Complications

Effect of Physical Exercise to FFA, Pancreatic Beta Cell Function and Expression of GLUT4 in Diabetic Rats

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Type 2 Diabetes, Hyperglycemia, Metabolic diseases, Insulin.

Introduction
Type 2 Diabetes Mellitus is a metabolic disease characterized by insulin resistance and pancreatic β cell deficiency, which decreases insulin levels so that glucose remains high in the blood which lasts chronic. Initially as high glucose compensation in the blood, the pancreas will produce more insulin so that hyperinsulinemia occurs, but over time there is fatigue and damage to the pancreas so that it is unable to produce enough insulin to control glucose to remain normal in the blood. As a result there is a decrease in insulin levels and glucose remains high in the blood [1-3].

Hyperglycemia and hyperinsulinemia at the beginning which then becomes a decrease in insulin levels will also affect the levels of FFA (Free Fatty Acid) in the blood of people with type 2 DM. In this condition there is an increase in FFA in the blood and accumulation of fat metabolites (Fatty acyl-CoAs, Diacylglycerols and ceramides) that arise due to a decrease in fatty acid oxidation in the cell. This fat metabolite will activate PKC (Protein Kinase C) which inhibits phosphorylation (tyrosine will decrease, serine will increase) and inhibit activation of IRS-1 (Insulin Receptor Substrate), so GLUT4 (Glucose Transporter 4) cannot translate to the surface of the cell membrane to enter glucose into the cell. This long-acting glucotoxicity and lipotoxicity will pose a risk to all organs ranging from the brain, cardiovascular, liver, kidneys, eyes, extremities and others so that it will endanger lives [2-4].

Management of Type 2 Diabetes Mellitus begins with Life style Education, arranges Diet, Exercise, Anti Diabetes Medicine, to replace the pancreas, which aims to control blood glucose by reducing insulin resistance and improving pancreatic β cell function. Besides that the role of physical exercise in Type 2 Diabetes Mellitus can also improve insulin resistance through 5-AMPK (Adenosine Monophosphate Protein Kinase) activation and phosphorylation of AS 160, so that later it will increase the expression and translocation of GLUT4 to the membrane surface to enter glucose into muscle cells. Physical exercise will also reduce the accumulation of fat metabolites in muscle cells, and reduce levels of FFA in the blood [5-9].

In clinical Practically to assess the presence of pancreatic beta cell damage in type 2 diabetes mellitus can be done by examining HOMA B. HOMA B can be used to determine whether there is an improvement in pancreatic β cell function. Normal value of HOMA B is 70% - 150%. If HOMA B is found below 70%, it shows that there has been a decline in function in pancreatic β cells [10-12].

Can Physical Exercise improve HOMA B, reduce FFA, increase Expression of GLUT-4 in skeletal muscle cell membranes of diabetic rats and improve insulin resistance? In 4 studies carried out in humans and 1 study conducted in mice, with the ivGTT method that inserted glucose and insulin through an IV tube to measure insulin sensitivity, proved that physical exercise improved pancreatic beta cell function and insulin resistance. The 5 studies did not see the mechanism for improving pancreatic beta cells and insulin resistance [13-17]. The aim of this study is to prove that physical exercise will improve HOMA B, improve insulin resistance by decreasing FFA levels and increasing GLUT-4 expression on diabetic skeletal muscle cell membranes.

Material and Method
This research is Experimental Laboratories, where in this study allows researchers to measure the effect of intervention in group I and II experiments and determine the extent of the change, by comparing the two groups. 10 male Wistar Novergicus rats aged

6-8 weeks with a weight of 150 - 300 grams, were treated first for 7 days aiming for rats to adjust to the environment such as changes in their cages and meal times. During adjustment / adaptation all rats were given standard feed with a composition of chicken feed / PARS 66.6% and wheat flour 33.4%, and rats were put in one cage. Room temperature is 22˚C - 25˚C, and light is given for 12 hours alternating 12 hours in dark conditions.

Diabetes in rats can be induced by giving a high-fat diet for 9 weeks before STZ injection, with the composition of the mixture: 50% PARS cofeed, 25% flour, 5% duck egg yolk, goat fat 18.01, coconut oil 1.89% and colat acid 0.1%, with a composition of 58% fat, 25% carbohydrate, and 17% protein from total calories. Total calories needed (total calories = 4.73 kcal/g). Streptozotosine injection (STZ) 30 mg / kgBB (0.1 M citrate buffer, pH 4.5) intraperitoneal (IP) every week, for 2 weeks (week 4 and week 5) and a high-fat diet is still given until the week 9. At the end of week 9, at night the rats were fasted for 8-12 hours and blood samples were taken through the orbital vein for fasting insulin levels, fasting glucose levels and fasting FFA levels. TTGO was performed by giving glucose fluid load of 2 grams / kg body weight, blood glucose level was checked at 0, 30, 60 and 120 minutes with Easy Touch GCU Glucose Test Strips through a tail stabbed with lancet, after administering a liquid glucose load of 2 grams / kg. Diabetes is established based on the results of blood glucose levels fasting ≥ 140 mg / dL and / or random glucose levels ≥ 200 mg / dL or blood glucose level ≥ 200 mg / dL after OGTT [18-21]. 10 male Wistar Novergicus rats of type 2 diabetes mellitus were divided into two groups: Group I was treated sedentary, group II was given physical exercise treatment using a treadmill rat (rodent-treadmill).

Chronic exercise is a physical exercise done in stages by running on the rodent treadmill for 10 weeks. Whereas Moderate Intensity Physical Exercise is physical exercise with flat running without inclination (tilting) on rodent treadmills 6 days a week, starting with a speed of 10 meters per minute for 10 minutes per day and increasing gradually every 2 weeks to reach 1 hour per exercise day with a speed of 27 meters per minute [13,15,17,22-24].

Blood sugar levels and FFA levels were fasting blood sugar levels and fasting blood FFA taken at week 9 and week 19, then measured using the Fluorometry method. Whereas blood insulin levels are fasting blood insulin levels were also taken at week 9 and week 19 then measured by blood insulin levels using the ELISA method. HOMA B is a parameter to measure the strength of the function of pancreatic beta cells that produce insulin and is calculated by the formula = (360 x fasting insulin levels) / (glucose - 63)%. Normal value of HOMA B is 70% - 150% [10-12].

Rats killed (euthanasia) by means of cervical dislocation and surgery for blood and muscle sampling required. Blood samples are taken through a cardiac puncture for examination: fasting insulin levels and fasting blood glucose, FFA levels of fasting blood.

Expression of GLUT4 is GLUT4 expressed on the surface of the muscle tissue membrane of Gastrocnemius limb and abdominal rectus muscle. Calculation of GLUT4 expression using immunohistochemical methods. GLUT4 expression measurement with Convocal Laser Scanning Microscope (CLSM). Immunohistochemistry using primary antibodies and secondary antibodies, and paint with FITC then observed using the CLSM Olympus FV-1000. The results are reported as unit arbitrage which is the luminous intensity in the area of ROI minus luminescence on the background of ROI.

Statistical Analysis

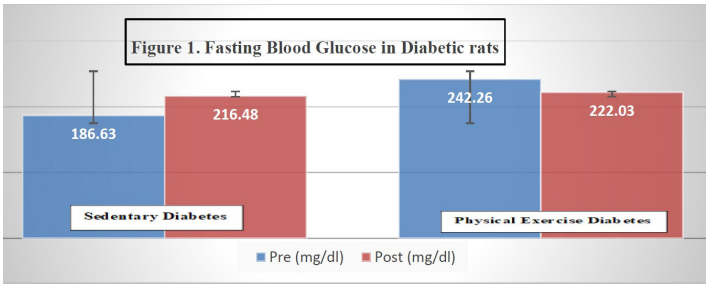
Data measured by fasting blood sugar levels, fasting blood insulin levels, fasting FFA levels, HOMA B calculations and GLUT4 expressions are shown in mean ± SD. If the data distribution is normal, the comparison hypothesis test uses paired T test. However, if the data distribution is not normal, after the transformation of the comparative hypothesis test data using Mann Whitney test is done. All data is analyzed and statistical test significance if the value is $p < 0.05$.

Result

In DM rats which received treatment for 19 weeks, an increase in fasting blood glucose levels from 186.63 mg / dL to 216.48 mg / dl, and a decrease in blood glucose levels in DM rats that received physical training from 242.26 mg / dL becomes 222.03 mg / dL (Table 1, Figure 1).

Group of rats	KGD Pre (mg/dl) Mean ± SD	KGD Post (mg/dl) Mean ± SD	p
Sedentary Diabetes	186.63 ± 7,9	216.48 ± 80,62	0.42
Physical Exercise Diabetes	242.26 ± 50,48	222.03 ± 9,64	0.63

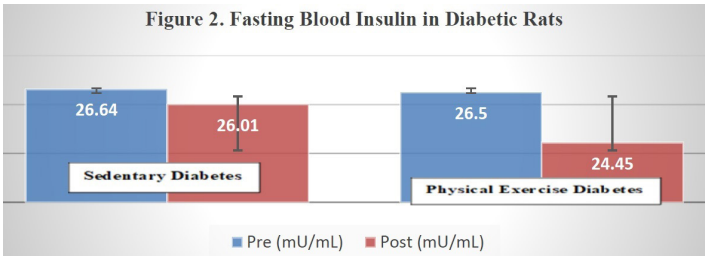
Table 1: Fasting Blood Glucose in Diabetic rats.



In table 2, figure 2, there has been a slight decrease in fasting blood insulin levels. Then after physical exercise in Diabetic rats there was a decrease in fasting blood insulin levels. In sedentary Diabetic rats it was found a significant increase ($p = 0.049$) fasting blood FFA levels. After 10 weeks of physical exercise there was a decrease in fasting blood FFA levels in Diabetic rats (Table 3, Figure 3).

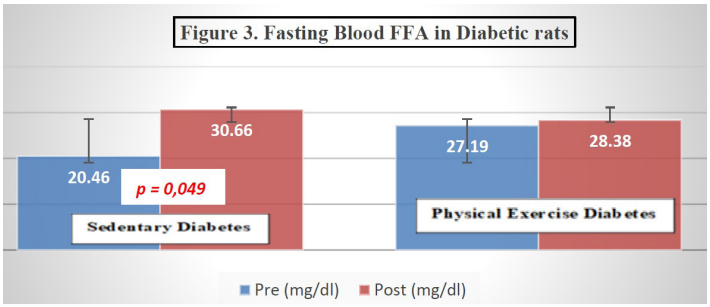
Group of rats	Insulin Pra (mg/dl) Mean ± SD	Insulin Post (mg/dl) Mean ± SD	p
Sedentary Diabetes	26.64 ± 1,50	26.02 ± 1,06	0.47
Physical Exercise Diabetes	25.6 ± 1,47	24.456 ± 0,42	0.132

Table 2: Fasting Blood insulin in Diabetic rats.



Group of rats	FFA Pre (mg/dl) Mean ± SD	FFA Post (mg/dl) Mean ± SD	p
Sedentary Diabetes	20.46 ± 1,63	30.66 ± 6,66	0.049
Physical Exercise Diabetes	27.19 ± 1,26	28.38 ± 0,86	0.164

Table 3: Fasting Blood FFA in Diabetic rats.



In table 4, figure 4, HOMA B in sedentary diabetic rats shows a significant decrease, which means that there is deterioration in pancreatic beta cell function from 94.83% to 61.05% (p = 0.033). Then after physical exercise there was a significant improvement in pancreatic beta cells as indicated by the increase in HOMA B from 47.06% to 65.86% (p = 0.005).

Group of rats	HOMA B Pra (%) Mean ± SD	HOMA B Post (%) Mean ± SD	p
Sedentary Diabetes	94.83 ± 22,01	61.05 ± 19,36	0.033
Physical Exercise Diabetes	47.06 ± 10,05	65.86 ± 4,11	0.005

Table 4: HOMA B in Diabetic rats.

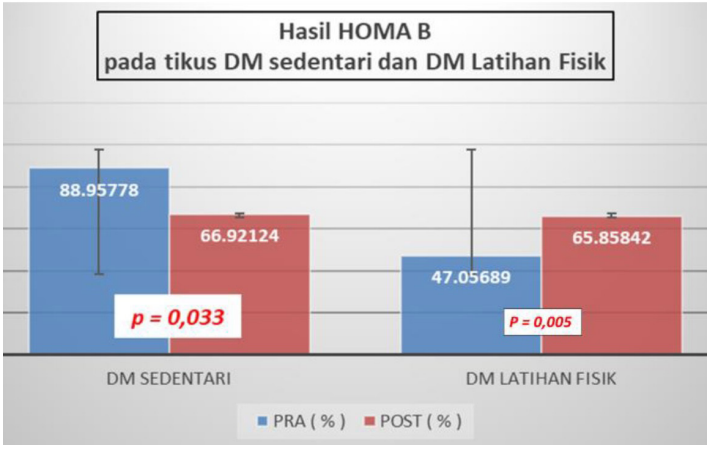


Figure 4: HOMA B in diabetic rats.

Expression of GLUT 4 on the membrane surface of skeletal muscle tissue in Diabetic rats was examined by the CLSM method. In GLUT 4 expression there was a significant increase in the number of GLUT4 expressions after receiving physical exercise (p =0.017) (Table 5, Figures 5 and 6).

Groups of rats	GLUT4 (Arbritrasi unit) Mean ± SD	P
Sedentary Diabetes	247,96 ± 130,6	0,017
Physical Exercise Diabetes	455,01 ± 387,8	

Table 5: GLUT4 expression in Diabetic rats by CLSM method.

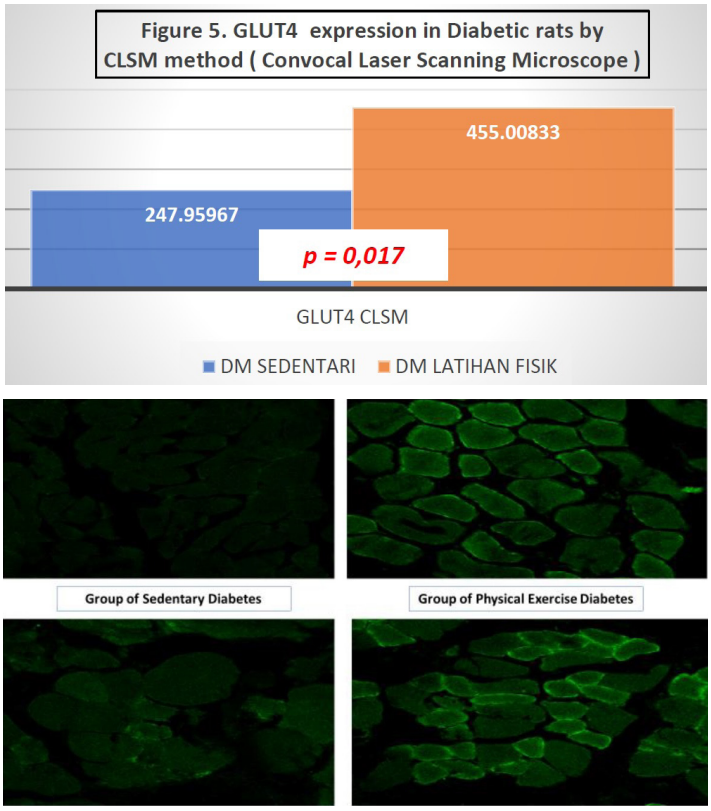


Figure 6: GLUT4 expression in Diabetic rats by CLSM method.

Discussion

Diabetes mellitus is characterized by a decrease in fasting insulin levels, increased fasting glucose levels, increased levels of FFA in the blood, decreased GLUT4 expression to the surface of cell membranes and pancreatic beta cell dysfunction / damage that is characterized by a decrease in HOMA B.

In this study in Sedentary DM rats were found to increase blood glucose levels, a slight decrease in blood insulin levels and a significant increase in blood FFA levels (p = 0.049). This proves that in diabetes there is insulin resistance, hyperlipidemia and hyperglycemia. This lipotoxicity and glucotoxicity give rise to dysfunction / damage to beta cells of the pancreas which is characterized by a decrease in HOMA B in sedentary DM rats (p = 0.033).

Physical exercise can improve pancreatic beta cell function and insulin resistance. This is characterized by increased insulin secretion, decreased FFA levels and improved GLUT4 expression

so that glucose uptake will improve followed by a decrease in blood glucose levels.

In this study it was found that physical exercise reduced blood glucose levels, which was caused by a significant increase in the number of GLUT4 expressions ($p = 0.017$) which caused glucose uptake into the cells to improve. Physical exercise in diabetic rats in this study has been shown to improve pancreatic beta cell function / damage as evidenced by a significant increase in HOMA B ($p = 0.005$).

In physical exercise there is an improvement in insulin resistance which is marked by a decrease in the level of FFA in the blood. Then Physical Exercise will also improve pancreatic beta cells, which is characterized by an increase in the amount of insulin in the blood. But this research did not find the above. Where there is a slight decrease in insulin levels and even a slight increase in the level of FFA in the blood. The possibility of this is due to an improvement in insulin sensitivity and improved insulin resistance so that pancreatic beta cell damage is also reduced. Lipolysis that occurs is also small, which means that there is little insulin resistance. How the exact and clear mechanism of the above needs further research.

This study proves that physical exercise is one of the DM treatments that must be done in all DM patients to be able to improve insulin resistance and prevent damage to pancreatic beta cell function.

Conclusion

In sedentary DM rats there was a decrease in blood insulin levels, an increase in blood glucose levels, a significant increase in FFA levels ($p = 0.033$) and worsening of HOMA B (pancreatic beta cell damage) which was significant with $p = 0.033$, whereas physical exercise in DM rats occurred decrease in blood insulin levels decreased blood glucose levels, increased blood FFA levels and significant improvement in HOMA B ($p = 0.005$), which meant a significant improvement in pancreatic beta cell function).

Physical exercise in DM rats decreased blood glucose levels, significant improvement in HOMA B ($p = 0.005$) and a significant increase in the amount of GLUT4 expression on the surface of muscle tissue membranes. Physical exercise is recommended as one of the treatments for diabetes mellitus that should be done regularly and continuously.

References

1. Fatimah RN. Diabetes Melitus tipe 2. J Major. 2015; 4: 93-101.
2. Choi K, Kim YB. Molecular mechanism of insulin resistance in obesity and type 2 diabetes. Korean J Intern Med. 2010; 25: 119-129.
3. Muoio DM, Newgard CB. Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol. 2008; 9: 193-205.
4. Baynest HW. Classification, Pathophysiology, Diagnosis and Management of Diabetes. J Diabetes Metab. 2015; 6: 1-9.
5. Richter EA, Hargreaves M. Exercise, GLUT4, and Skeletal Muscle Glucose Uptake. Physiol Rev. 2013; 93: 993-1017.
6. Putri NHK, Isfandiari MA. Hubungan empat pilar pengendalian dm tipe 2 dengan rerata kadar gula darah. J Berk Epidemiol. 2013; 1: 234-243.
7. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and Treatment of Type 2 Diabetes Perspectives on the Past, Present and Future. Lancet. 2014; 383: 1068-1083.
8. Merry TL, McConell GK. Skeletal muscle glucose uptake during exercise: A focus on reactive oxygen species and nitric oxide signaling. IUBMB Life. 2009; 61: 479-484.
9. Cortright RN, Dohm GL. Mechanisms by which insulin and muscle contraction stimulate glucose transport. Can J Appl Physiol. 1997; 22: 519-530.
10. Wallace TM, Levy JC, Matthews DR. Use and Abuse of HOMA Modeling. Diabetes Care. 2004; 27: 1487-1495.
11. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28: 412-419.
12. Song Y, Manson JE, Tinker L, et al. Insulin Sensitivity and Insulin Secretion Determined by Homeostasis Model Assessment (HOMA) and Risk of Diabetes in a Multiethnic Cohort of Women: The Women's Health Initiative Observational Study. Diabetes Care. 2007; 30: 1747-1752.
13. Gomes RM, Tofolo LP, Wilson R, et al. Moderate Exercise Restores Pancreatic Beta-Cell Function and Autonomic Nervous System Activity in Obese Rats Induced by High-Fat Diet. Cell Physiol Biochem. 2013; 32: 310-321.
14. Shih K, Kwok C. Exercise reduces body fat and improves insulin sensitivity and pancreatic β -cell function in overweight and obese male Taiwanese adolescents. BMC Pediatr. 2018; 18: 1-8.
15. Slentz CA, Tanner CJ, Bateman LA, et al. Effects of Exercise Training Intensity on Pancreatic B Cell Function. Diabetes Care. 2009; 32: 1807-1811.
16. Heiskanen MA, Motiani KK, Mari A, et al. Exercise training decreases pancreatic fat content and improves beta cell function regardless of baseline glucose tolerance a randomised controlled trial. Diabetologia. 2018; 61: 1817-1828.
17. Nieuwoudt S, Fealy C, Malin SK, et al. Functional High Intensity Training Improves Pancreatic β -cell Function in Adults with Type 2 Diabetes. AJP Endocrinol Metab. 2017; 313: E314-E320.
18. Tremblay F, Lavigne C, Jacques H, et al. Skeletal Muscle of High Fat – Fed Rats Is Associated with Alterations in Both Akt / Protein Kinase B and Atypicaln Protein Kinase C Activities. Diabetes. 2001; 50: 1901-1910.
19. Zhang M, Lv X, Li J, et al. The Characterization of High-Fat Diet and Multiple Low-Dose Streptozotocin Induced Type 2 Diabetes Rat Model. Exp Diabetes Res. 2008; 2008: 1-9.
20. Ho K. A critically swift response: Insulin-stimulated potassium and glucose transport in Skeletal Muscle. Clin J Am Soc Nephrol. 2011; 6: 1513-1516.
21. Abu AM, Ismail ZB, Alzaben KR, et al. Induction of Diabetes Mellitus in Rats Using Intraperitoneal Streptozotocin: A

Comparison between 2 Strains of Rats. Eur J Sci Res. 2009; 32: 398-402.
22. Norton K, Norton L, Sadgrove D. Position Statement in Physical Activity and Exercise Intensity Terminology. J Sci Med Sport. 2010; 13: 496-502.
23. Röhling M, Herder C, Stemper T, et al. Influence of Acute and Chronic Exercise on Glucose Uptake. J Diabetes Res. 2016; 2016: 1-33.
24. Malin SK, Rynders CA, Weltman JY, et al. Exercise Intensity Modulates GlucoseStimulated Insulin Secretion when Adjusted for Adipose, Liver and Skeletal Muscle Insulin Resistance. PLoS One. 2016; 11: 1-14.

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