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Exercise Intensity Alter *Insulin Receptor* Gene Expression in Diabetic Type - 2 Rat Model

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Abstract

AIM: To analyse the differential expression of the *insulin receptor (IR)* gene between moderate continuous and severe continuous training in the T2DM rat model.

METHODS: This was an experimental study. Healthy male Wistar was used in this study, which divided into sedentary, moderate continuous training, and severe continuous training. Treated groups were assigned to run on the treadmill three times a week for eight weeks consequently.

RESULTS: The result shown that expression of mRNA *IR* gene in treated groups decline compared to control. There was a difference mRNA *IR* gene expression after eight weeks of exercise between MCT and control, SCT and control so are MCT and SCT. *IR* expression on skeletal muscle in treated groups was different compared with control. The distribution of IR on skeletal muscles in treatment groups was significantly increased compared control, but there was no significant difference distribution between MCT and SCT. HOMA-IR post-test in SCT was lower than MCT but FBG post-test lower in MCT than SCT.

CONCLUSION: The intensity of exercise makes a difference in *IR* gene expression between moderate continuous training and severe continuous training after eight weeks of assigned exercise in T2DM rat models.

Introduction

Nowadays, Indonesia becomes a global epidemic of Type-2 Diabetes Mellitus (T2DM). International Diabetes Federation (IDF) predicted the prevalence of T2DM patients in Indonesia is going to increase about 16,2 million by 2040 [1]. According to Indonesian Endocrinology Society (PERKENI), the increasing of T2DM prevalence is related to lifestyle changes, i.e. dietary and limited physical activity [2]. There were many studies related to exercise and insulin resistance but still limited yet to apply intensity altering insulin receptor gene expression.

Mechanism of insulin resistance in T2DM patient that caused by insulin signalling impairment is possible to happen at pre receptor, receptor and post-receptor level [3], [4], [5]. Chronic hyperglycemia caused an impact on insulin receptor such as *downregulation* receptor on the membrane surface of the skeletal muscle, thus causing impairment of insulin sensitivity [6]. Insulin receptors were found most at skeletal muscle, liver and adipose tissue [7]. Insulin receptor impairment in the liver will lead to blood glucose homeostasis disturbance at rest; while in skeletal muscle, glucose transporter-4 (GLUT-4) fails to exocytosis up to membrane surface when activity [8].

Insulin receptor expression on the skeletal muscle membrane was determined by mRNA receptor gene expression. The mRNA receptor gene expression will increase when our body requires more receptors. There was a relationship between gene expression and metabolism. Metabolic status and enzyme metabolites are inter-related, thus increasing one will affect others significantly [9].

Exercise can increase muscle contraction to full fill energy needs. Energy for muscle contraction is obtained from aerobic and anaerobic metabolism [10]. Power stroke produces ATP [11]. ATP and creatine ratio reduction activated enzyme AMP Kinase. This enzyme will then stimulate mitochondrial biogenesis through PGC-1α [12], [13]. Mitochondrial enzyme altered gene transcription [14]. Sympathetic activities during exercise suppressed insulin secretion from beta pancreas cell and at the same time, enhance gluconeogenesis in the liver, causing the blood glucose level to increase [10], [11].

Decreasing insulin levels stimulates an increase in receptor density on the surface of the cell membrane to facilitate blood sugar entry to the cell [15]. Rate of cortisol during exercise stimulates transcription of gene receptor insulin. The more vigorous intensity of exercises was carried out, the more accelerate the metabolism of the cell, drawing an effect of significant proportional in between the intensity of training and gene expression and the amount of insulin receptor involved [16]. This study aims to analyse the differential expression of the insulin receptor gene between moderate continuous and severe continuous training in T2DM rat models.

Material and Methods

Healthy male Wistar rats, 150-180 gram in weight, age eight weeks, were used in this study. These rats were acclimatised for seven days. Four rats were placed in the same cage, in a room with a temperature of 22-250°C with a dark light cycle 12 / 12 h. After acclimatisation, the animal experiment was given meals with a high-fat diet for five weeks consequently. They were then undergone fasting for one night before they were induced with Streptozotocin 30 mg/kg/intra-peritoneum diluted in 0.1 citrates, Buffer of pH 4.5. A repeated induction was given a week later with a dosage of 45 mg/kg [17]. Laboratory fasting blood glucose was tested on the next week for individual rats by taking the blood samples from the tail's veins. Rat determined as T2DM if fasting blood glucose was > 200 mg/dl and HOMA-IR > 6,5. Samples then classified into three groups, i.e. the *sedentary* group (control), *moderate continuous training* (MCT), and *severe continuous training* (SCT). There was no exercise assignment for the sedentary group. MCT group was running on the treadmill with a speed of 25 m/min and 30 m/min for the SCT group.

Fasting blood glucose level and HOMA-IR were recorded before and after exercise carried out, while the expression of the IR gene and muscle-skeletal insulin receptor were recorded after all exercise protocol was done. IR gene expression from gastrocnemius muscle was analysed using Real-Time PCR and immunohistochemistry used to assessed insulin receptor expression.

Exercise Protocol

The intervention carried out in this study is parallel with the protocol of Huang et 2016 [18], which the samples were placed on exercising on the treadmill. This intervention was held 3 times a week with 30 minutes/session for 8 weeks long. Before the protocol was given, rats are used to walk on the treadmill before the exercise protocol has begun. After exercise procedure was complete, rats were executed under sedation with ketamine 30 mg intramuscular.

The procedure of fasting blood glucose test and HOMA-IR

Blood glucose and HOMA-IR were measured before and after each session of assigned exercise. The samples fasted for 12 hours, and the blood samples were taken from the lateral vein of the tail, with a volume of 3 ccs. Anaesthesia with local Xylocaine spray was given before each blood samples were taken. Blood serum was extracted and tested for the blood glucose level with a spectrophotometer using GOD-PAP method (wavelength of 500 nm, Hg 546 nm, and temperature of 20-25°C / 37°C). Fasting insulin was tested with Insulin INS-Rat kit (Qayee-Bio, Shanghai, China) by ELISA. The resistance of insulin was determined by calculation of HOMA-IR, using the equation of:

Fasting Insulin (mIU/L) x fasting blood glucose level (mg/dl) 405

The procedure of expression Insr gene test

As much as 30-40 mg of gastrocnemius muscles were inserted in an Eppendorf tube filled with RNA fluid. Isolation of RNA gene carried out by using the standard procedure of the RNeasy Mini Kit (Qiagen-German). The result of isolation samples was kept under a temperature of -20°C for further analysis. RT-PCR test was carried out using One-Step RT-PCR with the SYBR® Green RT-PCR Reagents Kit (Biosystem).

Primer IR- Rat Forward (IDT): 5'- GGC CAG TGA GTG CTG CTC ATG C-3'

Primer IR-Rat Reverse (IDT): 5'- TGT GGT

GGC TGT CAC ATT CC-3'

Primer B actin Rat Forward (IDT): 5'- CAC CCG CGA GTA CAA CCT TC– 3'

Primer B actin Rat Reverse (IDT): 5'- CCC ATA CCC ACC ATC ACA CC – 3'

Thermocycler (rotor gene) were arranged according to the conditions of RT incubation 42°C (5 minutes), early denaturation 92°C (5 minutes), denaturation 45°C (10 seconds), annealing /extension 60°C (30 seconds) for 40 cycles.

Data Analysis

Normality of data obtained was testified using Shapiro–*Wilk* ($p < 0.05$). The t-dependent test was used to determine the difference between HOMA-IR and blood glucose level before and after assigned exercise in this study. Expression of *Insr* gene, HOMA-IR posttest among groups analysed by ANOVA test with the value of $p < 0.05$ and Post Hoc analysis with Bonferroni test. Meanwhile, the Kruskal Wallis test was used in this study to analyse the differences of skeletal muscle receptor in all three sample groups.

Results

Dismounts of HOMA-IR and blood glucose level were noted after eight weeks of assigned exercise were carried out. Reduction in blood glucose level in the treated groups was significant. FGB in MCT was lower than SCT and control. However, the reduction of HOMA-IR in MCT is not considered statically significant (table 1). According to the ANOVA test, there were different expressions on mRNA IR gene for MCT group samples and SCT group samples compared to control samples.

Table 1: Expression of gene *Inst*, insulin receptor, HOMA-IR and Fasting blood glucose level for control and independent sample

	Expression of gene *Insr* (ΔCt) Mean 2 ± SB	Insulin receptor muscles (%) Mean2 ± SB	HOMA-IR Mean2 ± SB	P value	FGB (mg/dl) Mean2 ± SB	P value
Control	1.0 ± 0.3	60 ± 20	Pre = 107.1 ± 14.4 Post = 49.1 ± 9.5		Pre = 474,8 ± 24.9 Post = 214.9 ± 5.7	
MCT	4.5 ± 2.2	86 ± 5.5	Pre = 90.5 ± 40.5 Post = 43.3 ± 8.6	0.066	Pre = 339 ± 103,7 Post= 191.6 ± 5.4	0.014*
SCT	2.8 ± 0.5	88 ± 4.5	Pre = 81.3 ± 6.1 Post = 18.5 ± 6.4	0.000*	Pre = 396.8 ± 25.7 Post = 198.2 ± 75.0	0.009*

Note: MCT = *moderate continuous training*; SCT = *severe continuous training;* ΔCt = *cycling threshold* target gene - *cycling threshold* refrence gene; SD = *standard deviasion*; HOMA-IR = insulince resistence; FGB = Fasting Blood Glucose; $p < 0.005$.

Table 1 showed that eight weeks of exercise has an effect on HOMA-IR and FGB. Both exercise in moderate continuous and severe continuous can reduce the average of HOMA-IR and FGB after 8 weeks of exercise in the T2DM rat model. Even 8 weeks of exercise has not quite enough to reduce HOMA-IR statistically significant in the MCT group. HOMA-IR post-test was found lower in SCT than MCT. But FGB was lower in MCT than SCT. mRNA IR gene cycling threshold and IR gene distribution on skeletal muscle increased both MCT and SCT.

Cycling threshold mRNA gene in MCT and SCT higher than control (Figure 1). Means that gene expression in MCT and SCT underwent eight weeks of exercise are found to have lower expression compared to control which are not given any exercise (MCT vs SCT vs Control = 4.5 ± 2.2 vs 2.8 ± 0.5 vs 1.0 ± 0.3; $p = 0.006$).

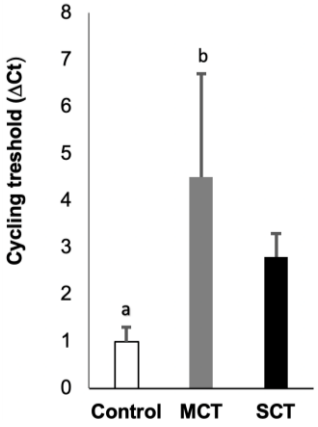

Figure 1: Insulin Receptor mRNA Gene Expression

Based on the Bonferroni post hoc test, there was a significant difference IR gene expression between the control group and MCT ($p = 0.002$), control and SCT ($p = 0.033$). IR gene expression in the MCT group also had a significant difference with IR gene expression in SCT ($p = 0.047$).

MCT gene *Insr* is found to have lower expression compared to SCT samples ($p = 0.047$) In the meanwhile, the amount of insulin receptor at skeletal muscle are found to rise at the group samples if compared to the control samples (MCT vs SCT vs Control = 86 ± 5.5 vs 88 ± 4.5 vs 60 ± 20; $p = 0.009$) (Figure 2) while group samples K3 having the most insulin receptor among all ($p=0.690$). A depression of expression *Insr* gene and rise in the amount of insulin receptor at the surface of skeletal muscle was noted, following by the reduction of HOMA-IR posttest within both group samples (control vs MCT vs SCT = 49.1 ± 9.5 vs 43.3 ± 8.7 vs 18.5 ± 6.4) (Figure 3). According to the ANOVA test, HOMA-IR, after eight weeks of assigned exercise, gave a significant reduction ($p = 0.001$) for the group SCT.

There was no significant difference in IR expression on the skeletal muscle between MCT and SCT, but both treated group were significantly differenced with control.

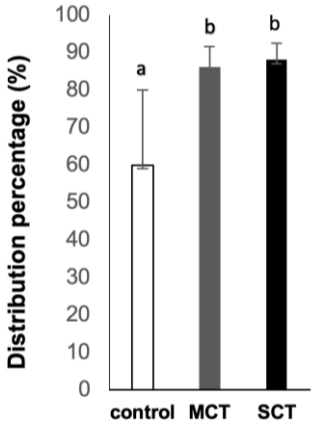

Figure 2: Insulin Receptor on Skeletal Muscle

HOMA-IR in MCT and SCT were reduced, but there was no statistical difference HOMA IR between MCT and control after 8 weeks of exercise. But in SCT group reduction of HOMA-IR after 8 weeks exercise was statistically different compared with control and MCT.

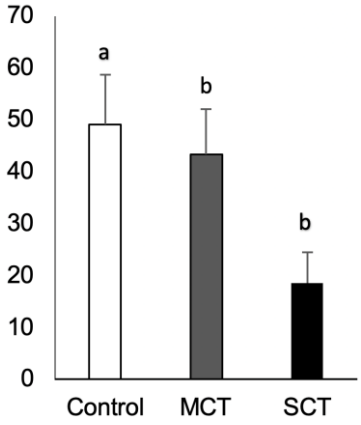

Figure 3: HOMA-IR; (**) note: groups with the same alphabet marked no statistically different, meanwhile group with different alphabet marked significantly different

Discussion

The purpose of this study was to analyse the difference *Insr* gene expression due to metabolic state in moderate and severe training in the T2DM rat model. We treated the group with the same condition between moderate and severe training group but the difference in intensity.

The result of this study shows that gene *Insr* expression, which underwent eight weeks of exercise, are found lower compared to the controlled samples, with a remarkable rose in cycling threshold (Δct) in the group samples that carried out the exercise assignment (Figure 1) Severe and moderate continuous aerobic exercise are found to have a significant effect on gene *Insr* expression (Figure 2). During exercise, ATP ratio and creatine will decrease, causing activation of enzyme AMP Kinase. The other metabolic enzyme that is mTOR also active. This enzyme plays a role as an integrated intracellular and extracellular signalling. Both AMPK and mTOR play together as a predominant enzyme in post-transcriptional gene and their activity can altered gene transcription [14]. Although the secretion of insulin have been depressed by sympathetic activity, by activating phosphorylation of AMPK — ACC, Ca2+ moduli skeletal muscle will stimulate translocation of GLUT-4 to the cell surface [15], [19].

State of metabolism highly affects the transcription of a gene [20]. A relationship was found among the state of metabolism, enzyme metabolic and transcription of a gene [9]. In the body of DM Type-2 patient, there is an interference of insulin signalling process causing the failure of translocation of GLUT-4 to the cell surface, resulting in an obstruction of glucose to diffuse into the cell [21]. Cellular stress is also one of the direct causes for the deficient of glucose in the cell by stimulating the secretion of cortisol in the adrenal cortex [11], [22]. Cortisol is essential in the gluconeogenesis process. Increase in plasma cortisol indicated that there would be stimulation to transcription of the gene insulin receptor, thus synthesising the insulin receptor [23]. Insulin synthesis will decelerate when insulin receptor is fully bonded, and the body is no longer in need of it.

Cortisol secreted during exercise will increase the rate of transcription of a gene. According to E.E. Hill et al., in a study, the frequency of cortisol in blood plasma increases significantly during exercise with the intensity of moderate and severe [24]. Metabolism among enzymes will stimulate biogenesis of mitochondria by activating PGC-1 of alpha mitochondria. High level of cortisol, enzyme metabolism rate and activation of PGC-1 alpha will stimulate the transcription of gene receptor insulin [9], [25]. The exercise that had been carried out eight weeks consecutively increase the amount of insulin receptor (Figure 2), increase in the sensitivity of insulin receptor, as well as decrease the insulin resistance (Figure 3), which results in the change of metabolic state. The difference in a metabolic state highly related to the decrease in gluconeogenesis in the liver causing a drop in gene transcription.

However, there were no such limitations observed in sedentary group samples, the limited amount of exercise carried out by sample, also causing a low glucose transportation rate in the cells. Increase in the rate of cortisol, produced by the compensated mechanism, will eventually stimulate the transcription of the gene to be continuously rising.

In this study, we found that there was a difference in the expression of gene *Insr* in rat models T2DM between moderate continuous exercise and severe continuous exercise. Samples which are given moderate continuous exercise have shown a higher

cycling threshold in mRNA gene *Insr* compared to the severe continuous exercise group. It is proven by there is a lower expression of gene expression in the moderate continuous exercise group compared to severe continuous, after eight weeks of exercise (Figure 1). Metabolisms of the cells in severe continuous exercise group samples are higher compared to the moderate continuous due to their intensity. According to E.E. Hill rate of cortisol in vigorous-intensity was higher compared to moderate one.

Reduction in insulin resistance and blood glucose level of the samples were found after eight weeks of exercise assignment. However, the decrease in insulin resistance in moderate continuous exercise group samples was not significant enough compared to the control groups, although there was a substantial reduction in blood glucose level compared to pretest.

In conclusion, exercise with high intensity will affect the expression of mRNA gene receptor and the synthesis of insulin receptor at the surface of muscle cells. Although a more drastic rise in the amount of receptor was recorded in severe continuous exercise group samples after eight weeks of exercise, a more precise consideration should be applied to the rate of cortisol and blood glucose level, appointed to T2DM patients. For those moderate continuous exercise individuals, it is recommended to carry out the more frequent exercise with the same consistency, in looking forward to a result of a significant decrease in insulin resistance.

Ethics

The study protocol was reviewed and approved by the Health Research Ethical Committee, Medical Faculty of Universitas Sumatera Utara, Medan, Indonesia (no. 263/KOMET/FK USU/2016).

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