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Effect various type of exercise to *Insr* gene expression, skeletal muscle insulin receptor and insulin Resistance on Diabetes Mellitus Type-2 model Rats

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Abstract

Physical activity is one of type-2 Diabetes mellitus(T2DM)management. Previous studies proved that aerobic training could improve insulin receptor sensitivity and insulin resistance. Aerobic training can be continuous and interval training. This study aimed to analyse the effect of various type of exercise to *Insr*gene expression, skeletal muscle insulin receptor, and HOMA-IR changes. It was an experimental study, using twenty male Wistar, aged eight weeks, weight 150-180 gram, T2DM model. We divided Rats into five groups, one group as control (sedentary) and four groups as treated groups, i.e., moderate continuous training (MCT), severe continuous training (SCT), slow interval training (SIT) and fast interval training (FIT). All treatment groups ran on the treadmill three times a week for eight weeks. Blood glucose level and HOMA-IR were checked before and after the exercise assignment. *Insr* gene expression was assessed by qPCR, skeletal muscle insulin receptor determined by IHC, fasting insulin was examined by ELISA, and blood glucose level was checked by using a spectrophotometer.The result found that *Insr* gene expression decline, Insulin receptor distribution on skeletal muscle increased, insulin resistance and fasting blood glucose decreasedin treated groups after eight weeks of exercises compared with control ($p<0,05$).In continuous groups, MCT was better than SCT to decline *Insr*gene expression, while SCT tends to better than MCT to increase skeletal muscle insulin receptor and decreased insulin resistance. In interval groups, FIT was tending to a better model than SIT to decreased *Insr*gene expression and increased skeletal muscle insulin receptor, while SIT tends to better decreased insulin resistance than FIT. Among the four models, SIT has the lowest insulin resistance at the post-test. The lowest *Insr*gene expression and highest skeletal muscle insulin receptor distribution were found in the FIT group.
In conclusion, there was the effect of type of exercise to *Insr* gene expression, skeletal muscle insulin receptor, insulin receptor and fasting blood glucose in type-2 DM model rats. Fast interval training was the best model to decline *Insr*gene expression and increased skeletal muscle insulin receptor. Slow interval training was the best model for decreased insulin resistance.

Keyword: aerobic training, *Insr* gene expression, insulin receptor, HOMA-IR, Type-2 DM, rat

Introduction

Since Indonesia become an epidemic in type-2 diabetes mellitus (T2DM), world health organization (WHO) predicted the prevalence of T2DM would become increased dramatically by 2030 [1,2]. Lifestyle change in diet and physical activity effect to cell metabolism and DNA methylation lead to insulin resistance [3]. The mechanisms of insulin resistance involving cellular metabolic disturbances lead to the management of T2DM should be followed by proper dietary regulation and measurable and regular exercise[4]. American Diabetes Care recommended aerobic exercise for people with type 2 diabetes [5] Aerobic exercise can be done continuously or interval.

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[6]The previous study found that both continuous training or interval training can improved insulin sensitivity on skeletal muscle, mitochondrial function, and promote glucose transporter (GLUT) – 4 exocytoses to the membrane surface, so that insulin resistance and fasting blood glucose level of the patient remain stable[7,8,9,10]. Insulin hormone is a sensor for the insulin receptor. Hyperinsulinemia caused down regulation of insulin receptor, while hypoinsulinemia promotes receptor insulin density. In T2DM patient, hyperglycemia due to diminished insulin receptor sensitivity lead to hyperinsulinemia. Chronic effect of hyperglycemia and hyperinsulinemia caused down-regulation insulin receptor [11]. Exercise spurred mitochondrial activity. Mitochondrial activity enzyme such as AMPK which increase by exercise and metabolism hormone effect to gene transcription [12] Different type of exercise will produce a different effect on gene transcription as a different effect on cell metabolism.

Few studies have been done to determine AMPK, mitochondrial biogenesis, insulin resistance, and blood glucose level as a result from exercise, but the effect of different type of exercise to insulin receptor expression still unknown. The study aimed to compare effect various type of exercise to *Insr* gene expression, insulin receptor on skeletal muscle, and insulin resistance.

Material and Methods

Animal model and exercise protocol

This Experimental study used twenty healthy male Wistar rat, aged 8 weeks, 150-180 gram in weight, housed in cages with room temperature, 12h/12h light-dark cycle T2DM model was made by providing high-fat diet 41% fat, 41% carbohydrate, dan 18% protein for 5 weeks and injecting twice of low dose streptozotocin (NacalaiTesgueInc) modified from Zhang etal protocol[13]. T2DM rats model was determined when fasting blood glucose was >200mg/dl and insulin resistance determined with HOMA-IR. Groups were divided into sedentary, continuous groups i.e moderate continuous training (MCT), severe continuous training (SCT) and interval groups i.e slow interval training (SIT), fast interval training (FIT). Exercise protocol was performed for 8 weeks, 3 times per week. Exercise protocol followed Huang et al (table.1)[14].Fasting blood glucose (FBG), fasting insulin and HOMA-IR recorded before and after exercise protocol was assigned. T2DM model rats were execution under sedation (ketamine 30 mg i.m), *musculus gastrocnemius* was taken for *Insr* gene expression. mRNA expression analysis by quantitative real-time PCR and insulin receptor distribution on skeletal muscle was determined by immunohistochemistry.

Elisa Examination

About 10 µlof blood serum taken from the vein tail is required for insulin examination. The examination of Insulin by Elisa procedure followed INS- Rat kit Qayee Bio. Insulin level was red by Elisa reader in 450 nm wavelength.

mRNA*Insr* gene expression

RNA from about 20-30 mg *musculusgastrocnemius* of each sample has been isolated by using RNeasy Mini Kit (cat Nos .74104 dan 74106) from Qiagen (Germany) and procedure protocol followed the instruction within. KAPPA SYBR MM 10 µl, primer IR forward 0,4µl, primer IR reverse 0,4 µl, KAPPA RT Mix 0,2µl Template RNA 2µl, and ddH2O 7µl mix in 0,2 ml PCR microtube. βactin gene acts as a reference gene. Primer IR F 5'-GGC CAG TGA GTG CTG CTC ATG C-3'. Primer IR R 5'-TGT GGT GGC TGT CAC ATT CC-3'. β actin F 5'-CAC CCG CGA GTA CAA CCT TC–3', β actin R 5'-CCC ATA CCC ACC ATC ACA CC –3' mRNA *Insr* gene expression then analyzed with quantitative real-time PCR (Rotor gene) incubation 42^0C 5 minute, denaturation start with 92^0C 5 minute and continued 45^0C 10 seconds, Annealing/extension 60^0C 30 seconds. Real-time PCR running for 40 cycles. PCR product was analyzed with LivaskMethode determined with ΔΔCt.

Immunihitochemistry

Immunohistochemical examination was initiated by deparination of the rats*gastrocnemius* tissue with xylol I and II respectively for 5 minutes. Dehydrated with ethanol absolute and ethanol 90% and 70%. Sample preparation was soaked in a phosphate buffer saline (PBS) 10mM citrate buffer pH 6 and heated in a high-temperature microwave. The primary antibody was dripped after the preparation was incubated at room temperature, dripped with blotto solution and passed three times with PBS. To clarify the reading of IHC, preparations are added to secondary antibodies.

Statistic Analysis

Data normality is determined by Shapiro Wilk (p>0,05). HOMA–IR pre and post-test each treatment groups was analyzed with *pair t-test*. To analyze differences in *Insr* gene expression and HOMA-IRamong groups we used *one-way ANOVA*, and to analyze differences percentage of insulin receptors distribution on skeletal muscleswe used *Kruskal Wallis*. Data was significant if p-value <0,05.

Ethical Approval

This study has been approved by the local ethics committee at Faculty of Medicine of Universitas Sumatera Utara and Adam Malik Hospital Medan after full board presentation in front of all committee memberswith ethical number 263/KOMET/FK USU/2016.

Table. 1 Exercise protocol for moderate continuous training, severe continuous training, slow interval training, and fast interval training

Groups	Speed	Duration
MCT	25 m/minutes	30 minutes
SCT	30 m/minutes	30 minutes
SIT	25 m/minutes	2 minx10, with 1 min active rest
FIT	30 m/minutes	30sec x 15, with 1min active rest

Note :*MCT = moderate continuous training, SCT= severe continuous training, SIT= Slow interval training, FIT= fast interval training*

Results

The purpose of this study was to analyze the different effects of various types of exercise on insulin receptor gene expression, skeletal muscle insulin receptors and insulin resistance in type-2 DM model rat. Each treatment group was given a different type of exercise throughout 8 weeks. Gastrocnemius muscle and sample blood from both sedentary group and treatment groups were taken to assess mRNA gene expression, insulin receptor distribution percentage, insulin resistance (HOMA-IR) and blood glucose level.

To determine the effect of FIT, SIT, SCT and MCT compared to sedentary on decreasing expression of *Insr* gene in the T2DM model rat after exercise assignment, ANOVA assay was performed by comparing the measurement results of the *Insr* gene expression among groups. The result of the ANOVA test showed that there was a difference effect of FIT, SIT, SCT and MCT on the mRNA *Insr* gene expression compared to the control (p= 0,03). Furthermore, to see the difference between the four types of exercises we used LSD post hoc test. The different effect between groups on mRNA *Insr* gene expression can be seen in the figure.1

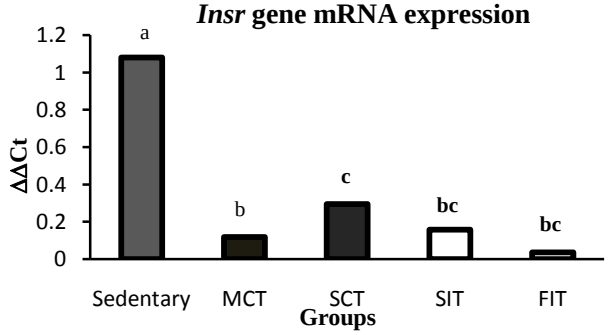

Figure. 1*Insr* mRNA gene expression sedentary group compared treatment groups after 8 weeks exercise in T2DM model Rat. MCT and SCT were continuous training, while SIT andFIT were interval training. Group with the same code (a, b, c) hasa significant difference

Figure .1 showed that mRNA expression of *Insr* gene in treatment groups were lower than sedentary group (p=0,003) and the lowest expression was found in the FIT group. mRNA expression of *Insr* gene in SCT group slightly increased compared to the other three groups.

From the figure above, seems that intensity and type of exercise influence gene expression. Continuous training in severe intensity, promote *Insr* gene expression increased slightly compared to moderate intensity (p=0,047) although it remained lower than control. However, in interval training groups, increased intensity did not give a different effect to mRNA *Insr* gene expression. This study found there were no significant differences between SIT and FIT (p=0,851)). Interval training groups gave the same effect to mRNA *Insr* gene expression both carried out slow intervals (SIT) and fast intervals (FIT).

Insulin receptors expression on skeletal muscle were determined by the percentage of insulin receptor distribution. Kruskal Wallis test was performed by comparing the results of skeletal muscle insulin receptor distribution percentage in MCT, SCT, SIT, and FT compared to control. The results test found that all treatment group insulin receptor were rose following eight weeks exercise and percentage of distribution was more than a control group (p=0,002) (figure.2) But there was no significant difference insulin receptor distribution on skeletal muscle among treatment groups (p>0,05). The FIT group has the most percentage distribution insulin receptor on skeletal muscle compared to other groups.

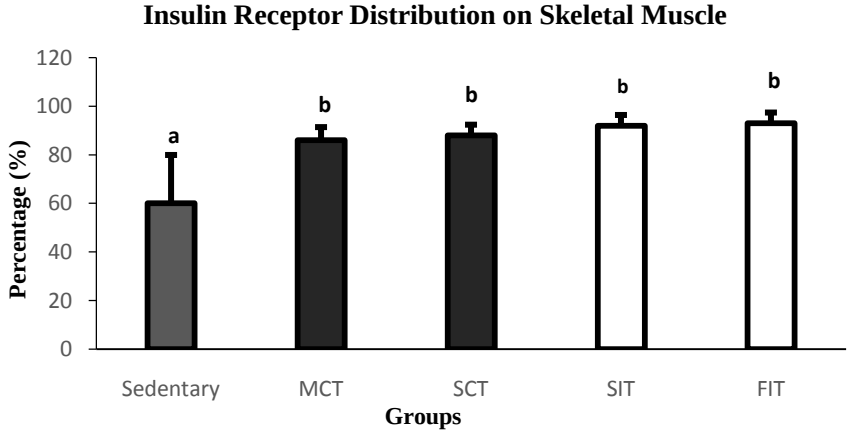

Figure. 2 Insulin receptor distribution on skeletal muscle after eight weeks exercise.MCT and SCT were continuous training, while SITdanFIT were interval training.Group with the same code (a, b, c) hasa significant difference

Changing in *Insr* expression and insulin receptor distribution as the effect of exercise followed by insulin resistance reduction. Based on the ANOVA test, there was significant different insulin resistance between sedentary group and treatment groups (p=0,009). Post Hoc LSD test result found SCT, SIT and FIT showed significant differences compared to sedentary group and MCT group, whereas we have not found significant decreasing of insulin resistance in moderate continuous training (MCT) after eight-week exercise compared to sedentary (p= 0,066)

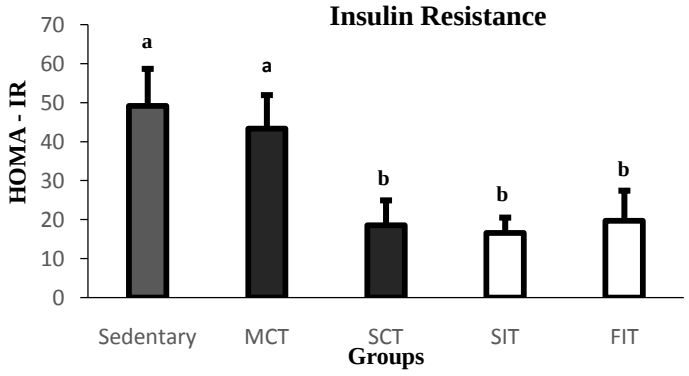

Figure 3. Insulin resistance in treatment groups compared sedentary group.

Discussion

The mechanism of insulin resistance can occur at pre-receptor, receptor and post-receptor. The arrangement at the receptor level corresponds to the amount of the receptor density on the cell surface and the receptor polymorphism. Receptors are needed as insulin ligand for insulin to activate insulin-signaling so that the translocation of Glut-4 to the surface of the cell membrane occurs [15]. Exercise can improve insulin resistance by a modified neurohormonal system so that insulin receptor promoted to increase [16,17]. The purpose of this study was to analyze the effect of various type of exercise to *Insr* gene expression, insulin receptor on skeletal muscle, and HOMA-IR changes.

In this study, we found that there was a significant difference in mRNA *Insr* gene expression in T2DM model rat between exercise groups and sedentary group whereas mRNA *Insr* gene expression of skeletal muscle in exercise groups shown lower expression compared to the inactive group after eight weeks of exercise. There was a significant difference mRNA *Insr* gene expression among continuous training group with different intensity. Meanwhile, there was no significant difference mRNA *Insr* gene expression among the interval groups. Moderate continuous training and severe continuous training were high volume exercise but difference intensity, while SIT and FIT was low volume with high energy. The recent study has shown that exercise intensity influence *Insr* gene expression.

Insulin receptor expression on the skeletal muscle is mRNA receptor insulin gene translation product, but mRNA that has being transcripted does not always continue to mRNA translation. In this study, all treatment groups showed increased insulin receptor distribution on skeletal muscle of T2DM rat model. This suggested that there was an effect of exercise to insulin receptor protein expression so that insulin density on skeletal muscle was increased.

Exercise affects the metabolism of muscle cells. Cell metabolism produces the energy needed for muscle contraction. The higher the intensity, the more power in metabolic will required [18].Metabolism and gene expression are interrelated. From a kinetic model that was successfully compiled by Vital-Lopez et al (2013) showed an accurate mechanistic link between gene expression and cell metabolism[19]. Metabolism and metabolite enzymes affect gene transcription, and conversely, gene transcription affects metabolic status. Metabolic state affects mitochondrial action, metabolic enzymes, and gene expression. Several factors influence gene expression, namely extracellular signals, steroid hormones, metabolic enzymes, and chromatin modulators. Extracellular signals such as hormones activate transduction signals giving direct transcriptional responses to gene expression to alter metabolic status. Similarly, steroid hormones bind to the receptors in the nucleus [20].

Previous studies found that glucocorticoid and insulin hormones influence the expression of insulin receptors on the cell surface [21]. Glucocorticoid hormone increases insulin receptor biosynthesis by increasing the transcription of mRNA from the insulin receptor gene until the mRNA reaches a steady state.

In patients with T2DM, insulin resistance causes the body's cells to lack energy due to disturbed mitochondrial oxidation. For mitochondrial oxidation to continue, secretion of glucocorticoid hormone (cortisol) from the adrenal cortex increases to free up replacement energy from glucose reserves, fat reserves and protein reserves through gluconeogenesis. However, exposure to cortisol for a long time can damage metabolism and insulin action due to interference with glucose uptake and use of *free fatty acid* (FFA) as energy. The increased FFA stimulates the release of proinflammation. Meanwhile, cortisol also stimulates insulin secretion, but hyperinsulinemia causes receptor down-regulation [21,22]. That is why suggested in this study the mRNA expression of the insulin receptor gene increased in the sedentary group but was not followed by an increase in the number of receptors.

During exercise, acute response during exercise suppressed insulin secretion by sympathetic nerve work, insulin clearance and Insulin-degrading enzyme (IDE) from the liver were elevated resulting in hypoinsulinemia. [23]Low plasma insulin levels were a weak signal for receptors to stimulate the upsurge of receptor regulation[24]

In this study, the distribution of skeletal muscle insulin receptors was more in SCT group than in MCT group, as were the distribution of skeletal muscle insulin receptors more in the FIT group than in SIT group. This phenomena in line with insulin concentration alteration, insulin signaling, and insulin receptors are evident in moderate-to-severe exercise [24, 25].

Increased metabolism in exercise with higher intensity causes an increase in cortisol levels from the adrenal cortex, promoted increases in gene expression and the number of receptors on the surface of skeletal muscle. [26]However, long-term improvements in cortisol levels when exercise on severe intensity continuous training can increase blood sugar levels that are higher than moderate intensity even lower than sedentary group, so that mRNA expression still increases even though insulin receptor distribution has upregulated in recent study. While in training intervals, periodic intervals caused a decrease in expression and an increase in the number of receptors and gave no significant difference between slow intervals and fast interval.

Conclusion

Type and intensity of exercise affect insulin receptor gene expression, percentage distribution of insulin receptors in skeletal muscle and changes in insulin resistance in the rat model of type-2 diabetes mellitus. Exercise for eight weeks decreases mRNA expression of the insulin receptor gene and increases the distribution of insulin receptors in the skeletal muscle. Fast interval training reduces the expression of insulin receptor genes lowest than other treatment groups and slow interval training reduced insulin resistance lowest among other treatment groups. Interval training models can be used as alternative exercise models for patients with T2DM.

Conflict of Interest

There was no conflict of interest in this study.

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