

Effect of Exercise Intensity in *Glut4* Expression on Type 2 Diabetes Mellitus Rat

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ABSTRAK Glucose transporter4 (GLUT4) merupakan transporter glukosa utama pada otot rangka. Gangguan ekspresi GLUT4 berperan dalam gangguan homeostasis glikemik seperti Diabetes Mellitus Tipe-2 (DMT2). Olahraga dapat meningkatkan ekspresi GLUT4 pada otot rangka sehingga glukosa dapat masuk ke dalam sel lebih cepat. Intensitas olahraga merupakan faktor penting dalam menurunkan kadar glukosa darah pada pasien DMT2. Penelitian ini menggunakan bahan biologis simpanan otot gastrocnemius dari 15 tikus Wistar model DMT2. Tikus dibagi menjadi tiga kelompok perlakuan yaitu P0 (tanpa latihan, kontrol), P1 (latihan terus menerus dengan intensitas sedang), dan P2 (latihan terus menerus dengan intensitas kuat), dan diberi perlakuan selama 8 minggu. Ekspresi *glut4* ditentukan oleh real time-PCR. Ekspresi relatif gen *Glut4* dihitung menggunakan rumus Livak. Latihan intensitas sedang meningkatkan ekspresi *Glut4* sebesar 2,39 kali sementara latihan intensitas tinggi meningkatkan ekspresi *Glut4* 2,56 kali dibandingkan dengan kontrol. Latihan terus menerus dengan intensitas tinggi menunjukkan ekspresi *Glut4* yang lebih tinggi daripada latihan berkelanjutan dengan intensitas sedang.

Kata Kunci: DM Tipe 2, *Glut4*, Latihan, Tikus.

ABSTRACT *Glucose transporter4 (GLUT4) is the main glucose transporter in skeletal muscle. Impaired GLUT4 expression plays a role in the disorders of glycemic homeostasis such as Type-2 Diabetes Mellitus (T2DM). Exercise can increase the GLUT4 expression in skeletal muscles so that glucose can get into the cells faster. The intensity of exercise is an important factor in reducing blood glucose levels in patients with T2DM. This study used stored biological material of gastrocnemius muscle from 15 T2DM models of Wistar rats. Rats were divided into three treatment groups namely P0 (no exercise, control), P1 (moderate-intensity continuous exercise), and P2 (vigorous-intensity continuous exercise), and treated for 8 weeks. *Glut4* expression was determined by real time-PCR. The relative expression of the *Glut4* gene was calculated using the Livak formula. Moderate-intensity exercise increased *Glut4* expression by 2.39 times while vigorous-intensity exercise increased *Glut4* expression 2.56 times compared to control. The vigorous-intensity continuous exercise expressed higher *Glut4* expression than the moderate-intensity continuous one.*

Keywords: Exercise, *Glut4*, Rat, Type 2 DM.

PENDAHULUAN

Glucose is the main fuel for skeletal muscle and impaired glucose metabolism induces various metabolic diseases such as

insulin resistance and Type-2 Diabetes Mellitus (T2DM). Glucose transporter4 (GLUT4) is the main glucose transporter in skeletal muscle.¹ Insulin stimulates glucose uptake in skeletal muscle by translocating intracellular vesicles containing GLUT4 to integrate into the plasma membrane.² In the T2DM patients muscles, there is a decrease of around 30% in the amount of GLUT4.³ Carvalho et al. (2001) also found that GLUT4 expression decreased by about 60% in patients with T2DM.⁴ GLUT4 overexpression may improve glycemic control. The amount of GLUT4 correlates positively with glucose uptake. During muscle contraction in mouse skeletal muscle, *Glut4* overexpression increases glucose uptake by 35%, but muscle-specific loss of *Glut4* impaired glucose uptake.⁵ Therefore, disturbances in GLUT4 expression are the focus in the management of impaired glycemic homeostasis.

Lifestyle modifications, including exercise, can reduce the incidence of T2DM.⁵ Exercise improves glucose control by increasing glucose uptake through both insulin-dependent and insulin-independent mechanisms. Exercise also increases the expression of GLUT4 in skeletal muscle so that the amount of GLUT4 protein increases and glucose can enter cells faster.^{7,8,9}

Exercise intensity is an important factor in reducing risk factor of metabolic syndrome.¹⁰ However, another study showed that there was no difference between low and vigorous-intensity exercises in GLUT4 expression.¹¹ Therefore, this study will examine the effect of moderate-intensity exercise and vigorous-intensity exercise on *Glut4* expression in T2DM model Wistar rats.

METODE

This study used stored biological materials derived from 15 male Wistar rats treated into T2DM model rats previously.¹² The rat muscles were stored in -80°C freezer.

Modeling of T2DM in rats was carried out by providing a high-fat diet for 5 weeks and then injected with 30 mg/kgBW and 45 mg/kgBW streptozocin. T2DM model rats were determined when fasting glucose levels were >200 mg/dL and insulin resistance was determined by the HOMA-IR value > 6.5.¹²

Rats were divided into 3 groups, namely P0; not given exercise as a control group, P1; group with moderate-intensity continuous exercise (running on a treadmill with a running speed of 20 m/minute, for 30 minutes), and P2; group with vigorous intensity continuous exercise (running on a treadmill with a running speed of 24-33 m/minute, for 30 minutes). Exercise was done every two days for 8 weeks.

RNA was extracted from gastrocnemius muscle tissue. To determine the quality of the biological material stored in this study, the purity of RNA extraction were examined using the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Newington, NH, USA). The results of the RNA extraction were then

Table 1. Primers Sequences

Gene	Sequences
<i>β actin</i>	F 5'-AGGCCCTCTGAACCTAAG-3' R 5'- ATGTCACGCACGATTCCT-3'
<i>Glut4</i>	F 5'- GGCCGGGACACTATACCCTA-3' R 5'- GGAGGAAATCATGCCACCCA-3'

Table 2. RNA Purity of stored T2DM Gastrocnemius Muscle

	Mean ± SD
RNA Purity	1,86±0,08

Table 3. Relative *Glut4* Gene Expression

Group	Average Δ Ct	$\Delta\Delta$ Ct	Relative gene expression	Up/Down regulation
P0	4,72	0	1	
P1	3,46	-1,26	2,39	upregulation
P2	3,36	-1,35	2,56	upregulation

P0, not given exercise (control); P2, moderate intensity continuous exercise; P3, vigorous intensity continuous exercise; Relative gene expression, $2^{-\Delta\Delta Ct}$; Δ Ct = Ct *Glut4* - Ct Beta actin; $\Delta\Delta$ Ct = Δ Ct (treatment group) - Δ Ct (control group).

SIMPULAN

Since this study used stored biological material then it is necessary to determine the tissue quality by measuring the RNA purity. RNA purity is based on the ratio of 260 nm and 280 nm waves. If the ratio of 260/280 gives a value

continued with cDNA construction. Real time-PCR reaction amplified *Glut4* and rat *beta actin* using the designed primers (Table 1).

HASIL PENELITIAN DAN PEMBAHASAN

Data was processed using IBM SPSS Statistics software version 23. Data was presented with mean and standard deviation. The relative expression of the *Glut4* gene was calculated using the Livak formula, $Rq = 2^{-\Delta\Delta Ct}$ [$\Delta\Delta$ Ct = Δ Ct (target) - Δ Ct (β -actin); Δ Ct = Ct (sample) - Ct (control)].¹³

The mean purity of RNA extraction in this study were 1.86 ± 0.08 (Table 2). Exercise for 8 weeks was found to increase the expression of *Glut4* in rats of T2DM model. Moderate-intensity exercise increased *Glut4* expression by 2.39 times and vigorous-intensity exercise increased *Glut4* expression 2.56 times compared to control (Table 3).

in the range of 1.8-2.0, then this is one indicator that RNA is classified as good for use in research.¹⁴ In this study, the RNA purity was quite good with a mean of 1.86 ± 0.08 .

GLUT4 is the main glucose transporter in skeletal muscle.¹ Analysis of the GLUT4 gene promoter showed that myocyte enhancer factor (MEF2) is important for normal expression of GLUT4 in skeletal muscle. Decreased MEF2 expression was associated with decreased GLUT4 expression. In the resting state, MEF2 binds to the histone deacetylase 5 (HDAC5) transcriptional repressor. HDAC5 makes the chromatin structure very dense so that it suppresses the action of MEF2. This leads to

repression of MEF2-dependent DNA transcription.¹⁵

In this study, it was found that moderate and vigorous intensity exercise increased Glut4 expression compared to the control group. During exercise, Adenosine Monophosphate activated Protein Kinase (AMPK) is activated and causes phosphorylation and removal of HDAC5 from the nucleus, so that peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1 α) as coactivator binds to MEF2. This stimulates MEF2 activity and increases GLUT4 expression.^{9,16} In addition, aerobic exercise for 6 weeks caused HDAC4/5 dissociation from the GLUT4 promoter thereby increasing transcription of the GLUT4 gene.¹ Brief exercise increases GLUT4 transcription temporarily and the expression returns to pre-exercise levels within 18-24 hours. Therefore, prolonged exercise may increase

GLUT4 protein as a result of the cumulative effect of transient increases in GLUT4 mRNA, which stimulates GLUT4 protein synthesis over a long period of time.¹⁶ Previous study showed that moderate intensity continuous exercise and severe intensity continuous exercise in T2DM rats significantly reduce fasting blood sugar.¹⁷

During exercise, glucose uptake in contracting muscles increases 7-20 times compared to basal conditions, depending on the intensity of the exercise performed.¹⁸ In this study, the vigorous intensity exercise group increased Glut4 expression higher than the moderate intensity exercise group. This is in line with the research of Cunha et al. (2015) in T2DM rats model, given low-intensity and high-intensity swimming exercise for 8 weeks. The result is that high-intensity exercise is more effective in increasing the amount of GLUT4 than low-intensity exercise.¹⁹ Greater exercise intensity activates Ca²⁺/Calmodulin-dependent protein kinase (CaMK) and, subsequently, AMPK will increase GLUT4 expression higher than low intensity exercise.⁹ AMPK and CaMK activation as well as HDAC phosphorylation in human skeletal muscle increased after high-intensity exercise and did not increase in low-intensity exercise.²⁰

Moderate and vigorous intensity continuous exercise increased Glut4 expression in T2DM model rats and the vigorous-intensity continuous exercise group increased Glut4 expression higher than the moderate-intensity continuous exercise group.

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