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**Thesis of Master of Science**

**The Effects of Resistance Exercise on Muscle  
Fiber Type Composition and GLUT4 Expression  
in Zucker Diabetic Rat Skeletal Muscle**

**8주간의 저항성 운동이 제 2형 당뇨 쥐(Zucker rat)의  
근섬유 조성과 GLUT4 발현에 미치는 영향**

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## ABSTRACT

**Backgrounds:** Skeletal muscle is a major organ that control glucose uptake and utilization, and its metabolic and contractile properties are dependent on its fiber type and mass. In this study, we investigate whether 8 weeks of resistance training could result in an alteration of muscle fiber size, fiber type composition, and glucose transporter protein 4 (GLUT4) which could ameliorate the impaired glucose tolerance in Zucker diabetic fatty (ZDF) rats before and after onset of diabetes.

**Methods:** Five weeks old male Zucker rats were divided into Zucker Lean Control (ZLC-Con, n=7), non-exercised Zucker Diabetic Fatty (ZDF-Con, n=7), and exercised Zucker Diabetic Fatty (ZDF-Ex, n=7) rat groups. ZDF-Ex conducted 8 weeks of ladder climbing resistance training followed by non weight bearing adaptation period for one week. We conducted immunohistochemistry to investigate effect of exercise intervention on muscle fiber size, fiber type composition, and GLUT4 expression in gastrocnemius (GAS) muscle. Western blotting was also performed to detect the changes of GLUT4 expression level in the same muscle. Intraperitoneal glucose tolerance test (IPGTT) was also evaluated before and after diabetes onset.

**Results:** Fasting glucose level and area under the curve (AUC) responses to an IPGTT were significantly increased after onset of diabetes in the ZDF-Con compare to ZLC-Con, while it was decreased in the ZDF-Ex. There was no difference between ZLC-Con and ZDF-Con in GLUT4 expression but ZDF-Ex was

increased by 88.5%. The proportion of MHCI was lower, and MHCII was higher in the ZDF-Con than ZLC-Con while there was no alteration of muscle fiber type distribution in the ZDF-Ex.

**Conclusion:** Resistance training improves muscle glucose tolerance in ZDF rats result from up-regulating GLUT4 expression without any changes in muscle fiber type composition. However, muscle hypertrophy and composition were not altered by exercise training. Thus, we concluded that resistance training could be an effective treatment for promoting muscle glucose tolerance and glucose uptake capacity independent of muscle morphology in ZDF rats.

### **Key words**

Diabetes Mellitus, Zucker, Laddering resistance exercise, GLUT4, Muscle fiber type, Muscle fiber size

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## **LIST OF ABBREVIATIONS**

<b>T2DM</b>	<b>Type 2 Diabetic Mellitus</b>
<b>GLUT 4</b>	<b>Glucose Transporter Protein 4</b>
<b>MHC</b>	<b>Myosin Heavy Chain</b>
<b>ZDF</b>	<b>Zucker Diabetic Fatty</b>
<b>ZLC</b>	<b>Zucker Lean Control</b>
<b>Ex</b>	<b>Exercise</b>
<b>Con</b>	<b>Control</b>
<b>GAS</b>	<b>Gastrocnemius</b>
<b>IPGTT</b>	<b>Intraperitoneal Glucose Tolerance Test</b>
<b>WB</b>	<b>Western Blot</b>
<b>IHC</b>	<b>Immunohistochemistry</b>
<b>AUC</b>	<b>Area Under the Curve</b>
<b>BSA</b>	<b>Bovin Serum Albumin</b>
<b>IACUC</b>	<b>Institutional Animal Care and Use Committee</b>

## I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is rapidly becoming a worldwide epidemic. The International Diabetes Federation has been reported that at least 177 million people in the world have diabetes and approximately 90–95% of people among them are diagnosed with T2DM (Wang, Simar et al. 2009). Moreover, World Health Organization estimated that the population of T2DM patients will rise to 300 million by 2025 (Wang, Simar et al. 2009). This phenomenon is resulted from high nutrient intake and sedentary lifestyles and may accompany not only an accumulation of adipose tissue but also less muscle mass and strength which contributes to impaired glucose tolerance and insulin sensitivity.

Exercise and physical activity have been considered as one of the most protective manner for alleviating insulin resistance and glucose intolerance in T2DM. There has been substantial evidence to indicate the effect of aerobic exercise, but less attempts to conduct resistance or strength training methods (Wang, Simar et al. 2009). Therefore, the question of which exercise type would be the ultimate manner for alleviating T2DM is becoming focal interests.

Exercise training has several important adaptations to prevent and

treat insulin resistance, impaired glucose and T2DM (Boule, Haddad et al. 2001; Umpierre, Ribeiro et al. 2011; Bacchi, Negri et al. 2012). Both aerobic and resistance exercise training can lead to loss of body fat mass and therefore contribute significantly in preventing and ameliorating insulin resistance (Bacchi, Negri et al. 2012). Likewise, the importance of resistance exercise training also underlines preventive effect through muscle development (Dunstan, Puddey et al. 1998; Baldi and Snowling 2003; Fenicchia, Kanaley et al. 2004).

Skeletal muscle is the major organ of glucose utilization and lipid metabolism. Therefore improving metabolic capacity of skeletal muscle is to be highly regarded. The metabolic characteristic in skeletal muscle is dependent on its mass and fiber type (Chalmers and Edgerton 1989; Hirofuji, Nakatani et al. 2000; Nakatani, Nakashima et al. 2000). Adult skeletal muscle demonstrates plasticity and able to transform its fiber types in response to a variety of external stimuli, including chronic contractile activity such as endurance exercise training, loading state, motor neuron activity, and environmental and pathological conditions (Booth and Thomason 1991; Jarvis, Mokrusch et al. 1996; Pette 1998; Chibalin, Yu et al. 2000; Olson and Williams 2000; Hood 2001; Hawley 2002;

Fluck and Hoppeler 2003). For example, resistance training induces a decrease in the percentage of myosin heavy chain (MHC) Iib isoform and increase in MHC Iia (Charette, McEvoy et al. 1991; Adams, Hather et al. 1993; Andersen and Aagaard 2000).

Mature rat skeletal muscle fibers are generally classified as four different MHC isoforms, type I, Iia, Iix and Iib. Type I MHC isoform represents the slowest oxidative form and type Iib MHC isoform represents the fastest glycolytic form. Muscle fiber type transition generally occurs in I ↔ Iia ↔ Iix ↔ Iib, with the adjacent isoform (Schiaffino and Reggiani 1996; Buonanno and Fields 1999; Pette and Staron 2001).

It has been suggested in several animal studies that muscle fiber insulin sensitivity follows an order of type I > type Iia > type Iib (Berchtold, Brinkmeier et al. 2000; Olson and Williams 2000). Additionally, there is sufficient evidence to indicate that hyperinsulinemia or diabetes causes muscle fiber type transition from slow-oxidative twitch (type I MHC) to fast glycolytic twitch (type II MHC) (Houmard, O'Neill et al. 1999). Recently, it has been shown that individuals with T2DM and offspring of those with T2DM have proportionally less Type I fibers, and thus oxidative enzyme activity are diminished compared with normal individuals

(Marin, Krotkiewski et al. 1994; Hickey, Carey et al. 1995; Nyholm, Qu et al. 1997; Oberbach, Bossenz et al. 2006). Insulin sensitivity seems to correlate with the proportion of slow-oxidative fibers (Lillioja, Young et al. 1987). Furthermore, insulin-stimulated glucose transport (GLUT4) is greater in skeletal muscle mostly composed of slow muscle fibers (Henriksen, Bourey et al. 1990; Song, Ryder et al. 1999; Daugaard, Nielsen et al. 2000), thus, promotion of the slow muscle fibers may protect against the deterioration of insulin resistance and T2DM (Ryder, Bassel-Duby et al. 2003).

GLUT4 protein expression is diminished in type I fibers of T2DM patients and appears to increase with resistance exercise training (Gaster, Staehr et al. 2001; Wang, Simar et al. 2009). Therefore, alterations in muscle fiber type distribution could influence glucose tolerance (Schofield, Rehrer et al. 2012). GLUT4 protein expressed in the slow muscle fibers resulted in enhanced glucose uptake capacity compared with fast muscle fibers (Gaster, Poulsen et al. 2000). These are important characteristics for improving insulin sensitivity and muscle glycogen storage (O'Gorman, Karlsson et al. 2006; Christ-Roberts, Pratipanawatr et al. 2004).

Thus, increasing the proportion of slow-oxidative muscle fibers

will overcome the glucose intolerance and metabolic defects associated with insulin-resistant states. Additionally, inducing alteration in skeletal muscle fiber type transition from fast-to-slow could therapeutic adjustment for type 2 diabetic mellitus.

Therefore, the purpose of this study was to investigate the effect of 8-week ladder climbing resistance exercise on muscle fiber type size, fiber type distribution, and GLUT4 protein expression in medial gastrocnemius (GAS) muscles of Zucker diabetic rats.

## II. LITERATURE REVIEW

### 1.1. Zucker Diabetic Fatty (ZDF) rat

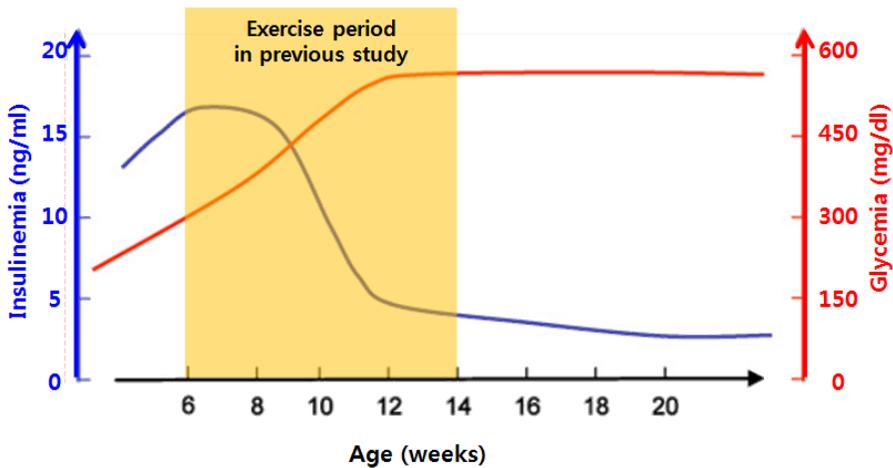


Figure 1. Age-dependent growth of glucose metabolism in ZDF rats (data from Pelvipharm and modified by Ji-Yeon Kim. 2013)

Zucker diabetic fatty (ZDF) rat is one of commonly used model of T2DM because the animal shows characteristics similar to T2DM in humans (Corsetti, Sparks et al. 2000)(Etgen and Oldham 2000). The progression of diabetic severity in ZDF rats is defined by several stages in age-dependent fashion through its life span. Hyperglycemic condition is rapidly progressed from 4 weeks of age to 12 weeks of age in the animal model (Etgen and Oldham 2000). The hyperglycemia which characterized these animals is due to the

increased hepatic production of very low density lipoproteins. Adipocytes are increased both in size and in number with the subcutaneous fat depot showing the largest increase in the number of fat cells. From the 7 weeks old to 8 weeks old period, insulin insufficiency is presented in the ZDF rat, and impaired glucose disposal and hepatic glucose output suppression are considered to be factors in the diabetes progression.

## **1.2. Muscle fiber type plasticity result from exercise**

Mature rat skeletal muscle fibers are generally classified as four different MHC isoforms, type I, IIa, IIx and IIb. These isoforms display marked differences according to contraction, metabolism, energy resources and enzyme activity (Booth and Thomason 1991; Berchtold, Brinkmeier et al. 2000; Olson and Williams 2000). MHC isoforms are widely used to measure the direction and extent of fiber type switching, with type I MHC representing the slowest form and type IIb MHC as the fastest one. During transition, the general direction is I → IIa → IIx → IIb, with the extent of transition depending on the intensity and duration of the stimuli. Adult skeletal muscle demonstrates plasticity and able to transform its fiber types in response to a variety of external stimuli, including chronic contractile activity such as endurance exercise training, loading state, motor neuron activity, and environmental and pathological conditions (Booth and Thomason 1991; Jarvis, Mokrusch et al. 1996; Pette 1998; Chibalin, Yu et al. 2000; Olson and Williams 2000; Hood 2001; Hawley 2002; Fluck and Hoppeler 2003). For instance, resistance training induces a decrease in the percentage of MHC IIb isoform and increase in MHC IIa isoform

(Charette, McEvoy et al. 1991; Adams, Hather et al. 1993; Andersen and Aagaard 2000). This transition of muscle fiber especially from type IIb to type IIa and type I is likely to be modulated by a  $\text{Ca}^{2+}$  signaling pathway that involves calcineurin, calmodulin-dependent kinase, and the transcriptional cofactor Peroxisome proliferator-activated receptor gamma coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) (Naya, Mercer et al. 2000; Olson and Williams 2000; Wu, Kanatous et al. 2002).

### **1.3. GLUT4 expression in type 2 diabetic mellitus**

Skeletal muscle in type 2 diabetes is insulin resistant, but is still insulin sensitive to exercise (DeFronzo, Simonson et al. 1982; Dohm, Tapscott et al. 1988). In the obese Zucker rat, a rodent model of type 2 diabetes that demonstrates muscle insulin resistance (Crettaz, Prentki et al. 1980; Sherman, Katz et al. 1988), impaired glucose uptake capacity is predominantly due to the failure of GLUT4 to translocate in response to insulin stimulation (King, Horton et al. 1992; Brozinick, Etgen et al. 1994). In terms of exercise, glucose uptake and GLUT4 translocation also developed in obese Zucker rat skeletal muscle (Sherman, Katz et al. 1988). Numerous factors determine the rate of glucose uptake during and after exercise, and one of the most important regulatory responses is an increase in blood flow to the contracting skeletal muscles (Goodyear and Kahn 1998).

### **III. MATERIALS AND METHODS**

#### **1.1. Animal model**

Male Zucker diabetic fatty (ZDF) and non-diabetic Zucker lean control (ZLC) rats (5 weeks old) were used for present experiment. All rats were housed in a controlled environment with a 12:12-h light-dark cycle with room temperature maintained at 22°C. All rats were provided with water and food ad libitum as recommended by Genetic Models Co. (Purina, St. Louis, MO, U.S.A.). The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals issued by Institute of Laboratory Animal Resources, USA, 1996, and a protocol for it was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University. All of the experiments were conducted to minimize the number of animals utilized and the suffering caused by the procedures of the present study.

## **1.2. Experimental design**

The animals were randomly assigned to each of the following groups: Zucker lean control (ZLC-Con; n=7) group; Zucker diabetic fatty control (ZDF-Con; n=7); Zucker diabetic fatty exercise (ZDF-Ex; n=7). IPGTT was conducted at the first, fourth, eighth week of training followed by 6 hours of fasting. ZDF-Ex group were forced to climb vertical ladder (length of 1m, incline of 85°) 3 times a week for 8 weeks. 48 hours after exercise training, animal sacrifice was performed to minimize the effect of the last bout of exercise. Following sacrifice, the medial gastrocnemius muscles were surgically removed and weighted. After muscle weight was measured, muscles were either embedded in 4% paraformaldehyde (PFA) for immunohistochemistry or snap frozen in liquid nitrogen and stored at -80°C.

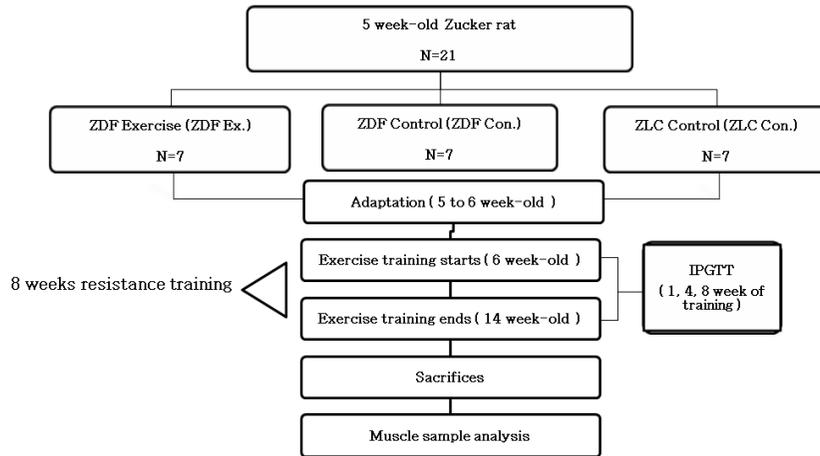


Figure 2. Experimental design

### 1.3. Exercise protocol



Figure 3. Ladder climbing resistance exercise

The rats in the ZDF-Ex group were forced to climb vertical ladder 3 day/wk for 8 weeks. Training was accomplished utilizing a 1-m ladder with 2-cm grid steps and inclined at 85°. Initially, rats were familiarized with the ladder by practicing climbing the ladder without weight from the bottom to the top for 3 days in the adaptation period. The only encouragement necessary was an occasional hands clapping at the bottom of the animal's tail. No

food reward was required nor any torturable stimulation, such as electric shock or forced air, in order for the rats to perform the exercise.

The initial weight attached to each animal's tail was 50% of its body weight. Rats were positioned at the bottom of vertical ladder and motivated to climb the apparatus. When the rats reached the top of the ladder, they were allowed to rest for 2 min. After the rest period, additional 20g were added in the weight, and the rats were returned to the bottom of the ladder for subsequent climbs. If a rat was able to climb the ladder with previous loads, additional 20g were placed in the weight for each subsequent climb. This procedure was repeated until ten climbs were achieved or until the rat failed to climb the entire length of the ladder. The training session was stopped when the rat succeed to climb 10 repetitions. Following exercise initial intensities were set at the 50 % of the maximal weight lifted in the previous day during first to third week of training, 70% of the maximal weight during fourth to sixth week, and 80% of the maximal weight during the rest of exercise periods.

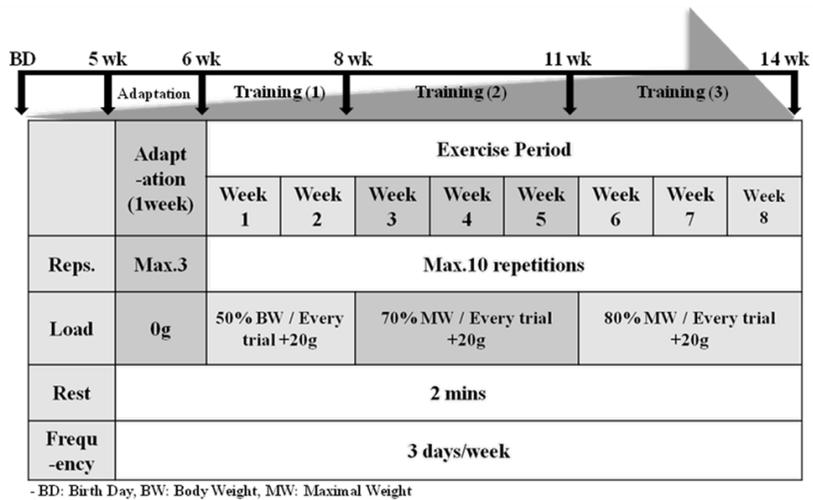


Figure 4. Exercise program

#### **1.4. Grip strength test**

Grip strength test was performed by allowing the animals to grasp a thin bar attached to the force gauge. This was followed by pulling the animal away from the gauge until the combined hind and forelimb released the bar. This provides a value for the force of maximal grip strength. The force measurements were recorded in three separate trials, and the maximum strength results were used in analyses. The grip strength was measured using a Grip Strength Meter (Bioseb, France), and a modification of a previously reported method (Meyer et al., 1979). The grip strength was measured at the last week of the exercise period. The animal was brought to all limbs made contact with and grasped the grid initially and pulled back gently but steadily until the grip was released. The maximum force (g) achieved by the animal before releasing its grasp from grid was recorded.

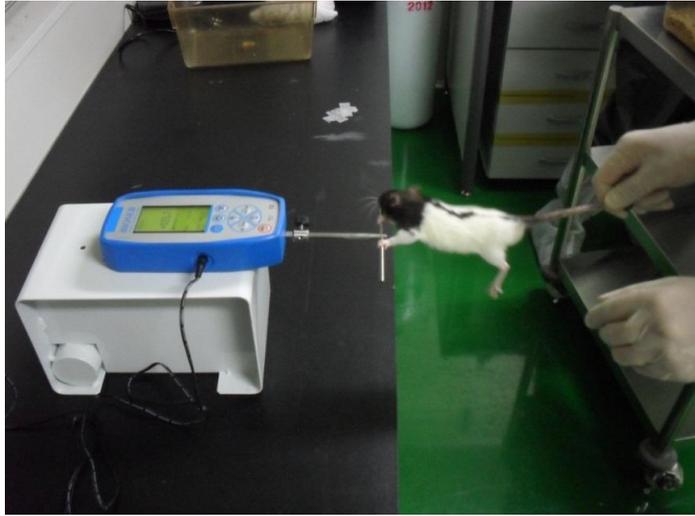


Figure 5. Grip strength test

### **1.5. Intraperitoneal glucose tolerance test (IPGTT)**

Glucose tolerance was assessed by intraperitoneal glucose tolerance test (IPGTT) after 1, 4 and 8 weeks of training, respectively. Rats were fasted for 6 h following intraperitoneal injection of 50% D-glucose solution (2g/kg). Blood samples were drawn from the tail vein at 0, 15, 30, 60, 90, and 120 min after glucose administration. Blood glucose levels were measured using a Glucometer (Accu-check, Germany).

### **1.6. Immunohistochemistry(IHC) Staining**

Animals in each group were anesthetized with 30 mg/kg Zoletil 50 (Virbac, Carros, France) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by overnight embedding with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) at room temperature. Muscles were postfixed in sucrose until it completely sank to the bottom. Several cross-sections of 4 $\mu$ m thick paraffin embedded samples were cut with a microtome (Leica

Microsystems, Germany). They were then incubated with diluted mouse anti-Myosin fast skeletal antibody (MY-32) (1:1,000, Novus), rabbit anti-Glucose Transport GLUT4 antibody (1:2,000, Novus), overnight at 4°C and subsequently exposed to biotinylated goat anti-mouse IgG and mouse anti-rabbit secondary antibodies (1:200, Vector, Burlingame, CA). They were then visualized by reaction to 3,3'-diaminobenzidine tetrachloride (Sigma) in 0.1 M Tris-HCl buffer (pH 7.2) and mounted on gelatin-coated slides. The sections were mounted in Canada Balsam (Kanto, Tokyo, Japan) following dehydration. Images were captured using an inverted microscopy system (ECLIPSE E100; Nikon, Kanagawa, Japan; <http://www.nikon.com>) and the cross-sectional area of each fiber was analyzed using a computer-assisted image processing system, INFINITY lite software (Innerview 2.0, Lumenera, Canada; <http://www.LUMENERA.com>).

### **1.7. Western Blot**

After euthanasia, the medial gastrocnemius muscles were rapidly removed, and stored at -80°C for the analysis of GLUT4 expression.

homogenised in 1.0 ml extraction buffer (RIPA). The extracts were then centrifuged at 12,000 rpm at 4°C for 40 min to remove the insoluble material. Determination of protein concentrations in the supernatants quantification of protein assay were performed using the Pierce BCA protein assay kit (Thermo scientific). The proteins were treated with Laemmli sample buffer containing dithiothreitol and boiled for 5 min before loading onto 8% SDS-PAGE gels in a Bio-Rad miniature slab gel apparatus. Aliquots containing similar amounts of protein (2.5µg/µl) were subjected to SDS-PAGE as described elsewhere (Carvalho et al. 1997). Electrotransfer of proteins from the gel to the nitrocellulose was performed for 3 h at 160 V (constant voltage) in a Bio-Rad miniature transfer apparatus. Nonspecific protein binding to the nitrocellulose was reduced by pre-incubation for 1 h at 22°C in blocking buffer (5% non-fat dry milk, 10 mM Tris, 150 mM NaCl and 0.02% Tween 20). The nitrocellulose membranes were incubated overnight at 4°C with antibodies against GLUT4 and  $\alpha$ -Tubulin (obtained from Novus Biotechnology; Novus, CO, USA) diluted in blocking buffer with 1% bovine serum albumin (BSA) and then washed for 30 min in blocking buffer without BSA. The blots were subsequently incubated with a peroxidase-conjugated secondary antibody for 1 h

at 22°C and processed for enhanced chemiluminescence to visualize the immunoreactive bands. The intensity of each band was quantified by densitometry and analyzed with Multi Gauge Version 3.0 software (Fuji PhotoFilm, Tokyo, Japan).

### **1.8. Statistical Analysis**

Statistical analyses were performed using SPSS version 18.0 software. Data were represented as mean  $\pm$  SEM. Statistical comparisons between subgroups were assessed by one-way analysis of variance (ANOVA) and Turkey's post-hoc was conducted. The IPGTT results were compared between groups by repeated measured ANOVA. P less than .05 was considered statistically significant.

## **IV. RESULTS**

### **1.1. Body weight and medial gastrocnemius muscle weight changes**

In the present study, we observed an increase in body weight of ZDF-Con group compared with ZLC-Con group. However, body weight was not altered after 8 weeks of resistance exercise training. Medial gastrocnemius muscle weight was not statistically different in all groups although relatively modest 10.1% of muscle weight gain was observed in ZDF-Ex group. Muscle wet weight divided into body weight showed significant difference in ZLC-Con and ZDF-Con group (Table 1).

Table 1. Body weight and gastrocnemius muscle characteristic

Variable	ZLC-Con	ZDF-Con	ZDF-Ex
	Mean $\pm$ S.E.M.		
Body weight (g)	272 $\pm$ 5.35	364.5 $\pm$ 13.57*	371.5 $\pm$ 17.42
		(.002)	(.925)
Muscle wet weight (mg)	586.25 $\pm$ 17.60	505 $\pm$ 15.14	556.25 $\pm$ 30.85
		(.070)	(.285)
Muscle wet weight/body weight (mg/g)	2.16 $\pm$ 0.09	1.39 $\pm$ 0.03**	1.50 $\pm$ 0.18
		(.000)	(.512)

Values are mean  $\pm$  S.E.M.; n=4/group; \* significantly different from ZLC-Con group (p<0.05); \*\* significantly different from ZLC-Con group (p<0.001).

## 1.2. Exercise performance

Exercise performance was progressively increased during the 8 weeks of training session. At the last week of training, ZDF rats were capable of lifting weight by 756g which is reached to the 2.05 fold of the body weight (Table 2).

Table 2. Weekly exercise performance changes

	1 wk	2 wk	3 wk	4wk	5wk	6wk	7wk	8wk
Variable	Mean $\pm$ S.E.M.							
Maximal weight lifted (g)	198.34 $\pm$ 11.59	304.17 $\pm$ 24.96	402.50 $\pm$ 31.46	482.50 $\pm$ 37.80	585.0 $\pm$ 31.75	665.0 $\pm$ 27.94	713.33 $\pm$ 23.14	756.67 $\pm$ 5.77
Maximal weight lifted /body weight (g/g)	1.46 $\pm$ 0.17	1.49 $\pm$ 0.09	1.71 $\pm$ 0.04	1.77 $\pm$ 0.08	1.99 $\pm$ 0.04	2.12 $\pm$ 0.03	2.06 $\pm$ 0.01	2.05 $\pm$ 0.08

Values are mean  $\pm$  S.E.M.; n=4/group

### 1.3. Grip strength

Our results revealed significant differences between ZLC-Con and ZDF-Con groups ( $p=.009$ ) which showed approximately 36% decreased muscle strength power in diabetic rats. Although 30.3% increased muscle strength was observed after 8 weeks of resistance exercise training, we fail to meet the statistic significance.

Table 3. Grip strength test

Variable	ZLC-Con	ZDF-Con	ZDF-Ex
	Mean $\pm$ S.E.M.		
Grip strength (g)	1099.48 $\pm$	704.74 $\pm$	918.09 $\pm$
	50.55	53.73* (.009)	99.05 (.141)
Grip strength/muscle weight (g/g)	1877.39 $\pm$	1389.86 $\pm$	1671.94 $\pm$
	78.67	64.22 (.072)	210.02 (.344)
Grip strength/body weight (g/g)	4.04 $\pm$ 0.18	1.93 $\pm$ 0.08**	2.52 $\pm$ 0.37
		(.000)	(.248)

Values are mean  $\pm$  S.E.M.; n=4/group; \* significantly different from ZLC-Con group ( $p<0.05$ ); \*\* significantly different from ZLC-Con group ( $p<0.001$ ).

#### **1.4. Glucose tolerance**

The changes of fasting blood glucose level at the first, fourth, eighth week of training in all groups are shown in Table 4. At the beginning of the exercise intervention, there were no differences of fasting blood glucose level between groups. At the last week of the exercise intervention, we found the significantly increased fasting blood glucose level in ZDF-Con group compared with ZLC-Con group which was ameliorated by 8 weeks of resistance exercise training.

Table 4. Changes of fasting blood glucose level

Variable	ZLC-Con	ZDF-Con	ZDF-Ex
	Mean $\pm$ S.E.M.		
Fasting blood glucose level of 1 <sup>st</sup> week of training(mg/dL)	97 $\pm$ 3.03	108.5 $\pm$ 12.71 (.564)	111.5 $\pm$ 2.87 (.959)
Fasting blood glucose level of 4 <sup>st</sup> week of training (mg/dL)	100.25 $\pm$ 3.54	217.25 $\pm$ 71.69 (.169)	115.75 $\pm$ 2.63 (.247)
Fasting blood glucose level of 8 <sup>st</sup> week of training (mg/dL)	102.75 $\pm$ 4.48	272 $\pm$ 23.56** (.000)	134.75 $\pm$ 8.87 <sup>##</sup> (.000)

Values are mean $\pm$  S.E.M.; n=4/group; \* significantly different from ZLC-Con group (p<0.05); \*\* significantly different from ZLC-Con group (p<0.001); <sup>##</sup> significantly different from ZDF-Con group (p<0.001).

The results of intraperitoneal glucose tolerance test (IPGTT) in all groups are shown in Figure 6. In ZDF-Con group, blood glucose level was remained significantly higher level after 120 min of glucose injection. However, ZDF-Ex group showed significantly lower level of blood glucose than ZDF-Con group at the each time point. These results suggest that 8 weeks of resistance exercise training could effectively controlled glucose intolerance in diabetic rats.

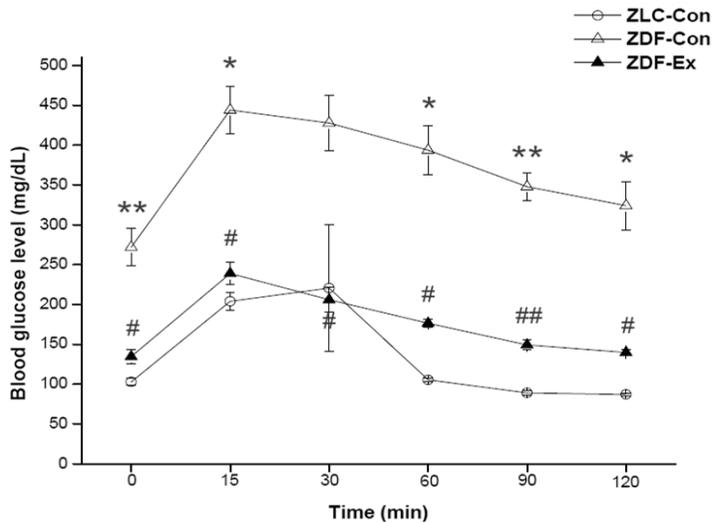


Figure 6. Blood glucose level changes during IPGTT at 8 weeks of exercise training

Values are mean± S.E.M.; n=4/group; \* significantly different from ZLC-Con group ( $p<0.05$ ); \*\* significantly different from ZLC-Con group ( $p<0.001$ ); # significantly different from ZDF-Con group ( $p<0.05$ ); ## significantly different from ZDF-Con group ( $p<0.001$ ).

AUC responses to an IPGTT at week 1 and week 8 showed similar results with blood glucose level except the ZDF-Con which showed statistically increased AUC level than the other groups at the beginning of the exercise training (Table 5 and Fig. 7). These results indicate that the severity of diabetes has progressed as exercise training carried out, thus, exercise training could be a preventive intervention to treat T2DM.

Table 5. Changes of glucose area under the curve (AUC)

Variable	ZLC-Con	ZDF-Con	ZDF-Ex
	Mean $\pm$ S.E.M.		
AUC of 1 <sup>st</sup>			
week of	13310.63 $\pm$	19282.5 $\pm$	14814.38 $\pm$
training	418.66	2203.49* (.027)	468.36 (.094)
(mg/dL·min)			
AUC of 4 <sup>st</sup>			
week of	13957.5 $\pm$ 268.49	28005 $\pm$	19556.25 $\pm$
training		3180.01* (.001)	844.31 <sup>#</sup> (.029)
(mg/dL·min)			
AUC of 8 <sup>st</sup>			
week of	15931.88 $\pm$	45429.38 $\pm$	21099.38 $\pm$
training	1880.11	3099.8** (.000)	701.63 <sup>##</sup> (.000)
(mg/dL·min)			

Values are mean  $\pm$  S.E.M.; n=4/group; \* significantly different from ZLC-Con group (p<0.05); \*\* significantly different from ZLC-Con group (p<0.001); # significantly different from ZDF-Con group (p<0.05); ## significantly different from ZDF-Con group (p<0.001).

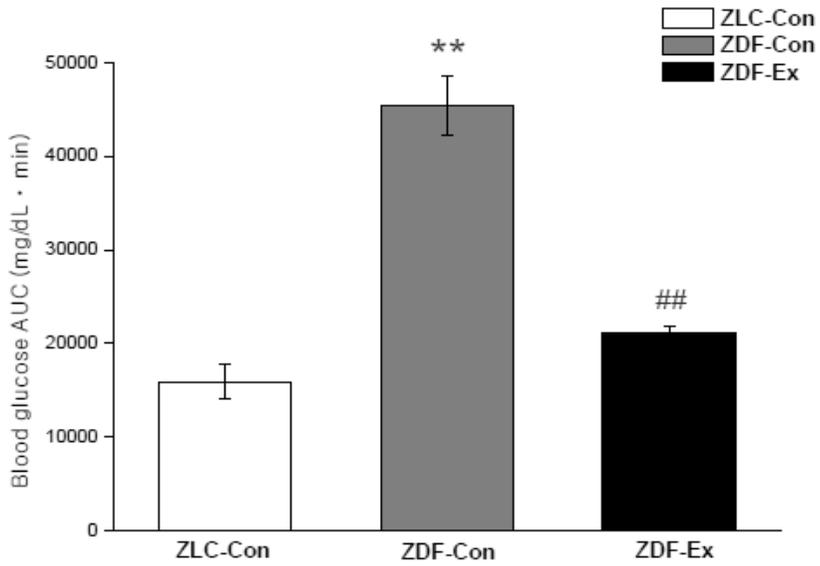


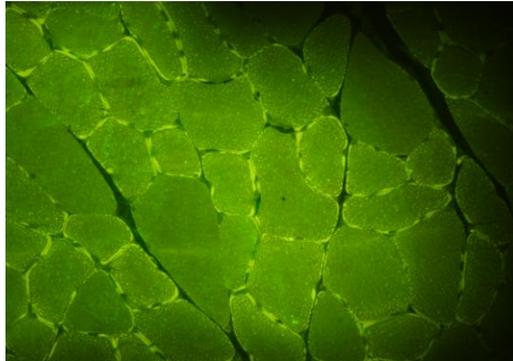
Figure 7. Blood glucose AUC at 8 weeks of exercise training

Values are mean  $\pm$  S.E.M.; n=4/group; \*\* significantly different from ZLC-Con group ( $p < 0.001$ ); ## significantly different from ZDF-Con group

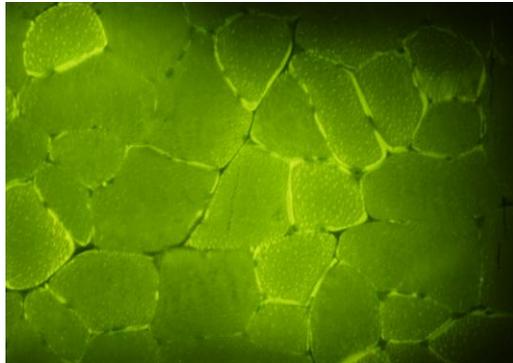
### 1.5. GLUT4 expression

Figure 8. Immunofloresence staining for GLUT4 in medial gastrocnemius

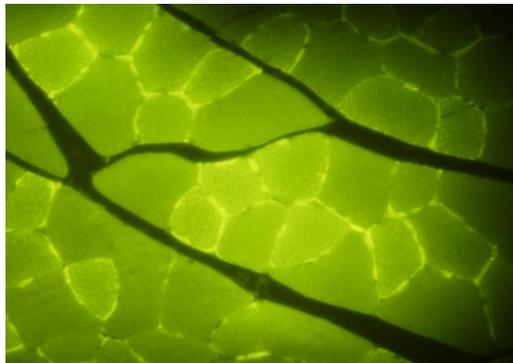
**A. ZLC-Con**



**B. ZDF-Con**



**C. ZDF-Ex**



## 1.6. Western blot

The density of GLUT4 protein in the cross sectional area of gastrocnemius muscle was shown in Figure 9. We also performed western blot for verifying the relative expression of GLUT4 protein content. In the ZDF-Con, a 25.6% decrease of GLUT4 density was seen (ZLC-Con;  $10167.15 \pm 1781.58$  vs. ZDF-Con;  $7565.01 \pm 824.26$ ), but did not achieve statistical significance. Interestingly, GLUT4 protein content was markedly increased by 8 weeks of exercise training ( $14259.26 \pm 2073.51$ ,  $p = 0.44$ ).

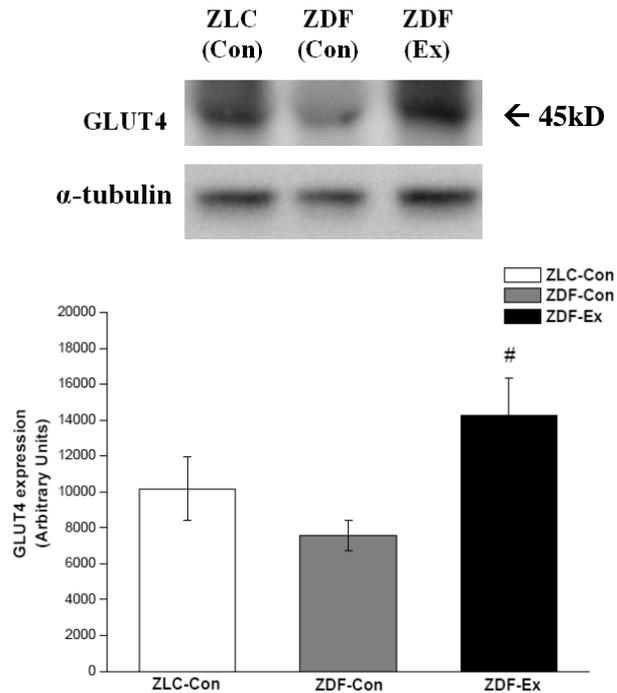


Figure 9. GLUT4 protein expression in medial gastrocnemius

Values are mean  $\pm$  S.E.M.;  $n=4$ /group; # significantly different from ZDF-Con group ( $p<0.05$ )

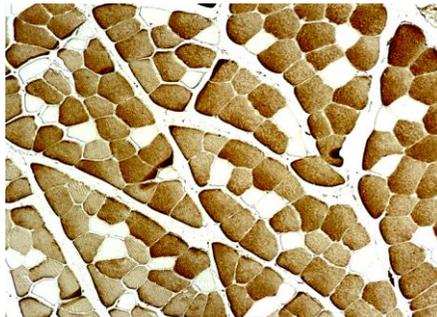
### 1.7. Immunohistochemistry staining

Figure 10. Immunohistochemistry staining of fast myosin heavy chain (MY-32) in medial gastrocnemius muscles

**A. ZLC-Con**



**B. ZDF-Con**



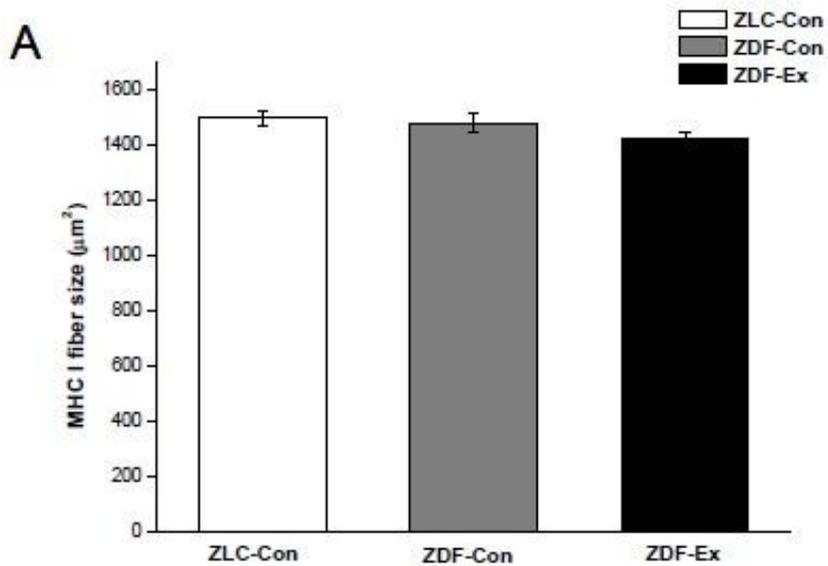
**C. ZDF-Ex**

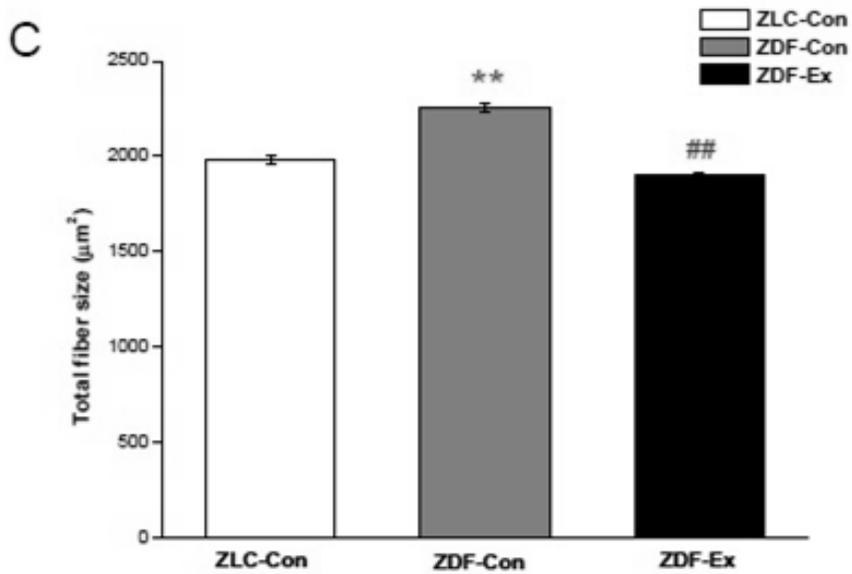
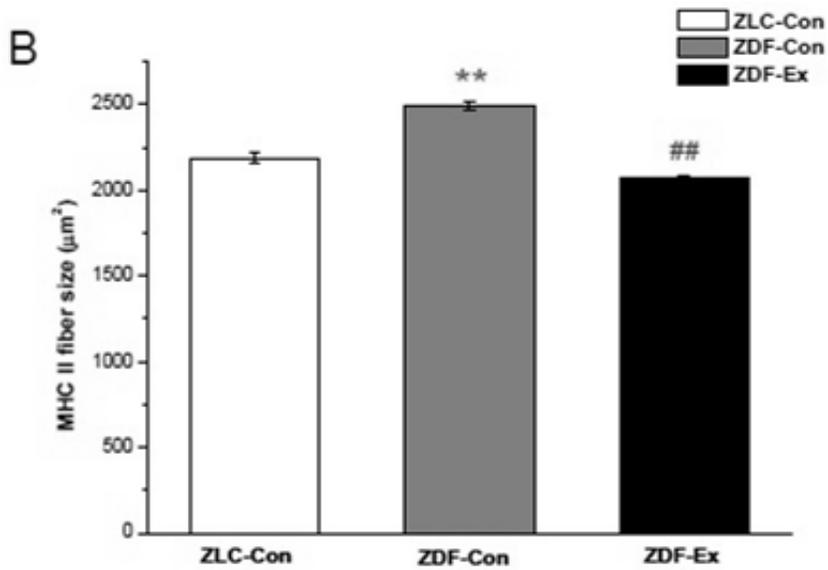


Type I MHC was presented unstained area (white), and type II MHC was presented stained area (brown).

### 1.7.1. Muscle fiber size

MHC I fiber size was significantly decreased after resistance training in ZLC rats and MHC II fiber size was also significantly decreased after resistance training in both rat groups. Total fiber size was significantly decreased after resistance exercise in both rat groups, whereas it was increased in ZDF-Con group compared with ZLC-Con group. There was no exercise effect on muscle fiber hypertrophy in both muscle fiber types.





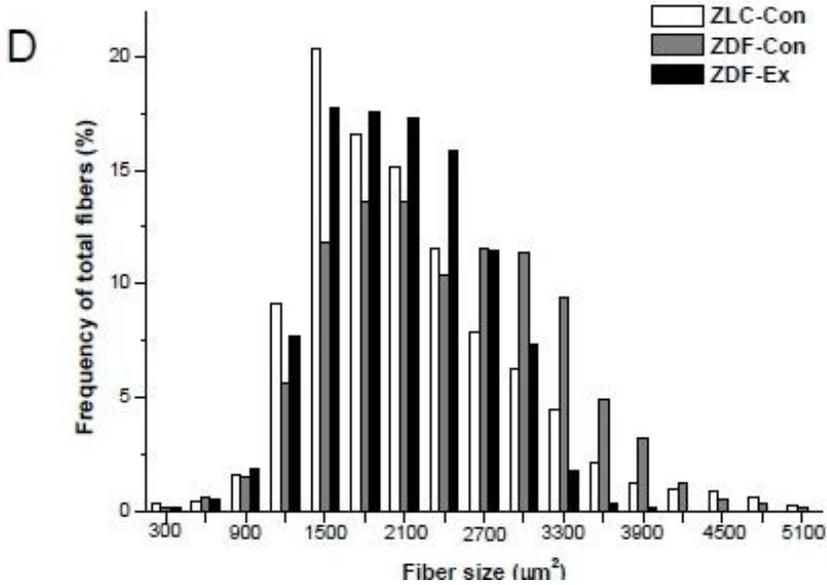
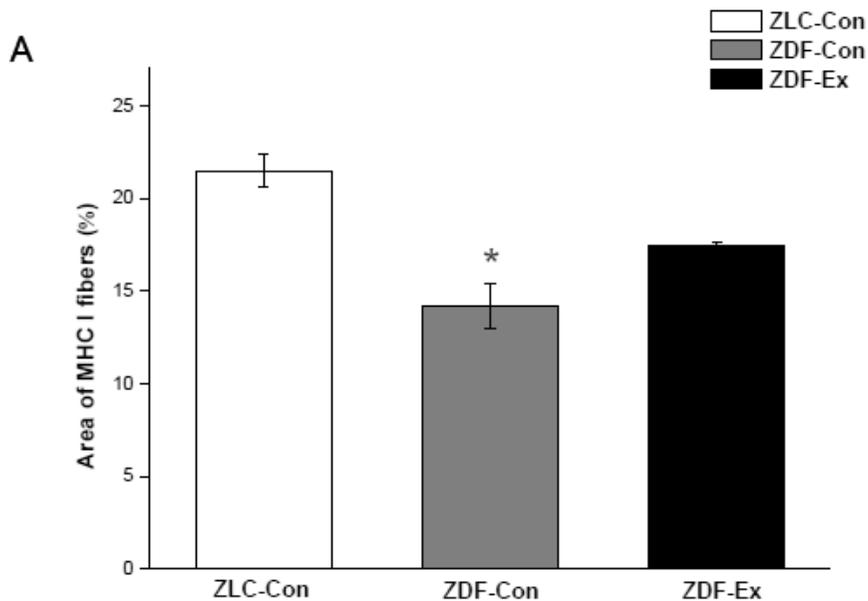


Figure 11. Muscle fiber size and frequency counted from medial gastrocnemius muscles (calculated more than 800 fibers in each group)

Values are mean± S.E.M.; n=4/group; \*\* significantly different from ZLC-Con group (p<0.001); ## significantly different from ZDF-Con group (p<0.001)

### 1.7.2. Muscle fiber type

Area of MHC I fibers was significantly decreased in ZLC-Ex group as well as ZDF-Con group than ZLC-Con group. However, there was little tendency of increased area of MHC I fibers in ZDF-Ex group, but fail to meet statistical significance ( $p=.109$ ). Area of MHC II fibers was significantly increased in ZLC-Ex group as well as ZDF-Con group than ZLC-Con group.



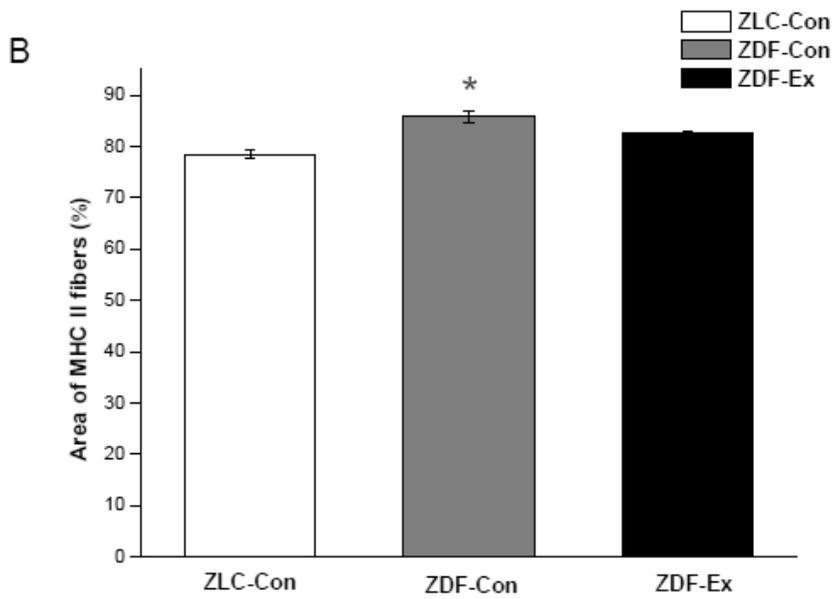


Figure 12. Percent area of slow and fast myosin heavy chain muscle fibers

Values are mean  $\pm$  S.E.M.; n=4/group; \* significantly different from ZLC-Con group (p<0.05)

## V. DISCUSSION

The main findings of this study were that progressive resistance training, thrice weekly at relatively high intensities, led to significant 1) improvements in muscle glucose tolerance, and 2) increases in muscle glucose transport protein expression. Although failed to reach the statistical significance ( $p=0.091$ ) in alteration of medial gastrocnemius muscle fiber distribution accounting for the exercise training, 28 % increased MHC I fiber and 8 % decreased MHC II fiber were observed in the resistance exercise training group compared with the non-exercise diabetic group.

These observations suggest that three sessions per week of laddering resistance exercise training could serve as a potential adjunct therapy in the management of type 2 diabetes in Zucker diabetic rats. It may have an important practical relevance for the optimal design of exercise training programs for animal model with type 2 diabetes.

In the present study, there was significant difference on body weight between normal Zucker lean control (ZLC-Con) group and non-exercised Zucker diabetic fatty (ZDF-Con) group. However 8 weeks of resistance exercise didn't lead to change in both body

weight between ZDF-Con group and exercised Zucker diabetic fatty (ZDF-Ex) group (Table 1). Similarly, gastrocnemius muscle wet weight was 13.9% diminished in the ZDF-Con compared with ZLC-Con, as well as 10.1% of increased muscle weight has been observed after exercise intervention, but all results were not statistically significant even though weekly exercise intensities were progressed of initial lifted weight from 198g to 756g (Table 1 and 2).

To investigate whether muscle strength would be improved after heavy resistance exercise, we performed grip strength test. In diabetic group, we found significant decreased muscle strength (35.9% lower than ZLC-Con group) indicates that the possibility of diabetic rats have poor glucoregulation. It seems to ameliorate in ZDF-Ex group (30.3% higher than ZDF-Con group), however it also fail to meet the statistical significance (Table 3).

For measure the changes of blood glucose levels during training period, we conducted Intraperitoneal glucose tolerance test (IPGTT) at week 1, 4, and 8 of training session (Table 4 and 5). Fasting blood glucose level at the 1<sup>st</sup> and 4<sup>st</sup> week was not significantly difference between all groups, but as reached to the 8<sup>st</sup> week of training ZDF-Con group showed significantly higher level of

fasting blood glucose level, and it was corrected by resistance exercise. This result implicated that degree of diabetic status was progressively worsened and positively alleviated by the resistance exercise training.

Previous study indicates that resistance exercise on its own is able to significantly improve glucose tolerance without any changes in body weight or muscle weight (Ibanez, Izquierdo et al. 2005). According to Tuomilehto et al. any type of physical activity such as sports, house hold work, gardening, or work-related physical activity can be equally beneficial in preventing diabetes (Tuomilehto, Lindstrom et al. 2001). It is possible to achieve primary prevention of type 2 diabetes patients by means of a nonpharmacologic treat that can be implemented as one of the effective health care.

In skeletal muscle cells, the most important glucose transporter is glucose transporter protein 4 (GLUT4), which is thought to be responsible for insulin- and contraction-stimulated glucose transport (Richter, Jensen et al. 1998). Many animal models have been used to study the characteristics and causes of muscle insulin resistance, but the obese Zucker rat is one of the more popular. Not only has this animal model proven valuable for detecting cellular

defects related to muscle insulin resistance, but it has yielded significant information on the mechanisms by which exercise training alleviates muscle insulin resistance. The effect of the mechanical load put on the muscle on the GLUT4 content is less clear. In accordance with these studies, GLUT4 contents in skeletal muscle of the obese Zucker rat was increased after exercise training in the present study which was not differ from non-diabetic state, indicates that exercise training could enhance impaired systemic glucose uptake and insulin sensitivity by over expressing GLUT4 (Fig. 8 and 9).

Despite an increased ability of the rats to lift progressively heavier loads, this heavy resistance training model did not induce gross muscle hypertrophy of the gastrocnemius muscles. In addition, even though this exercise regimen is sufficient to cause significant improvement in glucose tolerance and insulin sensitivity by overexpressing GLUT4, it was not severe enough to cause alteration of skeletal muscle fiber composition in the gastrocnemius of Zucker diabetic rats.

These results are attributed to the reason that resistance training methods for animal model were yet ideally established so that, to achieve the expected benefit of resistance exercise, such as muscle

hypertrophy, could be difficult to identify. Also in this study, lacking N number and large statistical variation could be an obstacle to meet the anticipated result to reach to the statistical significance in spite of exercise effect existed.

In conclusion, we have demonstrated that the 8 week of ladder climbing resistance training induces muscle glucose tolerance and insulin sensitivity in obese Zucker rats. Furthermore, muscle GLUT4 contents were increased after training. However, muscle hypertrophy and composition were not altered by exercise training. These results suggest that resistance training could be an effective remedy for promoting muscle insulin sensitivity and glucose uptake capacity in type 2 diabetic rats.

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## 국 문 초 록

### 8주간의 저항성 운동이 제 2형 당뇨병 쥐(Zucker rat)의 근섬유 조성과 GLUT4 발현에 미치는 영향

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골격근은 당을 흡수하고 사용하는 주요 기관으로서 근육의 대사적, 기능적 특성은 근질량과 근섬유의 종류에 따라 변화한다. 성장이 이루어진 쥐의 골격근섬유는 흔히 type I, IIa, IIx, IIb의 네가지로 분류할 수 있으며, 이러한 종류에 따라 효소의 활성이나 수축속도, 근섬유 면적 등에서 차이를 나타낸다. 따라서, 본 연구에서는 8주간의 저항성 사다리 운동이 제 2형 당뇨병 모델인 Zucker rat의 근섬유에 미치는 형태적 변화와 GLUT4 단백질 발현의 변화에 대하여 관찰하였다.

본 연구에서 사용된 Zucker rat은 정상군(Zucker Lean Control; ZLC-Con), (Non-exercised Zucker Diabetic Fatty; ZDF-Con), 그리고 당뇨병운동군(Exercised Zucker Diabetic

Fatty; ZDF-Ex)으로 분류되었으며, 당뇨운동군은 총 8주간의 저항성 사다리 운동을 실시하였다. 운동 1주, 4주, 8주차에 복강내당부하검사를 실시하여 내당능력의 변화를 관찰하였으며, 운동 종료 후 모든 그룹에서 비복근을 적출하여 근섬유 조성을 관찰하기 위한 면역조직화학법과 GLUT4의 발현을 관찰하기 위한 Western Blot 실험을 진행하였다.

그 결과, 8주간의 저항성 사다리 운동은 당뇨쥐에서 내당 능력을 개선시키고 GLUT4를 과발현 시킴으로써 인슐린 민감성을 증가시켰다. 하지만, 근섬유의 비대는 운동을 통하여 관찰할 수 없었으며, 근섬유 조성 또한 유의하게 변화하지 않음을 관찰할 수 있었다.

따라서, 저항성 운동은 근육의 형태적 변화와 독립적으로 인슐린 저항성 개선과 당흡수 능력을 향상시키는데 효과적인 치료방법으로 제시될 수 있을 것으로 사료된다.

주요어: 당뇨, Zucker, 저항성 사다리 운동, GLUT4, 근섬유 조성, 근섬유 크기

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