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Dissertation of Doctor of Philosophy

Effect of resistance exercise on myokines and
muscle functions in type 2 diabetes and aging

: Animal and human study

제2형 당뇨병과 노화에서 저항성 운동이 근기능과
myokine에 미치는 영향

February 2016

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Abstract

Skeletal muscle has recently been recognized as an endocrine organ that produces and releases various cytokines termed myokines that are involved in the regulation of several physiological and metabolic pathways. Myokines are identified as cytokines and other peptides that are produced, expressed and released by muscle fibers and exert autocrine, paracrine or endocrine effects. These findings that the muscle derived myokines provide a conceptual basis and a whole new paradigm for understanding how muscle communicates with other organs, such as adipose tissue, liver, pancreas, bone, and brain. However, there was not enough evidence regard to evaluate the effect of resistance exercise on myokines in diabetes and aging model. In this dissertation, three-part of experiments were performed as follows; 1) Resistance training in type 2 diabetes rats, 2) Resistance training in aging mice, and 3) Resistance training in aging adults. The purpose was to investigate the effect of resistance exercise on myokines (including irisin and interleukin-15) and muscle functions in diabetes and aging models. In part 1 study, muscle quality and glucose tolerance were significantly improved in training group compared to control group. And IL-15 and irisin levels were significantly higher in soleus muscle of exercise group compared to control group. In addition, there were positive correlations between grip strength and level of

myokines in soleus muscle. In part 2 study, muscle mass and muscle strength were increased following resistance training. IL-15 and irisin were significantly increased in soleus muscle and circulating level. In addition, level of myokines and muscle strength has positive association in aging mice model. In part 3 study, muscle strength and muscle functions were significantly improved in trained elderly compared to sedentary elderly. Circulating myokines were significantly increased after 12 weeks of resistance training in trained group compared to their control group. In addition, changes of myokines were positively correlated with improvement of muscle strength in aging participants. Taken together with these results of dissertation, resistance training for aging and diabetic population was the efficient intervention to enhance the myokines and improve the muscle quality.

I. Study Background

1.1 Identification of myokine

Skeletal muscle has recently been recognized as an endocrine organ that produces and releases various cytokines termed myokines that are involved in the regulation of several physiological and metabolic pathways. Myokines are identified as cytokines and other peptides that are produced, expressed and released by muscle fibers and exert autocrine, paracrine or endocrine effects. These findings that the muscle derived myokines provide a conceptual basis and a whole new paradigm for understanding how muscle communicate with other organs, such as adipose tissue, liver, pancreas, bones and brain. (Pedersen & Febbraio, 2012)

In summary, the characteristics of a myokine are as follows; (1) Myokines are cytokines or other peptides that are produced, expressed, and released by muscle fibers; (2) Myokines may exert autocrine, paracrine, or endocrine effects; (3) Myokines may balance and countact the effect of adipokines; (4) The muscle-cell secretome consists of several hundred secreted products; (5) Myokines may mediate protective effects of muscular exercise, with regard to diseases associated with a physically inactive lifestyle. (Pedersen & Febbraio, 2012)

Myokines are well known to be expressed and released during physical exercise. In response to muscle contraction following exercise, muscle fibers express myokines such as irisin, interleukin-15 (IL-15), leukemia inhibitory factor (LIF), brain derived neurotrophic factor (BDNF), fibroblast growth factor-21 (FGF-21), and secreted protein acidic and rich in cysteine (SPARC), which subsequently exerts their effect locally within the muscle or their target organs. In Fig. 1, skeletal muscle expresses and releases various myokines into circulation with their potential roles.

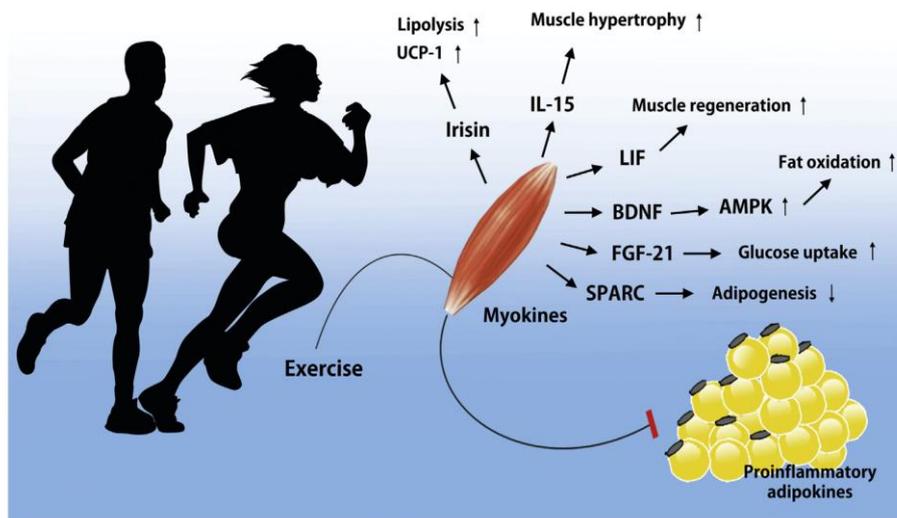


Figure 1. Potential role of exercise-induced myokines. (So, Kim, Kim, & Song, 2014) *adapted and modified with permission from authors

1.2 Role of myokine in aging

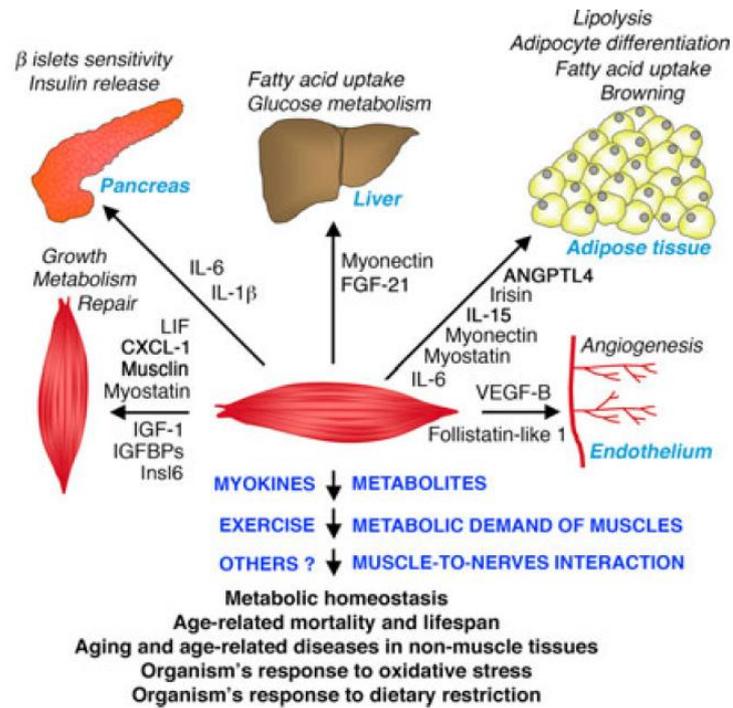


Figure 2. Systematic regulation of metabolism and aging by skeletal muscle and myokines (Demontis, Piccirillo, Goldberg, & Perrimon, 2013)
*adapted and modified with permission from authors

Skeletal muscle is one of the tissue in which age-related alternations are particularly prominent. As shown in Figure 2, the important role of skeletal muscle was highlighted in influencing metabolic homeostasis, life span, systematic aging, and the progression of age-related diseases. Skeletal muscle may crosstalk with other tissue via muscle derived cytokines and growth

factors (myokines). In aging process, myokines modulate several metabolic processes in the pancreas, liver, adipose tissue, endothelium, the muscle itself, and may affect on systemic aging and lifespan (Demontis et al., 2013). Because myokines show important role as an endocrine modulators or metabolic homeostasis, they are also likely to be important in aging progression and preventive strategy of age-related diseases.

For example, exercise-induced irisin secretion by contracting skeletal muscle fibers, which could have evolved from shivering-related muscle contraction, might be a potential target to control the body weight and metabolic profile in aging population. (Lee et al., 2014). According to this review article, targeting irisin and FGF-21, and particularly the key signaling pathway, the Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha), could identify new potent candidates to be included in the anti-aging armamentarium (Fig. 3) (Sanchis-Gomar, Pareja-Galeano, Mayero, Perez-Quilis, & Lucia, 2014)

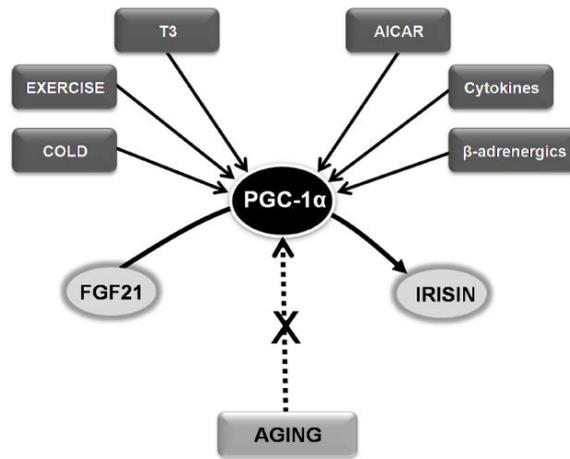


Figure 3. Hypothesizing the role of FGF-21-PGC-1 alpha-Irisin axis in age-related conditions and sarcopenia (Sanchis-Gomar et al., 2014) *adapted and modified with permission from authors

1-3. Role of myokines in diabetes

Skeletal muscle accounts for ~40% of body weight and constitutes the largest organ of the body in non-obese individuals. Recently, skeletal muscle is recognized as an endocrine organ, and proteins expressed by and released from skeletal muscle have been termed myokines. In previous reports using primary human myotubes and proteomics, hundreds of myokines have been identified, including more than 50 novel myokines (Eckardt, Gorgens, Raschke, & Eckel, 2014; Pedersen & Febbraio, 2008)

Exercise-induced myokines may play a key role in mediating the beneficial effects of physical exercise in metabolic disease. (Raschke, Eckardt, Bjorklund Holven, Jensen, & Eckel, 2013). And myokines are very likely to be involved in the crosstalk between skeletal muscle and other tissues. Therefore, their endocrine effects with regard to metabolic regulation are an important focus of therapeutic target, since they may help to overcome or prevent the metabolic impairment with insulin resistance.

It has been reported that IL-15 positively effects directly on glucose and fat metabolism as well as insulin sensitivity (Argiles, Lopez-Soriano, & Busquets, 2009; Barra, Chew, Holloway, & Ashkar, 2012; Nielsen et al., 2007; Quinn & Anderson, 2011). IL-15 treatment also decreases fasting glucose levels and improves insulin sensitivity (Barra et al., 2012) and glucose uptake

(Busquets, Figueras, Almendro, Lopez-Soriano, & Argiles, 2006) in animal models.

According the review article, myokines might affect to insulin secretion and glucose metabolism via crosstalk between skeletal muscle and pancreas (Kanzleiter et al., 2014).

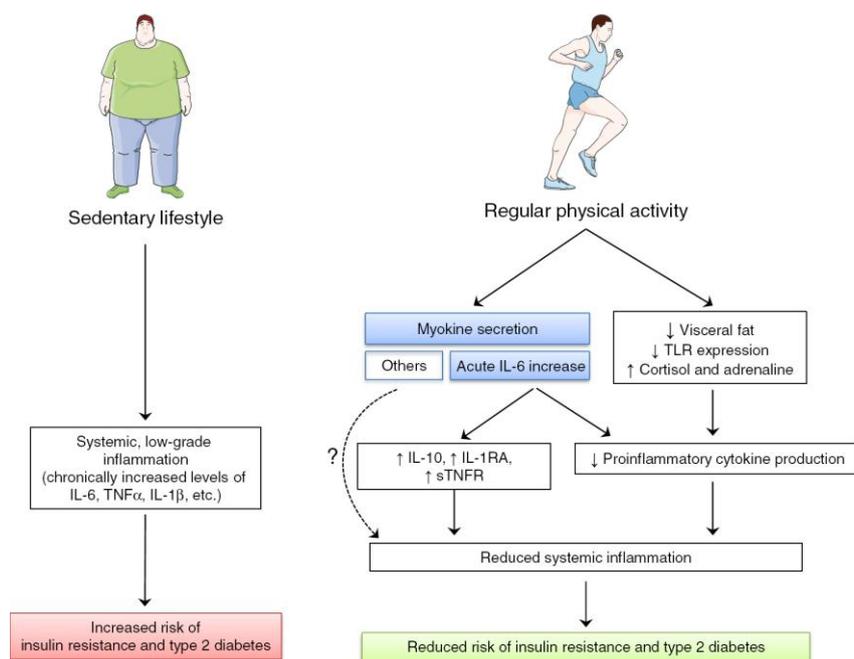


Figure 4. Impact of physical activity level on inflammatory statuses and risk of insulin resistance and/or type 2 diabetes (Kanzleiter et al., 2014) *adapted and modified with permission from authors

A sedentary lifestyle combined with obesity is often associated with systemic low-grade systematic inflammation and an increased risk of insulin

resistance and type 2 diabetes. On the other hand, an active lifestyle with physical exercise decreases the risk of development insulin resistance and type 2 diabetes. Cross-sectional studies have shown a strong inverse association between the level of physical activity and systemic low-grade inflammation (Abramson & Vaccarino, 2002; King, Carek, Mainous, & Pearson, 2003; Mattusch, Dufaux, Heine, Mertens, & Rost, 2000). These observations might be explained by a anti-inflammatory effect of physical exercise, which could be mediated via different mechanism including various myokine pathways. For example, IL-6 level was acutely increased after physical exercise and resulted in the enhanced release of anti-inflammatory cytokines such as IL-10, IL-10Ra, and sTNFR, which reduces systemic inflammation. However, it is not clearly evaluated whether other myokines may also directly or indirectly affect systemic inflammation (Eckardt et al., 2014)

1.4 Effect of resistance exercise on myokines

Myokines, as one of multiple health factors, constitute an important area of research in metabolic diseases. (Henriksen, Green, & Pedersen, 2012; Pedersen, 2011). Based on the previous reports regarding the role of myokines, exercise-induced myokines might be potential mediators to explain the beneficial effect of exercise on various tissues or organs in our body.

Although numerous studies demonstrated that exercise alters IL-15 protein level in serum or at the mRNA level, more evidences were required to elucidate the effect of resistance exercise on the IL-15 concentration in serum. The level of IL-15 level in plasma was significantly increased after resistance exercise (Lambert, Flynn, Sullivan, & Evans, 2004; Riechman, Balasekaran, Roth, & Ferrell, 2004); however, mRNA levels of IL-15 were decreased after 2 hours of intensive strength training. (Nieman et al., 2004). In addition, acute and chronic resistance exercise increase the IL-15 level in both serum and skeletal muscle in type 2 diabetic rats. (So et al., 2014)

In irisin studies, exercise increases circulating irisin level following exercise training in rodents and humans (Bostrom et al., 2012; Huh, Siopi, Mougios, Park, & Mantzoros, 2015). Recently, circulating irisin level was significantly increased in obese/overweight adults after 8 weeks of resistance training compared to control group, and these change of irisin was highly correlated with body composition (H. Kim et al., 2015).

II. Experimental Researches

In the present dissertation, three parts of experiments were investigated. In animal studies (Part I & II), levels of exercise-induced myokines were measured in various skeletal muscle tissues considering the muscle fiber type such as SOL, EDL, TA, and GAS in type 2 diabetes and aging animal. In human study (Part III), exercise-induced myokines were analyzed considering correlation between changes of myokines and improvement of muscle functions in elderly women.

1. Part I

Effect of resistance exercise on myokines in serum and skeletal muscles with improvement of glucose tolerance in type 2 diabetic rats

2. Part II

Effect of resistance exercise on myokines and muscle functions in aging mice

3. Part III

Effect of resistance exercise on myokines and muscle functions in elderly adults

PART I

**Effect of resistance exercise on myokines in
serum and skeletal muscles with improvement of
glucose tolerance in type 2 diabetic rats**

1. Introduction

The diabetes is accompanied by disorder of glucose and insulin resistance that can negatively affect functional status, including a decrease in muscle mass, strength, and functionality, termed sarcopenia. Increased expression of cytokines has been identified as one of the possible mechanisms of sarcopenia in diabetes. Physical exercise is considered as a cornerstone in the therapeutic and preventive intervention for patients with type 2 diabetes mellitus (T2DM), and the importance of physical exercise is underscored by the costs and side importance that accompany pharmacological intervention. Recently, resistance training has been found to be effective for T2DM and may provide the additional benefit of preventing musculoskeletal dysfunction associated with T2DM (Brooks et al., 2007; Cuff et al., 2003; Dunstan et al., 2002; Eves & Plotnikoff, 2006). Resistance exercise induces a range of changes facilitating muscle hypertrophy and whole body insulin action. Also, a robust finding in both human and rodent muscle in response to resistance exercise is an increase in the abundance of the glucose transporter GLUT4 isoform mRNA and protein (Hawley & Zierath, 2008). Additionally, resistance exercise appears to have an important impact on HbA1C significantly more than performing no exercise at all and similarly to aerobic exercise in T2DM patients (Irvine & Taylor, 2009).

It has been reported that IL-15 positively effects directly on glucose and fat metabolism as well as insulin sensitivity (Argiles et al., 2009; Barra et al., 2012; Busquets et al., 2005; Quinn & Anderson, 2011; Quinn, Anderson, Conner, & Wolden-Hanson, 2013). IL-15 treatment also decreases fasting glucose levels and improves insulin sensitivity (Barra et al., 2012) and glucose uptake (Busquets et al., 2006) in animal models. It is well established that regular exercise is accompanied positively effects of metabolic changes in skeletal muscle (Rinnov et al., 2014). Resistance exercise especially improve a negative energy balance, and decrease white fat mass and increase muscle mass, muscle strength and muscle function as well as reduce insulin resistance and metabolic dysfunction (Frayn, 2000).

In the previous studies exercise did not increase IL-15 in the plasma (Ostrowski et al., 1998) and skeletal muscles (Nieman et al., 2004) after endurance exercise. A few recent studies, however, showed that IL-15 mRNA and IL-15 protein increases following endurance running (Yang et al., 2013). In addition, resistance exercise transiently increased the concentration of plasma IL-15 (Lambert et al., 2004; Riechman et al., 2004), and IL-15 mRNA levels of skeletal muscle increase into the recovery period after a bout of resistance exercise (Nielsen et al., 2007). Although the anabolic effect of IL-15 was previously investigated, the results of IL-15 expression by exercise have been conflicting and the influence of resistance training on IL-15

expression in T2DM skeletal muscle has not been reported yet. Furthermore, little is known about physical function changes in exercise induced expression of IL-15 in diabetic rat skeletal muscle.

Fibroblast growth factor-21 (FGF-21) and irisin were known to be peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 alpha) related potential therapeutic targets for metabolic diseases from recent studies. FGF-21, one of the members of the fibroblast growth factor, has been well-recognized as a metabolic and a promising target in managing metabolic diseases. Skeletal muscle induced-FGF21, which is regulated by a PI3K/Akt1 pathway dependent mechanism, protects against obesity and insulin resistance, induces the browning of white adipose tissue, and protects against cardiac hypertrophy. With its protecting role against obesity, insulin resistance and even hypertrophy, irisin has been known as an exercise-induced myokine that is secreted into circulation following proteolytic cleavage from its cellular form, fibronectin-type III domain-containing 5 (FNDC5) (Bostrom et al., 2012). It reverses diet-induced obesity and diabetes by stimulating thermogenesis in rodents through increasing brown adipocyte-like cell abundance within white fat (Petrovic et al., 2010; Wu et al., 2012).

Neurotrophins are well known to regulate several neuronal processes primarily through Trk receptor tyrosine kinases. The mammalian family of

neurotrophins consists of nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), and brain-derived neurotrophic factor (BDNF). Among these neurotrophins, BDNF and its receptor TrkB are the most widely and abundantly expressed in the brain (Huang & Reichardt, 2001). Other studies suggest that BDNF could be involved in peripheral metabolism. Wisse and Schwartz (2003) reported that BDNF has been identified as a key modulator of the hypothalamic pathway that controls body composition and energy homeostasis (Wisse & Schwartz, 2003). Also, BDNF has been thought to be a regulator of metabolism in skeletal muscles (Matthews et al., 2009) and an enhancer for glucose utilization in diabetic skeletal muscles (Yamanaka et al., 2007).

2. Purpose of study

Although the roles of myokines were well-demonstrated in metabolic disease including obesity and type 2 diabetes, there were no reports to investigate the effect of resistance exercise on myokine in diabetic skeletal muscles with improvement of muscle quality and glucose intolerance. Therefore, this study investigated the change of myokines including IL-15, FGF-21, irisin, and BDNF levels in various skeletal muscles and their association with muscle strength following 8 weeks of resistance training in Zucker diabetic fatty rats.

3. METHODS AND METATERIALS

3.1 Animals

Male and female Zucker diabetic fatty (ZDF, *fa/+*) rats were purchased from Genetic Models (Indianapolis, ME, USA) and allowed to mate. They were housed in conventional cages under adequate temperature (23°C) and humidity (60%), control with a 12-hour light/12-hour dark cycle, and free access to food and water. Purina 5008 rodent diet (7.5% fat) was provided as recommended by Genetic Models Co. (Purina, St. Louis, MO, USA). The *fa* gene genotype was determined using the strategy described in our previous study [17]. Twenty-four male lean (ZDF lean control, ZLC, *+/+*) and diabetic (ZDF, *fa/fa*) Zucker rats (8-week-old) were separated into three groups, lean control (sedentary ZLC, ZLC-Con, *n*=8), diabetic control (sedentary ZDF, ZDF-Con, *n*=8), and diabetic exercise-trained (exercised ZDF, ZDF-Ex, *n*=8).

The training began after one week of adaptation. At 6 weeks (pre-exercise) and 14 weeks (post-exercise) of age, all animals' body weight (Mettler instrument AG CH-8606, Switzerland), grip strength (Bioseb, France), and fasting plasma glucose level (Roche Diagnostics LTD., Mannheim, Germany) were measured. The procedures for handling and caring for the animals adhered to the guidelines that are in compliance with the current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication

No. 85-23, 1985, revised 1996), and protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (SNU-131007-1). All of the experiments were conducted to minimize the number of animals used and the suffering caused by the procedures used in the present study.

3.2 Progressive resistance exercise training

The rats in the exercise-trained group (ZDF-Ex) were trained to climb a 1-m vertical (85 degrees inclined) ladder (Figure 5) with weights secured to their tail. In the first week, rats were familiarized with climbing up to the top of the cage with and without weight on their tails. The training sessions, from the second week, were commenced with intensity at 50% of each rat's body weight; the weight was applied by a conical tube filled up with iron pellets securely attached to the tail using plastic belt and tape. Rats were put at the bottom of the ladder and forced to climb to the top. When they reached the top, 2 min of rest was given and the next trial was followed. Subsequent trials were performed from the bottom, and weight of 20 g was added to the prior weight in every trial. If a rat was able to climb with increased weights for 10 times, training session was considered as being completed. In the case where a rat fail to complete the climbing with increasing weight as it was planned, the rat was forced to complete 10 trials with the previous successful weight, with no

further attempts with increased weight allowed. (Figure 5)

	Exercise Period							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Reps.	Max.10							
Load	50% BW / Every trial +20g				70% MW / Every trial +20g			
Rest	2 min							
Frequency	3 days/week							



Figure 5. Ladder climbing exercise for rats (100×12 cm, 2 cm grid, 85° incline)

3.3 Grip strength test

Grip strength was conducted using a Grip Strength Meter (Figure 6) adapted with a dual grip bar connected to two separate strain gauges to allow separate measurements of force with the two forepaws simultaneously. On each trial, the rat was held around the abdomen and lowered at an angle perpendicular to the bar until it gripped the two bars, one with each forepaw, and with its rear paws standing on the inclined surface of the apparatus. The rat was then pulled gently by the base of the tail, in a rearward direction, away

from the bars. Rats instinctively clung to the bar to the point until they can no longer resist the pull. The applied force at the point at which the rat releases its grip for each paw was recorded by two separate strain gauges connected to a digital readout. Grip strength was tested in sessions of five trials separated by approximately 1 min between each trial. Mean of measurements for each rat was used.



Figure 6. Grip strength meter

3.4 Tissue collection

Skeletal muscle tissue collection was performed 2 days after the last session of the exercise training program. Rats were anesthetized by Zoletil 50 (intraperitoneal injection, 10 mg/kg; Vibac Laboratories, Carros, France) and sacrificed. The tissues were collected from the soleus (SOL) and the gastrocnemius medial (GM) muscles. The samples were quickly weighed, frozen with liquid nitrogen, and then stored at -80°C until used. Each tissue

was homogenized in radio immune-precipitation assay buffer. The homogenized tissues were then centrifuged at 14,000 rpm for 20 minutes at 4°C, and the total protein concentration of the supernatant was determined using the Bradford assay.



Figure 7. Skeletal muscle sampling

3.5 Intraperitoneal glucose tolerance test

Glucose tolerance was assessed using the intraperitoneal glucose tolerance test (IPGTT) in rats during the last week of the training session. IPGTT was performed by injecting glucose (2 g/kg in 20% solution) intraperitoneally in overnight-fasted rats. Blood samples were obtained by cutting the tail tip before and 0, 15, 30, 60, 90, and 120 minutes after glucose administration. Blood glucose concentration was measured using an Accu-Chek glucose analyzer (Roche Diagnostics Ltd., Mannheim, Germany).

3.6 Skeletal muscle volume analysis

PET/CT imaging was carried out with a total of 18 animals for measurement of hindlimb muscle volume. The rats were imaged at the age of 14 weeks. All imaging procedures were performed under inhalation anesthesia with isoflurane (Isoba vet., Essex Pharma, Germany) at a concentration of 4% for induction of anesthesia and 1–2% for maintenance. The rats were placed in prone position on a temperature controlled bed at 39°C (T/Pump, Gaymar, Orchard Park, NY, USA) allowing changes between imaging modalities without repositioning and isoflurane was supplied via a nose cone (Summit Anesthesia Solutions, Bend, OR, USA). The animal respiration was spontaneous, and the breathing was monitored continuously using a small pressure transducer (Biovet, m2m imaging, Newark, NJ, USA). Breathing was maintained at a rate between 60 and 100 per minute. After image data acquisition the recovery time of the animals from anesthesia was usually less than five minutes. Overall the procedures were well tolerated.

3.7 Analysis of myokines

The skeletal muscle levels of IL-15, FGF-21, irisin, and BDNF were measured by enzyme-linked immunosorbent assays (ELISA), according to the specifications of the manufacturer considering their detection range. All

samples were determined in duplicate to guarantee the precision of the results. And all samples were run within the range of the standard curve. The results were expressed as concentration of myokines (pg/ml) read from standard curves.

3.8 Statistical analysis

Statistical analysis was performed using SPSS 18.0 software package. Data was analyzed using two samples t-test to examine the level of myokines in skeletal muscles. Data are presented as means \pm S.E.M. with significance set at $p < 0.05$. Associations of myokines expression in skeletal muscle with grip strength were calculated with Pearson's correlation coefficient.

4. Results

As shown in figure 8A, ZDF-Con rats were markedly heavier from second week of training than their lean littermates. At the 7 and 8-week exercise program, body weight was lower ($p < 0.05$) in ZDF-EX than ZDF-Con. Exercise performance (lifted tail weight) was progressively increased during the training period (Figure 8B). At the end of training, the rats were able to lift 660 g, a weight 1.97-fold heavier than their body weight.

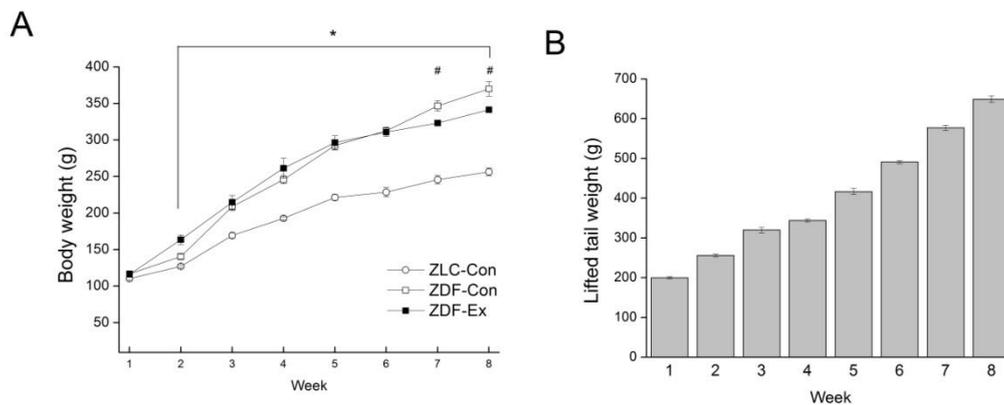


Figure 8. Changes of body weight and lifted tail weight during training program

Glucose tolerance was tested with IPGTT before and after training program. In pre-test, increased blood glucose concentration induced by dextrose injection was decreased to baseline (fasting level) within 120 minutes in all of 3 groups (Figure 9A). In post-test, however, the significant glucose intolerance was found in ZDF-Con group (Figure 9B). In ZDF-Con group, fasting blood concentration and area under the curve (AUC) of IPGTT were significantly higher than ZLC-Con group at post-test. In ZDF-Ex group, fasting blood glucose and AUC of IPGTT were significantly decreased compared to ZDF-Con at post-test (Figure 9C and 9D).

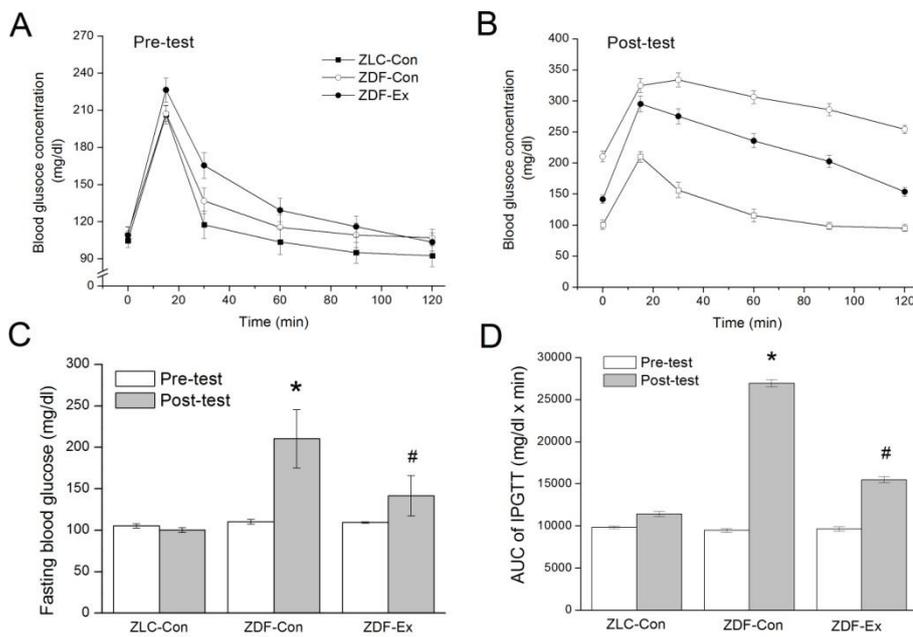


Figure 9. Effect of 8 weeks of resistance exercise on glucose tolerance and fasting blood glucose level in diabetic rats

The total volume of skeletal muscle in entire leg was measured using PET-CT in order to estimate the muscle quality. In ZDF-Con rats, the total volume of skeletal muscle was significantly decreased compared to ZLC-Con rats. Whereas, there was no significant difference between the ZDF-EX rats and ZDF-Con rats after resistance training (Figure 10A). Muscle quality was calculated by taking the ratio of grip strength (g) entire skeletal muscle volume (cm^3) of leg. Although muscle volume of ZDF-Con was reduced significantly, the muscle quality did not have statistical differences. However, the significant improvement (18.2%, $p < 0.05$) of muscle quality following the resistance training was found in ZDF-Ex rats compared to ZDF-Con rats (Figure 10B).

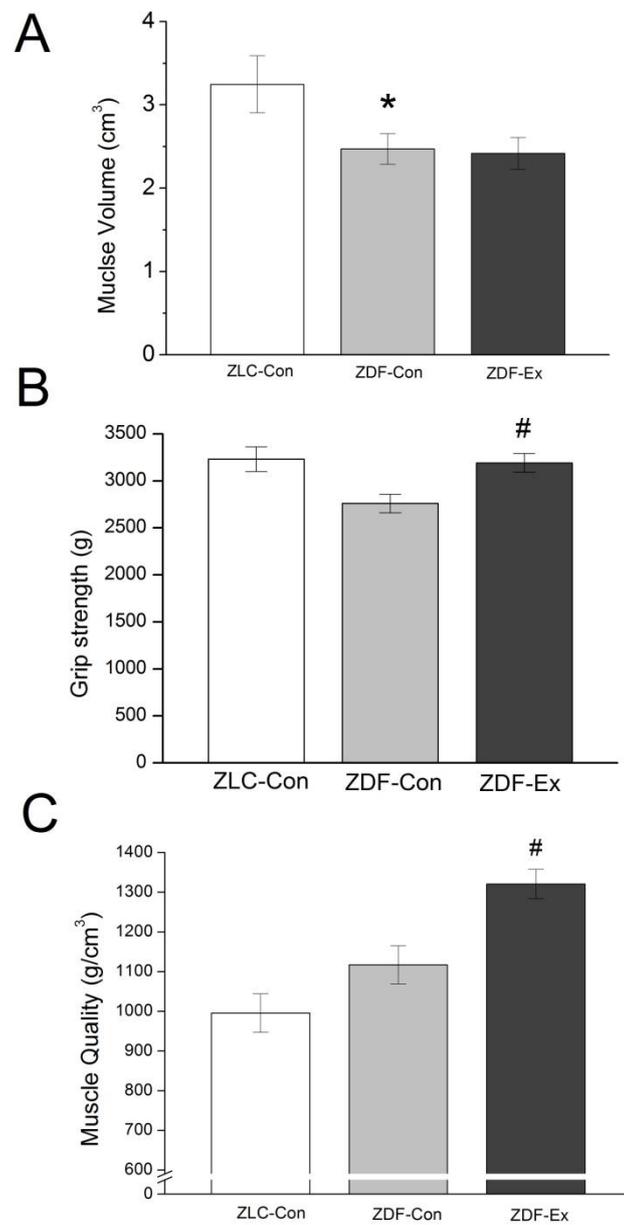


Figure 10. Effect of resistance exercise on muscle volume, muscle strength, and muscle quality

There was a main effect of 8 week of resistance training on IL-15 level in SOL ($F=10.53$, $p<0.01$). IL-15 level in soleus of ZDF-Ex rats was 44.1% higher following resistance training when compared to ZDF-Con rats ($p<0.05$, Figure 11A). However, there were no significant alternation of IL-15 level affected by diabetes or exercise in other skeletal muscles including EDL, GM, and TA. In addition, there was a positive association between the IL-15 level in SOL and muscle quality ($r=0.687$, $p=0.001$; Figure 11B).

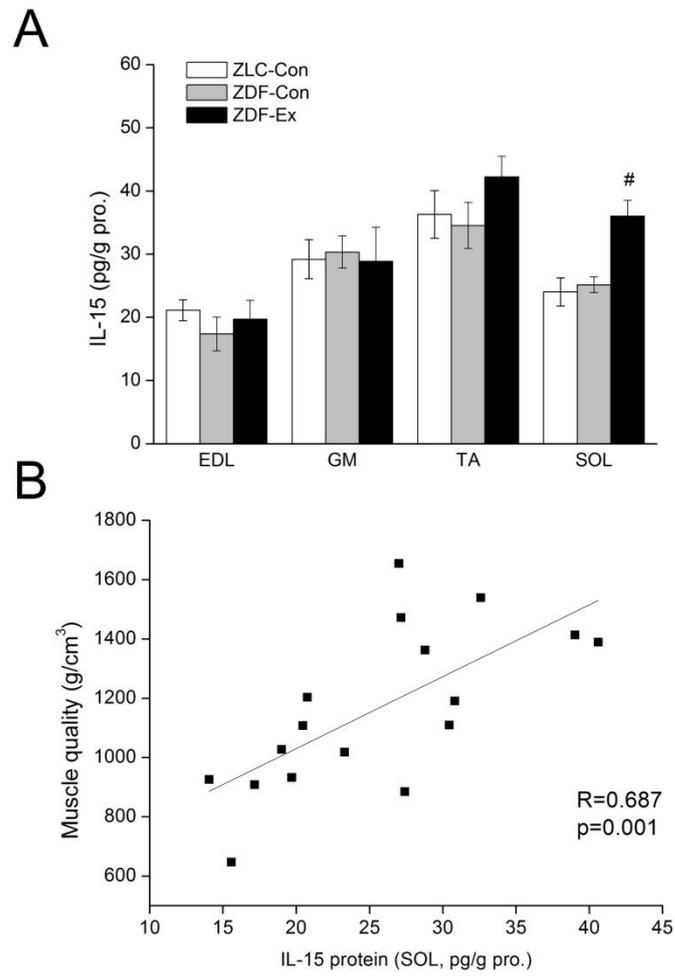


Figure 11. Effect of resistance exercise on IL-15 level in skeletal muscle and correlation with muscle quality

The levels of FGF-21 and irisin in SOL, EDL, TA, and GAS were measured at the end of the 8 weeks progressive resistance training. In SOL and EDL muscles, there were no significant differences of FGF-21 expression between ZLC-Con and ZDF-Con. And the expressions of FGF-21 in SOL and EDL muscles of ZDF-Ex were higher ($p<0.05$) compared to ZDF-Con (Figure 12A). In addition, we found a significantly higher level of irisin expression in SOL muscle of ZDF-Ex compared to ZDF-Con (Figure 12B). However, no significant effect of diabetes and resistance training was detected in TA and GAS muscles. The association between levels of exercise-induced myokines and grip strength was evaluated in SOL muscles of three-experimental groups. We found that a level of FGF-21 ($R=0.532$, $p=0.02$) and irisin ($R=0.498$, $p=0.03$) has significant correlations with grip strength (Figure 12C and D).

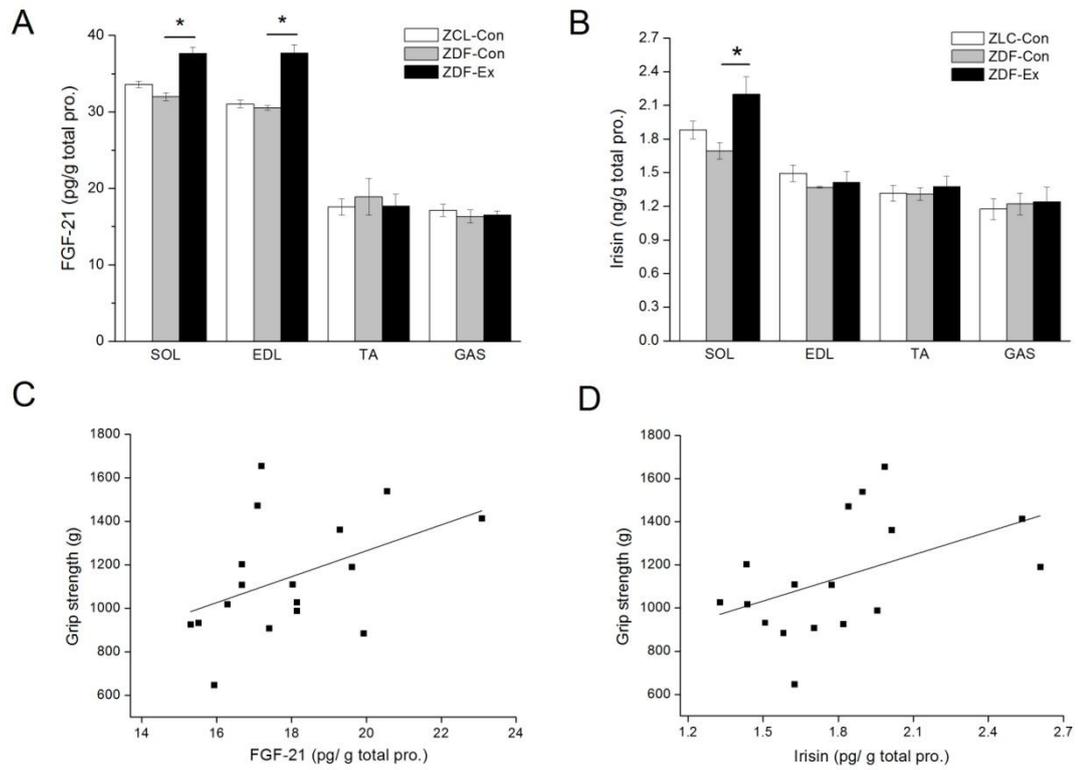


Figure 12. Effect of resistance exercise on FGF-21 and irisin levels and their correlations with grip strength

There was a main effect of 8 week of resistance training on BDNF in SOL ($F=10.53$, $p<0.01$). In soleus muscle, BDNF in ZDF-Con was 39.9% higher compared to ZLC-Con. And BDNF in soleus of ZDF-Ex rats was 40.1% lower following resistance training when compared to ZDF-Con rats ($p<0.05$, Fig. 13). Although there was no significant change of BDNF expression in TA, GAS, and EDL between ZLC-Con and ZDF-Con, skeletal muscle derived BDNF was significantly lower in TA ($p<0.05$, 28.7% reduction) and EDL ($p<0.05$, 13.1% reduction) muscles following 8 weeks of resistance training (Fig. 13)

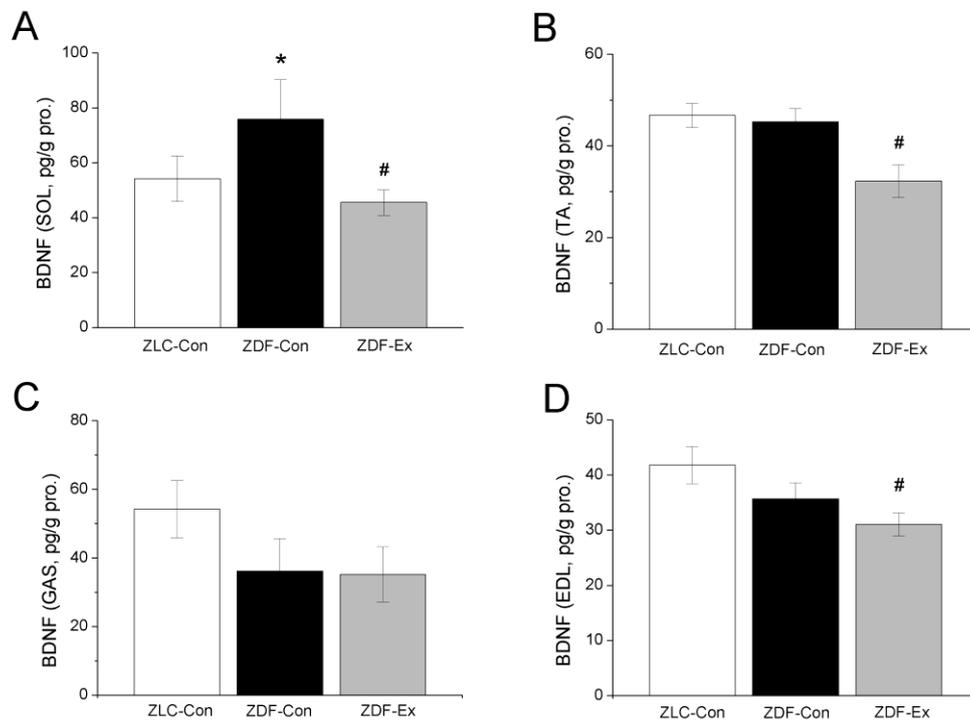


Figure 13. Effect of resistance exercise on BDNF levels in skeletal muscles

There was a positive association between the levels of plasma glucose and the BDNF in soleus muscle (n=17; r=0.612; p=0.008; Fig. 14A). And there was a negative association between the values of grip strength and the BDNF in soleus muscle (n=17; r=-0.657; p=0.004; Fig. 14B).

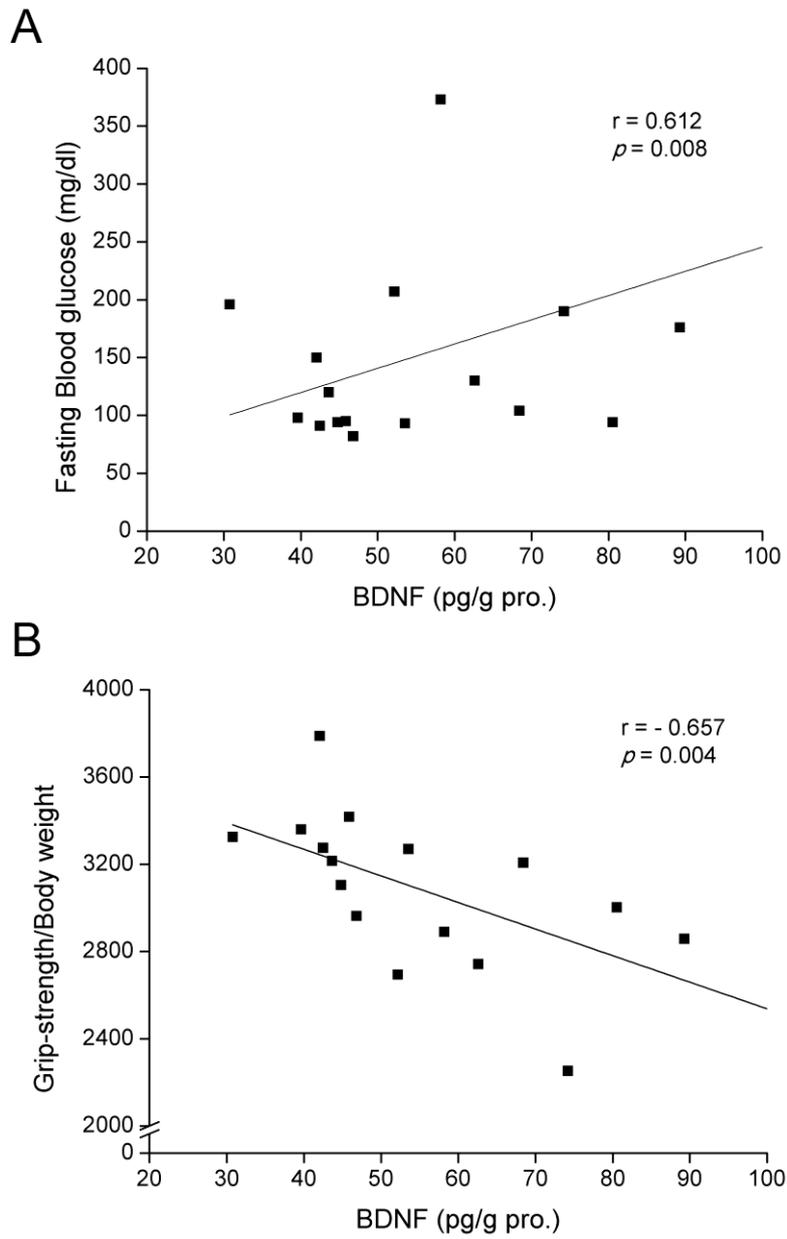


Figure 14. Association of BDNF and plasma glucose, grip strength

5. Discussion

Effect of resistance exercise on BDNF and glucose tolerance

In the present dissertation, the effect of progressive resistance training on BDNF and its relation to muscle strength in ZDF rat skeletal muscle was investigated. The main findings of the study are that the body weight and fasting plasma glucose concentration were following 8 weeks of resistance exercise training. And grip strength and muscle strength were significantly improved in trained diabetic animals. In SOL muscles of ZDF rats, elevated BDNF was significantly decreased and negatively correlated with grip strength. To our knowledge, this is the first study to investigate the effect of resistance training on the muscle-derived BDNF with concomitant improvement of muscle strength in transgenic diabetic animals. The result showed the body weight reduction in ZDF-Ex rats than that in ZDF-Con. Based on observation, there was no significant difference in food and water intake between exercise group and non-exercise group (data was not shown). Additionally, there was no visible symptom of under-nutrition in entire resistance training program. In the previous reports, resistance training leded to reductions in HbA1c and fasting insulin (Baldi & Snowling, 2003), improvement of insulin sensitivity (Ibanez et al., 2005). Thus, in our results, the significant changes in body weight and grip strength with improvement glucose intolerance indicate that

the resistance training was well performed. Recently, the number of studies to demonstrate the effects of resistance training on glycemic control and insulin sensitivity in patients with insulin resistance has substantially increased. Resistance training has been found to be effective for managing T2DM patients and may provide the additional benefits for preventing or limiting muscular dysfunction related with T2DM (Wood & O'Neill, 2012). Additionally, resistance training increased protein contents of GLUT4, insulin receptor, glycogen synthase (GS) and GS total activity (Holten et al., 2004). In our previous animal study, expression of GLUT4 protein was increased in skeletal muscles following same protocol as present study, high-intensity progressive resistance training using ladder and tail weight (our unpublished data). Also, in the present study, our resistance training protocol was suitable for T2DM model considering muscle strength and muscle quality were significantly increased concomitant with improvement glucose intolerance in trained diabetic rats. Taken together, our results suggest that progressive resistance training shown preventive effect on impairment of glucose metabolism and maintenance of muscle quality in T2DM patients.

It has been suggested that older adults with T2DM tend to have greater muscle loss, worse muscle quality, reduced upper and lower limb strength than their healthy, age-matched counterparts (Gregg et al., 2000; Gregg et al., 2002; Hovanec, Sawant, Overend, Petrella, & Vandervoort, 2012;

Park et al., 2006; Park et al., 2007). Muscle quality and strength gains followed by resistance training may result in greater physical activity participation (Fiatarone et al., 1994; Nelson et al., 1994; Rall & Roubenoff, 1996), with more effective mobility function, and glycemic profile improvements in T2DM patients (Boule, Haddad, Kenny, Wells, & Sigal, 2001)]. In the present study, muscle strength was significantly improved following 8 weeks of resistance training in diabetic animals. These results might indicate that resistance training could provide beneficial effects to diabetic patients.

In the previous study, increased BDNF mRNA in soleus muscle of diabetic rats compared to age-matched control proposed that elevation of BDNF may act to protect the distal nerve from denervated muscle of diabetic rat (Funakoshi et al., 1993; Koliatsos, Clatterbuck, Winslow, Cayouette, & Price, 1993). In the present study, on the same line as previous study, increase of BDNF expression in soleus muscle of ZDF-Con compared to ZLC-Con was observed and BDNF expression was significantly decreased following resistance exercise in SOL, TA, and EDL muscles as shown in Fig. 3A. At age 15 weeks, ZDF rats showed peripheral neuropathy involved decrease in motor/sensory nerve conduction velocities, myelin thickness, axon diameter, and axon thickness compared with the age-matched ZLC rat (Shevalye et al., 2012). Studies in the diabetic rat show that the levels of BDNF mRNA in soleus

muscle were elevated beginning at 4 weeks with a maximal six-fold increase by 6 weeks (Fernyhough, Diemel, Brewster, & Tomlinson, 1995). In the follow-up study, they demonstrated that increase in BDNF mRNA in the ipsilateral soleus muscle was dependent upon muscle activity (Fernyhough, Maeda, & Tomlinson, 1996). It was proposed that elevation of BDNF mRNA in diabetic skeletal muscle was an endogenous protective and/or repair mechanism induced by denervation of gastrocnemius muscle (Koliatsos et al., 1993). In addition, muscle fiber damage in the diabetic soleus muscle results in an upregulation of BDNF mRNA in muscle fiber with activation of satellite cells. According to previous reports and our data, BDNF in skeletal muscles might be expressed as a compensatory neurotrophic factor against diabetic neuropathy or myopathy.

Peripheral neuropathy and myopathy are common complications in patients with diabetes, lead to reduced muscle strength (Andersen, 1996; Andersen, Nielsen, Mogensen, & Jakobsen, 2004) due to progressive muscular atrophy (Andreassen, Jakobsen, Flyvbjerg, & Andersen, 2009). Previous study shown that aerobic exercise training can ameliorate autonomic nerve dysfunction and diabetic nerve regeneration (Malysz et al., 2011) in diabetic rats (De Angelis et al., 2000; Harthmann et al., 2007; Souza et al., 2007) and provide beneficial effect in experimental diabetic peripheral neuropathy (Selagzi, Buyukakilli, Cimen, Yilmaz, & Erdogan, 2008). However, the effects

of resistance exercise training on diabetic neuropathy including motor neuron denervation or reinnervation are little known. In the present study, BDNF in soleus muscles was positively correlated with surrogate factor of diabetes (fasting blood glucose) and negatively correlated with surrogate factor of physical fitness (muscle strength) in Fig. 4. These results demonstrated that resistance training applied to T2DM model induced not only increase of BDNF level in skeletal muscle, but also improvement of major variables for T2DM management including body composition, muscle strength, and fasting glucose level. Considering skeletal muscle derived BDNF has been identified as a novel contraction-induced cytokine that may contribute to the multiple health benefits associated with exercise (Pedersen et al., 2009), exercise induced BDNF in skeletal muscles might be an important mediator to elucidate the preventive effect of exercise for patients with T2DM.

Limitation of the present study includes lack of evaluation the neuropathy and myopathy in diabetic animals. In the present study, also, the collected skeletal muscles contain intramuscular nerve endings, Schwann cells, vascular endothelial cells and connective tissue, in addition to skeletal muscle fibers. As *in situ* hybridization was not performed to determine the localization of BDNF expression, we cannot conclude which cells contributed to the increase of BDNF response to resistance exercise in skeletal muscles of diabetic rats. The previous studies using *in situ* hybridization have confirmed

that BDNF was expressed in muscle fibers of both of human (Kust, Copray, Brouwer, Troost, & Boddeke, 2002) and rats (Coprav et al., 2000), and Schwann cells surrounding motor neurons (Griesbeck, Parsadonian, Sendtner, & Thoenen, 1995). Our results reflected not only alternation of BDNF in skeletal muscles, but the complicated interaction in other cell types within skeletal muscle. Therefore, further study would be needed to elucidate the alternation of BDNF derived from various cell types in diabetic skeletal muscles.

Effect of resistance exercise on IL-15

In previous studies, the role of IL-15 on metabolism was reported that IL-15 stimulated glucose uptake and lipid oxidation in muscle tissue (Almendro et al., 2006; Busquets et al., 2006). Furthermore, systemic injection of IL-15 reduced fat deposition in normal and obese rodent, associated with inhibition of lipogenesis in liver and adipose tissue (Alvarez et al., 2002; Carbo et al., 2001). Also, the circulating IL-15 protein can regulate body composition including adipose tissue deposition (Quinn, Anderson, Strait-Bodey, Stroud, & Argiles, 2009). Recent study reported that IL-15 improves glucose homeostasis and insulin sensitivity in obese mice (Barra et al., 2012). In this study, we found the increase of IL-15 expression in SOL muscle with concomitant improvement of glucose tolerance in diabetic animals followed

by training. Although it is not known whether exercise-induced IL-15 has a systemic effect on improvement of glucose metabolism, IL-15 may be a potent mediator to elucidate the beneficial effect of exercise on metabolic diseases such as obesity or type 2 diabetes.

Having an important role in the determination of carbohydrate and lipid metabolism, skeletal muscle is affected by insulin resistance in diabetic conditions. In patients and animals with type 2 diabetes, skeletal muscles showed lower oxidative enzyme activity than normal (Marin, Andersson, Krotkiewski, & Bjorntorp, 1994) (Hickey et al., 1995) (Nyholm et al., 1997). Also, the alternation of muscle fiber types and fiber specific oxidative enzyme activities in skeletal muscles of patients with type 2 diabetes have been reported (Oberbach et al., 2006). In various type 2 diabetic animals including OLETF (Otsuka Long Evans Tokushima Fatty), GK (Goto-Kakizaki) and ZDF (Zucker diabetic fatty) rats, skeletal muscle have a lower percentage of type IIA fibers compared to age-matched non-diabetic rats (Yasuda et al., 2002) (Yasuda et al., 2006). Additionally, Yasuda et al., reported that SOL muscle in ZDF has a lower percentage of type IIA fibers than lean control (Adachi et al., 2007). Due to different metabolic properties of the muscle fibers classification, type I (slow-twitch, oxidative) and type II (fast-twitch, glycolytic), skeletal muscles are differently affected by diabetes with controversial results.

Effect of resistance exercise on irisin and FGF-21

In the present study, we found the increase of FGF-21 and irisin levels in skeletal muscles (both in soleus muscle) following 8 weeks of progressive resistance training in type 2 diabetic rats. In addition, these increases of FGF-21 and irisin level in soleus showed a significant correlation with grip strength after resistance training. To our knowledge, these descriptive results were the first report to evaluate the effect of resistance training on FGF-21 and irisin levels in skeletal muscles using type 2 diabetic animal models.

Physical activity or exercise training plays important roles in preventing and treating metabolic diseases. The secretion of some myokines from skeletal muscle with endocrine manner has been treated as a potential mediator or modulator released by exercise (Pedersen & Febbraio, 2012). Previous studies regarding effect of acute exercise on FGF-21 and irisin in circulating level were reported. In normal mice and healthy humans, increment of serum FGF-21 level was induced by a single bout of treadmill (K. H. Kim et al., 2013) and by 2 weeks of treadmill training (Cuevas-Ramos et al., 2012). In addition, FGF21 circulating level after 1h recovery of a single bout of exercise was significantly increased prior to exercise, while the level of FGF-21 secreted from high-intensity exercise was higher than that of mild intensity exercise (K. H. Kim et al., 2013).

Recent research indicated that circulating irisin was significantly lower in T2DM subjects compared to non-diabetic controls (Pedersen & Febbraio, 2012) (Liu et al., 2013). Also, Choi et al. demonstrated significantly decreased circulating irisin levels related to glucose tolerance status in normal group and type 2 diabetes group (Choi et al., 2013). Furthermore, high-intensity exercise increased irisin response compared to low-intensity exercise with similar energy expenditure (Tsuchiya et al., 2014). In addition, exercise-induced changes of irisin levels were not different between healthy controls and subjects with metabolic syndrome (Huh et al., 2015). However, based on the previous reports to evaluate the change of irisin level response to exercise training, most types of exercise intervention were limited to aerobic exercise and it seems to be needed to investigate the effect of resistance training or compare the different effect from aerobic and resistance exercise in metabolic diseases.

The role of FGF-21-PGC-1 α -irisin axis in age-related condition including type 2 diabetes was proposed with possible PGC- α activator such as exercise (Sanchis-Gomar et al., 2014). Lee et al., (2014) suggested that targeting PGC-1 α , e.g., using endocrine activators of fat browning such as FGF-21 and irisin, might benefit in treating metabolic diseases (Lee et al., 2014). However, there was no report to investigate the effect of exercise type (aerobic and resistance exercise) and intensity (vigorous and moderate

intensity) on FGF-21 and irisin in metabolic diseases. In this regard, our results might be fundamental for establishing the standard of exercise.

PART II

Effect of resistance exercise on myokines and muscle functions in aging mice

1. Introduction

Skeletal muscle has recently been recognized as an endocrine organ that produces and releases various cytokines termed myokines that are involved in the regulation of several physiological and metabolic pathways. Discovery of myokines has emphasized the role of muscle as an important source of exercise-induced hormones to communicate information and interact with other tissues, including fat, liver, and pancreas, to alter metabolism (Huh et al., 2012). Irisin, a novel myokine peptide identified recently (Bostrom et al., 2012) is a cleaved and secreted fragment of fibronectin type III domain-containing protein 5 (FNDC5). It is up-regulated by peroxisome proliferator-activated receptor (PPAR)- γ co-activator 1 alpha (PGC-1 α). Irisin has been suggested to mediate some beneficial effects of exercise on fat metabolism by a process called adipocyte browning through inducing uncoupling protein 1 (UCP1) (Bostrom et al., 2012). Brown adipose tissue has positive functions such as anti-obesity and anti-diabetes in murine as well as positive effect in humans as a thermogenic factor (Harms & Seale, 2013; Huh et al., 2014; Qian et al., 2013; Rothwell & Stock, 1979). Recent studies have suggested that brown and beige fat can regulate fat metabolism in human (Harms & Seale, 2013).

According to previous reports, targeting irisin and irisin-related

pathway, particularly the key signaling molecule responsible for their secretion, could identify new candidates in the anti-aging armamentarium (Sanchis-Gomar, 2012). Lower baseline circulating irisin was recently reported in old human subjects compared to young adults (Huh et al., 2014). The role of FGF21-PGC-1 α -Irisin axis in age-related conditions and sarcopenia has been hypothesized recently (Sanchis-Gomar et al., 2014). In aged condition, reduced mitochondrial biogenesis has been shown to be associated with abnormal PGC-1 α signaling (Sanchis-Gomar & Derbre, 2014). Maintaining normal PGC-1 α reactivity might help prevent age-related loss of mitochondrial biogenesis in skeletal muscle (Derbre et al., 2012). Taken together, using activators of brown fat thermogenesis like irisin to target PGC-1 α might prevent age-related loss of muscle and metabolic disorders (Lee et al., 2014).

Recently, irisin has been reported to respond to aerobic and resistance training. Previous human studies have demonstrated immediate increase of circulating irisin expression after acute exercise (Aydin et al., 2013; Daskalopoulou et al., 2014; Huh et al., 2012; Kraemer, Shockett, Webb, Shah, & Castracane, 2014; Norheim et al., 2014; Pekkala et al., 2013). It has been reported that exercise-induced irisin secretion is independent of age or physical fitness level. Increased irisin may directly modulate muscle

metabolism through AMPK activation (Daskalopoulou et al., 2014). However, the effect of exercise with different type (resistance or endurance exercise), intensity (high- or moderate-intensity exercise), and duration (acute or chronic exercise) on circulating and muscle irisin expression seems controversial. Although resistance training was considered as effective intervention to improve age-related problems, the effect of resistance training on irisin expression in aging population has not been reported yet. Therefore, the objective of this study was to determine the effect of resistance training on irisin expression in aged mice and human subjects.

2. Purpose of study

Although resistance training was considered as effective intervention to improve age-related problems, the effect of resistance training on irisin expression in aging population has not been reported yet. Therefore, the objective of this study was to determine the effect of resistance training on irisin expression in aged mice and human subjects. In the animal experiment, we evaluated the change of irisin expression in circulating level and various tissues of skeletal muscle following 8 weeks of progressive resistance training using ladder and tail weight.

3. Methods and materials

3.1 Animal and experimental design

Fourteen 19 months old male C57BL/6 mice were obtained from Biomedical Mouse Resource Center, Korea. All mice were housed in a controlled environment with a 12:12 light-dark cycle at room temperature (22°C). They were provided with adequate food and water. These 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. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University. All experiments were conducted to minimize the number of animals utilized and the suffering caused by the procedures of the study. The experimental mice were randomly assigned into two groups: Old Control group (OCON, n=7) and Old Resistance exercise group (ORT, n=7). Weight of chow consumed was measured weekly to observe food intake and possible stress the animals might experience during the experiment.

3.2 Exercise protocol

For the exercise group, resistance ladder climbing exercise was performed 3 days per week for 12 weeks. Ladder climbing exercise was

conducted by using 1 m ladder with 1.5 cm grids. The ladder was set to attain 85 degree angle with the ground. One week adaptation was conducted by letting mice to climb up the ladder without any resistance. To reduce stress, outsource stimulus such as food reward and electrical stimulation were not given to the mice during the ladder climbing exercise. The mice were positioned at the very bottom of 1 meter ladder and motivated to climb up the ladder. When mice reached the very top of the ladder, 2 minute rest was given before the next trail of ladder climbing. After the one week adaptation, resistance at 10 % of body weight was given to the mice by adding weight on the tail. The loads were increased gradually as the exercise sessions preceded. To progressively increase exercise intensity, 2 grams of additional weights were applied after four successful trails. However, because the animals were very old and fragile, intensity was carefully adjusted for each mouse at each exercise session.

Training period	12 weeks
Frequency	3 days/week
Repetition	Maximal 8 times per day
Intensity (load)	with tail weight (10% to 100% of body weight)



Figure 15. Progressive resistance training using ladder and tail weight

3.3. Body composition and grip strength

At 48 hours after the last exercise session, body composition was measured by using DXA (Discovery W, Hologic, USA). Whole body animal DEXA modulation was used to measure body composition. By using Standard Software (QDR for Windows XP Operating System, Hologic, USA), hind limb region was carefully selected and analyzed. Every two weeks, grip-strength was measured by using Grip Strength Meter (Bioseb, France). Briefly, mice were allowed to grasp steel wired grid attached to the force gauge. Mice were then pulled back from the gauge. The force was recorded when mice released the grid. For each measurement, five trails were conducted. The maximal values were taken for analysis.

3.4 Protein extraction and irisin quantification

After the DEXA measurement, anesthetized mice's left and right soleus (SOL), tibialis anterior (TA), gastrocnemius (GAS), and extensor digitorum longus (EDL) muscle were rapidly removed and stored at -80°C until protein analysis. To extract proteins from skeletal muscles were homogenized in 500 µl of extraction buffer (RIPA) with protease inhibitor. The solution was then centrifuged at 12,000 rpm at 4°C for 15 minutes to precipitate insoluble materials. Protein concentrations in the supernatants were quantified using Bradford Protein Assay Kit (Bio-Rad, USA). Quantification and measurement of irisin was performed using Irisin ELISA Kit (Phoenix Pharmaceuticals, CA, USA) and micro-reader.

3.5 Muscular endurance capacity and mobility

The wire hanging test was performed to examine the muscular endurance capacity of mice. It began with the position that an animal being put on the top of the elevated grid. The mice were placed on the top of the grid with 10 g weight attached to their tail. Then, the grid was inverted and suspended above the cage with sponge in order to prevent any possible damage to animals. When the mice fell within 10 seconds, additional trials were performed again.

Mobility was measured to observe physical activity level of mice. Physical activity chamber was made 100 cm x 100 cm x 16 cm with transparent acrylic panels and 10 cm x 10 cm lined-square was drawn on top plate. After 4 hours adaptation for physical activity chamber, each group of mice was put in the chamber and recorded their activity by a video camera fixed above the chamber in a dimmed-room light setting for 30 minutes without supervisor present in the same room. Activity score of 1 was counted when the body of mouse crossed the line and the average counted number of each group was used for an analysis.

2.3. Data analysis

Statistical analysis was performed using the SPSS 18.0 software (SPSS Inc.). Data were analyzed using independent t-test to compare the body composition, BMC, BMD, food intake, muscle strength, physical function and irisin expression in the mice study. Two-way ANOVA with repeated measurement was used to compare the basic characteristics of participants, irisin expression and muscle strength and physical functions in the human study. Statistical significance was considered when p value was less than 0.05.

4. Results

As shown in Table 1, there was no significant difference in body weight, fat mass, or lean mass between the Old-Con group and the Old-Ex group. In addition, although the wet weight of skeletal muscle in the hindlimb including SOL, EDL, TA, and GAS muscles was measured after exercise training, we could not find any significant alternations in wet weight (data was not shown). Bone quality including BMC and BMD was not changed following resistance training in old mice.

Table 1. Basic characteristic of aging mice

	Old-Con (n=6)	Old-Ex (n=7)	<i>p</i> -value
Body Weight (g)	35.35 ± 0.798	35.586 ± 1.208	0.872
Lean Mass (g)	26.76 ± 0.969	28.357 ± 1.052	0.196
Fat Mass (g)	15.25 ± 0.764	15.014 ± 0.542	0.341
Bone mineral contents (g)	0.692 ± 0.044	0.77 ± 0.056	0.538
Bone mineral density (g/cm ²)	0.115 ± 0.002	0.111 ± 0.005	0.847
Food Intake (g)	3.043 ± 0.047	2.889 ± 0.042	0.124

Values are presented as mean±SEM

As shown in Figure 16A-B, there were no significant differences in body weight and food intake between Old-Con and Old-REX group following 12 weeks of resistance training. In this progressive resistance training using ladder and tail weight, the tail weight was gradually increased (added 2 g of tail weight) after successful trial. The initial tail weight was approximately 3.0 g and the tail weight of final session was 43.14 ± 0.91 which was relatively 122.09% of body weight (Figure 16 C and D).

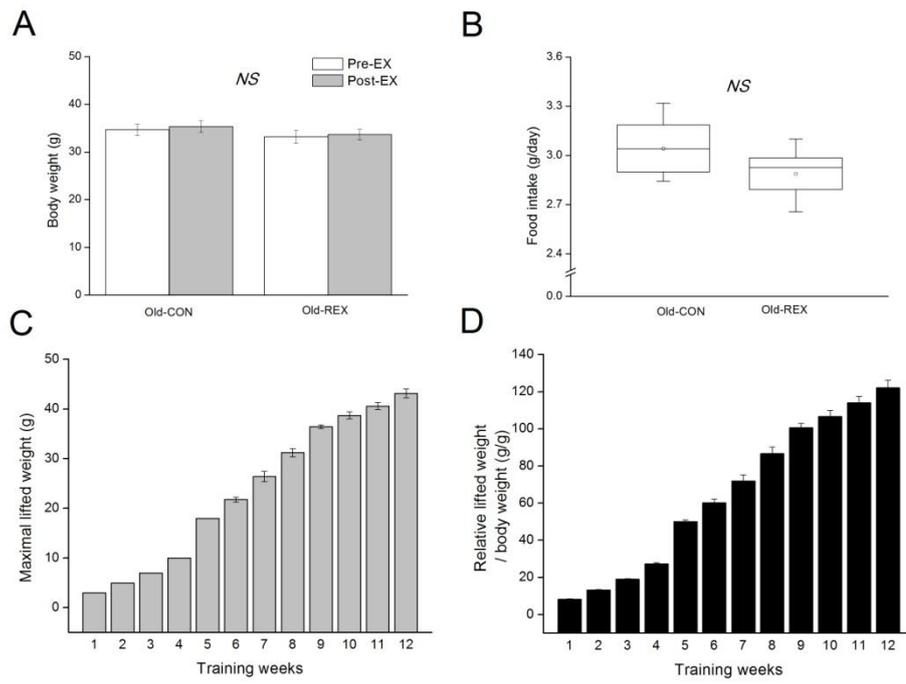


Figure 16. Effect of resistance training on the body weight, food intake, and lifted tail weight.

The muscle wet weights of SOL, EDL, TA and GAS were measured after 12 weeks of training. The significant increase of muscle weight muscle was found only in SOL muscle ($p < 0.05$), but not in other muscles. And we found similar trend in relative muscle weight which was adjusted by body weight (table 2). In addition, we analyzed the histological alternation after resistance training using H&E staining in GAS muscle. Although there was no significant change in muscle wet weight in GAS muscle, we found the centralized nuclei in muscle fiber (indicated with arrow in figure 17) that was the marker of muscle regeneration (Figure 17). However, we did not find any increase of cross sectional area in Old-Rex group compared to Old-Con group (data was not shown).

Table 2. Muscle wet weight and relative muscle weight adjusted by body weight

	SOL	EDL	TA	GAS
<i>Muscle wet weight (mg)</i>				
Old-Con	10.01 ± 0.26	12.85 ± 0.34	54.06 ± 1.31	140.64 ± 3.20
Old-REX	10.94 ± 0.35*	12.58 ± 0.21	54.35 ± 0.87	139.53 ± 1.99
<i>Relative muscle weight (mg/g)</i>				
Old-Con	28.42 ± 0.88	36.34 ± 0.44	153.05 ± 2.25	398.22 ± 5.15
Old-REX	32.41 ± 1.27*	37.27 ± 1.02	160.90 ± 3.95	413.20 ± 10.08

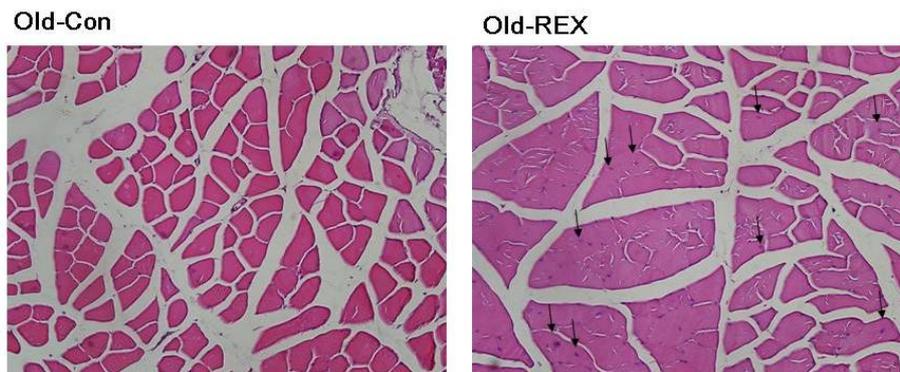


Figure. 17 H&E staining for evaluating the muscle regeneration

Grip strength of old mice was significantly increased (14.85%, $p=0.02$) in the Old-Ex group compared to that in the Old-Con group (Figure 18A). The total lean mass of skeletal muscle in both of fore- and hind-limb was measured using DEXA in order to estimate muscle quality. Muscle quality was calculated by taking the ratio of grip strength and total lean mass. Significant improvement (16.34%, $p=0.03$) of muscle quality following resistance training was found in the Old-Ex mice group compared to that in the Old-Con mice group (Figure 18B). However, there was no significant change in muscular endurance measured by hanging test with 10% of tail weight. In addition, the mobility was significant increased in training group compared to control group. (Figure 18C and D)

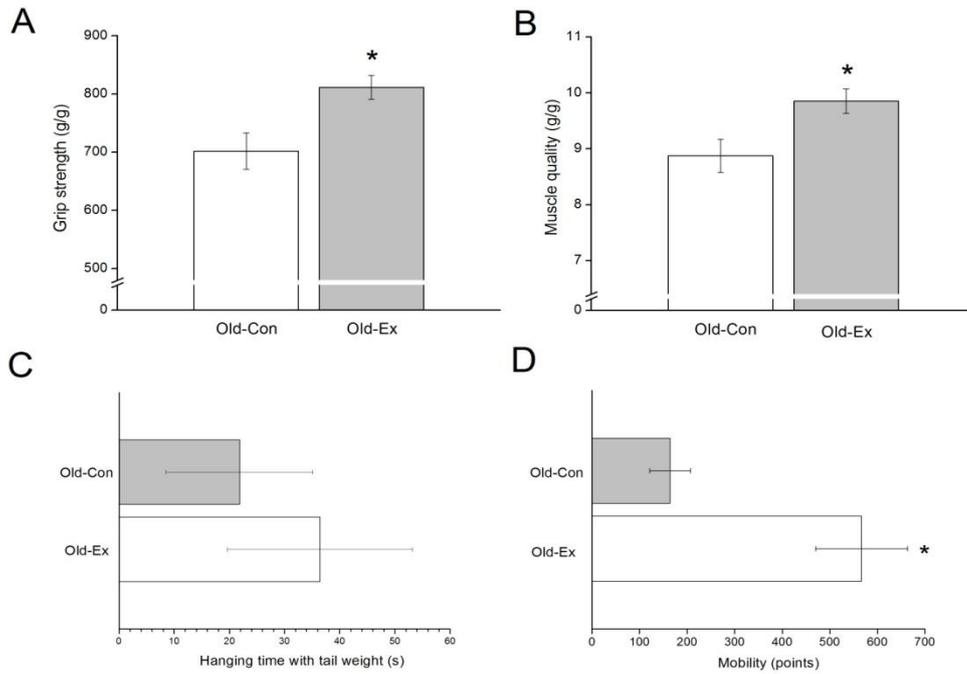
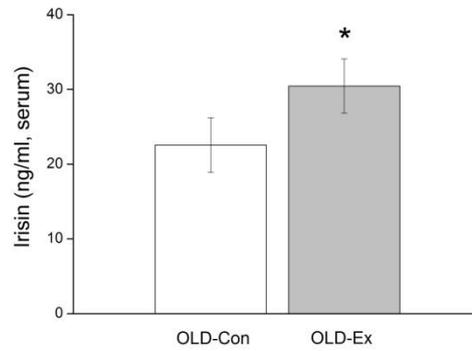


Fig. 18. Effect of resistance exercise on improvement of physical functions in aging mice. Physical functions including grip strength, muscle quality, muscle endurance, and physical activity (mobility) were measured after 12 weeks of progressive resistance exercise. (A) Grip strength was tested with both fore- and hind-limb of mice. (B) Muscle quality was calculated by taking the ratio of grip strength and total lean mass of mice. (C) Wire-hanging time was measured to evaluate muscle endurance capacity with 10 g of tail weight. (D) Physical activity was evaluated by counting the number of cross the line in activity chamber. * $p < 0.05$ compared with OLD-Con group. Values are mean \pm S.E.M. for OLD-Con (n=6) and Old-Ex (n=7).

The expressions of irisin in serum and skeletal muscle including SOL, EDL, TA, and GAS were measured at the end of 8 weeks resistance training. In circulating level, irisin protein level in the serum of the Old-Ex mice group was higher ($p=0.02$) compared to that in the Old-Con mice group (Figure 19). The significant increase of irisin protein was found in SOL muscle of Old-Ex mice compared to Old-Con mice. However, there was no significant change in EDL, TA, or GAS muscles (Figure 19).

A



B

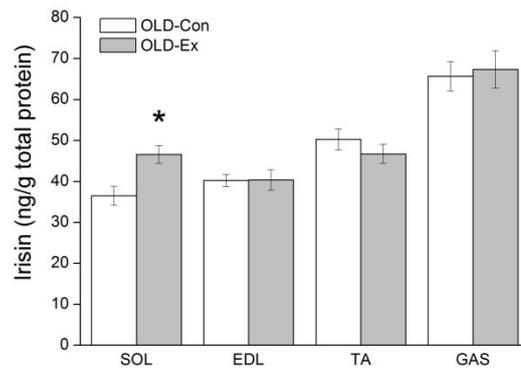


Figure 19. Irisin levels in serum and skeletal muscles. The expressions of irisin were measured in (A) serum and (B) skeletal muscles including SOL, EDL, TA, and GAS after 12 weeks of progressive resistance exercise. Irisin expression in skeletal muscle was calculated by dividing total protein of each muscle tissues. * $p < 0.05$ compared with OLD-Con group. Values are mean \pm S.E.M. for Old-Con (n=6) and Old-Ex (n=7).

We evaluated the level of IL-15 in serum and various skeletal muscles including SOL, EDL, TA, and GAS. The significant increase of circulating IL-15 level was found in Old-REX compared to Old-Con (Figure 20A). In skeletal muscles, IL-15 level was significantly increased in SOL muscle after 12 weeks of resistance training, but not in other muscles (Figure 20B). In addition, level of IL-15 in SOL muscle was highly correlated with muscle weight of SOL ($R^2=0.603$, $p=0.032$). However, there was no relationship between IL-15 level in SOL and grip strength ($R^2=0.324$, $p=0.473$, Figure 21).

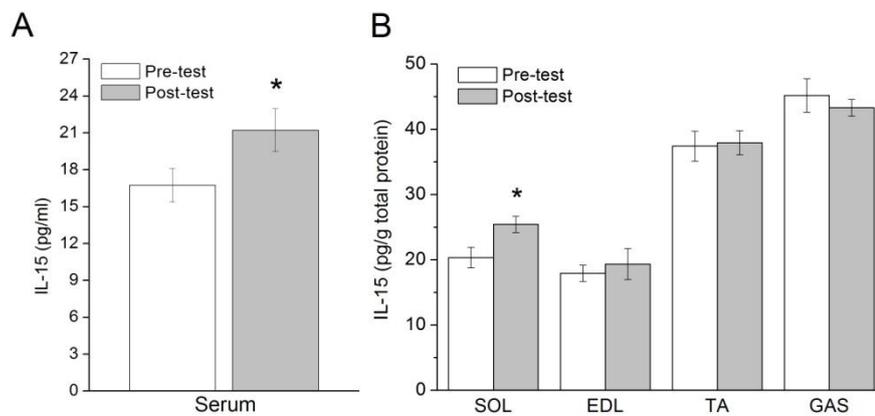


Figure 20. Effect of resistance exercise on IL-15 levels in serum and skeletal muscle.

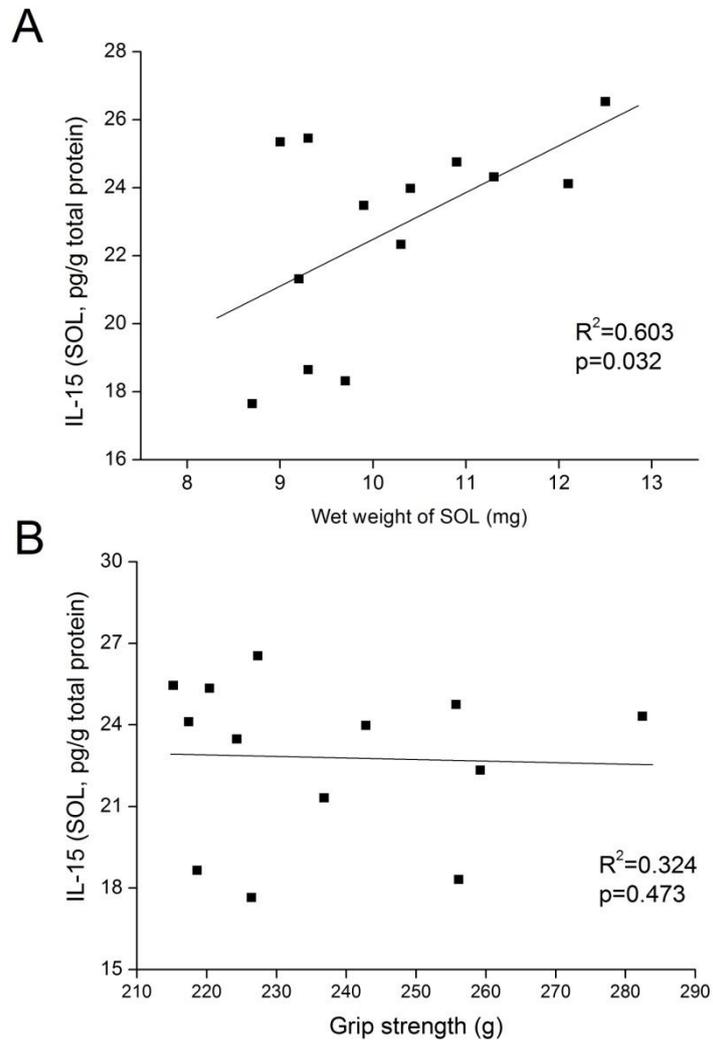


Figure 21. Association of IL-15 with muscle weight and grip strength

5. Discussion

This study revealed that circulating irisin protein was significantly increased concomitant with improvement in muscle strength following resistance training in aging mouse. In the aging mice study, irisin expression in skeletal muscle (especially in the soleus muscle) was increased in the 12-weeks of resistance training group.

In the animal study, body weight and food intake were not different between the exercise group and the non-exercise group. Based on our observation, there was no visible symptom of under-nutrition or over-training in the entire resistance training program. In our results, improvement in muscle function including muscle strength, and mobility indicated that the resistance training was well performed.

In the aging mice model, irisin was present in skeletal muscle tissue and serum under resting conditions (Brenmoehl et al., 2014). Immunohistochemical analysis confirmed the presence of irisin in murine skeletal muscle. Its predominant location in the extracellular space between muscle cells is in agreement with a proposed mechanism of cleavage of the extracellular domain of transmembrane protein FNDC5 (Bostrom et al., 2012). In a previous report, the expression of irisin was increased in femoral skeletal muscles, whereas there was no significant change in heterogeneous crus

muscle including soleus, extensor digitorum longus, or gastrocnemius after acute treadmill exercise (Brenmoehl et al., 2014). In the present study, we measured the expression of irisin in distinctive individual skeletal muscle such as soleus, extensor digitorum longus, tibialis anterior, and gastrocnemius. In our results, irisin protein level was significant increased, especially in soleus muscle after 12 weeks of resistance training. Interestingly, we found changes of myokine (such as IL-15, LIF, BDNF etc.) after resistance training, especially in the soleus of various experimental animal models (normal, diabetic, and aging mice, unpublished data). These results together with previous reports support that soleus might be the source of irisin induced by resistance exercise in aging skeletal muscle.

Resistance training is essential to counter age-related declines in muscle mass, strength, and power in the aging population. Resistance training for the elderly can provide a broad range of systemic benefits, including moderating the development of sarcopenia that aerobic-based exercise training cannot achieve. Representative benefits for frail elderly include: 1) prevents muscle mass loss and improves muscle strength/function; 2) improves modestly cardiorespiratory fitness; 3) decreases risk of fall but increases bone mineral density and tendon strength; and 4) improves various cardio metabolic risk factors in the absence of weight loss. Moreover, skeletal muscle is increasingly recognized as an endocrine organ that can release a variety of

signaling molecules and regulating cytokines called myokines, including irisin that regulates several aging-related physiological and pathological processes. In the present study, physical functions including muscle strength and muscle quality were significantly increased with the increase of irisin expression. Therefore, resistance training could be potent intervention for frail elderly to improve their muscle strength and function by increasing the expression of irisin. Exercise might have induced irisin secretion by contracting skeletal muscles. This might be a potential target of therapies to optimize weight control and metabolic profile (Lee et al., 2014). Targeting irisin and PGC-1 α , the key signaling molecules for its secretion, could identify new candidates in the anti-aging armamentarium named “FGF21-PGC-1 α -Irisin axis” (Sanchis-Gomar, Lippi, Mayero, Perez-Quilis, & Garcia-Gimenez, 2012; Sanchis-Gomar et al., 2014). Hur et al., reported that circulating irisin was negatively associated with age, insulin, cholesterol, and adiponectin levels, indicating a possible compensatory role of irisin in age-related metabolic regulation (Huh et al., 2012). Similarly, circulating irisin level was correlated with age and pulse pressure in patients with type 2 diabetes (Liu et al., 2013). In addition, Rana et al. (Rana et al., 2014) recently reported that circulating irisin proteins were positively associated with telomere length related to aging and risk of myocardial infarction. Irisin may prove to be beneficial in monitoring or treating obesity and diabetes characterized by an imbalance between energy

demand and expenditure (Hojlund & Bostrom, 2013; Sanchis-Gomar et al., 2012). Taken together with our results, exercise-induced irisin as an endocrine activator of brown fat function might benefit the treatment of other age-related conditions, particularly metabolic diseases.

PART III

Effect of resistance exercise on myokines and muscle functions in elderly adults

1. Introduction

Age-related frailty in the elderly is loss of muscle mass and concomitant reduction of muscle strength and functional ability. (Marcell, 2003; Sayers & Gibson, 2010) A decrease of capacity to develop high velocity movement and lessened responsiveness to prevent falls are risks of disability and injury (Hakkinen, Alen, Kallinen, Newton, & Kraemer, 2000; Pereira, Izquierdo, Silva, Costa, Bastos, et al., 2012; Pereira, Izquierdo, Silva, Costa, Gonzalez-Badillo, et al., 2012). Decreases of muscle mass and changes in muscle properties such as type II muscle fiber loss, atrophy, and slow nerve conduction velocity may accelerate the age-related reduction of muscle power, the product of force x velocity (Norris, Shock, & Wagman, 1953; Sayers & Gibson, 2010). In addition, rapid force characteristics such as rate of torque development may be more functionally relevant compared to maximal force capacities (Lockie, Murphy, Knight, & Janse de Jonge, 2011; Thompson, Ryan, Sobolewski, Conchola, & Cramer, 2013). A decline in rapid force production across the life span may therefore lead to large decrements in functional and independent living abilities and increase injury risks associated with common daily activities (Thompson et al., 2013). Therefore, maintenance of muscle power is a key factor in usual life performance, including stair climbing, normal gait, as well as decreasing the risk of falls, especially in older women

(Arnold, Warkentin, Chilibeck, & Magnus, 2010; Pereira, Izquierdo, Silva, Costa, Bastos, et al., 2012).

Recently, the effective resistance training for the elderly was demonstrated to overcome age-related reduction of muscle power. The effectiveness of traditional resistance training in older population has been questioned, especially for functional tasks (Ramirez-Campillo et al., 2014; Raymond, Bramley-Tzerefos, Jeffs, Winter, & Holland, 2013), since these training protocols do not improve fast and explosive activities (Izquierdo et al., 2001; Pereira, Izquierdo, Silva, Costa, Gonzalez-Badillo, et al., 2012). Of various resistance training methods for the elderly, a number of studies have demonstrated that high-speed power training (HSPT) in the elderly may improve muscle power or functional performance compared to traditional resistance training (Fielding et al., 2002; Henwood, Riek, & Taaffe, 2008; Miszko et al., 2003; Newton et al., 2002; Sayers & Gibson, 2010). There is a critical difference between classical resistance training and HSPT considering the application of principle for resistance training. In classical resistance training was applied with 1:1 or 1:1.5 ratio of contraction and extension time. In contrast, HSPT was performed the concentric phase of each repetition as fast as possible, paused for 1 second, and performed the eccentric portion of the contraction over 2 seconds with lower exercise intensity (Sayers & Gibson, 2010). Based on results of previous reports, HSPT may be considered as a

potential muscle power training to promote therapeutic developments in the elderly (Fieo, Watson, Deary, & Starr, 2010; Pereira, Izquierdo, Silva, Costa, Gonzalez-Badillo, et al., 2012; Reid & Fielding, 2012). In addition, elastic band exercise training has become increasingly popular as an alternative to machine-based resistance training because of its safety and portability in the elderly (Yamauchi et al., 2005). In previous reports, elastic band-based training was utilized by older adults of various fitness levels, including independently living older adults (Rogers, Sherwood, Rogers, & Bohlken, 2002; Yamauchi et al., 2005).

2. Purpose of study

Although the effectiveness of power training and band-based exercise was well understood, the effect of HSPT using elastic band to improve physical functions of the elderly has not been reported. Therefore, the objective of this study was to determine the effect of HSPT using elastic band on muscle strength (including rate of force development) and physical functions (including balance, pick-up task, and escape task) in community-dwelling older adults.

3. Methods and materials

3.1. Participants

Between February and March of 2013, participants were recruited from silver academy college of Y-church in Seoul. A total of 50 women with age over 65 years were enrolled in this study. Those who were able to walk 10 m without a walking aid and those who did not have a habit of exercise were subjected to further inclusion criteria. Exclusion criteria were: unstable cardiac disease, cerebrovascular disease, musculoskeletal impairment, presence of other medical conditions with psychiatric disorder, or neurologic disorder. Before randomization, we conducted health related education to participants. Of the 50 enrolled participants, 7 participants were excluded to fulfill the exclusion criteria at the first visit of the experiment and 43 patients were randomly divided into two-experimental group (exercise group=22, control group=21). Finally, 8 participants (1 in the exercise group and 7 in the control group) were dropped out from the study during the experiment due to personal reasons (lack of motivation) or for health reasons unrelated to the study. Informed written consents to participate the study were obtained. This study and consents were approved by the Seoul National University Ethics Committee (SNUIRB No.1305/001-009).

3.2. Supervised- and home-based exercise program

The exercise group used HSPT protocol and performed the concentric phase of each repetition as fast as possible, paused for 1 second, and performed the eccentric portion of the contraction over 2 seconds.(Sayers & Gibson, 2010) The exercise program consisted of 12 weeks of 1-h exercise session 2 days a week on Tuesday and Thursday. Additionally, they were encouraged to perform home-based exercise at home on Monday, Wednesday, and Friday. Each session had 10 min of warm-up exercises, 40 min of elastic band training, and 10 min of cool down exercises. Resting periods of one min were allowed between sets and two min between exercises. Participants were supervised by a qualified instructor who was also certified for cardio-pulmonary resuscitation (CPR) and automatic external defibrillation (AED).

For the exercise group, the elastic band was green. Participants were instructed to perform exercise training at a perceived exertion intensity of 12-13.(Borg, 1998) Participants in the exercise group performed 2-3 sets of 12-15 repetitions of this exercise per session. By applying progression principle, the intensity of the sessions was progressively increased by the number of sets and the number of repetitions. In addition, by shortening the length of the band through grasp different position, the intensity of exercise was progressively increased throughout the intervention period. The exercise program followed the recommended guidelines for older adults by the American College of

Sports Medicine. For the non-exercise (balance and tone) group, participants were asked to continue their routine daily activities and perform stretching (static, dynamic) once a week for one hour. Participants in the non-exercise group also had taken health education.

The home-based exercise program was consisted of flexibility and resistance exercise. In addition to the supervised exercise session, participants were instructed to perform exercise three times per week. In contrast to a standardized supervise resistance exercise program, a home-based exercise targeted major muscle groups of the upper and lower extremities were provided for exercise group. Provided home based exercise program was relatively light enough to perform at home. For instance, home based exercise program includes sit and stand while watching television or weight bearing exercise. Instructor provided exercise program guidebook every month and checked whether participants performed home-based exercise at home. After exercise intervention, instructor provided feedback to continue exercise regularly.

3.3. Body composition

The body composition was measured at the beginning and the end of the exercise training. Subjects were asked to fast overnight and normal

physical activity. The anthropometric parameters were screened by the same examiner. The height was evaluated by using an extensometer. Body weight, BMI, fat mass, percent body fat, fat free mass, and skeletal muscle mass were measured by bioimpedance analysis using Inbody 370 (Biospace, Korea).

3.4. Functional performance

3.4.1. Short Physical Performance Battery (SPPB)

Short Physical Performance Battery (SPPB) is a method for assessing physical performance of older patients (Guralnik, Seeman, Tinetti, Nevitt, & Berkman, 1994). Tests in SPPB were used to assess the balance, walking, strength, and endurance by examining the capacity to stand with feet side by side, semi tandem and tandem, to perform a walk on 4 meters and get up and sit 5 times in a row. Each test provides a performance score. SPPB has a total of 12 points, including 4 points of chair stand test, 4 points of balance test, and 4 points of gait speed test. According to Perera and colleagues, SPPB score change of 1.0 point is a substantially meaningful (Perera, Mody, Woodman, & Studenski, 2006).

3.4.2. Timed-Up-and-Go Test (TUG)

The purpose of Timed-Up-and-Go Test (TUG) is to measure dynamic balance and agility (Shumway-Cook, Brauer, & Woollacott, 2000). TUG test

requires a subject to stand up from a chair, walk 2.44 meters to a cone, turn, walk back, and sit down. Time taken to complete the test is strongly correlated with level of functional mobility. Participants were requested to sit with their back against a chair. At the command of 'go,' they needed to stand upright and then walk as fast as possible to the cone in front of them, turn around, return to the chair, and sit down. A stopwatch was started on the word 'go' and stopped when the subject returned to the starting position.

3.4.3. Balance test

Balance test was used to evaluate the participants' ability to maintain functional (single-legged) balance, as ability to maintain balance has been shown to be a predictor of falls in older adults.(Baczkowicz et al., 2008) Center of pressure (COP) measures were completed using a force platform (Advanced Medical Technology, Inc., USA). Participants performed the eyes-open single-legged stance. Each condition was repeated three times with 30 seconds rest between sessions. The results were recorded as second.

3.4.4. Pick-up test

Subjects performed pick-up test to measure their balance ability during functional performance. All subjects were ready in a natural position with shoulder width of feet outline on the force-platform (AMTI, USA). They

were then asked to pick up a basket as fast as possible after reacting to a LED signal from the lamp 3 m ahead. The basket laid 30 cm ahead. The baskets were 28×21×14 cm at 600 g. Subjects performed a total of three trials. LED signal was manipulated by a researcher to minimize subject's predictable reaction using a trigger. We synchronized the start time using a synchronizer (Visol, Korea) that simultaneously sent signal to the main computer's QTM (Qualysis Track Manager, Qualysis, Sweden) software. Also, analogue data of ground reaction force (GRF) from force-platform were converted to digital using A/D board and sent to QTM. Through this process, we could synchronize LED signal with GRF data. GRF data were collected at a frequency of 1000Hz. These data were used to collect subject's center of pressure (COP) during the trials and measure the time from signal to finish. Intra-class correlation coefficient (ICCs) was evaluated to examine test-retest reliability of pick-up test. ICCs for pick-up test was 0.934 (data was not shown).

3.4.5. Escape test

Subjects performed Escape test to measure their ability of self-defense against danger during emergency situations in life. All subjects were ready in a natural position with shoulder width of feet outline. They were asked to escaped above 13 cm height blocks that placed at three locations (2.2 m ahead,

right side and behind from the start location) as fast as possible after reacting to LED signal from the lamp 3m ahead. During the task, all processes was captured by eight infrared cameras (Oqus500, Qualysis, Sweden) to calculate subjects' foot location by those reflective markers attached onto heels with 3D motion analysis method. All subjects performed a total of three trials and the fastest one was used for analysis. LED signal was manipulated by a researcher to minimize subject's predictable reaction using a trigger. We collected the start time signal using synchronizer (Visol, Korea) that simultaneously sent signal to the main computer's QTM (Qualysis Track Manager, Qualysis, Sweden) software. Also, collected kinematical data of markers were sent to QTM. Through this process, we could synchronize LED signal with subjects' movement data. 3D motion capture data was collected at a frequency of 100Hz. We attached two additional markers on each blocks located at a 2.2 m distance. The end event was defined when one foot crossed the line between those markers attached on the block. The total time from LED signal to the end event was recorded. Intra-class correlation coefficient (ICCs) was evaluated to examine test-retest reliability of escape test. ICCs for escape test was 0.914 (data was not shown).

3.5. Strength assessment

3.5.1. Leg muscle strength

The strength of the lower limbs was measured by isokinetic dynamometer (Humac Norm, CSMi, USA). Isokinetic dynamometer provides objective measures of concentric dynamic strength. It provides optimal and efficient loading of muscles and joints through range, thereby minimizing potential risk of injury. The knee extension and flexion peak torques of each lower limb were evaluated for both isokinetic contraction tests. Before starting the test, subjects were required to have two to five light contraction practice. After 10 s of resting period, subjects performed five repetitions of knee extension trial at 60°/sec. Following a two to five minutes rest, they did fifteen repetitions of extension trial at 180°/sec.

3.5.2. Hand grip strength

The hand grip strength test was measured by using digital hand grip dynamometer (my-5401, TAKEI, Japan). Subjects were asked to stand at a neutral position of arm and wrist. They were measured two times (left and right hand in turn). Mean values were recorded.

3.6. Statistical analysis

Baseline characteristics between groups were compared by using

independent t-test. The treatment effects were determined by Two-way repeated ANOVA. All data were presented as mean \pm SD. All analyses were performed using SPSS version 18.0 software (SPSS Inc.). Significant difference was considered when p value was less than 0.05. Associations of pick-up time and physical functions were calculated with Pearson's correlation coefficient.

4. Results

The participant's attendance rate of exercise training class averaged 94.18% (mean of 22.6 completed sessions of the total 24 planned sessions). Participant's characteristics at pre- and post-test of exercise training are summarized in Table 3. There was no significant change in body composition (BMI, skeletal muscle mass, percent fat mass, and waist hip ratio) or blood lipid profiles (fasting blood glucose, total cholesterol, triglycerol, LDL- , and HDL-cholesterol, Table 3).

Table.3 Changes in characteristics of participants Values are presented as mean±SEM. Interaction with p value is evaluated by RM-ANOVA (time x group interaction).

Characteristics of participants	CON (n=8)		EX (n=22)		Interaction (p)
	Pre-test	Post-test	Pre-test	Post-test	
Age (yrs)	76.05 (2.01)		74.45 (0.62)		
Weight (kg)	55.56 (4.32)	54.5 (4.27)	58.24 (1.28)	57.32 (1.30)	0.696
Height (cm)	151.81 (2.53)	151.74 (2.53)	151.75 (1.17)	151.72 (1.16)	0.576
BMI (kg/m ²)	23.85 (1.06)	23.49 (1.04)	25.28 (0.46)	24.88 (0.44)	0.828
Skeletal muscle mass (kg)	18.35 (1.25)	18.81 (1.32)	19.08 (0.61)	19.75 (0.52)	0.613
Percent body fat (%)	35.53 (2.77)	34.06 (1.27)	37.91 (1.56)	34.84 (1.10)	0.113
Waist Hip Ratio (WHR)	0.89 (0.02)	0.97 (0.02)	0.89 (0.02)	0.88 (0.01)	0.445
Arm circumference (cm)	27.38 (1.05)	27.01 (1.07)	29.05 (0.53)	27.57 (0.45)	0.063
Thigh circumference (cm)	49.88 (1.58)	49.25 (2.15)	51.77 (0.77)	50.59 (0.81)	0.683
Systolic blood pressure (mmHg)	140.30 (3.22)	139.40 (3.01)	141.36 (3.6)	136.41 (2.95)	0.058
Diastolic blood pressure (mmHg)	82.38 (2.01)	84.25 (2.15)	85.45 (2.71)	77.18 (2.07)	0.052
Fasting blood glucose (mg/dl)	104.10 (5.12)	109.10 (6.68)	97.23 (3.41)	104.62 (2.60)	0.838

Total cholesterol	183.70	179.30	185.05	187.77	0.655
(mg/dl)	(6.94)	(9.34)	(9.70)	(7.48)	
Triglycerides	115.3	117.20	121.63	118.04	0.351
(mg/dl)	(14.71)	(10.43)	(10.85)	(10.56)	
LDL cholesterol	114.90	114.34	114.63	113.18	0.945
(mg/dl)	(6.77)	(9.24)	(7.54)	(6.89)	
HDL cholesterol	56.75	49.63	57.59	53.18	0.573
(mg/dl)	(4.26)	(3.08)	(3.20)	(2.29)	

Isokinetic muscle strength was measured with knee extension and flexion at 60°/sec and 180°/sec (Table 4). The peak torque of knee extension at 60°/sec was significantly (time x group interaction, $p=0.019$) improved (from baseline to 42.14%) in the HSPT group compared to the control group. There were no significant changes in knee extension at 180°/sec. There were main effects (time x group interaction) in knee flexion at both of 60°/sec and 180°/sec. Interestingly, the peak torque in knee flexion at both of 60°/sec and 180°/sec were dramatically decreased after 12 weeks of training period in the control group. In the HSPT group, however, the values of knee flexion peak torque were relatively maintained from baseline compared to the control group (Table 4). In addition, the grip strength was significantly (time x group interaction, $p<0.001$) increased (from baseline to 27.01%) in the HSPT group (Table 4).

Table 4. Effect of high speed power training on knee extension/flexion and hand grip strength. Data are presented as means (SD). Interaction with p value is evaluated by RM-ANOVA (time x group interaction). * $p < 0.05$ compared to pre-test, and ** $p < 0.001$ compared to pre-test.

Isokinetic contraction (peak torque, N)	CON		EX		Interaction (p)
	Pre-test	Post-test	Pre-test	Post-test	
<i>Knee extensor (60 degree/sec)</i>					
Right	104.03 (6.65)	115.62 (9.86)	80.77 (5.74)	114.80 (6.78)**	0.019
Left	95.53 (7.98)	107.56 (8.63)	91.86 (5.99)	90.86 (4.31)	0.191
<i>Knee flexor (60 degree/sec)</i>					
Right	74.52 (5.34)	56.88 (5.68)**	69.45 (3.81)	68.41 (3.36)	0.002
Left	70.25 (6.16)	53.54 (5.06)**	65.41 (3.31)	59.73 (3.83)*	0.019
<i>Knee extensor (180 degree/sec)</i>					
Right	57.63 (4.29)	66.54 (5.81)	51.87 (3.70)	69.33 (3.61)	0.070
Left	70.25 (6.16)	53.58 (5.07)	51.19 (3.32)	64.28 (3.67)	0.572
<i>Knee flexor (180 degree/sec)</i>					
Right	53.63 (2.95)	34.88 (4.07)**	48.09 (2.83)	39.25 (3.02)**	0.028
Left	51.38 (4.05)	33.01 (4.04)	46.41 (2.67)	38.14 (2.67)	0.144
Hand grip strength (kg)	18.70 (3.37)	19.75 (2.74)	18.48 (3.25)	23.47 (3.44)**	<0.001

The value of rate of torque development (the values of peak torque divided by time to peak torque) was calculated to evaluate the rapid torque characteristics in isokinetic knee extension and flexion at 60°/sec (Table 5). The rate of torque development in extensor of left leg at 60°/sec was significantly (time x group interaction, $p=0.014$) increased (from baseline to 24.07 in the HSPT group). The rate of torque development in flexor of both right (time x group interaction, $p=0.015$) and left legs (time x group interaction, $p=0.007$) was dramatically decreased (right leg: 32.86%, left leg: 36.59% from baseline) in the control group, similar to the decline of peak torque in knee flexion (Table 5). Although preventive effect of HSPT was found in the exercise group, the decline of rate of torque development was relatively diminished compared to the control group.

Table 5. Effect of high speed power training on rate of torque development (peak torque/ time to peak torque). Data are presented as means (SD).

Interaction with p value is evaluated by RM-ANOVA (time x group interaction).

* $p < 0.05$ compared to pre-test, and ** $p < 0.001$ compared to pre-test.

Rate of torque development (N/sec)	CON		EX		Interaction (p)
	Pre-test	Post-test	Pre-test	Post-test	
Extensor Right	122.80 (61.09)	124.48 (64.82)	95.50 (36.16)	112.84 (44.20)	0.341
	114.71 (45.46)	108.75 (49.39)	98.11 (40.34)	121.73 (49.38)**	
Flexor Right	90.09 (49.32)	60.48 (30.64)*	69.69 (24.92)	66.34 (21.30)	0.015
	86.95 (39.40)	55.13 (29.73)*	70.90 (31.17)	67.35 (28.41)	

Various functional tests were performed before and after the exercise training to measure the improvement of physical functions in usual life. SPPB score for measuring physical function of elderly was significantly (time x group interaction, $p=0.011$) improved (from baseline to 33.86%) in the exercise group compared to the control group (Figure 23A). Of 22 participants in the HSPT group, 20 (90.91%) had improvement over 0.5 point in SPPB score. However, there was no significant change (time x group interaction, $p=0.192$) in the result of timed up and go test (Figure 23B).

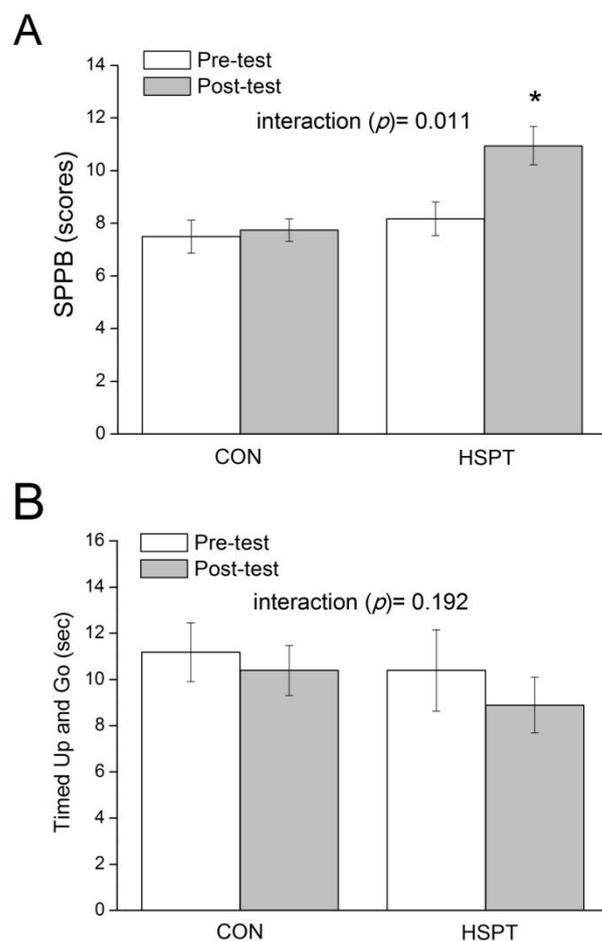


Figure 23. Physical function test. Effect of 12 weeks of HSPT on (A) SPPB test, and (B) Timed up and go test. SPPB score was significantly improved (from baseline to 33.86%) in the exercise group compared to the control group. And there was no significant change in the result of timed up and go test. All values are mean \pm SD. * p <0.05 pre vs. post-training within the group.

The time of pick-up the 600 g of small basket was significantly ($p<0.05$) decreased (time x group interaction, $p=0.006$) in the HSTP group compared to the control group (Figure 24A). In addition, path of center of pressure (COP) was significantly ($p<0.05$) shorter (time x group interaction, $p=0.014$) in the HSPT group compared to the control group (Figure 24B). However, there was no significant improvement in escape time (reaction ability) or balance test (Figure 24C and D). Interestingly, change of pick-up time was significantly correlated with improvement of SPPB ($R=-0.514$, $p=0.002$) and leg muscle strength ($R=-0.508$, $p=0.002$), especially in knee flexion at $60^\circ/\text{sec}$ (Fig 25).

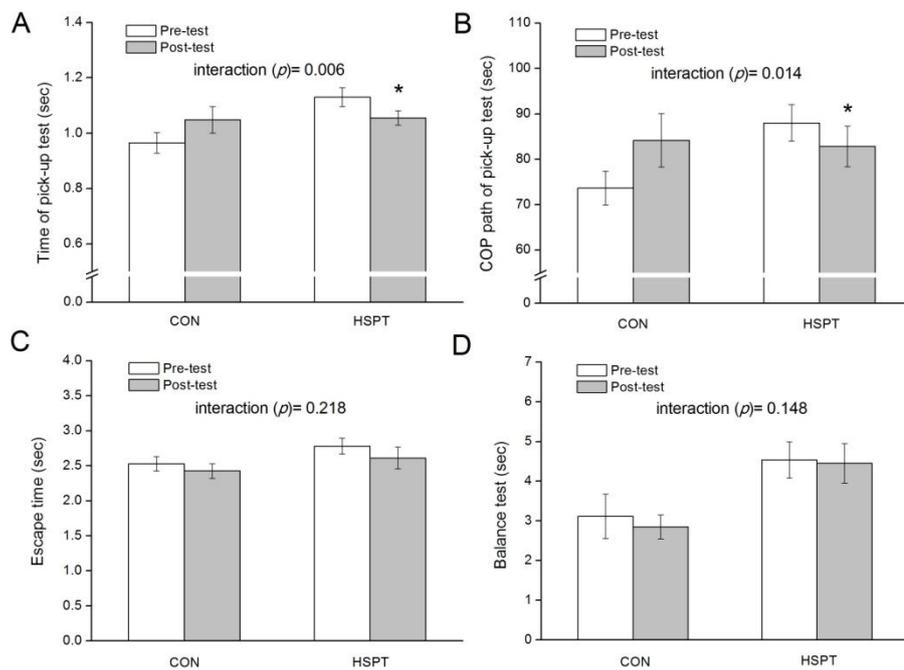


Figure 24. Effect of 12 weeks of HSPT on physical function test (A) time of pick-up test, (B) COP path of pick-up test, (C) escape test, and (D) balance test. The time of pick-up test was significantly decreased in the HSTP group compared to the control group. Path of center of pressure (COP) was significantly shorter in the HSPT group compared to the control group. However, there was no significant improvement in escape time and balance test. All values are mean \pm SD. * $p < 0.05$ pre vs. post-training within the group.

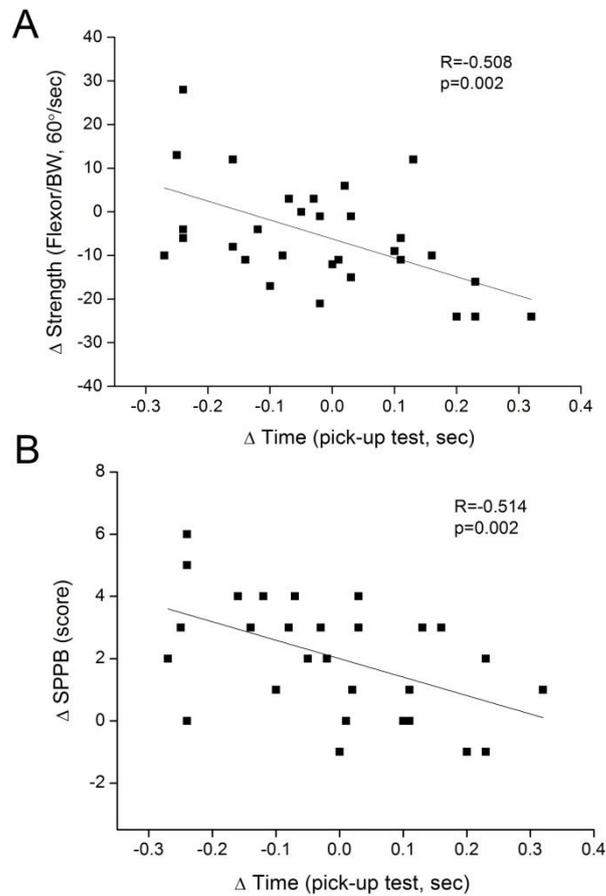


Figure 25. Association between improvement of physical function test (pick-up time) and improvement of leg strength (A) and SPPB score (B). Change of pick-up time was significantly correlated with improvement of SPPB ($R=-0.514$, $p=0.002$) and leg muscle strength ($R=-0.508$, $p=0.002$).

After 12 weeks of resistance training, circulating irisin protein level in the exercise group was significantly ($p < 0.05$) increased compared to that in the control group (Figure 26A). There were significant correlations between improvement in muscle strength (both of upper- and lower-body) and increase of circulating irisin proteins. We found positive relation between improvement in grip strength and increase of serum irisin level ($R = 0.526$, $p = 0.002$, Figure 26B). In addition, improvement of isokinetic leg strength (knee extension, 60°/sec) showed positive correlation with increase of serum irisin level ($R = 0.414$, $p = 0.003$, Figure 26C).

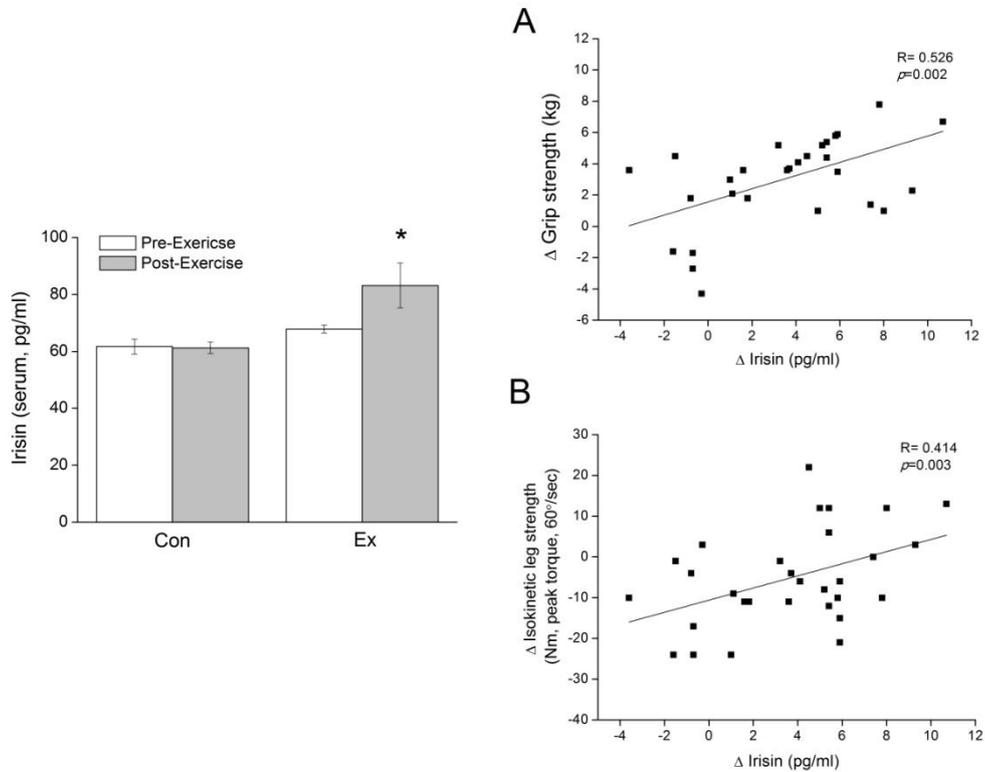


Figure 26. Effect of resistance exercise on serum irisin expression and its correlation with improvement of muscle strength in aging human (A) The expression of irisin in serum was measured after 12 weeks of resistance exercise training. The change of irisin expression in serum has positive correlations with improvement of grip strength (B) and isokinetic leg strength (B). Values are mean \pm S.E.M. for Old-Con (n=14) and Old-Ex (n=18).

In addition, circulating IL-15 level was significantly increased in exercise group compared to control group. Similar to result of irisin, positive correlation between change of irisin level and improvement of leg muscle strength was found after 12 weeks of resistance training (Figure 27).

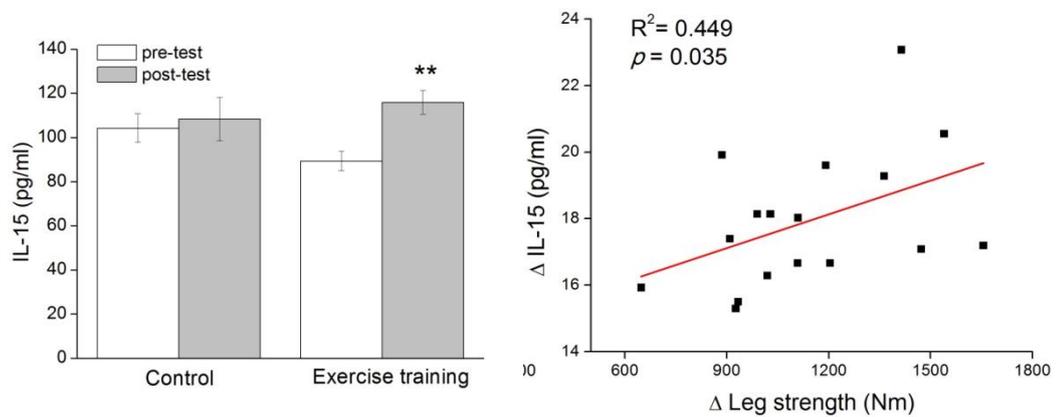


Figure 27. Effect of resistance training on circulating IL-15 level and its correlation with improvement of muscle strength.

5. Discussion

This study revealed that muscle strength (both of grip and leg strength) and physical functions including SPPB and pick-up task were significantly improved after 12 weeks of elastic band-based high-speed power training in elderly women. In addition, improvement of pick-up time was significantly correlated with increase of muscle strength (knee extension, flexion at 60°/sec) and SPPB scores. To our knowledge, this is the first study to evaluate the effect of elastic band-based high speed power training on the improvement of physical functions closely related to usual life in the elderly.

In the present study, 12 weeks of exercise training program was consisted of supervised exercise (community-based, 2 days per week) and home-based exercise (3 days per week). As motivating older adults to perform exercise on a regular basis is an important factor to achieve high attendance to maintain the effects of exercise, instructors tried to communicate with participants via cell phone text message and individual consultation to check the daily assignment. The attendance rate was 94.18%. Most participants mentioned that they were satisfied with the group-exercise program. After completion of the training program, participants continued to exercise. They currently attend self-directed exercise group twice a week. These results suggest that community-based exercise classes in a group setting have positive

physical and psychological effects.

Resistance training is essential to counter the age-related declines in muscle mass, strength and power in the aging population. Traditional resistance training with a strengthening component has been shown to improve muscle performance and physiological characteristics of skeletal muscles (Wolters, 2000). In addition, traditional resistance training regularly exerts significant increases in muscle strength and hypertrophy (type I, II) and whole muscle (Charette et al., 1991; Hikida et al., 2000; Jubrias, Esselman, Price, Cress, & Conley, 2001; Pereira, Izquierdo, Silva, Costa, Bastos, et al., 2012). Constant resistance and moderate velocity training protocols have been reported to significantly improve muscle strength in older adults (Frontera, Meredith, O'Reilly, Knuttgen, & Evans, 1988; Henwood et al., 2008). Various resistance exercises using weight bearing, exercise machines, and elastic bands can improve the muscle strength and muscle size in older adults (Chen, Tseng, Huang, & Li, 2013; Hess & Woollacott, 2005). Especially, elastic band based training is recognized as safe, convenient, inexpensive, and effective strategy to enhance the neuromuscular system and improve muscle strength and power, therefore increasing the ability to perform functional tasks (Chen et al., 2013; Galvao & Taaffe, 2005). In addition, a modest intensity stretching exercise using elastic band increased the range of joint motions and flexibility of seniors (Chen et al., 2013; Swank, Funk, Durham, & Roberts, 2003). In the

present study, beneficial effect of high-speed power training using elastic band was found to be safe and efficient resistance exercise program suitable for frail adults. Improvement of strength (both of upper and lower body) and functional performance was found in the high speed power training group. Taken together, our results suggest that the elastic band based high speed power training may be an efficient resistance training program to improve physical function of frail population.

According to previous reports on leg flexor in the population, more investigations are needed on age-related alternation in leg flexor muscle strength, especially age-related alternation in rate of torque development (Thompson et al., 2013). The leg flexors have been shown to be important contributors for a variety of locomotor-related functional movement, such as jumping, agility, and balance. These functions are important for daily living and independence in the aging population (Bento, Pereira, Ugrinowitsch, & Rodacki, 2010; Bobbert, van der Krogt, van Doorn, & de Ruitter, 2011; Delecluse, 1997; Houck, 2003; Ramirez-Campillo et al., 2014). The decline in leg flexor strength, especially in the rapid torque production, may explain some of the early decline in functional performance often regarded as a consequence of aging. Thus, development and evaluation of exercise training programs to improve the age-dependent decline in both maximal and rapid force production of leg flexors may have profound impact on the functional

performance and physical limitation of older adults. In the present study, the decline of maximal strength and rate of torque development at leg flexor was significantly improved, concomitant with the improvement of physical function.

The increase of circulating irisin was found after endurance training in human subjects. Bostrom et al. (2012) demonstrated a two-fold increase of circulating plasma irisin in healthy adults compared to non-training group after 10-weeks of endurance training (Bostrom et al., 2012). In addition, circulating irisin was increased immediately after endurance training (Huh et al., 2014; Norheim et al., 2014). Resistance or strength training did not affect circulating irisin proteins in healthy male (Bang et al., 2014) or hemodialysis patients (Moraes et al., 2013). Our recent laboratory unpublished data found the increase of circulating irisin only in the resistance training group. A positive correlation was found between the change of circulating level and the change of muscle mass following resistance training in obese adults. In the present study, circulating irisin was significantly increased after resistance training in both aging mice and human. Considering the benefits of resistance training in improving muscle strength and function for the frail elderly, resistance training could be an efficient intervention strategy to increase circulating irisin expression in the aging population.

A possible limitation of the present study was the lack of comparison

group to the high-speed power training (i.e, low speed power training or traditional band training). Therefore, we could not compare high speed power training with traditional band training (or traditional resistance training). The participants in the control group (non-exercise, balance and tone group) had shown high level of drop-out rate (about 68%) because of the difficulties in time commitment as well as loss of motivation. Therefore, the additional comparison between drop-out subjects and exercise training participants was performed. However, there were no significant differences in body composition, blood lipid profiles, blood pressure, and physical functions. Additionally, a relatively small sample size and disproportion of sex were limitations of the study. Therefore, in further studies, the beneficial effects of the high speed power training should be tested in a large randomized sample with various physiological measurements to better understand the underlying mechanism of training-induced adaptation and alternation.

III. Overall discussion

In the present dissertation, effects of resistance training on myokines and their relationship with improvement of muscle functions were investigated in aging and diabetic models, the results that resistance training increases the myokines in serum or skeletal muscles may be importance evidences considering the resistance training was thought to be an efficient intervention for prevent or therapeutic strategy to manage the age-related sarcopenia or metabolic disease.

In animal study, exercise-induced myokines including IL-15, irisin, FGF-21 were significantly increased in soleus muscle after resistance training in both aging and type 2 diabetic models. In this dissertation, myokines were analyzed with ELISA within various skeletal muscle tissues such as SOL, EDL, TA and GAS muscle. Interestingly, increased myokines were especially found in SOL muscle response to resistance training in aging and type 2 diabetic animals. The unsolved inquiry of the result is that why dose exercise-induced myokines increase SOL muscle which was type I fiber dominant muscle rather than other muscles such as TA or GAS after resistance training. The possible reasons were as follows; 1) SOL muscle might have more potential ability than other muscles to produce the myokine response to resistance training. And 2) the biomechanical movements of ladder climbing

exercise which were applied as resistance exercise in this experiment stimulates especially soleus muscle rather than other muscles.

SOL muscle for the source of exercise-induced myokines

The SOL muscle is a powerful muscle and located in superficial posterior compartment of the leg. It is closely connected to the gastrocnemius muscle. The SOL muscle specifically plays an important role in maintaining standing posture and locomotion. In addition, SOL muscle is the most effective muscle for plantarflexion in a bent knee position.

There are many researches reporting increase of cytokines or protein synthesis in SOL muscle following resistance exercise with normal or diabetic animal models. Farrell et al., (1999) reported that resistance training (mimicking the hindlimb squat using electrical shock) induced increase of SOL muscle weight with increase of protein synthesis in diabetic rats (Farrell et al., 1999). In genomics study using microarray, response to aerobic exercise increased gene expressions in various genes related to cell cycle, metabolism, and transcription factors. In that report, SOL muscle and GAS muscle showed different patterns in gene expression response to same exercise bout (McKenzie, Goldfarb, & Kump, 2011). Oh et al., (2014) reported that IL-15 concentration in response to acute bout of resistance exercise was markedly increased ($p < 0.05$) and peaked 1 hour after exercise compared to resting level,

then returned to the resting level at 6 hours after resistance exercise in both SOL and TA (Oh et al., 2014). The significant higher concentration of IL-15 in SOL was found compared to the CON ($p < 0.05$) after 8 weeks resistance training. In animal study using type 1 diabetes rats, resistance training preserved FHL muscle weight in diabetic rats and increased IL-15 protein levels in both the soleus and FHL muscles (Molanouri Shamsi et al., 2015). Therefore, based on the previous reports and results of this dissertation, SOL muscle might be a potent origin tissue to produce myokines response to resistance exercise.

Ladder climbing exercise stimulates especially SOL muscle

In the experiments of this dissertation, ladder climbing exercise with tail weight was applied as a resistance exercise for rodents. According to Lee et al., reports, ladder climbing exercise induces the hypertrophy in FHL muscle rather than other muscles. Surprisingly, the more commonly used plantar flexor muscles including soleus (1%), plantaris (2.9%) and gastrocnemius (0%) did not appear to be affected by resistance training. In addition, Duncan et al., (1998) applied resistance exercise using ladder (40 cm) with tail loads (Duncan, Williams, & Lynch, 1998). They reported that increased mass of SOL muscle (also EDL muscle) and cross-sectional area was found in trained (4 days/week) compared to control group. Moreover,

Hornberger and Farrar (2004) reported 23% of absolute increase in the FHL muscle in ladder climbing exercise trained rats (Hornberger & Farrar, 2004). Hutchison et al., analyzed the amplitude and temporal interrelationships of the EMG signals from the rat SOL and GAS muscles during standing, locomotion on a treadmill at various speeds and inclines (Hutchison, Roy, Hodgson, & Edgerton, 1989). With increasing treadmill speed and/or incline, there was a greater probability of an enhancement in amplitude of the GAS than SOL muscle. However, in the previous studies, there was no report regarding muscle activation during the ladder climbing movement of rat or mouse. When the rodents were climbing the ladder with tail weight, the plantar flexor muscles including SOL, FHL, plantaris, and GAS were recruited to maintain the movement. According the EMG patterns from inclined treadmill locomotion (which is similar biomechanical movement pattern), ladder climbing exercise might stimulate the common plantar flexor muscles but not SOL muscle specific responses.

IV. Conclusion

In this dissertation, effect of resistance training was investigated focused on the myokine productions and their correlations with muscle functions and muscle mass using aging animal and human models. The descriptive measurement of myokines performed in various skeletal muscles following resistance exercise in aging and diabetes animals. In addition, circulating myokines in blood was measured with improvement of muscle functions after resistance training in aging adults. The summary of the results regarding effect of resistance training on myokines in diabetes and aging were as follow;

- 1) Myokines including IL-15, irisin, FGF-21 were significantly increased after resistance training in SOL muscle with improvement of fasting glucose levels (or glucose intolerance) and muscle strength in diabetes animals.
- 2) Myokines including IL-15 and irisin were significantly increased after resistance training in SOL muscle with improvement of muscle functions in aging mice.
- 3) Circulating myokines including IL-15 and irisin were significantly increased after resistance training and showed high correlations with muscle

functions in elder women.

The potent myokines such as IL-15, irisin, FGF-21 and BDNF for therapeutic target to prevent or overcome the aging and metabolic syndrome were measured. These myokines are part of a complex network that mediates communication between muscle, liver, adipose tissue, brain and other organs. Moreover, myokines were could be a potent mediator to bring about multiple benefits for human health maintenance and/or improvement. For the further study, considering the important role of exercise induced myokines in aging and diabetes, there are needed to elucidate the role of exercise-induced myokines compared to exogenous myokines in modulating muscular metabolism using siRNA, receptor agonist/antagonist, histology technique (for muscle fiber type shifting) etc. in diabetes and aging process. In addition, the optimal exercise protocol regard with exercise type, intensity, duration, and frequency have to be establish for the efficient modulation of exercise-induced myokines.

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국문초록

제2형 당뇨병과 노화에서 저항성 운동이 근기능과 myokine에 미치는 영향

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최근 골격근은 중요한 내분비기관으로 여겨지며 다양한 사이토카인(cytokine)들을 만들어내고 분비하는 것으로 보고되었다. 이러한 수축하는 근육에서 분비되는 사이토카인을 마이오카인(myokine)이라고 명명하였고, 운동 시 분비되는 마이오카인은 여러 질병 및 대사증후군에서 중요한 역할을 할 것으로 기대되고 있다. 하지만, 지금까지 연구에 의하면 마이오카인의 중요성은 상대적으로 연구가 많이 되었지만 제2당뇨와 노화 과정에서 저항성 운동에 따른 마이오카인의 변화에 관한 근거들은 부족하다. 따라서 본 학위논문에서는 제2형 당뇨병 및 노화 모델에서 저항성 운동에 따른 마이오카인의 변화과 근기능 향상을 확인하고자 한다. 이를 위해 총 3가지의 연구로 나누어 동물, 인체 실험을 아래와 같이 진행하였다. 1) 제2

형 당뇨병 쥐에서 저항성 운동의 효과 확인, 2) 노화 쥐에서 저항성 운동의 효과 확인, 3) 노인에서 저항성 운동의 효과 확인.

첫번째, 제2형 당뇨병 쥐에서 8주간 저항성 운동이 마이오카인 및 혈당조절기능에 미치는 영향을 확인한 연구에서는 운동 그룹이 대조군에 비해 높은 근육 질(muscle quality) 및 혈당조절능력을 나타내었다. 동시에 가자미근(soleus muscle)에서 IL-15, irisin, FGF-21이 운동 그룹에서 높게 나타나는 것을 확인하였다. 두번째, 노화 쥐에서 12주간 저항성 운동이 마이오카인 및 근기능에 미치는 영향 연구에서는 운동그룹에서 IL-15와 irisin이 혈중 및 가자미근에서 높게 나타나는 것을 확인하였다. 증가된 마이오카인은 근기능 수준과 높은 상관관계를 보였다. 세번째, 65세 이상 노인에서 12주간 저항성 운동이 혈중 마이오카인 및 근기능에 미치는 영향 연구에서는 운동 프로그램에 참여한 노인이 대조군에 비해 높은 혈중 IL-15, irisin을 보였고, 유의한 근기능 향상을 보였다. 또한 증가된 마이오카인은 향상된 근기능과 높은 상관관계를 보였다. 이러한 결과를 요약해보면 저항성 운동은 제2형 당뇨병 및 노화 모델에서 혈중 및 골격근 (특히 가자미근)에서 마이오카인을 증가시키고 이러한 증가는 혈당조절능력 및 근력, 근기능의 향상과 밀접한 관계를 보였다. 본 연구 결과와 기존의 선행논문들의 보고를 종합해보면, 저항성 운동은 마이오카인 발현 증가 및 혈당조절 및 근기능 향상을 위해 제2형 당뇨병과 노화 모델에서 중요한 운동중재 방법이 될 것이다.

주요어: 마이오카인, IL-15, irisin, 저항성 운동, 제2형 당뇨병, 근육 노화
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