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BRIEF COMMUNICATION

Effects of an acute bout of resistance exercise on fiber-type specific *GLUT4* and *IGF-1R* expression

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Abstract: The effects of resistance exercise on fiber-type–specific expression of *insulin-like growth factor I receptor (IGF-1R)* and *glucose transporter 4 (GLUT4)* was determined in 6 healthy males. The expression of both genes increased in Type I fibers ($p < 0.05$), but only GLUT4 increased ($p < 0.05$) in Type II fibers. These data demonstrates that an acute bout of resistance exercise can up-regulate mechanisms of glucose uptake in slow and fast-twitch fibers, but the IGF signaling axis may not be as effective in fast-twitch fibers.

Key words: muscle adaptation, fast-twitch, slow-twitch.

Résumé : Cet article étudie chez six hommes en bonne santé l'effet d'un exercice contre résistance sur l'expression du *récepteur du facteur de croissance 1 analogue à l'insuline (IGF-1R)* et du *transporteur du glucose 4 (GLUT4)* en fonction du type de fibres. L'expression des deux gènes augmente dans les fibres de type I ($p < 0,05$), mais il n'y a que l'expression du GLUT4 ($p < 0,05$) qui augmente dans les fibres de type II. Ces observations révèlent qu'une seule séance d'exercice contre résistance peut réguler à la hausse les mécanismes de captation du glucose dans les fibres musculaires à secousse lente et à secousse rapide, mais que l'axe de signalisation de l'IGF ne serait pas aussi efficace dans les fibres à secousse rapide. [Traduit par la rédaction]

Mots-clés : adaptation musculaire, secousse rapide, secousse lente.

Introduction

Resistance exercise has been shown to increase whole muscle strength and elicit hypertrophy (Fry 2004). It has repeatedly been shown that fiber-type–specific responses to exercise and training between slow- (Type I) and fast-twitch (Type II) muscle fibers are different (Raue et al. 2012; Tupling et al. 2007; Yang et al. 2006). For example, the level of expression of heat shock proteins (Tupling et al. 2007), hypertrophic (Raue et al. 2012), and proteolyitic genes (Yang et al. 2006) are different between slow and fast-twitch muscle following an acute bout of exercise. However, there is little information regarding the differences in expression of genes involved in metabolism and muscle hypertrophy between fast- and slow-twitch muscle fibers following resistance exercise. In this study we examined the expression of *insulin-like growth factor I receptor (IGF-1R)* and *glucose transporter 4 (GLUT4)* following an acute bout of resistance exercise in isolated single muscle fibers utilizing a novel technique (Wacker et al. 2008).

The transmembrane receptor IGF-1R is a tyrosine kinase that is expressed in many cell types, including skeletal muscle (Philippou et al. 2007). The binding of insulin and IGF (I and II) to IGF-1R results in a variety of responses, such as cell proliferation, differentiation, and inhibition of apoptosis, depending on the cell type and cross talk from other signaling pathways (Denley et al. 2005). Furthermore, IGF-1R can replace insulin receptor in insulin receptor-deficient animals (Baudry et al. 2001). Most studies show that activation of IGF-1R signaling pathway in skeletal muscle is required for hypertrophy and the maintenance of muscle mass (Adams and Haddad 1996; DeVol et al. 1990; Quinn et al. 2007); however, hypertrophy without the contribution of IGF signaling has been observed (Spangenburg et al. 2008). While fiber-type comparisons in IGF-1R levels have been seen in whole muscles (e.g., soleus vs. extensor digitorum longus), to date, there is little data on single fiber-type expression of *IGF-1R* following exercise. Resistance exercise has been shown to increase the expression of genes associated with hypertrophy (Raue et al. 2012) and proteolysis (Yang et al. 2006) to a greater extent in fast-twitch muscles compared with slow-twitch muscles.

The GLUT4 isoform is responsible for glucose transport following insulin and muscle contractions by translocating to the cell surface following stimulation (Furtado et al. 2002). The majority of studies show an increase in GLUT4 levels following endurance training (Green et al. 2008; Houmard et al. 1991; Hussey et al. 2012; Kraniou et al. 2006; Leick et al. 2010; Stuart et al. 2010) and a few have found similar responses following resistance training (Derave et al. 2003; Holten et al. 2004; Tabata et al. 1999). Several studies have shown that a greater basal level of GLUT4 in slow-twitch compared with fast-twitch fibers (Daugaard et al. 2000; Stuart et al. 2010). This relationship is consistent with the fact that insulin stimulated glucose uptake is positively correlated with the percentage of Type I fibers and inversely related to the percentage of Type IIb fibers (Andersen et al. 1993; Lillioja et al. 1987). Thus, it is reasonable to predict that *GLUT4* expression will be greater in Type I compared with Type II fibers.

To our knowledge, no one has examined the fiber-type–specific changes in *IGF-1R* or *GLUT4* gene expression in human single skeletal muscle fibers following resistance exercise. Thus, the purpose of this investigation was to determine the effects of an acute bout of resistance exercise on the expression of *IGF-1R* and *GLUT4* in slow- and fast-twitch muscle fibers in humans. For this study, we utilized an innovated technique to analyze the expression of single muscle fibers prior to and following an acute bout of resistance exercise (Wacker et al. 2008). Because of the type of exercise and the role that IGF-1R plays in both glucose and protein metabolism, we hypothesized that resistance exercise would increase

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the expression of *IGF-1R* in fast-twitch fibers. Based on previous studies (Hussey et al. 2012; Kraniou et al. 2006; Leick et al. 2010), we hypothesized that resistance exercise would increase the expression of *GLUT4* in slow-twitch muscles fibers.

Materials and methods

Subjects

Six healthy males volunteered to serve as subjects in this investigation (age, 22.2 ± 0.6 years; weight, 76.1 ± 1.3 kg; height: 180.5 ± 0.9 cm). All volunteers were nonobese (body mass index <28 kg·m^{-2}), normotensive, nonsmokers, and were recreationally trained in both resistance and endurance activities for 4–6 h·week^{-1}. Subjects were fully informed of the risks associated with the study prior to giving their consent to participate in accordance to the guidelines set by the Human Subjects Committee at the University of Kansas and the Declaration of Helsinki.

Experimental design and timeline

Diet

The day before each trial, subjects consumed a weight, height, and age-adjusted number of calories based on the Harris–Benedict formula: [655 + (9.6 × weight kg) + (1.8 × height cm) − (4.7 × age years)] × 1.4. The diet consisted of 55% carbohydrates, 30% fat, and 15% protein. Subjects were required to report to the Applied Physiology Laboratory (APL) for each meal. Subjects reported to the APL on the day of testing with a minimum 8-h fast.

Resistance exercise

Subjects performed a bout of resistance exercise that consisted of 3 sets (2 sets of 10 repetitions and 1 set to failure, 8–12 repetitions) of knee extensor resistance exercise (Cybex, Medway, Mass., USA) at 90% of their 10-repetition maximum (maximum amount of weight that the subjects could lift 10 times). The concentric and eccentric motions of each lift that lasted 2 s and there was a 2-min rest period between each set. Each subject's 10-repetition maximum was determined 7–10 days prior to his first trial.

Muscle biopsies

Percutaneous needle muscle biopsies (Bergstrom 1962) were obtained in the morning of each trial following an 8–10-h fast. Muscle biopsies were obtained from the vastus lateralis muscles pre-exercise (Pre), and 3.5 h following exercise (Post). Subjects rested in a prone position for 30 min prior to the Pre biopsy. Each biopsy was obtained from the mid-belly region of the muscle approximately two-thirds proximal the length of the thigh. The Post biopsy was taken approximately 5 cm proximal of the Pre biopsy. The muscle samples were immediately placed in an RNAlater (Qiagen, Valencia, Calif., USA) and stored at –20 °C.

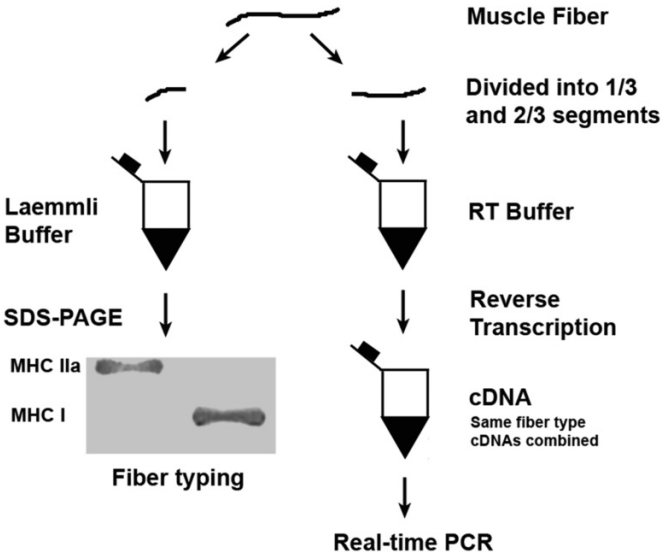
Single fiber isolation

Small sections of each of the muscle bundles were separated while in the RNAlater solution. The separated muscle section was then placed in phosphate-buffered saline and single muscle fibers were isolated from the muscle bundle using fine tweezers and a stereo-microscope (Baush and Lomb, Rochester, N.Y., USA). Connective tissue and other debris was removed from the fiber and one-third of each fiber was placed in 10 μL of SDS sample buffer for fiber typing and the other two-thirds of each fiber was placed into 5 μL of reverse transcriptase buffer. Following fiber typing, each subject's fibers were pooled in a fiber-type–specific manner. An illustration of the procedures is displayed in Fig. 1. For each subject, 20 Type I and 30 Type II fibers were analyzed. This technique is described in greater detail in a previous study (Wacker et al. 2008).

Reverse transcription (RT)

The RT buffer (Invitrogen, Carlsbad, Calif., USA) contained 2.5 μL of the RT master mix buffer, 0.5 μL Superscript III, and 2 mL

Fig. 1. Technique for gene expression analysis from single muscles fibers of the same fiber type. One-third of individual fibers were placed in SDS sample buffer for SDS-PAGE analysis of myosin head size (fiber typing). Two-thirds of a fiber was placed directly into reverse transcription buffer for complementary DNA (cDNA) conversion. cDNA products of the same fiber types were pooled for real-time polymerase chain reaction (PCR) gene expression.



RNase/DNase-free water. RT reactions were incubated at 25 °C for 10 min, followed by 55 °C for 30 min in a PTC 200 thermocycler (MJResearch; Waltham, Mass., USA). Once the fiber types were known, cDNA from the each fiber type were combined for a given subject.

Quantitative polymerase chain reaction (PCR)

Each PCR reaction contained 5 μL Platinum Taq qPCR Supermix (Invitrogen, Grand Island, N.Y., USA), 0.5 μL of MgCl2, 0.5 μL of primer/probe, 2.5 μL of cDNA reaction, and 1 μL of water. PCR reactions were set up using an automated liquid handling system (CAS-1200; Corbett Robotics/Qiagen, Valencia, Calif., USA) and real-time PCR was performed using the Corbett-Research Rotor Gene 3000 (Corbett Robotics/Qiagen). Each primer/probe set was run in triplicate for each fiber type with each subject. The threshold line for determining cycle threshold (CT) values was set at the first indication of amplification above the background noise in the exponential phase of the curve.

Primer/probe sets

Primer/probe sets for *GAPDH* (Hs99999905_m1), *beta-2 microglobulin (B2M)* (Hs99999907_m1), *IGF-1R* (Hs00609566_m1), *GLUT4* (Hs00168966_m1), and *pyruvate dehydrogenase kinase 4 (PDK4)* (Hs00176875_m1) were obtained as pre-made 20× mixtures from Applied Biosystems (Foster City, Calif., USA). These primer/probe sets were designed utilizing TaqMan chemistry and span an exon–exon junction. Reaction efficiencies were previously tested for these primer/probe sets and were found to be close to 100% (Wacker et al. 2008).

Controls

Two negative and 1 positive control tubes were run with each subject's slow- and fast-twitch fiber samples. One negative control tube was run exactly the same as the experimental tubes except that a muscle fiber was not placed into the buffers (no template control tube). Another negative control tube was run the same (including adding the muscle fiber) except that the RT enzyme was not included into the RT mixture (no RT control tube). PDK4 was analyzed as a positive control. Previous research has shown that

the expression of *PDK4* increases to a great extent following both resistance and endurance exercise (Yang et al. 2005; Wacker et al. 2008).

Fiber-type determination

The myosin heavy chain (MHC) protein expression of the one-third section of dissected fiber was determined using SDS-PAGE, as described previously (Williamson et al. 2001). The fibers were solubilized in 10 μL of 10% SDS sample buffer (10% SDS, 6 mg·mL^{-1} of EDTA, 0.06 mol·L^{-1} of Tris (pH 6.8), 2 mg·mL^{-1} of bromphenol blue, 15% glycerol, and 5% β-mercaptoethanol). These samples were stored at −80 °C until analyzed for MHC content using SDS-PAGE (SE 600 series, Hoefer, San Francisco, Calif., USA). Samples were loaded on a 3.5% loading and a 5% separating gel, and run 12 h at 4 °C. The gels were then silver stained, revealing the MHC isoform profile for each individual fiber. Only MHC I fibers were used for the type I analysis. Hybrid fibers that were classified as MHC I/IIa were not analyzed. MHC IIa and IIa/IIx fiber were pooled together for the type II fibers. We did not detect or analyze any pure type IIx fibers.

Data and statistical analysis

Three housekeeping genes (*β-Actin*, *B2M*, and *GAPDH*) were tested for stability between pre- and post-treatment. *B2M* and *GAPDH* both changed less than 1 cycle, while *β-Actin* changed more than 1 cycle on average. Therefore, CT values were normalized to *GAPDH* and *B2M* (ΔCT). The ΔCT values from these 2 housekeeping genes were then averaged to use as the final ΔCT (Vandesompele et al. 2002). Fold changes in gene expression were then calculated using $2^{-\Delta\Delta CT}$, comparing Pre and Post exercise numbers. Statistical procedures were performed using Graphpad Prism 5.0. Data are presented as means ± SE. Data were compared using a one-way ANOVA. Significance was set at the $p < 0.05$ level. When necessary, the one-way ANOVA was followed up with a modified Bonferroni's multiple comparison post hoc test.

Results

To verify that this technique has the sensitivity to measure differences in gene expression, the levels of *PDK4* were measured in isolated muscle fibers Pre and Post resistance exercise. We saw a 7.4-fold increase ($p < 0.05$) in expression of *PDK4* in Type I fibers and an 8.1-fold increase ($p < 0.05$) in *PDK4* gene expression in Type II fibers, demonstrating that the exercise bout was sufficiently stressful to elicit an increase of a commonly expressed gene (Fig. 2). We saw no amplification of genes in the 2 negative control samples (no template control and no RT control) for any of the subjects (data not shown).

We found that an acute bout of resistance exercise increased the expression of *IGF-1R* by 2.4-fold ($p < 0.05$) and increased the expression of *GLUT4* 2.2-fold ($p < 0.05$) in Type I fibers (Figs. 3 and 4, respectively). Interestingly, resistance exercise did not increase the expression of *IGF-1R* in the Type II fibers, but induced the expression of *GLUT4* by 2.8-fold ($p < 0.05$).

Discussion

The current study investigated the expression of IGF-1R and GLUT4 in slow- and fast-twitch skeletal muscle fibers in response to an acute bout of resistance exercise. We found that there were no differences in *IGF-1R* or *GLUT4* between Type I and Type II fibers prior to exercise. Interestingly, *IGF-1R* only increased in Type I fibers after resistance exercise. Following resistance exercise, *GLUT4* was shown to increase in both fiber types.

Previous studies have shown that resistance exercise increases *IGF-1R* (Drummond et al. 2009; Wilborn et al. 2009). However, Drummond et al. (2008) found no change in *IGF-1R* expression 3 h following a bout of low-intensity resistance exercise. This discrepancy in *IGF-1R* expression is likely due to the differences in intensity of the exercise. Subjects in the studies that found significant

Fig. 2. Fold change in *pyruvate dehydrogenase kinase 4 (PDK4)* mRNA expression before and 3.5 h after an acute bout of resistance exercise in slow- (Type I) and fast-twitch (Type II) muscle fibers. Twenty Type I and 30 Type II fibers were isolated and the complementary DNA from the each fiber type were combined for a given subject. Each sample was run in triplicate for each fiber type with each subject. Cycle threshold values were normalized using the average of *GAPDH* and *B2M* housekeeping genes. Fold change was calculated using the 2-ΔΔCT method comparing pre- and postexercise levels of expression. *, $p < 0.05$.

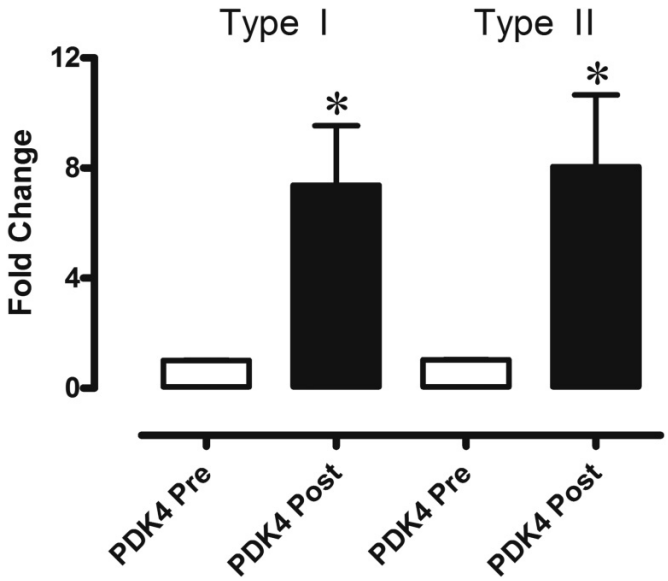


Fig. 3. Fold change in *insulin-like growth factor I receptor (IGF-1R)* mRNA expression before and 3.5 h after an acute bout of resistance exercise in slow- (Type I) and fast-twitch (Type II) muscle fibers. Twenty Type I and 30 Type II fibers were isolated and the complementary DNA from the each fiber type were combined for a given subject. Each sample was run in triplicate for each fiber type with each subject. Cycle threshold values were normalized using the average of *GAPDH* and *B2M* housekeeping genes. Fold change was calculated using the 2-ΔΔCT method comparing pre- and postexercise levels of expression. *, $p < 0.05$.

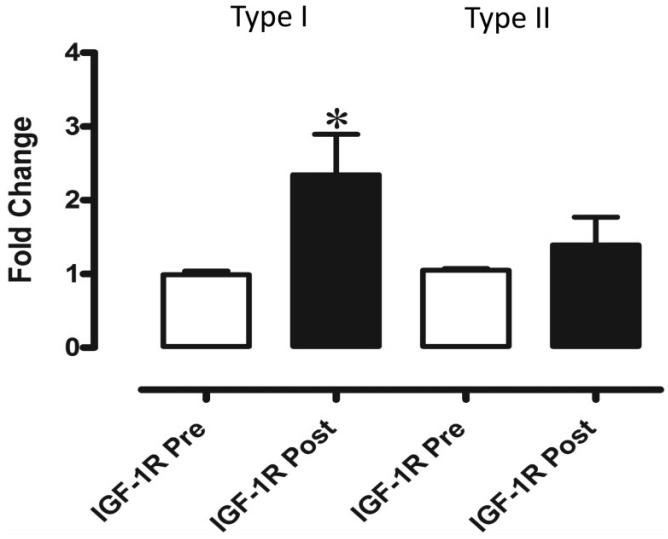
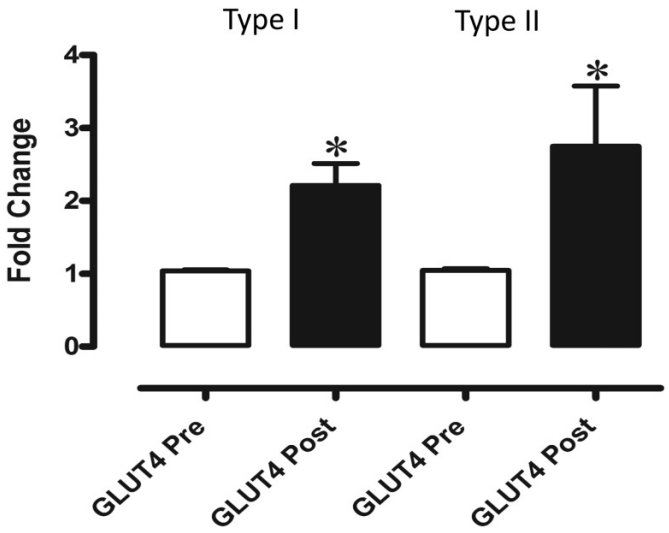


Fig. 4. Fold change in *glucose transporter 4 (GLUT-4)* mRNA expression before and 3.5 h after an acute bout of resistance exercise in slow- (Type I) and fast-twitch (Type II) muscle fibers. Twenty Type I and 30 Type II fibers were isolated and the complementary DNA from the each fiber type were combined for a given subject. Each sample was run in triplicate for each fiber type with each subject. Cycle threshold values were normalized using the average of *GAPDH* and *B2M* housekeeping genes. Fold change was calculated using the 2-ΔΔCT method comparing pre- and postexercise levels of expression. *, $p < 0.05$.



changes in *IGF-1R* performed repetitions at 60%–80% of their one-repetition maximum (1RM); whereas, subjects in the study finding no change in *IGF-1R* performed 1 set of 30 repetitions followed by a second set of 15 repetitions, both at 20% of their 1RM. Contrary to our hypothesis, we found an increase in *IGF-1R* in Type I fibers, but not Type II fibers following resistance exercise. However, there was a low subject number in the current study. It may be that with more subjects we would have seen significant increases in *IGF-1R* following resistance exercise. To our knowledge, no studies have examined the fiber-type–specific expression of *IGF-1R* following resistance exercise. Although not an exercise study, Oudin et al. (1998) saw no differences in *IGF-1R* expression between breast and leg muscles in chickens.

The regulation of *IGF-1R* in adult skeletal muscle is not yet completely understood. However, in various cell lines, IGF-1 has been shown to decrease the expression of *IGF-1R* and fibroblast growth factor has been shown to increase *IGF-1R* expression (Hernandez-Sanchez et al. 1997; Rosenthal et al. 1991). The increased expression of *IGF-1R* in Type I fibers seen in the current study may be a compensation for the lack of IGF "stimulation". It has been suggested that *IGF-1a*, but not *IGF-1c*, expression is inhibited in skeletal muscle during the first few hours of recovery following resistance exercise (Psilander et al. 2003). Differences between fiber-types were not examined; thus, it is not known if the decrease in IGF-1a was primarily in slow-twitch fibers. There are other transcription factors that have been shown to regulate *IGF-1R* expression in various tissues, including signal transducers and activators of transcription, nuclear factor-κB, and early growth response-1 (Kavurma et al. 2008; Shalita-Chesner et al. 2004; Wu et al. 2010). Further research is needed to discern the role that these transcription factors play in adult skeletal muscle, particularly in response to different types of exercise.

We saw no difference in *GLUT4* mRNA between fiber-types, which is inconsistent with previous research. Previous studies have shown GLUT4 protein content to be approximately 13%–40% higher in Type I fibers compared with Type II fibers (Daugaard et al. 2000; Gaster et al. 2000; Stuart et al. 2010). One explanation for this difference may be due to the fact the earlier studies analyzed protein levels and we analyzed mRNA levels. Generally, mRNA levels are correlated with the level of proteins (Guo et al. 2008; Pascal et al. 2008). However, factors including transcriptional splicing, post-transcriptional splicing, and mRNA degradation may alter the relationship between transcription and translation.

In the current study, *GLUT4* increased in both Type I and Type II fibers following resistance exercise. Many studies have seen an increase in *GLUT4* expression in muscle homogenates following exercise (Hussey et al. 2012; Kraniou et al. 2006; Leick et al. 2010). Previous investigations on fiber-type–specific GLUT4 protein expression following exercise have shown incongruent results (Daugaard et al. 2000; Stuart et al. 2010; Xiao et al. 2012). It should be noted that nearly all of the previous studies have employed endurance exercise to induce the increase in GLUT4 expression. Thus, an increase in GLUT4 is not unexpected in those studies. Few studies have examined the effects of resistance training on GLUT4. Holten et al. (2004) saw an increase in GLUT4 content following 6 weeks of resistance training in diabetic subjects, but not in health control subjects. The resistance exercise employed in the current study involved an eccentric component. Eccentric exercise has been shown to decrease GLUT4 protein levels, possibly because of damage to the sarcolemma (Asp et al. 1995). Perhaps the eccentric component of the exercise protocol in the current study was not intense enough to elicit the damage needed to inhibit *GLUT4* expression. Another possible explanation for the results seen in the current study may be that the expression of *GLUT4* is upregulated as an adaptation to replace the loss in *GLUT4* protein.

There are several known transcription factors for *GLUT4*, including Peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, which stimulates mitochondrial biogenesis and causes fiber-type transition toward the oxidative, slow-twitch type (Mortensen et al. 2006; Zorzano et al. 2005). The regulation of *GLUT4* expression by PGC-1α occurs primarily by the binding to and co-activation of myocyte enhancer factor 2C (MEF2C) (Michael et al. 2001). Sakuma et al. (2008) found that the calcineurin inhibitor, cyclosporine A, inhibited MEF2C activation in overloaded soleus muscle, suggesting that calcineurin is needed for MEF2C activation. Interestingly, we saw *GLUT4* expression increase despite the fact that resistance exercise has been shown to inhibit calcineurin activity (Lamas et al. 2010). Thus, there most likely are other transcription regulators of *GLUT4* that are activated with resistance exercise. Zorzano et al. (2005) have written a thorough review of the regulation of *GLUT4* transcription.

In conclusion, the current study showed an increase in *GLUT4* expression in both slow-twitch (Type I) and fast-twitch (Type II) skeletal muscle fibers following resistance exercise. This study demonstrates that resistance exercise is an effective training modality to facilitate the mechanisms of glucose uptake in both slow and fast fiber-types. Interestingly, we saw an increase in *IGF-1R* expression in slow-twitch but not in fast-twitch muscle fibers. This may partially provide a mechanism for fast-twitch muscle fiber loss seen with aging (Trappe et al. 2003). The regulation of *IGF-1R* expression in skeletal muscle has yet to be fully elucidated. Given the role that skeletal muscle plays in health (Wolfe 2006), this knowledge would be beneficial for the determination of appropriate countermeasures to sarcopenia, cachexia, and other forms of muscle atrophy.

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