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A chronic increase in physical activity inhibits fed-state mTOR/S6K1 signaling and reduces IRS-1 serine phosphorylation in rat skeletal muscle

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Abstract

A chronic increase in physical activity and (or) endurance training can improve insulin sensitivity in insulin-resistant skeletal muscle. Cellular mechanisms responsible for the development of insulin resistance are unclear, though one proposed mechanism is that nutrient overload chronically increases available energy, over-activating the mammalian target of rapamycin (mTOR) and ribosomal S6 kinase 1 (S6K1) signaling pathway leading to increased phosphorylation of serine residues on insulin receptor substrate-1 (IRS-1). The objective of this study was to determine if increased physical activity would inhibit mTOR/S6K1 signaling and reduce IRS-1 serine phosphorylation in rat skeletal muscle. Soleus muscle was collected from fed male Sprague-Dawley sedentary rats (Inactive) and rats with free access to running wheels for 9 weeks (Active). Immunoblotting methods were used to measure phosphorylation status of mTOR, S6K1, IRS-1, and PKB/Akt (protein kinase B/AKT), and total abundance of proteins associated with the mTOR pathway. Muscle citrate synthase activity and plasma insulin and glucose concentrations were measured. Phosphorylation of mTOR (Ser₂₄₄₈), S6K1 (Thr₃₈₉), and IRS-1 (Ser₆₃₆₋₆₃₉) was reduced in Active rats ($p < 0.05$). Total protein abundance of mTOR, S6K1, IRS-1, 4E-BP1, eEF2, PKB/Akt and AMPK α , and phosphorylation of PKB/Akt were unaffected ($p > 0.05$). Total SKAR protein, a downstream target of S6K1, and citrate synthase activity increased in Active rats ($p < 0.05$), though plasma insulin and glucose levels were unchanged ($p > 0.05$). Reduced mTOR/S6K1 signaling during chronic increases in physical activity may play an important regulatory role in the serine phosphorylation of IRS-1, which should be examined as a

potential mechanism for attenuation of insulin resistance associated with increased IRS-1 serine phosphorylation.

Keywords

mTOR; S6K1; IRS-1; physical activity; skeletal muscle; insulin resistance

Introduction

There is a growing epidemic of insulin resistance and type 2 diabetes in the United States and worldwide, in part due to an increased prevalence of obesity and (or) reduced physical activity levels (Kahn and Flier 2000). Skeletal muscle is a major regulator of insulin resistance, since it is responsible for up to 75% of insulin-dependent glucose disposal in human subjects (Shulman et al. 1990). It is also clear that reduced caloric intake (McCurdy and Cartee 2005) and an increase in physical activity and (or) endurance exercise training can improve insulin sensitivity in skeletal muscle (Christ et al. 2002; Goodyear and Kahn 1998; Zierath 2002). Therefore, because physical exercise and activity uses skeletal muscle for locomotion, it is likely that the improved insulin sensitivity following exercise training is due (in part) to cellular adaptations within skeletal muscle, which improve insulin signaling (Jessen and Goodyear 2005; Kim et al. 2004).

The insulin signaling cascade has long been studied as a means to understand the molecular mechanisms of insulin resistance and type 2 diabetes (Cohen 2006). Insulin binds to its receptor and initiates the intrinsic tyrosine kinase activity of the receptor, which further phosphorylates cellular substrates including insulin receptor substrate 1 (IRS-1). IRS-1 is the predominant isoform of the insulin receptor substrate in skeletal muscle (Araki et al. 1994). Tyrosine phosphorylation of IRS-1 creates a scaffold for which the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K) can bind, further transducing the signal through the PI3K/Akt pathway. Insulin stimulation of PI3K is a necessary step in the insulin signaling pathway that leads to the uptake of glucose from the blood (Saltiel and Kahn 2001). Therefore, signaling defects in this pathway disrupt normal insulin signaling and have been proposed as involved in the pathogenesis of insulin resistance (Shulman 2000).

Recent evidence implicates an inhibitory role in insulin signaling for the mammalian target of rapamycin (mTOR) pathway via an increased serine phosphorylation of IRS-1 (Tremblay and Marette 2001; Tzatsos and Konstantin 2006; Um et al. 2004). Serine or threonine phosphorylation of different sites on IRS-1 can have many consequences, including dissociation of IRS proteins from the insulin receptor, blockage of certain Tyr phosphorylation sites of IRS, and inducing degradation of IRS protein (Paz et al. 1997; Pederson et al. 2001). Phosphorylation of IRS-1 at Ser₆₃₆₋₆₃₉ has been shown to be involved in insulin resistance in cases involving obesity-linked insulin resistance and type 2 diabetes (Bouzakri et al. 2003; Khamzina et al. 2005). Both mTOR and the ribosomal S6 kinase 1 (S6K1), a downstream effector of mTOR, appear to be involved in the Ser₆₃₆₋₆₃₉ phosphorylation of IRS-1 (Bouzakri et al. 2003; Khamzina et al. 2005; Tremblay and Marette 2001; Tremblay et al. 2005). In fact, S6K1 knockout mice are protected against diet-induced insulin resistance (Um et al. 2004). Furthermore, mice lacking the eukaryotic initiation factor 4E binding protein (4E-BP1) are highly susceptible to diet-induced insulin resistance, which implies that 4E-BP1 may be acting as a "metabolic brake" in the etiology of insulin resistance in skeletal muscle (Le Bacquer et al. 2007). A recently identified protein, SKAR (S6K1 Aly/REF-like target), was found to be an insulin-sensitive, novel binding protein and substrate of S6K1 that specifically affects cell size (Richardson et al. 2004). These recent findings provide more evidence that overactivation or deregulation of the mTOR signaling pathway is an important regulator of insulin signaling.

There have been numerous studies conducted in the area of insulin signaling and exercise over the past 30 years (Tomas et al. 2002), however, the effect of chronic physical activity and (or) exercise training on mTOR signaling and its role in regulating insulin signaling is not known.

Therefore, the purpose of the current study was to determine whether a chronic increase in physical activity alters expression and phosphorylation of regulatory proteins associated with both the insulin and mTOR signaling pathways. We hypothesized that an increase in physical activity would inhibit or attenuate fed-state mTOR/S6K1 signaling and reduce IRS-1 serine phosphorylation in rat skeletal muscle.

Materials and methods

Seventeen male Sprague-Dawley rats between 4 and 5 weeks old were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, Ind.). Weanling rats were assigned to either an Inactive group ($n = 8$) or Active group ($n = 9$). The Inactive rats were housed in standard polycarbonate rat cages and the Active rats were housed in cages with free access to running wheels for 9 weeks (Nalgene; Rochester, N.Y.). Daily voluntary running distance (i.e., total number of revolutions of the wheel) was monitored with an optical sensor attached to the side of the running wheel interfaced with a personal computer (activity wheel counter model 86060, Lafayette Instrument, Lafayette, Ind.) (Collins et al. 2005).

Both groups of rats were fed ad libitum throughout the study. Running wheels were removed from the exercise group cages 24 h before sacrifice of the animals to avoid any acute effects on muscle signaling proteins due to the prior exercise bout. We define fed-state as allowing the rats access to food (normal rodent diet) and water ad libitum. We sacrificed the rats at the same time of day (early morning) and thus we did not expect large differences in food intake between groups. Rats were sacrificed by i.p. injection with sodium pentobarbital. Both groups were sacrificed at approximately 3 months of age and soleus muscles from both legs were removed and quickly frozen in liquid nitrogen. Samples were stored at -80°C until analysis. To collect serum samples, a needle was inserted into the abdominal aorta and 3 mL of blood was withdrawn, centrifuged, and stored at -80°C until analysis.

Soleus muscle specimens were dissected free from blood and connective tissue and homogenized (1:9 w/v) in icecold buffer ($50\text{ mmol}\cdot\text{L}^{-1}$ Tris-HCL, $250\text{ mmol}\cdot\text{L}^{-1}$ mannitol, $50\text{ mmol}\cdot\text{L}^{-1}$ NaF, $5\text{ mmol}\cdot\text{L}^{-1}$ sodium pyrophosphate, $1\text{ mmol}\cdot\text{L}^{-1}$ EDTA, $1\text{ mmol}\cdot\text{L}^{-1}$ EGTA, 1% Triton X-100 (pH 7.4), $1\text{ mmol}\cdot\text{L}^{-1}$ DTT (dithiothreitol), $1\text{ mmol}\cdot\text{L}^{-1}$ benzamidine, $0.1\text{ mmol}\cdot\text{L}^{-1}$ PMSF (phenylmethylsulfonyl fluoride), $5\mu\text{g}\cdot\text{mL}^{-1}$ soybean trypsin inhibitor (SBTI); DTT, benzamidine, PMSF, and SBTI were added to the buffer immediately before use). Supernatant was collected after centrifugation at $6000\text{ r}\cdot\text{min}^{-1}$ (2012g) for 10 min at 4°C .

A Bradford protein assay was performed to determine protein content of samples. Except for aliquots for 4E-BP1, samples were then combined with 2 $\times$ sample buffer (SB) containing $125\text{ mmol}\cdot\text{L}^{-1}$ Tris (pH 6.8), 25% glycerol, 2.5% sodium dodecyl sulfate (SDS), 2.5% β -mercaptoethanol, and 0.002% bromophenol blue. Aliquots used to detect 4E-BP1 were initially boiled at 100°C for 10 min and spun for 30 min at $10\,000\text{ r}\cdot\text{min}^{-1}$ (5590g) before combining the supernatants with 2 $\times$ SB.

SDS-PAGE and Western blot analysis

Details of the immunoblotting procedures have been previously published (Dreyer et al. 2006). Samples containing $50\mu\text{g}$ of total protein per lane were loaded in duplicate and separated by SDS-PAGE for 60 min at 150 V using 7.5%, 10%, or 15% gels on a Criterion electrophoresis cell. A molecular weight ladder (BioRad, Precision Plus protein standard) was also included on each gel. Following SDS-PAGE, proteins were transferred to polyvinylidene difluoride

membranes (PVDF) (Hybond-P, Amersham Biosciences, Piscataway, N.J.) at 50 V for 1 h. Once transferred, PVDF membranes were placed in blocking buffer (5% non-fat dry milk (NFDM) in TBST (tris-buffered saline and 0.1% Tween-20) for 1 h. Blots were then serially washed twice in deionized water and twice more in TBST before incubating with primary antibody in 5% bovine serum albumin (BSA) in TBST overnight at 4 °C with constant agitation. The next morning, the blots were washed twice in TBST and incubated with secondary antibody (1:2000) for 1 h in 5% NFDM in TBST at room temperature with constant agitation. After secondary incubation the blots were washed for 15 min and then serially washed (4 × 5 min) with TBST. Blots were then incubated for 5 min with enhanced chemiluminescence reagent (ECL plus Western Blotting Detection System, Amersham Biosciences, Piscataway, N.J.) to detect horseradish peroxidase activity. Optical density measurements were obtained with a CCD camera mounted in a ChemiDoc XRS imaging system (BioRad, Hercules, Calif.). Once the appropriate image was captured, densitometric analysis was performed using Quantity One 1-D analysis Software v. 4.5.2 (BioRad, Hercules, Calif.). Data are expressed as raw value of the band minus a representative background sample from the membrane, divided by an internal loading control (50 µg/lane) loaded on every gel to ensure comparability across membranes.

The membranes described above were incubated in Restore™ Western blot stripping buffer (Pierce, Rockford, Ill.) for 20 min at 37 °C and re-probed with appropriate polyclonal antibodies for detection of the total expression levels of each protein. Equal loading of protein was determined spectrophotometrically and equal transfer confirmed by actin standard and (or) Ponceau S staining. Therefore, the immunoblotting data are expressed as protein phosphorylation status (in arbitrary units) because total protein abundance did not change.

Antibodies

The primary antibodies used were all purchased from Cell Signaling (Beverly, Mass.): phospho-mTOR (Ser₂₄₄₈; 1:1000); phospho-p70 S6K1 (Thr₃₈₉; 1:500); phospho-IRS-1 (Ser₆₃₆₋₆₃₉; 1:500); phospho-PKB/Akt (protein kinase B/Akt) (Ser₄₇₃; 1:1000); total-mTOR (1:1000); total-p70 S6K1 (1:500); total-IRS-1 (1:500); total-4E-BP1 (1:1000); total-eEF2 (eukaryotic elongation factor 2; 1:1000); total-PKB/ Akt (1:1000) total-AMPK α (AMP-activated protein kinase α ; 1:1000), and total SKAR (1:1000). Anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody was purchased from Amersham Bioscience (1:2000).

Insulin, glucose, and citrate synthase

Plasma insulin concentrations were determined by rat-mouse insulin ELISA kit (Linco Research, St. Charles, Mo.) as directed by the manufacturer. Plasma glucose concentrations were measured using an automated glucose and lactate analyzer (YSI, Yellow Springs, Ohio). Citrate synthase activity was determined using adapted methods previously described by Srere (1969).

Statistics

Data are presented as means $\pm$ SEM. An independent two sample Student's *t* test was used to assess differences in phosphorylation status and total protein content of each protein, muscle citrate synthase activity, body mass, soleus mass, and plasma insulin and glucose concentrations between groups. Significance was set at $p < 0.05$. Assumptions of the test included a normal distribution of the data, equal variances, and randomization of the independent sample groups.

Results

Peak running distance over the 9 weeks reached 5.4 ± 1.4 km/d between weeks 4 and 5. The masses of the rats prior to sacrifice were compared between groups. The Active group had significantly lower body mass as compared with the Inactive group (338 ± 5 g vs. 411 ± 11 g, $p = 0.00001$; Table 1). However, there was no difference in soleus muscle mass between the two groups (0.164 ± 0.004 g vs. 0.175 ± 0.005 g controls, $p = 0.11$; Table 1).

Insulin, glucose, lactate, and citrate synthase

There was no difference in plasma insulin or glucose concentrations between the two groups ($p = 0.82$, $p = 0.87$, respectively; Table 1).

Soleus muscle citrate synthase activity was significantly higher in the Active group ($p = 0.006$; Table 1) as compared with the Inactive group.

Western blot analyses

The phosphorylation status of mTOR (Ser2448) and S6K1 (Thr389) were significantly reduced in the Active rats ($p = 0.005$ and $p = 0.00001$, respectively; Figs. 1A and 1C), whereas total protein content for both proteins remained unchanged ($p = 0.95$ and $p = 0.13$, respectively; Fig. 1B and 1D). Total protein abundance of eEF2, 4E-BP1, and AMPK α was also not different between the Active and Inactive groups ($p = 0.999$, 0.533 , and 0.805 , respectively; Fig. 2). SKAR total protein content, a specific binding partner and substrate of S6K1, was significantly increased in the Active group ($p = 0.003$; Fig. 2B).

The phosphorylation status of IRS-1 (Ser₆₃₆₋₆₃₉) was significantly reduced in the Active group compared with the Inactive group ($p = 0.001$, Fig. 3A) with no change in total IRS-1 protein content ($p = 0.855$, Fig. 3B). Phosphorylation status and total protein content did not change between groups for PKB/Akt ($p = 0.603$ and $p = 0.661$ respectively; Fig. 3C and 3D).

Discussion

The primary and novel finding from our study was that an increase in physical activity was associated with a downregulation of the fed-state mTOR/S6K1 signaling pathway and a reduction in IRS-1 serine phosphorylation in rat skeletal muscle. Specifically, we found that mTOR Ser₂₄₄₈, S6K1 Thr₃₈₉, and IRS-1 Ser₆₃₆₋₆₃₉ phosphorylation were significantly reduced in the Active rats that were exposed to 9 weeks of free access to a running wheel. Overactivation of the mTOR signaling pathway has been proposed as a mechanism by which insulin signaling can be inhibited because IRS-1 is a substrate for both mTOR and S6K1 (Um et al. 2006). In fact, evidence is accumulating for a significant role of the mTOR pathway being involved in nutrient overload or diet-induced insulin resistance in skeletal muscle (Le Bacquer et al. 2007; Tremblay and Marette 2001; Um et al. 2004). Our study is the first to suggest a connection between chronic physical activity and improved insulin signalling by reduced mTOR signaling and thus reduced inhibitory IRS-1 serine phosphorylation in skeletal muscle.

It is well-known that exercise can stimulate insulin-independent GLUT4 translocation and glucose uptake within skeletal muscle (Brozinick et al. 1994; Goodyear and Kahn 1998). Insulin promotes GLUT4 translocation by binding to its receptor, enhancing IRS-1 tyrosine phosphorylation, and stimulating signaling to PI3K and PKB/Akt (Zierath 2002). Exercise is proposed to enhance insulin sensitivity both by insulin-independent stimulation of GLUT4 translocation and by the interaction of many signaling pathways (Jessen and Goodyear 2005; Zierath 2002). In our study, we found that mTOR signaling and IRS-1 serine phosphorylation were reduced following a period of increased physical activity in rats. Therefore, in addition to the other well-known effects of exercise on enhanced insulin signaling and glucose uptake

in skeletal muscle, we propose that reduced mTOR signaling may also be playing a role in decreasing IRS-1 Ser₆₃₆₋₆₃₉ phosphorylation in this model.

It has been demonstrated that the phosphorylation of serine residues 636-639 of IRS-1 are involved in insulin resistance (Bouzakri et al. 2003). Other studies show serine residue phosphorylation on the C terminus of IRS-1 prevents insulin-stimulated tyrosine phosphorylation (Paz et al. 1997). It is the tyrosine phosphorylation on the C-terminus of IRS-1 that recruits PI3-kinase, a key step in insulin signal transduction. There are several recent studies that support an important physiological role for mTOR signaling in the regulation of insulin resistance by the ability of mTOR and S6K1 to enhance IRS-1 serine phosphorylation (Khamzina et al. 2005; Patti et al. 1998; Tremblay and Marette 2001; Tremblay et al. 2005). In a recent study by Um et al. (2004) S6K1-deficient mice remained sensitive to insulin even with high levels of blood glucose and free fatty acids, and IRS-1 serine phosphorylation was reduced. In addition, Le Bacquer et al. (2007) have shown that 4E-BP1 deficient mice were more sensitive to diet-induced insulin resistance, suggesting that 4E-BP1 interacts with and regulates S6K1 within muscle. The primary mechanisms for activating mTOR within skeletal muscle are most likely elevated insulin and amino acid concentrations (Avruch et al. 2006; Fujita et al. 2007). Chronic exposure to nutrient overload would elevate circulating insulin levels and stimulate mTOR by upstream activation of Akt and hence reduce the suppression on mTOR by TSC2 (Avruch et al. 2005; Proud 2006). However, inhibition of insulin signaling is the common characteristic of insulin resistance at which point it appears that amino acid signaling (i.e., from excess amino acids present during nutrient overload) can chronically activate mTOR and S6K1 in muscle and thus further inhibit insulin signaling via an increase in IRS-1 serine phosphorylation (Khamzina et al. 2005; Patti et al. 1998; Tremblay and Marette 2001; Tremblay et al. 2005).

With the current study design, we were unable to measure insulin sensitivity with stable isotopic techniques or via a euglycemic-hyperinsulinemic clamp. There are, however, a few potential explanations for not seeing a difference in fed insulin and glucose levels. First, the Inactive group, although sedentary, may not have experienced a detectable insulin resistance. The fact that there was no detectable difference in the phosphorylation status or total PKB/Akt between groups supports this explanation. It has been hypothesized that the effect of exercise on insulin signaling and sensitivity is an acute effect and is rapidly reversed with the cessation of the exercise stimulus (Burstein et al. 1985; Hayashi et al. 1997). In fact, most control groups in rat physiological studies use sedentary rats with no detectable defects in insulin signaling. However, as recently suggested by Booth and Lees (2006), the use of sedentary, inactive controls may introduce a bias into the interpretation of exercise studies because it is assumed that sedentary rats are healthy. Young, sedentary rats, although not showing outward signs of clinical hyperinsulinemia, hyperlipidemia, or hyperglycemia, may not necessarily be considered healthy. Rats are normally very active animals and therefore maintaining rats in a small cage may be considered a type of inactivity model. Our results are consistent with this hypothesis, showing that the Inactive rats had higher levels of phosphorylation of mTOR, S6K1, and IRS-1 serine residues. Thus, the reduction in phosphorylation in the active rats may imply a return to a healthier overall status (i.e., improved insulin sensitivity) as would be predicted by the hypothesis presented by Booth and Lees (2006). Secondly, the fed response between groups may have masked any basal differences in insulin and glucose concentrations. We chose to study the rats in the fed state because we suspected it would be the best time to potentially detect differences in mTOR signaling due to the stimulation of feeding on this pathway. It has previously been shown that active rats following a similar protocol actually consume a greater amount of food than inactive controls (Cortright et al. 1997). Therefore, owing to a lack of differences in either fed plasma insulin or glucose levels between groups, we believe the feeding effect is negligible in analysis of the results.

SKAR, a novel binding protein and substrate of S6K1, is insulin sensitive and has been shown to affect cell size (Richardson et al. 2004). SKAR protein is phosphorylated by activated S6K1 and inhibition of both SKAR and S6K1 results in cells that are smaller (Richardson et al. 2004). An interesting finding from our study was that the expression of SKAR protein was increased in Active rats as compared with Inactive rats. The elevated SKAR protein content may be an adaptive response by the more physically active muscle; however, this is quite speculative, since the physiological function of SKAR is currently unknown. We also found that 9 weeks of free access to a running wheel did not alter the protein expression for mTOR, S6K1, 4E-BP1, PKB/Akt, AMPK α , eEF2, or IRS-1. Our results are consistent with previous studies that have reported that IRS-1 total protein expression does not change with exercise training (Christ et al. 2002) and that Akt concentrations were not altered (Bernard et al. 2005), though we do acknowledge that results may have been altered had we examined Akt isoforms 1 and 2 separately. Therefore, it appears that the increase in physical activity and overall energy expenditure was primarily responsible for the reduction in fed-state mTOR, S6K1, and IRS-1 serine phosphorylation.

It is well known that a chronic activation of AMPK is associated with an upregulation of citrate synthase and other mitochondrial oxidative enzymes in rat skeletal muscle (Winder et al. 2000). Endurance exercise and (or) muscle contractions can activate AMPK activity in both rodent and human skeletal muscle (Fujii et al. 2000; Rasmussen and Winder 1997). Recently, we and others have shown that AMPK activation (from both resistance exercise and endurance exercise protocols) in skeletal muscle is associated with a reduction in mTOR signaling (Dreyer et al. 2006; Williamson et al. 2006). AMPK is an important cellular energy sensor within muscle and it is therefore activated during cellular stress and increases in energy demand. The Active rats had a significantly reduced overall body mass as compared with the Inactive rats, suggesting that an increase in overall energy expenditure is an important component that could regulate mTOR signaling in muscle. Soleus muscle mass was not different between groups, implying that the reduced body mass in the Active rats was primarily due to a reduction in total body fat. Interestingly, citrate synthase activity was also significantly elevated (11%) in the Active rats. The modest increase in citrate synthase in our study, as compared with the much larger increases in citrate synthase activity reported in exercise-training studies (Taylor et al. 2005), suggests that a chronic increase in physical activity and energy expenditure (at an exercise intensity lower than that commonly used to induce an exercise-training effect) can have beneficial health effects on skeletal muscle mTOR signaling.

In summary, we report that rats allowed free access to running wheels for 9 weeks increased their overall physical activity, reduced whole-body mass, and lowered soleus muscle mTOR, S6K1, and IRS-1 serine phosphorylation as compared with sedentary, inactive rats. We conclude that reduced mTOR and S6K1 activation during chronic increases in physical activity may play an important regulatory role in the serine phosphorylation status of skeletal muscle IRS-1 and may have important implications for reducing insulin resistance associated with this feedback pathway. Future studies are required to determine the specific role reduced mTOR signaling may play in regulating the well-known effect of enhanced insulin sensitivity associated with exercise training.

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Fig. 1.

Soleus muscle mTOR phosphorylation at Ser₂₄₄₈ (A), total mTOR protein content (B), S6K1 phosphorylation at Thr₃₈₉ (C), and total S6K1 protein content (D). Data are expressed relative to an internal loading control and as mean $\pm$ SEM, $n = 8$ per group. Insert shows representative Western blot for duplicate samples for Inactive (left) and Active (right) rats. AU, arbitrary units. Asterisk (*) indicates significant difference from Inactive, $p < 0.05$.

Fig. 2.

Soleus muscle total eEF2 protein content (A), total SKAR protein content (B), total 4E-BP1 protein content (C), total AMPK α protein content (D). Data are expressed relative to an internal standard and as mean $\pm$ SEM, $n = 8$ per group. Insert shows representative Western blot for duplicate samples for Inactive (left) and Active (right) rats. AU, arbitrary units. Asterisk (*) indicates significant difference from Inactive, $p < 0.05$.

Fig. 3. Soleus muscle IRS-1 phosphorylation at Ser₆₃₆₋₆₃₉ (A), total IRS-1 protein content (B), PKB/Akt phosphorylation at Ser₄₇₃ (C), and total PKB/Akt protein content (D). Data are expressed relative to an internal loading control and as mean $\pm$ SEM, $n = 8$ per group. Insert shows representative Western blot for duplicate samples for Inactive (left) and Active (right) rats. AU, arbitrary units. Asterisk (*) indicates significant difference from Inactive, $p < 0.05$.

Table 1

Rat characteristics

Male Sprague-Dawley rats	Total body mass (g)	Soleus mass (mg)	Plasma insulin ($\mu\text{U}\cdot\text{mL}^{-1}$)	Plasma glucose ($\text{mmol}\cdot\text{L}^{-1}$)	Citrate synthase activity ($\mu\text{mol}\cdot\text{g}\cdot\text{min}^{-1}$)
Inactive ($n = 8$)	411 $\pm$ 10	175 $\pm$ 5	23.2	10.4 $\pm$ 0.5	29.9 $\pm$ 0.8
Active ($n = 9$)	338 $\pm$ 5 [*]	164 $\pm$ 4	24.3	10.6 $\pm$ 0.3	33.4 $\pm$ 0.9 [*]

^{*} Significantly different from Inactive, $p < 0.05$.