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Lean and Obese Zucker Rats Exhibit Different Patterns of p70S6kinase Regulation in the Tibialis Anterior Muscle in Response to High Force Muscle Contraction

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Abstract

Increased phosphorylation of the 70 kDa ribosomal S6 kinase (p70s6k) signaling is strongly correlated with the degree of muscle adaptation following exercise. Here, we compare the phosphorylation of p70s6k, Akt and mTOR in the tibialis anterior (TA) muscles of lean and obese Zucker rats following a bout of eccentric exercise. Exercise increased p70S6k (Thr 389) phosphorylation immediately after ($33.3 \pm 7.2\%$) and during recovery [1-hr; ($24.0 \pm 14.9\%$), and 3-hr ($24.6 \pm 11.3\%$)], in the lean TA and at 3-hr ($33.5 \pm 8.0\%$) in the obese TA. mTOR (Ser 2448) phosphorylation was elevated in the lean TA immediately after exercise ($96.5 \pm 40.3\%$) but remained unaltered in the obese TA. Exercise increased Akt (Thr 308) and Akt (Ser 473) phosphorylation in the lean but not the obese TA. These results suggest that insulin resistance is associated with alterations in the ability of muscle to activate p p70s6k signaling following an acute bout of exercise.

Keywords

Skeletal muscle; insulin resistance; obese Zucker rat; p70S6k; mTOR

INTRODUCTION

Type 2 (non-insulin dependent) diabetes mellitus (DM) is an emerging epidemic in Western cultures that is thought to afflict 150 million people worldwide 11. Insulin resistance is frequently accompanied by a variety of metabolic and cardiovascular abnormalities that include hypertension, glucose intolerance, Type 2 diabetes, dyslipidemia, atherosclerosis and central obesity. A number of studies that employ strength training regimens have been shown to improve glycemic control, increase skeletal muscle size and strength, and positively change body composition. They suggest that anaerobic exercise may be an effective strategy for the treatment of insulin resistance and type 2 diabetes 6,7,49,53. Recent reports have suggested that differences exist between normal and insulin resistant muscle in their adaptation to an exercise regimen 8,5,50,20,24,48. However, the direct effects of exercise on the phenotype of insulin resistant muscle have not been widely

studied. It is thought that the beneficial effects of exercise on muscle are mediated through activation of various signaling cascades which are involved in regulating changes in gene expression, glucose uptake, and protein synthesis 2. Whether insulin resistance alters exercise-induced signal transduction processes in muscle is unknown, but the existence of differences, if present, may help to explain why exercise-induced skeletal muscle adaptations may differ between normal and insulin resistant populations.

It is well established that increased muscle loading increases the rates of muscle protein synthesis 27. This increase in protein synthesis, at least in part, is thought to be regulated by the phosphorylation of the p70 ribosomal protein S6 kinase (p70S6k) 26, whose activation has been proposed to promote increased translation of messages that have a polypyrimide motif just downstream of the 5′ cap 45. It is thought that p70S6k activity is regulated by the mammalian target of rapamycin (mTOR), which functions as a growth factor and nutrient-sensing signaling molecule in mammalian cells 40. How mTOR activity is modulated is not clear; however, recent evidence suggests that mTOR is controlled by Akt or protein kinase B (PKB) which is activated in response to phospholipid products of the phosphatidylinositol 3-kinase (PI3K) reaction. It is well documented that binding of insulin to the membrane receptor stimulates a cascade of phosphorylation events resulting in activation of PI3K. It is likely that PKB/Akt directly increases mTOR activity by phosphorylating mTOR at Ser2448, and it has been hypothesized that this event is a critical point of control in the regulation of protein synthesis 4. It has been postulated that p70S6k signaling may be particularly important in mediating muscle adaptation given that the phosphorylation of this molecule following an exercise bout has been found to be strongly associated with the increase in muscle weight after 6 wk of chronic stimulation 1

The purpose of the present study was to determine whether insulin resistance alters p70S6k signaling after an acute episode of contractile activity. To investigate this possibility, muscle signaling was examined in 12-week old lean and obese Zucker rats, as it is widely accepted that the insulin resistance exhibited by these animals closely models the development of type 2 diabetes seen in humans 3,18,25,39. We hypothesized that insulin resistance would be associated with differences in how muscle contraction regulates the phosphorylation of the Akt/mTOR/p70S6k signaling cascade. To test this hypothesis, the contraction-mediated activation of Akt, mTOR and p70S6k was assessed either immediately after or 1 or 3h after a single bout of sciatic nerve stimulation. Taken together, our data suggest that insulin resistance alters contraction-induced p70S6k phosphorylation in skeletal muscle. These findings are consistent with the possibility that insulin resistance alters the way skeletal muscle "senses and responds" to contractile stimuli.

METERIALS AND METHODS

Animal Care

All procedures were performed as outlined in the Guide for the Care and Use of Laboratory Animals as approved by the Council of the American Physiological Society and the Animal Use Review Board of Marshall University. Young (10 week, n=12) male lean Zucker and young (10 week, n=12) male obese Zucker rats were obtained from the Charles River Laboratories. Rats were housed two to a cage in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved vivarium. Housing conditions consisted of a 12H: 12H dark-light cycle, and temperature was maintained at 22° ± 2°C. Animals were provided food and water *ad libitum* and allowed to recover from shipment for at least two weeks before experimentation. During this time the animals were carefully observed and weighed weekly to ensure that none exhibited signs of failure to thrive, such as precipitous weight loss, disinterest in the environment, or unexpected gait alterations.

Materials

Anti- p70S6k (#9202), Akt (#9272), mTOR (#2972), PTEN (#9552), TSC2/tuberin (#3612), raptor (#4978), phosphorylated Thr^{389} p70S6K (#9206), phosphorylated $Ser421/Thr424$ p70S6K (#9204), phosphorylated $Ser2448$ mTOR (#2971), phosphorylated $Thr308$ Akt (#9275), phosphorylated $Ser473$ (#9271) Akt, $Ser380/Thr382/383$ phosphorylated PTEN (#9554), phosphorylated $Thr1462$ TSC2/tuberin (#3611), Mouse IgG, and Rabbit IgG antibodies were purchased from Cell Signaling Technology (Beverly, MA). Enhanced chemiluminescence (ECL) western blotting detection reagent was obtained from Amersham Biosciences (Piscataway, NJ). Restore western blot stripping buffer was obtained from Pierce Company (Rockford, IL), and 3T3 cell lysates were from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were purchased from Sigma Biosciences (St. Louis, MO) or Fisher Scientific Company (Hanover, IL).

Contractile stimulation of skeletal muscles

The high-frequency electrical stimulation (HFES) model has been previously described 36 and was chosen on the basis of its efficacy in stimulating protein translation and muscle hypertrophy *in vivo*1. In order to produce maximal contractile stimulus, a voltage of 5–7V was applied to electrodes placed on the sciatic nerve and the sciatic nerve was stimulated with 1-ms pulses at 100 Hz, where all motor units(both fast and slow) are recruited. The contractionslasted 3 s and were followed by a 10-s rest, and after the sixth repetition there was an additional 50 s of rest. The HFES model used in the present study produced 10 sets of 6 contractions with an overall protocol time of 22 min. This protocol results in eccentric contraction of the TA. The TA muscle was chosen for analysis on the basis of previous studies demonstrating that HFES induces p70S6k phosphorylation and muscle hypertrophy in the TA muscle 15,30,47,54. Animals were killed by a lethal dose of pentobarbital sodium at baseline, immediately following, 1h or 3 h after HFES. Once excised, muscles were blotted dry, trimmed of visible fat and tendon projections, weighed, immediately frozen in liquid nitrogen, and stored at −80° C.

Preparation of protein isolate and immunoblotting

Muscles were pulverized in liquid nitrogen using a mortar and pestle until a fine powder was obtained. After washing with ice cold PBS, pellets were lysed on ice for 15 minutes in Tissue Protein Extraction Reagent (T-PER) lysis buffer composed of T-PER reagent (2mL/ 1g tissue weight) (Pierce, Rockford, IL), 1 mM EDTA (pH 8.0), 1 mM EGTA (pH 7.5), 1M magnesium chloride, 1 mM PMSF, 1μl protease inhibitor cocktail and 1 mM sodium vanadate to inhibit phosphatase activity and centrifuged for 10 minutes at 2000 × g to pellet particulate matter. This process was repeated twice, and the supernatants were combined for protein concentration determination using the Bradford method (Pierce, Rockford, IL). Samples were diluted to a concentration of 3 μg/ μl in SDS loading buffer and boiled for 5 minutes. 60 μg of protein were separated using 10% SDS-PAGE gels. Transfer of protein onto nitrocellulose membranes, verification of transfer and determination of equal loading between lanes and membranes was determined as outlined previously 33. Protein immuno-detection was performed as outlined by the antibody manufacturer while immunoreactive bands were visualized with Electrochemiluminescence (ECL) (Amersham Biosciences). Exposure time was adjusted at all times to keep the integrated optical densities (IODs) within a linear and non-saturated range, and band signal intensity was quantified by densitometry using a flatbed scanner (Epson Perfection 3200 PHOTO) and imaging software (AlphaEaseFC) after normalization to β-tubulin. Molecular weight markers (Cell Signaling) were used as molecular mass standards, and NIH 3T3 cell lysates were included as positive controls. To allow direct comparisons to be made between the concentration levels of different signaling molecules, immunoblots were stripped and re-probed with Restore western blot stripping buffer as detailed by the manufacturer (Pierce, Rockford, IL).

Statistical analysis

Results are presented as mean ± SEM. Data were analyzed by using the Sigma Stat 3.0 statistical program. Data was analyzed using a two-way ANOVA followed by the Student-Newman-Keuls post-hoc testing when appropriate. *P <0.05* was considered to be statistically significant.

RESULTS

Insulin resistance and muscle mass

The average body weight of the obese Zucker rats was ~82 % greater than that of the lean Zucker rats (597 ± 21.7 g vs. 328 ± 12.2 g; *P< 0.05*). Compared to lean animals, TA muscle mass was ~35% less (651 ± 9.0 mg vs. 479 ± 20 mg) in the obese Zucker rats (*P< 0.05*) (Table 1).

P70S6k pathway related protein levels and phosphorylation status are altered in the insulin resistant TA muscle

To investigate the effects of insulin resistance on the total amounts of p70S6k, mTOR, Akt, PTEN, TSC2 and raptor we performed SDS PAGE and western blot analysis using antibodies which recognize both the unphosphorylated and phosphorylated forms of these molecules. There were no differences in mTOR, raptor and TSC2 protein content (*P< 0.05*) (Figs.1, 5). The muscle content of p70S6k, and Akt in the obese zucker TA was 27.9 ± 3.6% and 28.2 ± 10.1% lower, respectively than that observed in their lean counterparts (*P< 0.05*) (Fig. 1). The expression of PTEN was higher in obese Zucker rats by 25.63 ± 4.2% when compared to the lean Zucker rats (*P< 0.05*) (Fig. 5). Immunoblot analysis using phospho-specific antibodies indicated that the basal phosphorylation level of p70S6k (Thr 389) was 26.1 ± 7.5% lower in the obese Zucker TA compared to that observed in lean animals (*P<0.05*) (Fig. 2). No differences were seen across groups in basal phosphorylation levels of p70S6k (Thr421/Ser424), mTOR (Ser 2448), Akt (Thr 308) Akt (Ser 473) and TSC2 (Figs. 2, 3, 4 and 5).

The contraction-induced phosphorylation of p70S6k is altered in the obese-Zucker rat

Phosphorylation of p70S6k, mTOR and Akt in exercised TA muscles was determined at 0-, 1-, and 3-hours after a bout of HFES and compared to control (unstimulated) muscles. Exercise-induced phosphorylation of these molecules was compared between lean and obese Zucker rats. In the case of each molecule examined, significant differences existed between lean and obese Zucker rats (Fig. 2, 3 & 4). In the lean rat TA, the phosphorylation of p70S6k (Thr 389) was found to be 33.3 ± 7.2%, 24.0 ± 14.9% and 24.6 ± 11.3% higher than in unstimulated control muscles at 0-, 1- and 3-hr post-HFES, respectively (*P< 0.05*) (Fig. 2). This response appeared to differ in the obese Zucker TA, where the phosphorylation of p70S6k (Thr 389) was 33.5 ± 8.0% higher than control 3hr post-exercise (*P<0.05*), but not immediately after or at the 1 hr post-HFES (Fig. 2). In lean Zucker TA, the Erk1/2-dependent phosphorylation of p70S6k (Thr 421/Ser 424) was 412.5 ± 37.2%, 331.0 ± 28.1% and 83.0 ± 20.7% higher than control at 0-, 1- and 3-hr, respectively (*P< 0.05*) (Fig. 2). Although different in magnitude compared to lean animals (Fig. 2) p70S6k (Thr 421/Ser 424) phosphorylation in the obese TA exhibited a similar pattern and was 294.5 ± 9.6%, 103.3 ± 35.5% and 182.0 ± 26.1% higher than baseline at 0-, 1- and 3-hours, respectively (Fig 2; *P< 0.05*)

Contraction-induced phosphorylation of potential p70S6k regulators is altered in the obese Zucker rat

It is thought that p70S6k phosphorylation is regulated by the mTOR complex and its upstream regulator Akt 4,40,41. mTOR phosphorylation (Ser 2448) was found to be $96.5 \pm 40.3\%$ higher than baseline immediately after exercise in lean animals ($P< 0.05$), while it was not different from baseline at 1 and 3 hr post-HFES (Fig. 3). This response differed in obese animals with no alteration in mTOR (Ser 2448) phosphorylation levels at any time point after HFES (Fig. 3). There was no significant difference between contraction-induced TSC2 (Thr 1462) phosphorylation in obese Zucker rats compared to their lean counterparts (Fig. 5). In the lean Zucker TA, Akt (Thr 308) phosphorylation was $31.8 \pm 16.2\%$, $29.8 \pm 7.5\%$ and $31.1 \pm 8.8\%$ higher than baseline at 0-, 1- and 3-hr post-HFES, exhibiting a biphasic response ($P<0.05$; Fig. 4). An altogether opposite response was observed in the obese Zucker TA, where Akt (Thr 308) phosphorylation was $25.3 \pm 6.2\%$, $44.6 \pm 9.0\%$ and $30.4 \pm 5.9\%$ lower than baseline at 0-, 1-, and 3-hours, respectively after HFES ($P<0.05$). In lean rats the phosphorylation of Akt (Ser 473) appeared to mirror what was seen at the Thr 308 residue with HFES and was $47.2 \pm 12.1\%$ and $43.7 \pm 11.7\%$ higher than baseline at 0- and 3-hours, respectively ($P< 0.05$) (Fig. 4). This pattern of response was essentially opposite in the obese Zucker rats, where the phosphorylation of Akt (Ser 473) was $18.7 \pm 6.4\%$, $46.3 \pm 5.5\%$ and $32.4 \pm 7.3\%$ lower than baseline at 0-, 1-, and 3-hours post-HFES, respectively ($P< 0.05$) (Fig. 4). Taken together these results suggest that the contraction-induced activation of p70S6k signaling may be altered with insulin resistance.

DISCUSSION

The obese Zucker rat is insulin resistant and has been used as a model of type 2 diabetes 10,22. In this report we demonstrate that the phosphorylation (activation) of the Akt/mTOR/p70S6k pathway in response to a maximal contractile stimulus appears to be altered in insulin resistant muscle.

Our results suggest that, compared to lean animals, the magnitude and time course of the contraction-induced phosphorylation of p70S6k (Thr 389) and p70S6k (Thr 421/ser 424) was significantly different in obese animals (Fig. 2). The p70S6k is a serine/threonine protein kinase, and it has been shown to play an important role in regulating protein synthesis. It is thought that p70S6k modulates protein synthesis, at least in part, by controlling the translation of numerous messenger RNA transcripts that encode components of the translational apparatus 38. Illustrating this fact is the finding that blockade of p70S6k activity results in significant inhibition of protein synthesis in multiple cell systems 12,23,9,32,55. In skeletal muscle, the contraction-induced phosphorylation of p70S6K has been found to be elevated following HFES 1,37,36 and is highly correlated with an increase in muscle mass following a resistance training program 1. To our knowledge, the influence of diabetes on p70S6k phosphorylation in response to muscle contraction has not been reported before. The physiological significance of these alterations remains unclear; however it is interesting to note that previous reports have suggested that the insulin-stimulated phosphorylation of p70S6k may be altered in diabetic rats 19,14,29. In the light of these studies, our data suggest that insulin resistance may affect how multiple stimuli may regulate the phosphorylation of p70S6k. Future studies that employ a combination of approaches to stimulate p70S6k phosphorylation will certainly be of value in determining how insulin resistance may affect the regulation of p70S6k in skeletal muscle.

To further explore how insulin resistance may be associated with alterations in the contraction-induced regulation of p70S6k phosphorylation, we assessed the effects of muscle contraction on mTOR. Like p70S6k, mTOR is thought to be involved in regulating several components of the translational machinery and in addition, it is thought to be an

upstream activator of p70S6k 16. Similar to previous studies, increased contractile activity appears to be a strong stimulus to increase the phosphorylation level of mTOR in non-diabetic muscle 37,36. Conversely, in the insulin resistant TA, HFES appears to be unable to alter the degree of mTOR phosphorylation (Fig. 3). This latter finding is consistent with our data that demonstrate that insulin resistant muscle exhibits a reduced ability to activate p70S6k following a bout of HFES. Taken together, these data suggest that insulin resistance-associated alterations in p70S6k regulation may be due, at least in part, to defects in the ability of diabetic muscle to activate mTOR following a contractile stimulus.

It is thought that mTOR phosphorylation of Ser 2448 is directly regulated by Akt/PKB 41,43,44. Akt is a serine/threonine kinase that has been shown to mediate certain types of muscle hypertrophy 4. Under non-stimulated conditions Akt is located in the cytoplasm. It is thought to translocate to the plasma membrane upon activation, where it is phosphorylated by phosphinositide-dependent kinases (PDK) on its two principal regulatory sites Thr 308 and Ser 47352. Like p70S6k and mTOR, HFES significantly increased the amount of Akt (Ser 308) and Akt (Ser 473) phosphorylation in non-diabetic muscle (Fig 4). Similar findings regarding the effects of contractile activity on Akt phosphorylation in non-insulin resistant muscle have been reported previously 34,42,51. In contrast to our findings in the non-insulin resistant animals, these events appeared to differ in insulin resistant muscle. This suggests that insulin resistance is associated with alterations in the ability of skeletal muscle to activate Akt signaling following increased contractile loading (Fig. 4). Given that Akt is thought to reside upstream of mTOR, this lack of Akt activation in insulin resistant muscle may provide an explanation as to why insulin resistance may be associated with defects in HFES induced mTOR and p70S6k phosphoryation.

In an attempt to explain differences in contraction-dependent signaling between models we also examined how HFES affected the regulation of raptor (a positive regulator of mTOR activity), the Akt inhibitor, phosphatase and tensin homolog (PTEN), and the mTOR repressor, tuberin (TSC2) 28. The ability of PTEN and TSC2 to inhibit downstream signaling is thought to be regulated by phosphorylation 57,56 while differences in raptor levels between models could influence mTOR activation. Our findings of no differences in the regulation of these three molecules with HFES between models suggests that raptor, PTEN and tuberin may not be responsible for the differences in Akt and mTOR phosphorylation that we observe between normal and insulin-resistant muscle subjected to HFES (Fig. 5). Although not investigated here, recent work has suggested that phospholipase D (PLD) may play a key role in regulating load-induced mTOR phosphorylation in skeletal muscle 21. Whether differences in PLD regulation with insulin resistance can explain the alterations in mTOR signaling we see between our experimental models will require further experimentation (Fig. 6).

The precise influence of insulin resistance on Akt regulation in muscle contraction remains unclear. It has been postulated that the degree of Akt activation following contractile activity may be dependent upon the type of contractile activity, contraction intensity, and/or the duration of stimulation 36. Given this contention, it is plausible that differences in the signaling response between models could be related to the time points chosen for evaluation. Future studies that employ other time points could yield different findings. Alternatively, it is possible that the two groups experienced a different amount of tension during the HFES protocol. Although this possibility exists, we consider it unlikely that differences in contractile intensity, if present, are solely responsible for the alterations in muscle signaling we observe. Indeed, given the nature of the HFES model (direct nerve stimulation), the type of contraction this model produces in the TA (maximal eccentric loading) and the conditions under which the loading occurred (identical in both groups), we suggest that our data are consistent with the notion that insulin resistance is characterized by alterations in contractile

signaling. The molecular mechanism(s) responsible for these differences are largely unknown. Exercise was found to have no effect on Akt as observed in isolated soleus muscle, suggesting perhaps that systemic factors and/or oxidative stress may also play a role in mediating the activation of Akt by exercise 46. Supporting this contention, hyperglycemia has been shown to directly decrease insulin-induced Akt phosphorylation on Ser473 in rat and human skeletal muscle 17,35. The factors which may regulate diminished Akt activation during hyperglycemia will require further experimentation. Similarly, it is also possible that reduced availability of circulating growth factors, decreased expression of local growth factors or alterations in cytokine levels may also play a role regulating Akt signaling 31. This latter possibility is an intriguing alternative that we are actively pursuing. Given the strong correlation between the phosphorylation level of p70S6k and degree of muscle hypertrophy seen by others, these data could suggest that insulin resistant muscle may be incapable of growth following a resistance-based exercise program. This is not in agreement with the findings of Farrell and colleagues who demonstrated that diabetic muscle is fully capable of undergoing muscle hypertrophy following 8 weeks of resistance training13. The reasons for this apparent discrepancy are not entirely clear, but they may lie in the difference in models and time points of the studies. Irrespective of the mechanism, it is likely that insulin resistance-associated differences in the ability of skeletal muscle to induce Akt → mTOR → p70S6K signaling could be of clinical importance, given the potential role that this signaling pathway may play in regulating protein synthesis and the adaptation of skeletal muscle to increased contractile loading (Fig. 6). Given the apparent linkage between exercise-induced increase in muscle mass and improvement in glucose disposal, we speculate that the data of the present study may have implications for the improvement of resistance based programs for the treatment of diabetes mellitus.

Acknowledgements

Abbreviations

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ECL	Enhanced Chemiluminiscence
HFES	High Frequency Electrical Stimulation
LZ	Lean Zucker
mTOR	mammalian Target of Rapamycin
PBS	Phosphate Buffered Saline
PTEN	Phosphatase and Tensin Homolog
OZ	Obese Zucker
p70S6k	70 kDa ribosomal S6 kinase
PKB	Protein Kinase B
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
TA	Tibialis Anterior
T-PER	Tissue Protein Extraction Reagent
TSC2	Tuberous Sclerosis Complex 2

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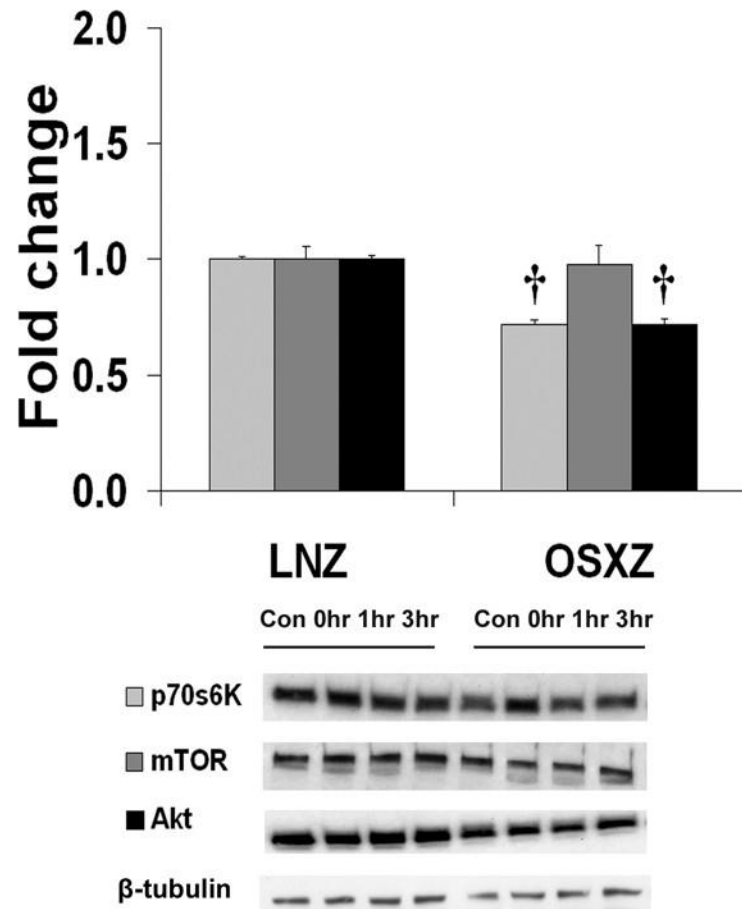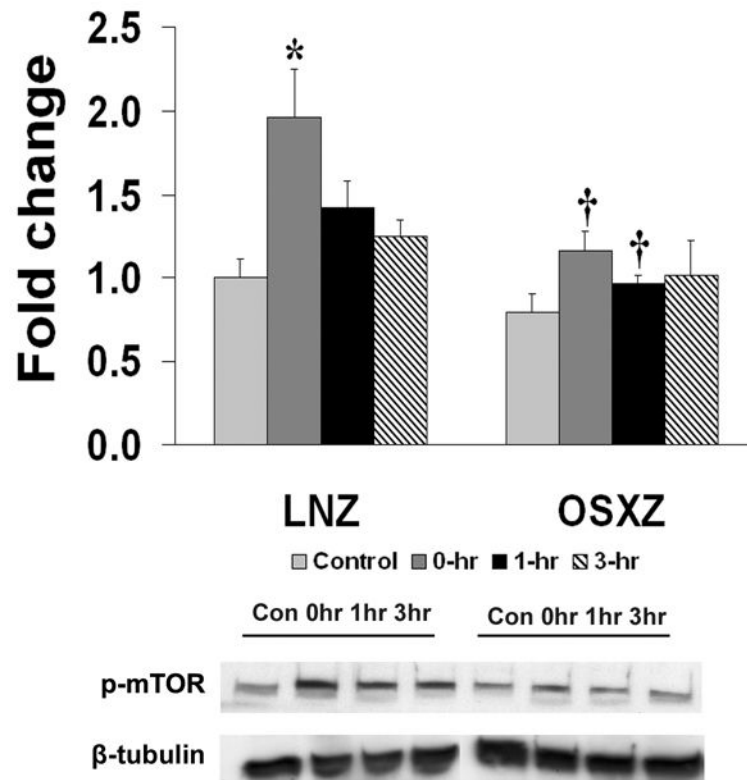
Figure 1.
TA muscles from lean (LNZ) and obese Zucker rats (OSXZ) were analyzed by Western blot analysis for diabetes-related changes in total p70S6k, mTOR and Akt protein expression. Protein quantifications were done after normalization by the abundance of β-tubulin protein. Results are expressed as a percent of the normal, LNZ value. An asterisk (*) indicates significant differences (P< 0.05) from the lean Zucker value.
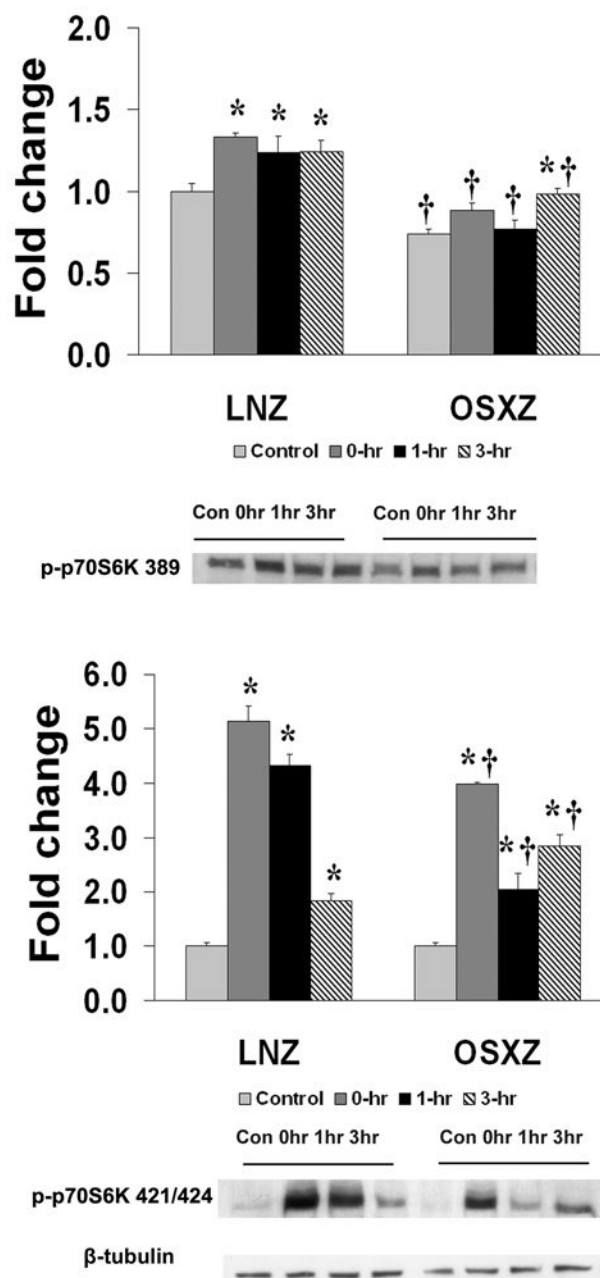
Figure 2. Contraction-induced p70S6k (Thr 389 & Thr 421/Ser 424) phosphorylation is altered with insulin resistance
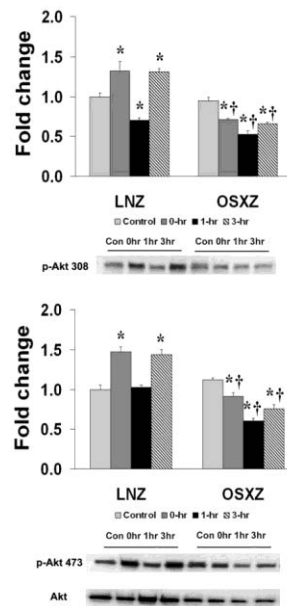The basal (control) and contraction-induced phosphorylation of p70S6k in TA muscles from lean and obese Zucker rats at 0, 1, and 3 hours after contractile stimulus. p70S6k (Thr 389 & Thr 421/ser 424) phosphorylation was determined by Western blot analysis and immunodetection for phosphorylation on Thr[389] andThr[421]/Ser[424]. All the protein quantifications were done after normalization by the abundance of β-tubulin protein. An asterisk (*) indicates a significant difference (P < 0.05) from the control within-animal model, and a cross (†) indicates significant difference (P < 0.05) at corresponding time points across animal models.

Figure 3. Contraction-induced mTOR (Ser2448) phosphorylation is altered with insulin resistance

The basal (control) and contraction-induced phosphorylation of the mTOR in TA muscles from lean (LNZ) and obese Zucker (OSXZ) rats at 0-, 1-, and 3-hours after HFES. Phosphorylation of mTOR was determined by immunodetection of phosphorylation on Ser2448. An asterisk (*) indicates significant difference ($P < 0.05$) from the control time point within-animal model, and a cross (†) indicates a significant difference ($P < 0.05$) at corresponding time points across animal models.

Figure 4. Effects of eccentric, maximal muscle contraction *in situ* on phosphorylation of Akt (Thr[308]/Ser[473])

The basal (control) and contraction-induced phosphorylation of the Akt in TA muscles from lean and obese Zucker rats at 0, 1, and 3 hours after contractile stimulus. Akt phosphorylation was determined by Western analysis and immunodetection for Akt phosphorylation on Thr308 and Ser473. An asterisk (*) indicates significant difference (P < 0.05) from the control within-animal model, and a cross (†) indicates significant difference (P < 0.05) at corresponding time points across animal models.
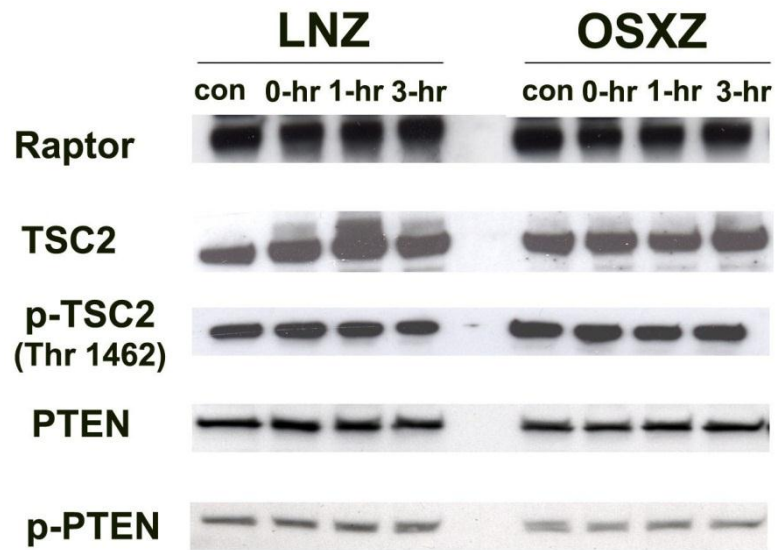
Figure 5.
Immunoblot analyses of Protein abundance of Raptor, TSC2 and PTEN and phosphorylation of Thr1462 on TSC2 and Phosphorylation of Ser380/Thr382/383 on PTEN in TA muscles from lean and obese Zucker rats at 0, 1, and 3 hours after contractile stimulus.
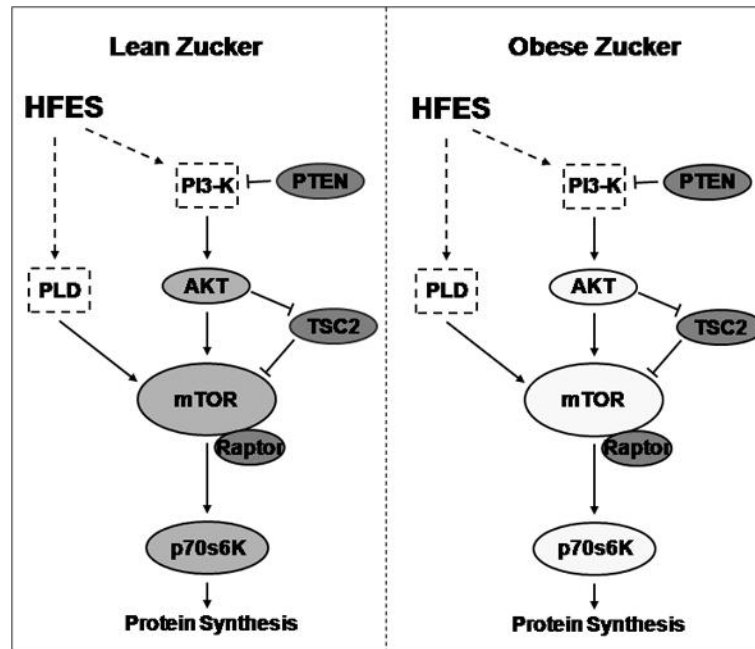
Figure 6.
Schematic diagram summarizing alterations in molecules of the signalling pathway leading to protein synthesis in Lean and Obese Zucker rats after High force electrical stimulation (HFES). Molecules shown in dark shaded, medium shaded and empty circles represent no change, complete and incomplete activation, respectively. Molecules shown in dashed rectangles were not measured in this study.

Table 1

Body weight and muscle mass of lean and obese (fa/fa) Zucker rats.

	Lean Zucker	Obese Zucker
Body mass, g	328 ± 12.2	597 ± 21.7*
TA mass, mg	651 ± 9.0	479 ± 20*

An asterisk (*) indicates significant difference (P < 0.05) from the lean Zucker value.