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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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Title

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 muscle loading results in phosphorylation of the 70 kDa ribosomal S6 kinase (p70s6k) signaling pathway and this event strongly correlates with degree of muscle adaptation following resistance exercise. Here, we compared the basal and contraction-induced phosphorylation of p70s6k, Akt and mTOR in tibialis anterior muscles of lean and obese Zucker rats. Immunoblotting demonstrated differences in level of basal p70S6k phosphorylation (Thr 389) in the normal and diabetic TA. HFES had an increase in p70S6k (Thr389) phosphorylation at 0-, 1- and 3-hr in lean TA and only at 3-hr in obese TA. mTOR (Ser 2448) Phosphorylation was elevated in lean TA immediately after HFES and remains unaltered in obese TA. HFES increased activity of both Akt (Thr 308) and Akt (Ser 473) in lean TA. These results suggest that diabetes is associated with alterations in the muscle content and ability to activate p70s6k signaling following an acute bout of exercise.

Key words: type 2 diabetes; skeletal muscle; p70S6k; mTOR; Akt; HFES

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ABBREVIATIONS

ADA	American Diabetes Association
ANOVA	One-way analysis of variance on ranks
BSA	Bovine serum albumin
DM	Diabetes Mellitus
ECL	Enhanced chemiluminiscence
GLUT4	Glucose transporter 4
HFES	High Frequency Electrical Stimulation
IOD	Integrated Optical Densities
LZ	Lean Zucker
mTOR	mammalian Target of Rapamycin
NHANES	National Health and Nutrition Examination Survey
NHIS	National Health Interview Survey
NIA	National Institute of Aging
OZ	Obese Zucker
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with 0.5% tween
PKB	Protein kinase B
PRT	Progressive Resistance Training
p70S6k	70 kDa ribosomal S6 kinase
SEM	Standard Error Mean
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
TA	Tibialis Anterior
TBS	Tris buffered saline
TBST	Tris buffered saline with 0.5% tween
T 2 DM	Type 2 diabetes mellitus

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Chapter 1

Introduction

It has been estimated that 171 million people worldwide have diabetes and it is estimated that this will increase to 366 million by 2030 [1]. An estimated 20.8 million people in the United States, about 7.0 percent of the population have diabetes. Diabetes mellitus (DM) is a serious, lifelong condition that is characterized high levels of blood glucose resulting from defects in insulin production, insulin action, or both. The obese Zucker (fa/fa) rat exhibits hyperinsulinemia, hyperlipidemia and hyperglycemia along with central adiposity. Since individuals with type 2 diabetes typically exhibit characteristics of metabolic syndrome, the genetically obese Zucker rat (fa/fa) is thought to be an appropriate model for diabetes related studies.

Exercise has long been recognized to have important health benefits for people with type 2 diabetes. The molecular events underlying exercise-induced adaptations in diabetic muscle remain to be unraveled. It is thought that beneficial effects of exercise on structural and functional adaptations of muscle are mediated through the activation of various signaling molecules. These signaling molecules, in turn, activate signaling cascades involved in regulating gene expression, glucose uptake, glycogen synthesis and protein synthesis. Similar to that seen with aerobic exercise modalities [2-4] , recent data suggest that anaerobic exercise may also be beneficial in the treatment of diabetes [5-12]. For example, progressive resistance training has been found to improve glycemic control, increase skeletal muscle size and strength, and positively change body composition by increasing lean body mass and decreasing visceral and total body fat [13-16]. Whether type 2 diabetes alters exercise-induced signal transduction

processes in muscle is not clear, but the existence of differences, if present, may help to explain why exercise-induced skeletal muscle adaptations may differ between normal and diabetic populations.

It is well established that strenuous exercise increases the rates of muscle protein synthesis [17]. This increase in protein synthesis is thought to be regulated, at least in part, by the phosphorylation of the p70 ribosomal protein S6 kinase (p70S6k) [18]. The activation of p70S6k has been proposed to promote increased translation of messages that have a polypyrimide motif just downstream of the 5' cap [19]. 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 [20]. How mTOR activity is modulated is not clear; however, recent evidence suggests that mTOR is controlled by protein kinase B (PKB) / Akt which is activated in response to phospholipid products of the phosphatidylinositol 3-kinase reaction. It is likely that PKB/Akt 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 [21]. Further, recent data suggests that p70S6k signaling may be particularly important in mediating muscle adaptation as 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 [22]. To our knowledge, whether differences exist between normal and diabetic muscle in this exercise induced activation of p70S6k has not been investigated.

PURPOSE

Our long term goal is to elucidate the contraction-induced intracellular signaling mechanisms thought to be involved in regulating skeletal muscle hypertrophy in diabetic skeletal muscle. The purpose of the present study was to determine whether type 2 diabetes alters Akt/mTOR/p70S6k pathway signaling after an acute episode of contractile activity.

SPECIFIC AIMS

Diabetes is the sixth leading cause of death in the US. The economic burden posed by diabetes is daunting with the total cost of diabetes in the U.S. in 2002 at an estimated $132 billion [23]. As such, it is clear that the scientific community must continue to search for the most effective methods for the treatment and prevention of this disease. Recent data have suggested that increased muscle loading regulates the rate of muscle protein synthesis and hypertrophy [17]. This increase in protein synthesis, at least in part, is thought to be regulated by the activation of the Akt/mTOR/p70S6k pathway [19-21]. No research has examined the response to a single contractile stimulus comparing normal and diabetic muscle. The working hypothesis of this study is that differences exist between normal and diabetic muscle in the maximal contraction induced activation of p70S6k pathway. To test this hypothesis and accomplish the purpose of this study the following specific aims are proposed;

Specific Aim #1: To determine if the expression and basal phosphorylation level of Akt/mTOR/p70S6k pathway proteins are altered in the Tibialis Anterior (TA) muscles of lean and obese Zucker rats with type 2 diabetes.

Hypothesis: Type 2 diabetes will be associated with alterations in the expression and basal phosphorylation level of Akt/mTOR/p70S6k pathway proteins in the Tibialis Anterior (TA) muscles.

Specific Aim # 2: To determine if the contraction induced activation/phosphorylation of Akt/mTOR/p70S6k pathway proteins are altered in the Tibialis Anterior (TA) muscles of lean and obese Zucker rats with type 2 diabetes.

Hypothesis: Type 2 diabetes will be associated with alterations in the contraction induced activation/phosphorylation of Akt/mTOR/p70S6k pathway proteins in the Tibialis Anterior (TA) muscles.

Chapter 2

Review of Literature

Introduction

A review of the pertinent literature concerning the present study will be presented in the following chapter. The following areas will be addressed: 1.) p70S6k pathway related proteins and the p70S6k regulation in skeletal muscle, and 2.) the obese syndrome X Zucker rat strain as an animal model for type 2 diabetes investigation.

p70S6k pathway related proteins and regulation of their activity in skeletal muscle

`The serine / threonine kinase, p70s6k, is believed to function in the regulation of protein synthesis [24]. The primary structure of p70s6k consists of four functional domains or modules. Module I extends from the N-terminus to the beginning of the catalytic domain and confers rapamycin sensitivity to p70s6k [25-27]. Module II contains the conserved catalytic domain, including the acute site of mitogen-induced phosphorylation in the activation T-loop [27]. Module III links the catalytic domain with the carboxyl tail, contains two additional sites of acute phosphorylation, and is conserved in many members of the second messenger family of Ser/Thr kinases [28]. Finally, Module IV contains the putative auto-inhibitory domain, which has significant sequence similarity with the substrate region of S6, and four closely clustered

phosphorylation sites [29]. The activation of P70s6k occurs in a hierarcal fashion through the sequential phosphorylation of each module. It is thought that P70s6k activation accelerates translation of mRNAs containing a terminal oligopolypyrimidine (TOP) track at the 5' end. This is important as the regulation of TOP containing proteins has been postulated to be a rate-limiting step in protein synthesis [30].

It is well known that exercise causes hypertrophy of the muscle and that a single bout of resistance exercise increases protein synthesis. Baar and Esser (1999) reported that a single bout of *in situ* contraction increased p70S6k activity in rat extensor digitorum longus (EDL) and tibialis anterior (TA) muscles [22]. In a similar study Hernandez et al., (2000) reported that resistance exercise causes an increase in the rate of protein synthesis, the rate of glucose uptake and the activity of p70S6k in rat skeletal muscle [31]. Other studies, using other exercise protocols, reported comparable results. For example, Nader et al., (2001) using high-frequency electrical stimulation, low-frequency electrical stimulation, or running exercise showed significant increase in p70S6k phosphorylation in the tibialis anterior and soleus muscles of rat [32]. To our knowledge how diabetes affects the exercise-induced regulation of p70S6k has not been examined.

Using humans, Koopman et al., (2006) reported that resistance exercise is associated with more pronounced phosphorylation of p70S6k in type II vs. type I muscle fibers [33]. Similarly, in rats, the phosphorylation of upstream regulators of p70S6k like PKB (or Akt) and mTOR was more pronounced in muscle tissue containing a greater proportion of type II muscle fibers [34, 35]. Taken together, these findings suggest that contraction may regulate p70S6k in an intensity- and time-dependent manner, and further that p70S6k stimulation in muscle may be fiber type-specific.

The Mammalian target of rapamycin (mTOR) functions as a growth factor and nutrient-sensing signaling molecule in mammalian cells. mTOR resides upstream of p70S6K (p70 ribosomal protein S6 Kinase) and is thought to be involved in regulation of a number of components of the translational machinery [36]. Insulin and activated Akt have been shown to induce phosphorylation of mTOR *in vivo* and *in vitro*. It is thought that the phosphorylation of Threonine 2446 and Serine 2448 regulates mTOR activity. Although the exact mechanism of this regulation is unknown, both *in vitro* and *in vivo* experiments have implicated Akt in the phosphorylation and activity of mTOR [37-39].

mTOR has been implicated in skeletal muscle remodeling using several models of altered muscle loading. For example, recent data by Bodine and colleagues (2001) demonstrated that overloading the rat plantaris muscle by synergist muscle ablation, increases mTOR Serine 2448 phosphorylation [21]. In contrast, unloading the gastrocnemius muscle by hind limb suspension, which promotes atrophy of the muscle, decreased Serine 2448 phosphorylation [37]. Similarly, a single bout of *in situ* muscle contractile activity in rats showed an increase in phosphorylation level of mTOR and its downstream target p70S6k [34, 37, 40]. Taken together, these studies suggest that mTOR phosphorylation is sensitive to muscle activity and may play a role in the exercise-induced regulation of p70S6k phosphorylation.

Akt (PKB) is the serine/threonine kinase that is required for the activation of mTOR. Akt plays an important role in executing multiple cellular metabolic pathways such as cell metabolism, cell survival and cell proliferation. In addition to these homeostatic functions, Akt is also thought to mediate the mitogenic effects of insulin and insulin-like growth factor-I [41-43]. Akt1 is the predominant isoform in most tissues [44] and, similar to many membrane proteins, it contains a pleckstrin homology (PH) domain that binds phospholipids [45]. Akt is activated in a

PI3K (Phosphoinositide-3 kinase) -dependent manner and is regulated by phosphorylation on Thr 308 and Ser 473. It appears that the phosphorylation of Thr 308 is dependent, at least in part, on the activity of PI3-kinase [41]. The kinase responsible for the phosphorylation on Serine 473 has not been identified. Phosphorylation on Serine 473 appears to be related to PI3-kinase activity, although PDK1 (3-phosphoinositide-dependent protein kinase 1) may also be involved [46]. Akt can also be activated by PI3K-independent mechanism, as is observed following growth hormone treatment [47], or after increases in intracellular calcium or cAMP levels [48, 49]. The mechanism for this PI3K-independent Akt activation is poorly understood.

Akt is thought to regulate skeletal muscle growth and metabolism. In Akt1/ PKBα-deficient mice, there is conspicuous impairment in organism growth [50] , whereas Akt2-deficient mice exhibit reduced insulin-stimulated glucose uptake in isolated EDL muscles [51]. *In situ* electroporation of constitutively active Akt has been examined in mouse skeletal muscle and recent data has suggested that this molecule plays a critical role in the progession of muscle hypertrophy [21]. The role of Akt as a signaling mediator in muscle contraction is not well understood. Using rodent models Lund and colleagues (1998) and Sherwood et al., (1999) following *in situ* muscle contraction reported no Akt kinase activation [52, 53]. Similar findings have been reported in humans. For example, Widegren and co-workers (1998) reported no activation of Akt following one-leg cycle ergometry at approximately 70% VO_2max in human skeletal muscle [54]. Conversely, Nader et al., (2001) reported different modes of exercise like a single bout of either a high-frequency electrical stimulation, a low-frequency electrical stimulation, or a running exercise protocol causes transient increase in Akt phosphorylation in the tibialis anterior and soleus muscles of rat [32]. Supporting these findings, Turinsky et al., (1999) reported that exercise *in vivo* is associated with activation of Akt1 but not Akt2 or Akt3

in contracting skeletal muscles [55]. The reasons for discrepancies between studies is not entirely clear however it is possible that differences in the model utilized, methods or time points may be involved. Taken together, these findings suggest that contraction may regulate Akt in an intensity- and time-dependent manner, or perhaps that Akt stimulation in muscle may be fiber type-specific. More research is necessary to clarify the pathway responsible for hypertrophy and increased protein synthesis rates in skeletal muscle.

Summary

The p70S6 kinase and its upstream regulators mTOR and Akt kinase, are proposed molecules involved in the contraction-mediated regulation of protein synthesis and hypertrophy in skeletal muscle. The exact molecular mechanism(s) regulating the activity of these molecules is/are largely unknown. Few studies have investigated differences in the activation of these signaling molecules in response to an acute bout of resistance exercise. Elucidating the differences between normal and diabetic muscle tissue at the molecular level may be beneficial for exercise and pharmacological interventions designed for the treatment of type 2 diabetes.

Overview of Diabetes Mellitus and Insulin resistance

Diabetes Mellitus

It has been estimated that 171million people worldwide have diabetes and it is estimated that this will increase to 366 million by 2030 [1]. An estimated 20.8 million people in the United States or about 7.0 % of the population have diabetes. Of those affliated, 14.6 million have been diagnosed while 6.2 million have yet to be. Diabetes mellitus (DM) is a group of diseases marked by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Insulin is a polypeptide hormone, secreted by the beta cells of the pancreas that

allows glucose to enter cells from the blood. A deficiency in this hormone, or the inability of cells to respond to it, causes abnormally high blood glucose levels, which if allowed to proceed unchecked can lead to numerous complications. The two major types of diabetes are type 1 and type 2. Type 1 diabetes mellitus can occur at any age and is characterized by the marked inability of the pancreas to secrete insulin because of autoimmune destruction of the beta cells [56]. This type of diabetes is typically diagnosed in children and has been referred to as juvenile onset diabetes or insulin dependent diabetes mellitus. Type 1 accounts for about five to ten percent of all cases of diabetes [56]. The major type of diabetes is type 2 diabetes mellitus (DM). Type II (non-insulin dependent) DM is an emerging epidemic in Western cultures that is thought to afflict 150 million people worldwide [57]. This type of diabetes accounts for ninety to ninety five percent of all diagnosed diabetic cases [58]. Type 2 diabetes is characterized by peripheral insulin resistance with an insulin-secretary defect (inability to increase beta-cell production of insulin) that varies in severity. In the progression from normal glucose tolerance to abnormal glucose tolerance, postprandial glucose levels first increase which eventually results in fasting hyperglycemia. Several genetic and environmental factors lead to the development of the disease in most patients [59]. To our knowledge research examining the signaling events thought to underlie exercise-induced muscle adaptation in type 2 diabetes has not been performed.

Insulin resistance

Insulin resistance is present when the biological effects of insulin are less than expected for glucose disposal in skeletal muscle [60]. Mutation of some members of the insulin signaling cascade (insulin receptor, insulin receptor-substrate) also leads to the manifestation of insulin resistance. Nevertheless, such mutations constitute a very rare cause of type 2 diabetes [61]. The

mechanism by which skeletal muscle becomes insulin resistant is unclear, but there is a strong correlation between insulin sensitivity and the levels of plasma free fatty acids and intra-muscular fatty acid metabolites (long chain acyl-CoA, diacylglycerol and triglycerides).

The obese Zucker strain as a model for type 2 diabetes investigation

Appropriate experimental models are essential tools for understanding the molecular basis of disease and the actions of therapeutic agents. Diabetic research has widely utilized animal models for experimentation. Thus far, most experiments have utilized rodent models [59] because of their short life span, the ability to manipulate animal genetics and economic considerations. There are numerous rodent models for the different types of diabetes. Similar to humans, Type 2 diabetes in rodents is a heterogeneous group of disorders characterized by insulin resistance and impaired insulin secretion. Many of these animal models have been developed using selective breeding which typically produces specific genetic mutations. Other methods for developing diabetic rodent models include molecular biology techniques such as gene targeting and transgenic techniques [59].

The Goto Kakizaki (GK) rat, developed by selective breeding of the Wistar rat, and is an example of a type 2 diabetic model. The GK rat develops relatively stable hyperglycemia and is characterized by insulin resistance and impaired insulin secretion. The GK rat, like other animal models of diabetes, develops some features that can be compared with the complications of diabetes seen in humans. These include renal lesions, structural changes in peripheral nerves and abnormalities of the retina. Similar to the GK rat the Kuo Kondo (KK) mouse was developed by selective breeding. Several different lines have been bred and they vary genetically and phenotypically [59]. The KK mouse becomes obese as an adult; developing insulin resistance,

islet cell hyperplasia, and mild hyperglycemia [59]. Conversely, the Nagoya-Shibata-Yasuda (NSY) mouse spontaneously develops diabetes in an age-dependent manner, but is not obese. This model is useful when studying age-related phenotypes [59]. P*sammomys obesus* (the Israeli sand rat) is vegetarian in its natural habitat, but when fed laboratory rat chow, becomes obese, insulin resistant and hyperglycemic [59]. Perhaps the most widely used is Obese Zucker (fa/fa) rat. The Obese Zucker is a model of monogenic obesity and diabetes along with the Ob/Ob mouse and db/db mouse. The db/db mouse and the fa/fa Zucker rat both have mutations in the hypothalamic receptor for leptin [59]. This mutation causes hyperphagia and is thought to be responsible for the obesity seen in this model. Animal models of diabetes have provided extremely valuable insight to the disease. The genetically obese Zucker (fa/fa) rat was selected for the present investigation as this model is widely used in obesity related diabetes studies.

The obese Zucker is developed through selective breeding and exhibits hyperinsulinemia, hyperlipidemia and hyperglycemia along with central adiposity. These characteristics are valuable for studying type 2 DM in the context of metabolic syndrome, and should provide useful insight to the disease since many type 2 diabetics possess these same conditions. Insulin resistance in obese Zucker rat is mainly associated with impaired insulin-stimulated GLUT-4 protein translocation [62, 63] and glucose transport activity, [64, 65] but does not appear to be associated with the amount of GLUT-4 turnover stimulated by insulin [62, 66, 67].

Previous studies have indicated differences in the skeletal muscle tissue between obese Zucker rats and their lean counterparts in response to exercise. Ardevol and others (1997) exercised female lean and obese Zucker rats in a short, intense treadmill protocol and oxygen consumption, carbon dioxide release, lactate and bicarbonate levels in venous and arterial blood were measured. It was concluded that fatigue appears earlier in obese rats due to loss of

buffering ability caused by massive extra-muscular glycolysis and lactate production triggered by exercise-induced adrenergic stimulation [68]. This same group studied differential substrate utilization by exercising and fatigued muscle in lean and obese Zucker rats [69]. It was found that lean rats managed their glycosyl units more efficiently than obese rats [69]. Taken together, these data suggest that metabolic differences exist between lean and obese Zucker rat muscle tissue in response to exercise.

The obese Zucker (fa/fa) rat model has been used in other investigations to demonstrate the potential benefits of exercise training. Cortez et al., (1991) reported significant increase in oral glucose tolerance while maintaining significantly lower plasma insulin concentrations in treadmill exercised female obese Zucker rats compared to sedentary control rats [70]. Similarly, Ivy et al., (1989) reported increased rates of glucose transport (assessed by measuring the rate of 3-O-methyl-D-glucose (3-OMG) accumulation) in obese Zucker rats after treadmill exercise [71]. The mechanism(s) underlying these improvements in glucose regulation are not entirely clear, however Brozinick et al.,(1993) demonstrated that obese Zucker rats, following *in vivo* muscle contraction, had significantly higher GLUT-4 protein concentration and citrate synthase activity than the control obese Zucker rats [71, 72]. Taken together, these studies suggest that exercise training decreases the skeletal muscle insulin resistance of the obese Zucker rat. Whether the mechanisms of muscle adaptation differ between normal and obese Zucker rats is not known. Elucidating the molecular events responsible for exercise induced muscle adaptation in diabetic muscle may be beneficial for exercise and pharmacological interventions designed for the treatment of this disease.

Summary

Type 2 diabetes is an emerging epidemic in Western cultures that accounts for ninety to ninety five percent of all diagnosed diabetic cases. The obese Zucker (fa/fa) rat is an appropriate animal model for studying diabetes at the molecular level in the context of metabolic syndrome. Few studies have investigated differences in diabetic and normal skeletal muscle in response to exercise. Elucidating the molecular events responsible for exercise induced muscle adaptation in diabetic muscle may be beneficial for exercise and pharmacological interventions designed for the treatment of this disease.

Chapter 3

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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Running Title*:* Diabetes alters contraction induced p70S6k signaling in skeletal muscle

ABSTRACT

Increased muscle loading results in phosphorylation of the 70 kDa ribosomal S6 kinase (p70s6k) signaling pathway and this event strongly correlates with the degree of muscle adaptation following resistance exercise. Whether differences exist between normal and diabetic muscle in the activation of the p70s6k pathway following a single episode of exercise remains unclear. Using an in situ high-frequency electrical stimulation (HFES), we examined the exercise-induced phosphorylation of p70s6k, Akt and mammalian target of rapamycin (mTOR) in the tibialis anterior (TA) muscles of lean and obese Zucker rats at 0, 1, and 3hr after HFES. Immunoblotting demonstrated differences in the content (27.9 ± 3.6% lower) and level of basal p70S6k phosphorylation (Thr 389) (26.1 ± 7.5% lower) in the normal and diabetic TA ($P<0.05$). p70S6k (Thr389) phosphorylation increased 33.3 ± 7.2% , 24.0 ± 14.9% and 24.6 ± 11.3% in lean TA at 0-, 1- and 3-hr post-HFES and increased 33.5 ± 8.0% in obese TA at 3-hr post-HFES ($P<0.05$). mTOR (Ser 2448) Phosphorylation was elevated in lean TA (96.5 ± 40.3%, $P< 0.05$) immediately after HFES and remains unaltered in obese TA with HFES. In lean TA, HFES led to increased phosphorylation of Akt (Thr 308) (31.8 ± 16.2% and 31.1 ± 8.8%) and Akt (Ser 473) (47.2 ± 12.1% and 43.7 ± 11.7%) at 0- and 3-hr time points respectively ($P<0.05$). Taken together, these data suggests that diabetes is associated with alterations in the muscle content and ability to activate the p70s6k signaling pathway following an acute bout of exercise. (250 words)

Key words: type 2 diabetes; skeletal muscle; p70S6k; mTOR; Akt; HFES

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 [57]. A number of studies employing strength training regimens have been shown to improve glycemic control, increase skeletal muscle size and strength, and positively change body composition suggesting that anaerobic exercise may be an effective strategy for the treatment of insulin resistance and type 2 diabetes [13-16]. Recent reports have suggested that differences exist between normal and diabetic muscle in their adaptation to an exercise regimen [8, 10, 72-75]. However, the direct effects of exercise on the phenotype of diabetic muscle have not been widely studied. It is thought that the beneficial effects of exercise on muscle are mediated through the activation of various signaling cascades which are involved in regulating changes in gene expression, glucose uptake, and protein synthesis [76]. Whether type 2 diabetes 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 diabetic populations.

It is well established that increased muscle loading increases the rates of muscle protein synthesis [17]. 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) [18], whose activation has been proposed to promote increased translation of messages that have a polypyrimide motif just downstream of the 5' cap [19]. 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 [20]. How mTOR activity is modulated is not clear; however,

recent evidence suggests that mTOR is controlled by protein kinase B (PKB) / Akt which is activated in response to phospholipid products of the phosphatidylinositol 3-kinase reaction. It is likely that PKB/Akt increases mTOR activity by phosphorylating mTOR at Ser 2448 and it has been hypothesized that this event is a critical point of control in the regulation of protein synthesis [21]. It has been postulated that p70S6k signaling may be particularly important in mediating muscle adaptation as 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 [22].

The purpose of the present study was to determine whether type 2 diabetes alters p70S6k signaling after an acute episode of contractile activity. We hypothesized that type 2 diabetes 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 in skeletal muscle from normal and diabetic rats either immediately after or 1 or 3h after a single bout of sciatic nerve stimulation. Taken together, the data indicate that diabetes alters contraction-induced p70S6k phosphorylation in skeletal muscle. These findings are consistent with the possibility that DM alters the way skeletal muscle “senses and responds” to contractile stimuli.

MATERIALS AND METHODS

Animals

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 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 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), phosphorylated Thr^{389} p70S6K (#9206), phosphorylated $^{Ser421/Thr424}$ p70S6K (#9204), phosphorylated ^{Ser2448} mTOR (#2971), phosphorylated ^{Thr308} Akt (#9275) and phosphorylated ^{Ser473} (#9271) Akt, Mouse IgG, and Rabbit IgG antibodies were purchased from Cell Signaling Technology (Beverly, MA). Enhanced chemiluminescence (ECL) western blotting detection reagent was from Amersham Biosciences (Piscataway, NJ). Restore western blot stripping buffer was obtained from Pierce (Rockford, IL) and 3T3 cell lysates were from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were purchased from Sigma (St. Louis, MO) or Fisher Scientific (Hanover, IL).

Contractile stimulation of skeletal muscles

The high-frequency electrical stimulation (HFES) model has been previously described [77] and was chosen on the basis of its efficacy in stimulating protein translation and muscle hypertrophy *in vivo* [22] . In order to produce maximal contractile stimulus, a voltage of 5-7V was applied to electrodes and sciatic nerve was stimulated with 1-ms pulses at 100 Hz, where all motor units (both fast and slow) are recruited. The contractions lasted 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 [78-81]. 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 isolates 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 T-PER (2 mL/1g tissue weight) (Pierce, Rockford, IL) and centrifuged for 10 minutes at 2000 X *g* to pellet particulate matter. This process was repeated twice and the supernants 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, boiled for 5 minutes, and 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 [82]. Protein immuno-detection was performed as outlined by the antibody manufacturer while immunoreactive bands were visualized with 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). 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 $\pm$ SEM. Data were analyzed by using the Sigma Stat 3.0 statistical program. Data were 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

Syndrome-X and muscle mass

Average body mass of obese Zucker rats (597 ± 21.7 *g*) was ~82 % greater than lean counterparts (328 ± 12.2 g; $P< 0.05$). Compared to lean animals (651 ± 9 mg), TA muscle mass was ~35% less in the obese Zucker rats (479 ± 20 *mg*; $P< 0.05$) (Table 1).

p70S6k pathway related protein levels and phosphorylation status are altered in the diabetic TA muscle.

To investigate the diabetic effects on the total amounts of p70S6k, mTOR and Akt, 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 protein content ($P< 0.05$; Fig. 1). 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). 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 significant differences were detected across groups in basal phosphorylation levels of p70S6k (Thr421/Ser424), mTOR (Ser 2448), Akt (Thr 308) and Akt (Ser 473) (Fig. 2, 3 & 4).

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 rat models (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 3 hr 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; $P< 0.05$)

The contraction-induced phosphorylation of potential p70S6k regulators is altered in obese-Zucker rat.

It is thought that p70S6k phosphorylation is regulated by mTOR and its upstream regulator Akt [20, 21, 37]. mTOR phosphorylation (Ser 2448) was found to be 96.5 ± 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). In lean Zucker TA, Akt (Thr 308) phosphorylation was 31.8 ± 16.2%, 29.8 ± 7.5% and 31.1 ± 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 ± 6.2%, 44.6 ± 9.0% and 30.4 ± 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 ± 12.1% and 43.7 ± 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, where the phosphorylation of Akt (Ser 473) was 18.7 ± 6.4%, 46.3 ± 5.5% and 32.4 ± 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 diabetes.

DISCUSSION

The obese Zucker rat is insulin resistant and has been used as model of type 2 diabetes [64, 83]. 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 diabetic 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 which plays an important role in regulating protein synthesis. p70S6k modulates protein synthesis, at least in part, by controlling the translation of numerous messenger RNA transcripts that encode components of the translational apparatus [84]. Illustrating this fact is the finding that blockade of p70S6k activity results in a significant inhibition of protein synthesis in multiple cell systems [85-89]. In skeletal muscle, the contraction induced phosphorylation of p70S6K is elevated following HFES [22, 34, 77] and is highly correlated with an increase in muscle mass following a resistance training program [22]. To our knowledge, the influence of diabetes on p70S6k phosphorylation in response to muscle contraction has not been reported. 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 [90-92]. In the light of these studies, our data suggest that diabetes affect how multiple stimuli regulate the phosphorylation of p70S6k. Future studies employing a combination of approaches to stimulate p70S6k phosphorylation will certainly be of value in determining how diabetes may affect the regulation of p70S6k in skeletal muscle.

To further explore how diabetes 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 the regulating several components of the translational machinery and in addition, is thought to be an upstream activator of p70S6k [36]. Similar to previous studies, increased contractile activity appears to be a strong stimulus to increase the phosphorylation level of mTOR in non-diabetic muscle [34, 77]. Conversely, in the diabetic TA, HFES appears unable to alter the degree of mTOR phosphorylation (Fig. 3). This latter finding is consistent with our data demonstrating that diabetic muscle exhibits a reduced ability to activate p70S6k following a single bout of exercise. Taken together, these data suggests that diabetes-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.

mTOR phosphorylation of Ser 2448 is regulated by Akt/PKB [37-39]. Akt is a serine/threonine kinase which mediates certain types of muscle hypertrophy [21]. Under non-stimulated conditions Akt is located in the cytoplasm and 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 Thr308 and Ser 473 [93]. Phosphorylation of both sites is essential for the activation of Akt. 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-diabetic muscle have been reported previously [32, 55, 94]. In contrast to our findings in the nondiabetic animals, these events appeared to differ in diabetic muscle, suggesting that diabetes is associated with alterations in the ability of skeletal muscle to activate Akt signaling following increased contractile loading (Fig. 4) and this latter finding may be important. Given that Akt is

thought to reside upstream of mTOR, this lack of Akt activation in diabetic muscle may provide an explanation as to why diabetes may be associated with defects in HFES induced mTOR and p70S6k phosphoryation. Although this possibility is promising, it should be noted that the functional role of Akt in regulating p70S6k signaling cannot be accurately assessed in the absence of further study to evaluate experimental manipulation of this protein. Additional studies perhaps employing strategies designed to directly inhibit or activate Akt signaling during HFES may prove to be useful in addressing these possibilities.

The precise influence of diabetes 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 [77]. Given this contention, it is plausible that reported differences in the signaling response between models could be related to the time points chosen for evaluation. Future studies employing other time points may yield different findings. Alternatively, it is possible that the two groups experienced 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 diabetes 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, but it occurs well *in vivo* and it is possible that systemic factors and / or oxidative stress may mediate the activation of Akt by exercise [95].

Supporting this contention, hyperglycemia has been shown to directly decrease insulin-induced Akt phosphorylation on Ser473 in rat and human skeletal muscle [96, 97]. The factors which may regulate diminished Akt activation during hyperglycemia will require further experimentation. Similarly, it is possible that a 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 [98, 99]. 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 diabetic muscle may be incapable of growth following a resistance based exercise program. This is not in agreement with the findings of Farrell and colleagues [3] who demonstrated that diabetic muscle is fully capable of undergoing muscle hypertrophy in rats following 8 weeks of resistance training. The reasons for this apparent discrepancy are not entirely clear, but may lie in the difference in models and time points between the studies and a more comprehensive examination is needed. Irrespective of the mechanism, it is likely that diabetes-associated differences in the ability of skeletal muscle to induce 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. 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.

Appendix

Appendix A

Tables and Figures

Table 1 Body weight and muscle mass of lean and obese (fa/fa) Zucker rats. An asterisk (*) indicates significant difference ($P < 0.05$) from the lean Zucker value.

	Lean Zucker	**Obese Zucker**
Body mass, g	328 ± 12.2	597 ± 21.7 *
TA mass, mg	651 ± 9	479 ± 20 *

Figure 1

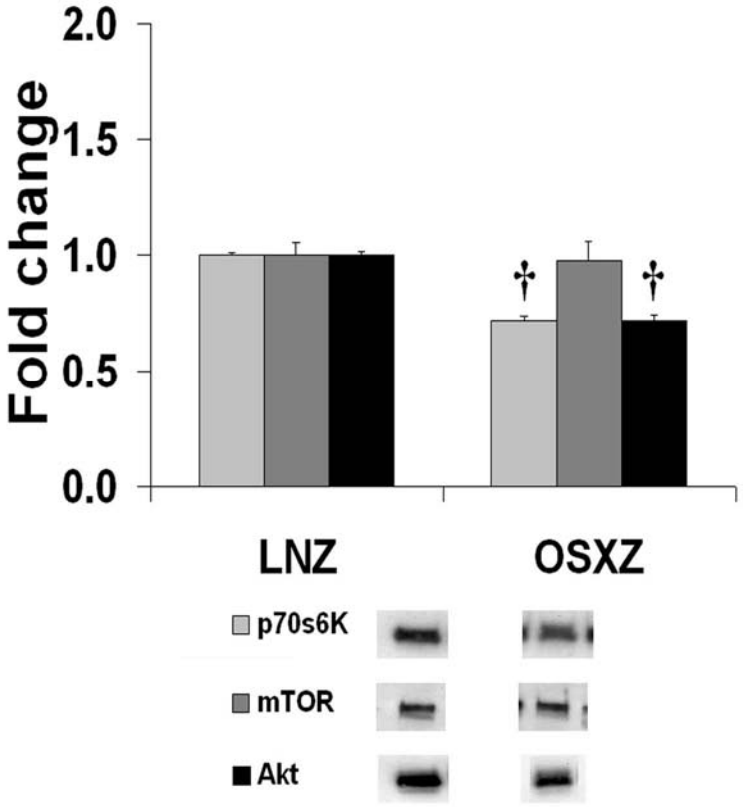
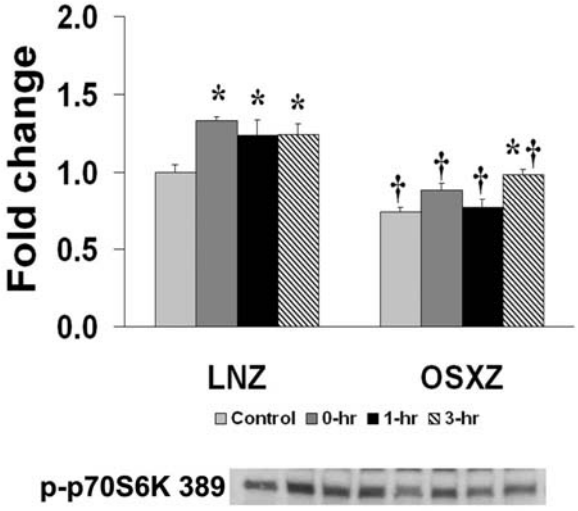


Figure 2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1.0
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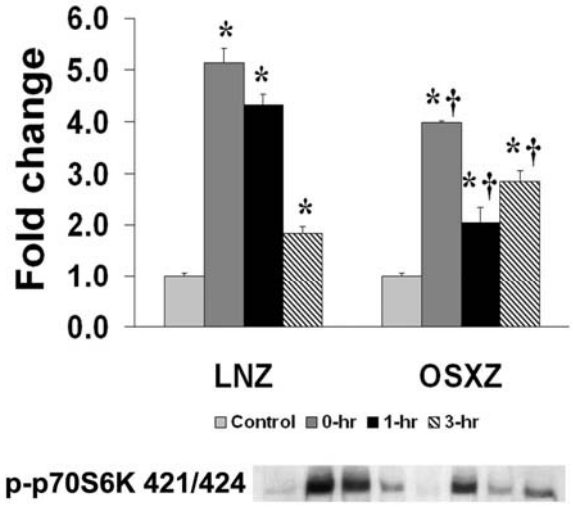
0.0
LNZ
OSXZ
Control 0-hr 1-hr 3-hr
p-p70S6K 389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3.0
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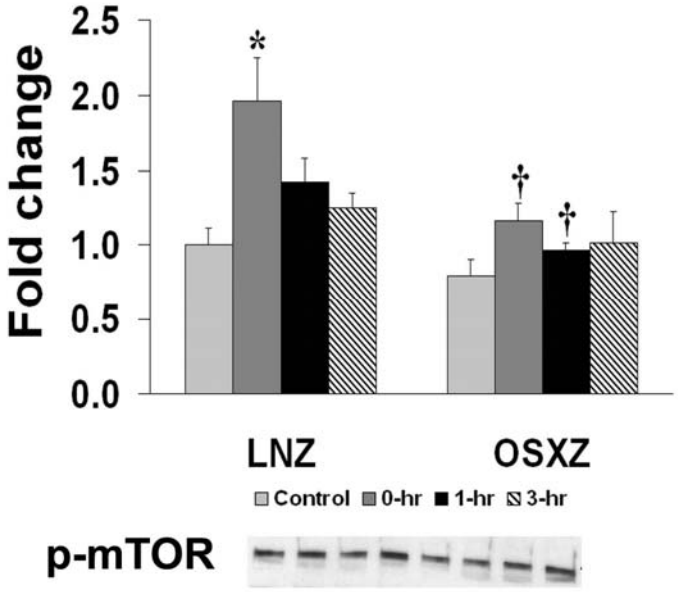
LNZ
OSXZ
Control 0-hr 1-hr 3-hr
p-p70S6K 421/424

Figure 3


Fold change
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2.0
1.5
1.0
0.5
0.0
*
†
†
LNZ
OSXZ
Control
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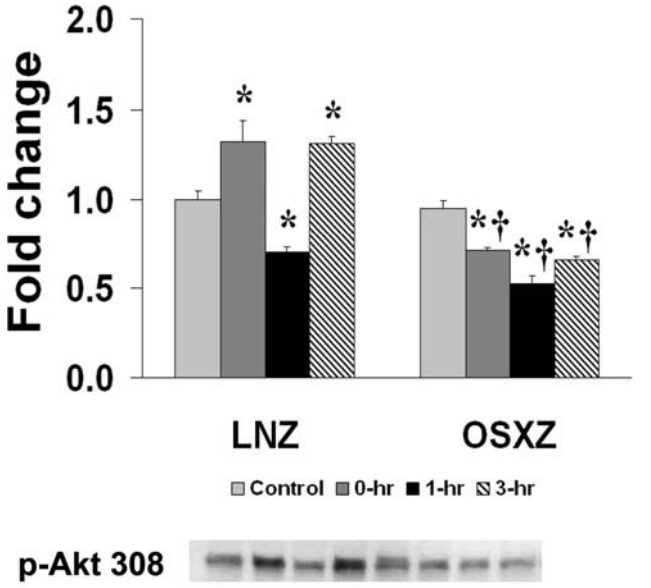
1-hr
3-hr
p-mTOR

Figure 4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LNZ
OSXZ
Control
0-hr
1-hr
3-hr
p-Akt 308

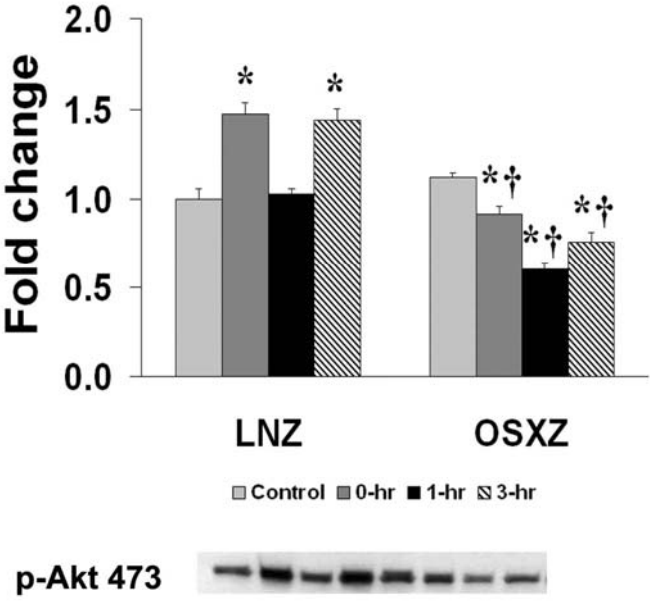

Fold change
2.0
1.5
1.0
0.5
0.0
LNZ
OSXZ
Control
0-hr
1-hr
3-hr
p-Akt 473

FIGURE LEGENDS

Fig. 1: Type 2 diabetes is associated with alterations in skeletal muscle p70S6k and its pathway related proteins. TA muscles from LNZ and obese (fa/fa) Zuckers (OSXZ) were analyzed by Western blot analysis for diabetes-related changes in total p70S6k, mTOR and Akt protein expression. Results are expressed as a percent of the normal, LNZ value. An asterisk (*) indicates significant differences ($P< 0.05$) from the lean Zucker value.

Fig. 2: Contraction-induced p70S6k (Thr 389 & Thr 421/Ser 424) phosphorylation is altered with type 2 diabetes. The basal (control) and contraction-induced phosphorylation of the p70S6k in TA muscles from lean and diabetic Zucker rats at 0, 1, and 3 hours after contractile stimulus. p70S6k (Thr 389 & Thr 421/ser 424) phosphorylation was determined by Western analysis and immunodetection for phosphorylation on Thr 389 and Thr 421/ser 424. 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.

Fig. 3: Contraction-induced mTOR (Ser 2448) phosphorylation is altered with type 2 diabetes. 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 Ser 2448. An asterisk (*) indicates significant difference ($P < 0.05$)

from the control time point within animal model, and a cross (†) indicates significant difference ($P < 0.05$) at corresponding time points across animal models.

Fig. 4: Effects of eccentric, maximal muscle contraction *in situ* on phosphorylation of Akt (Thr308 / Ser473). The basal (control) and contraction-induced phosphorylation of the Akt in TA muscles from lean and diabetic 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.

Appendix B
Tibialis Anterior (TA)
Film Properties Report for p70S6k (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

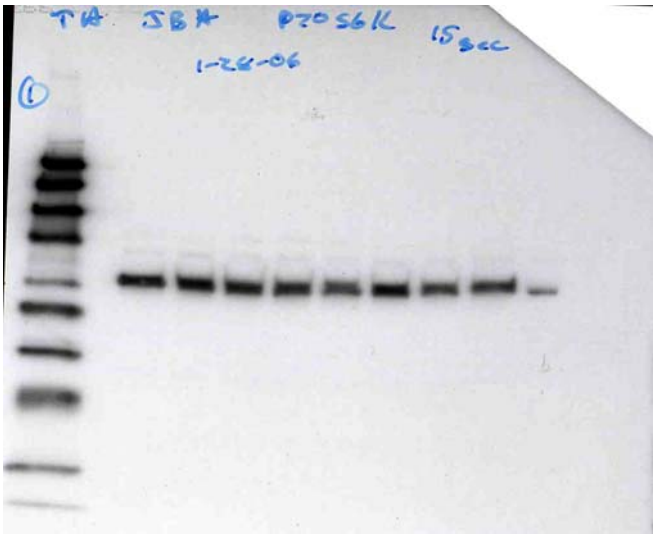
Primary Antibody: p70S6k (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution: 1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 15 seconds Molecular weight: 70 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p70S6k (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

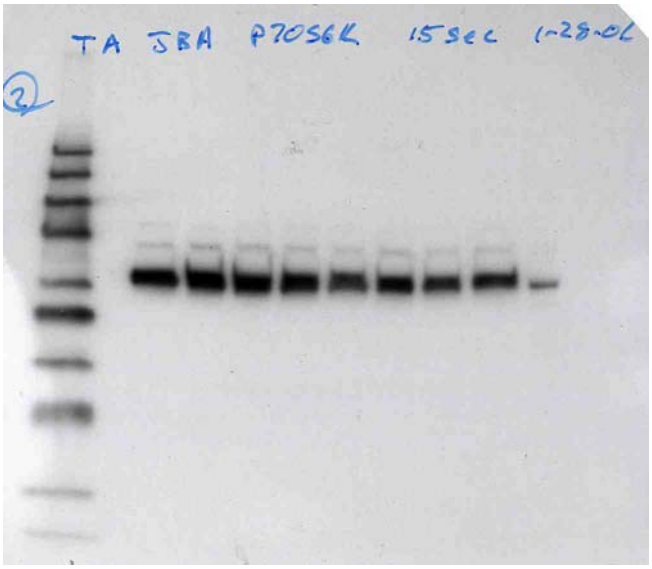
Primary Antibody: p70S6k (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution: 1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 15 seconds Molecular weight: 70 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p70S6k (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

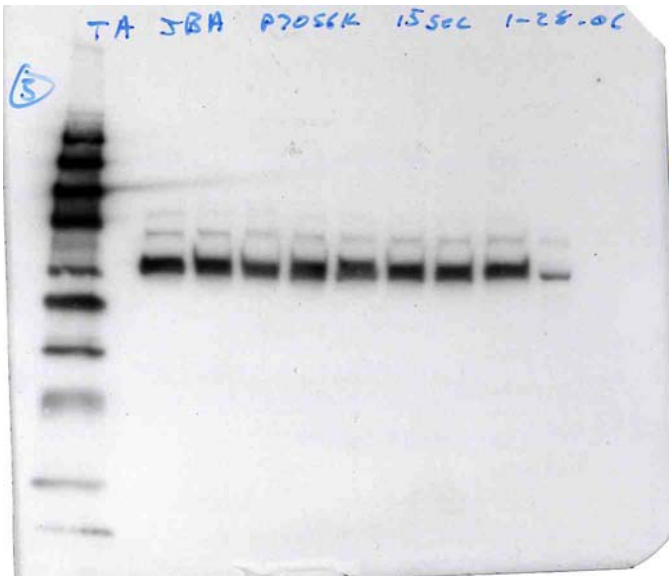
Primary Antibody: p70S6k (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 15 seconds Molecular weight: 70 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-p70S6k (Thr389) (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-p70S6k (Thr389) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 70 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

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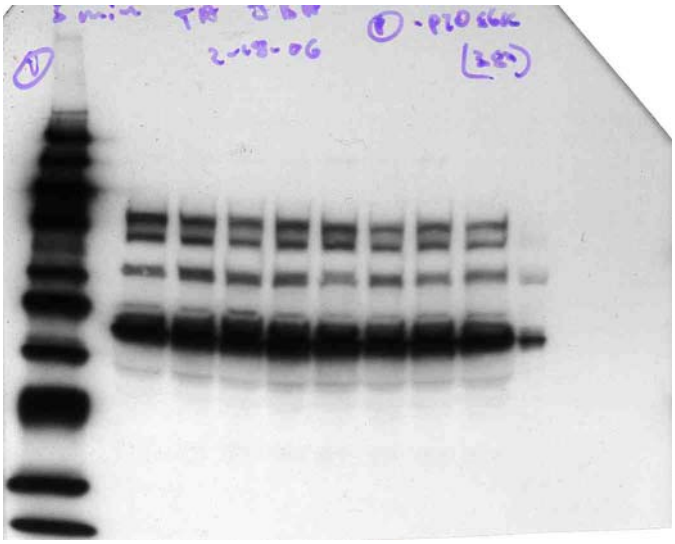
Lane 10: Obese Zucker 3 hour 20 µl



Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-p70S6k (Thr389) (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

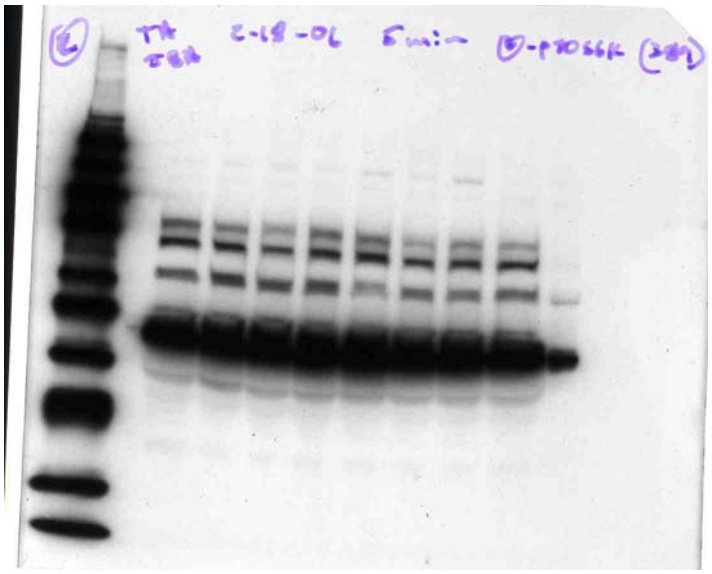
Primary Antibody: p-p70S6k (Thr389) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 70 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-p70S6k (Thr389) (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

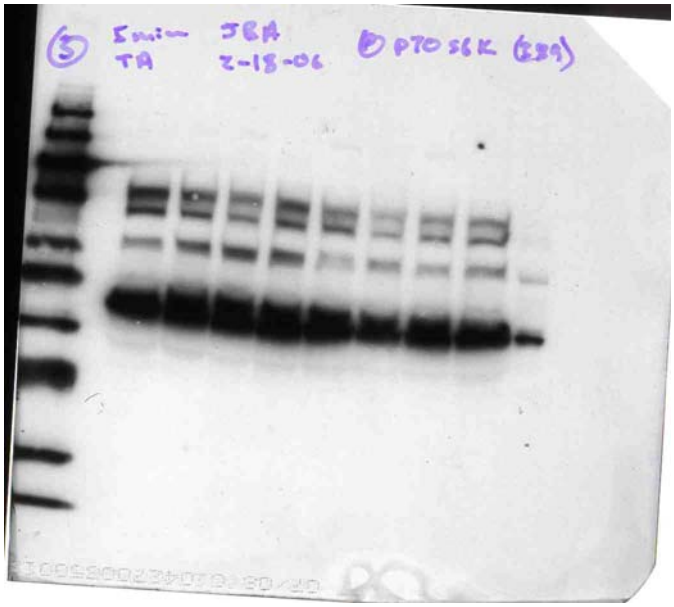
Primary Antibody: p-p70S6k (Thr389) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 70 kDa



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Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-p70S6k 421/424 (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-p70S6k 421/424 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 70 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

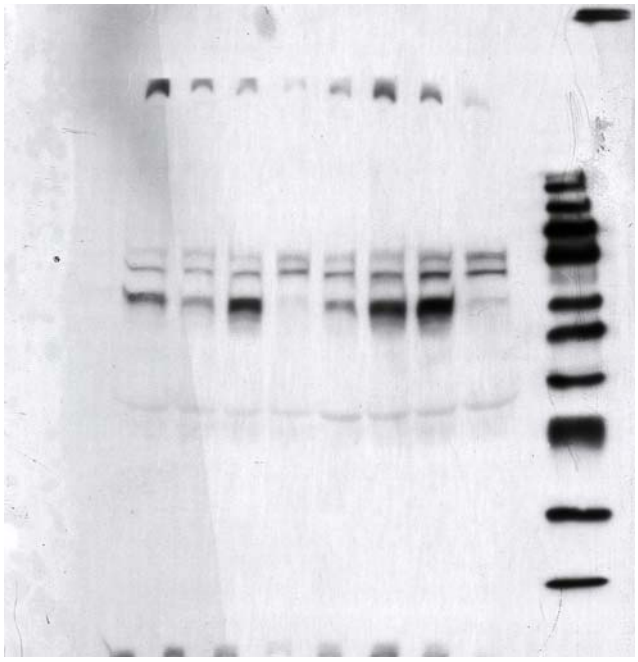
Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl



Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-p70S6k 421/424 (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-p70S6k 421/424 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 70 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

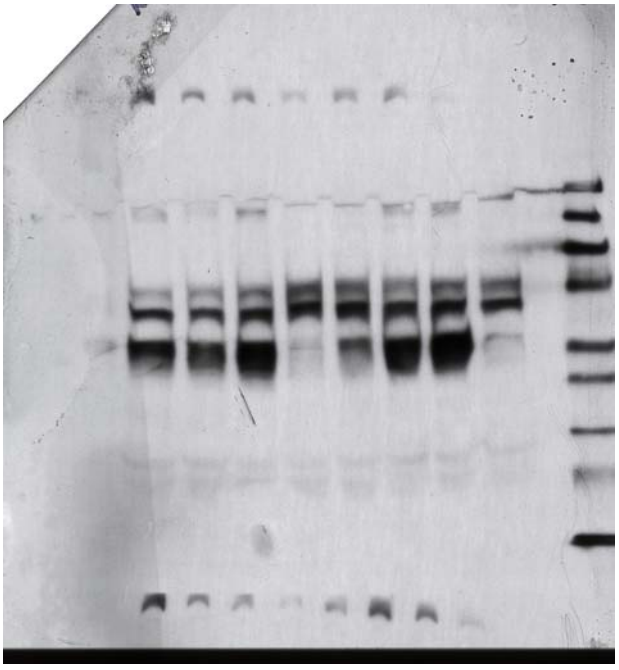
Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl



Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-p70S6k 421/424 (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

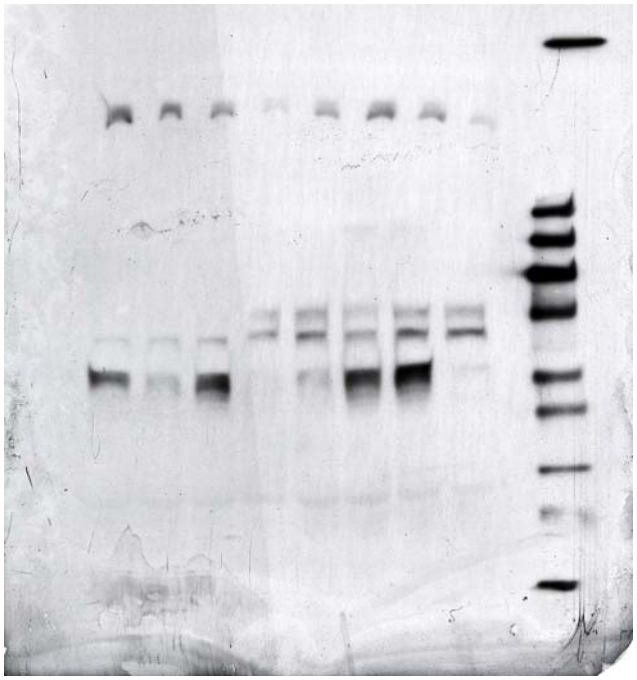
Primary Antibody: p-p70S6k 421/424 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 70 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for mTOR (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: mTOR (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution: 1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 2 min Molecular weight: 289 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

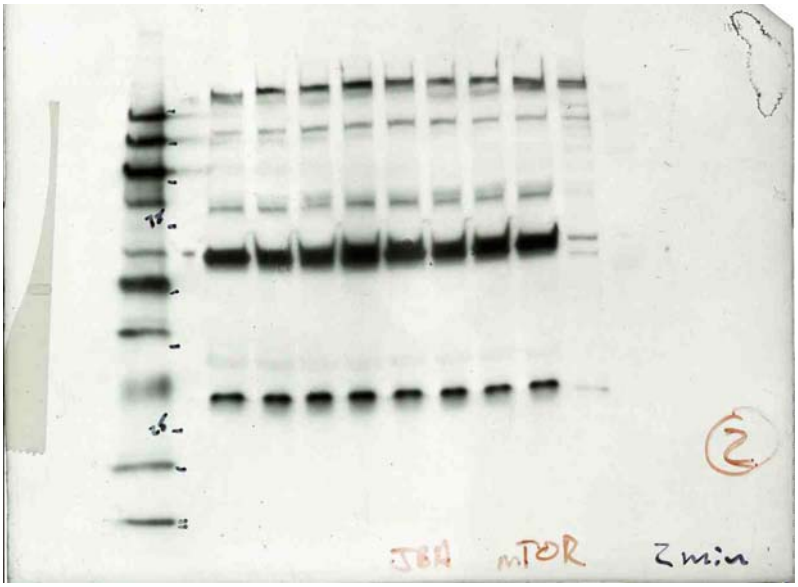
Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl



Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for mTOR (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: mTOR (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 1min Molecular weight: 289 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

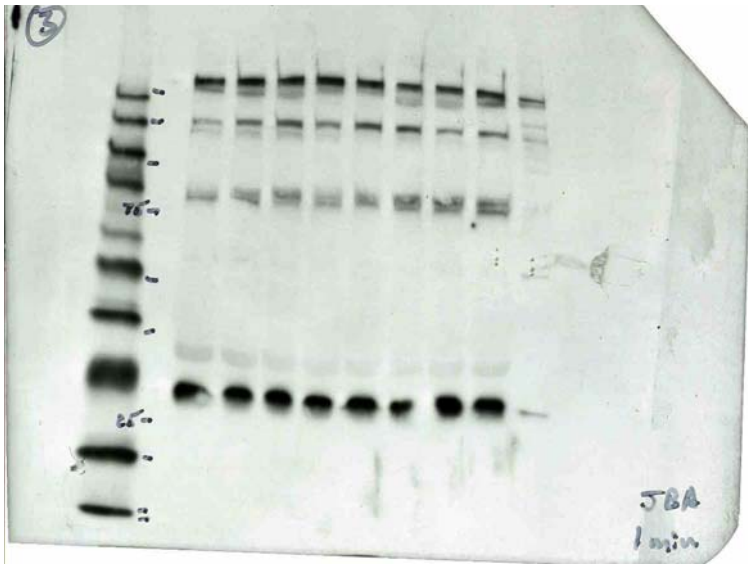
Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl



Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for mTOR (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: mTOR (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution: 1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 2 min Molecular weight: 289 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

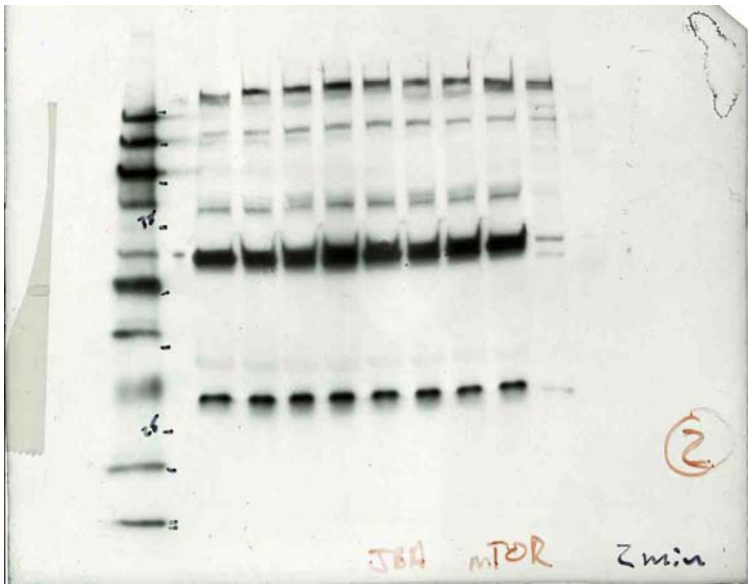
Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl



Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-mTOR (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-mTOR (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 289 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

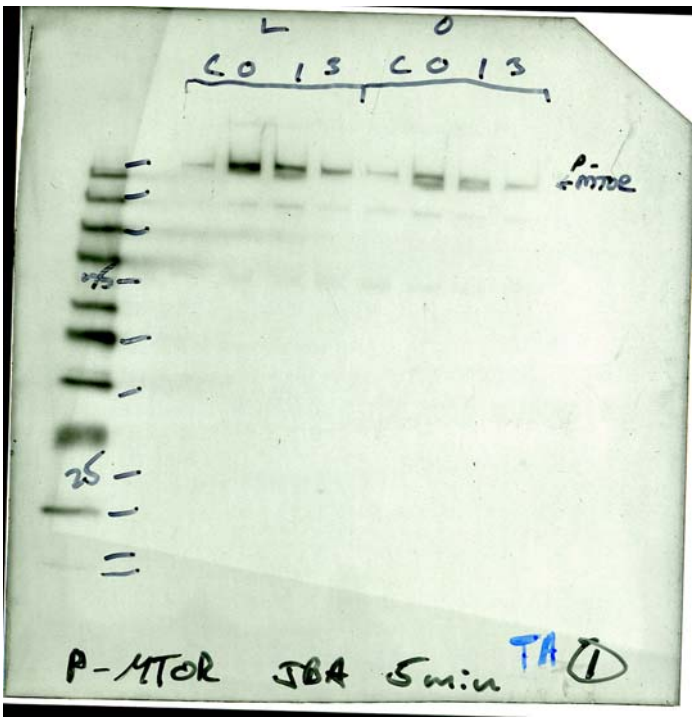
Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl



Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-mTOR (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-mTOR (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 2 min Molecular weight: 289 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

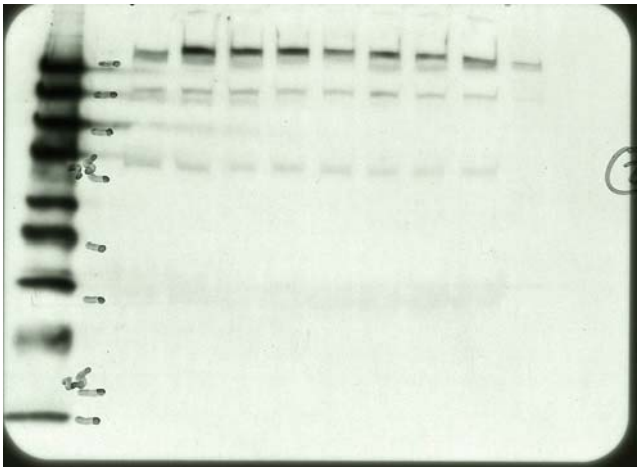
Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl



Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-mTOR (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

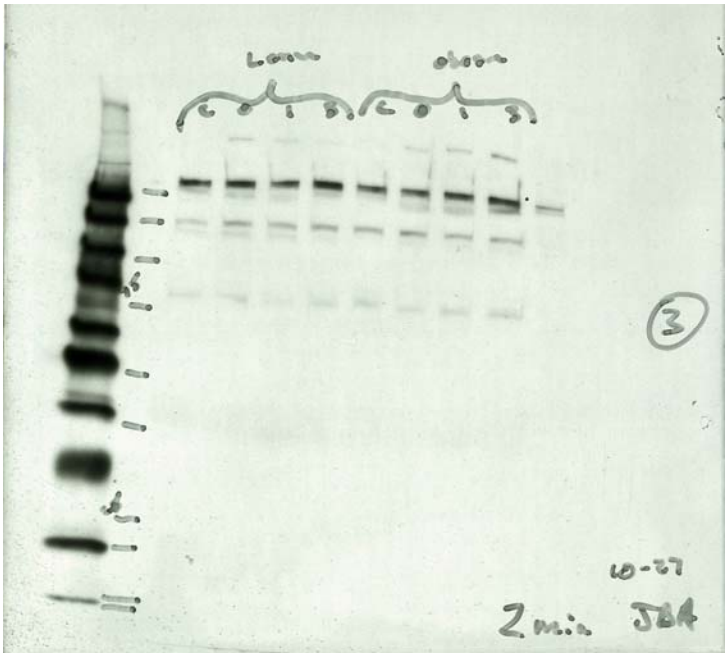
Primary Antibody: p-mTOR (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 2 min Molecular weight: 289 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for Akt (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

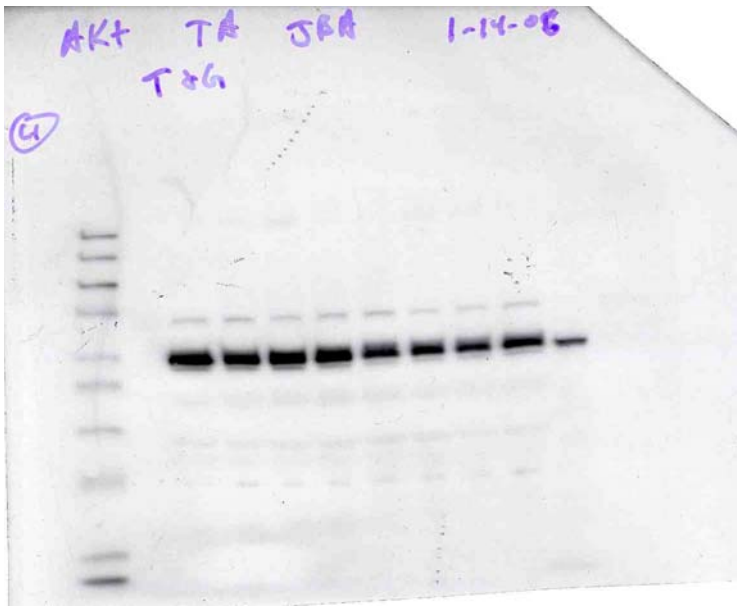
Primary Antibody: Akt (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution: 1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 seconds Molecular weight: 60 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for Akt (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

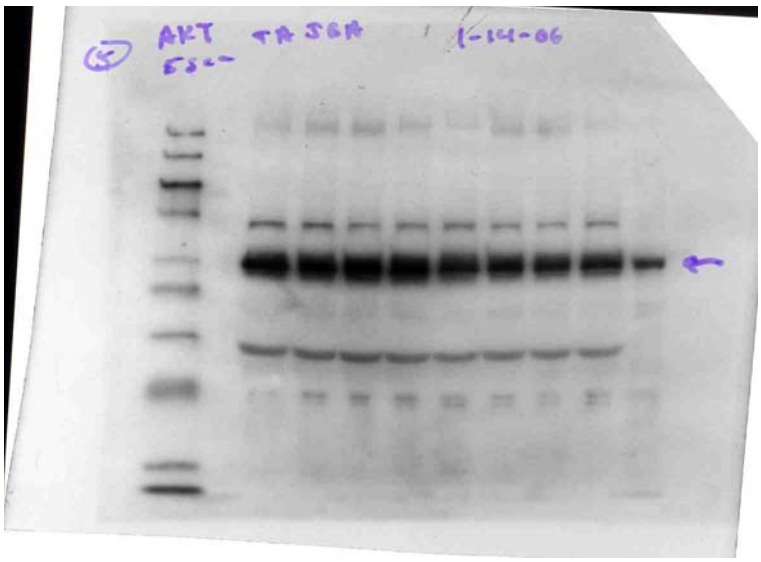
Primary Antibody: Akt (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution: 1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 seconds Molecular weight: 60 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for Akt (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

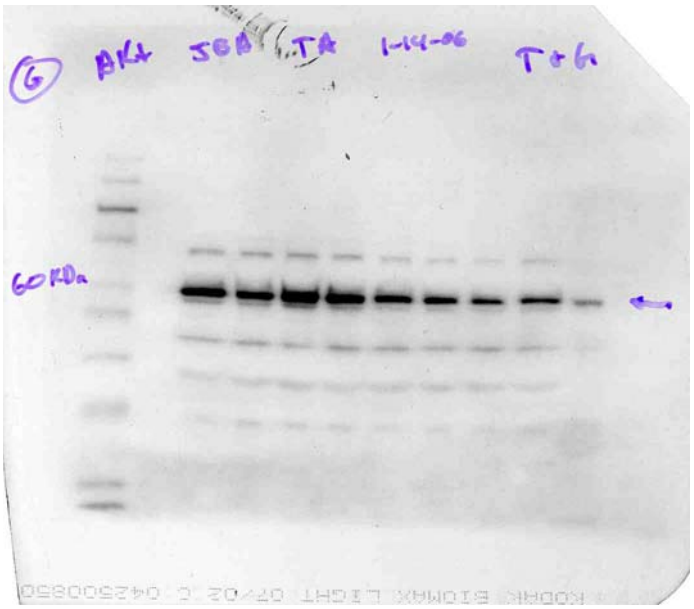
Primary Antibody: Akt (Cell Signaling) Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution: 1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 seconds Molecular weight: 60 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-Akt 308 (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-Akt 308 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 60 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

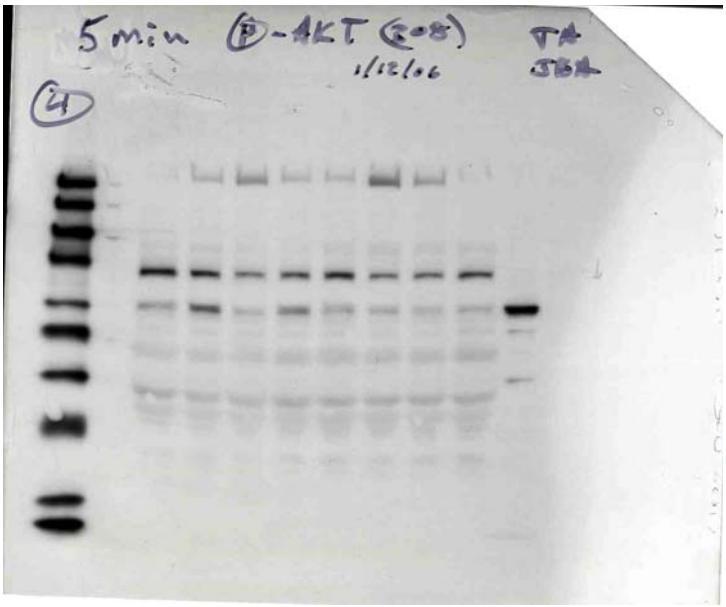
Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl



Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-Akt 308 (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

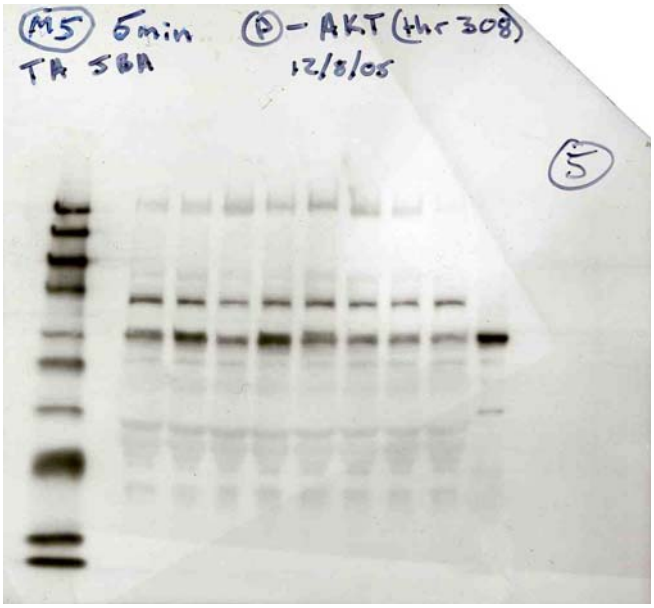
Primary Antibody: p-Akt 308 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 60 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-Akt 308 (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

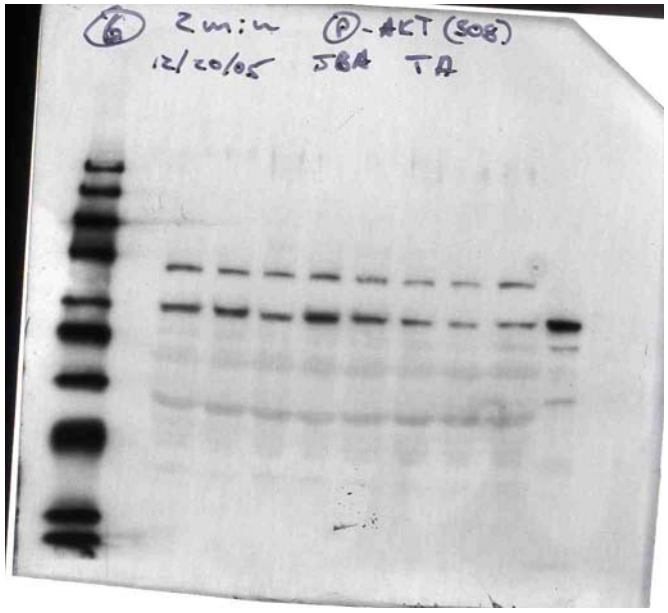
Primary Antibody: p-Akt 308 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 2 min Molecular weight: 60 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-Akt 473 (1)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

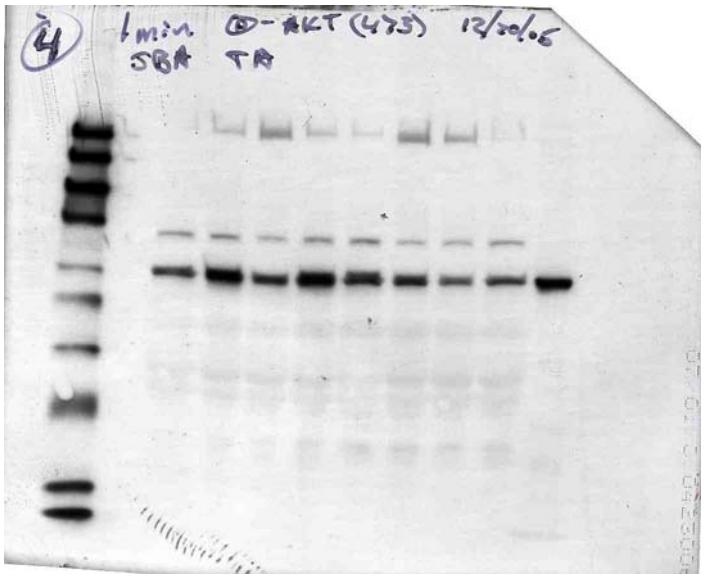
Primary Antibody: p-Akt 473 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 1min Molecular weight: 60 kDa



Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-Akt 473 (2)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-Akt 473 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 1 min Molecular weight: 60 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

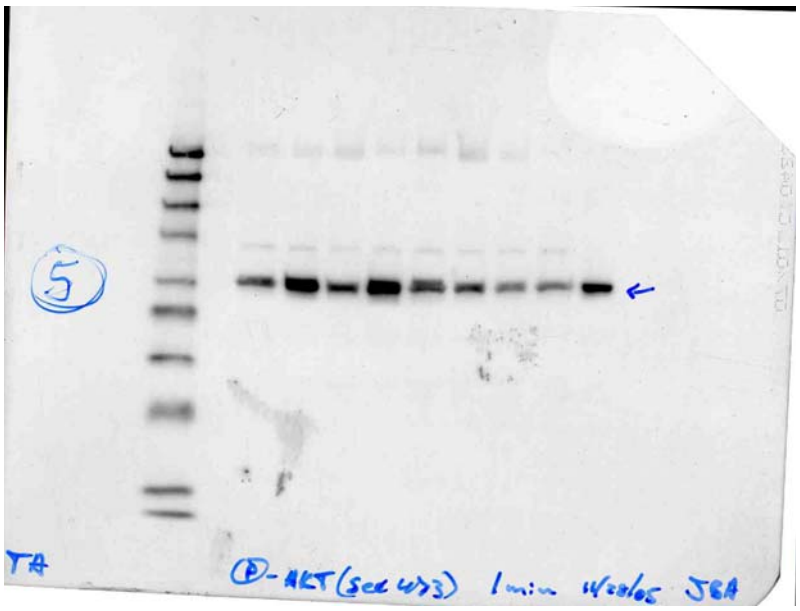
Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl



Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Tibialis Anterior (TA)

Film Properties Report for p-Akt 473 (3)

Experimenter: Anjaiah Katta

Muscle / Tissue: Tibialis Anterior (TA) Species: Rat (Zucker)

Protein conc.: 1.5µg/µl x 20µl = 30 µg Gel type: 10% Tris-HCL SDS_PAGE

Electrophoresis Voltage: 124V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-Akt 473 Primary Antibody Dilution: 1:1000

Incubation Time: overnight @ 4°C Medium: 5% BSA in TBS-T

Secondary Antibody: Anti-Rabbit Secondary Antibody Dilution:1:1000

Incubation Time: 1hr@room temp Medium: 5% milk in TBS-T

Exposure Time: 5 min Molecular weight: 60 kDa

Lane 1: Biotinylated Ladder 16µl

Lane 2: Mol Wt Marker 8 µl

Lane 3: Lean Zucker control 20 µl

Lane 4: Lean Zucker 0 hour 20 µl

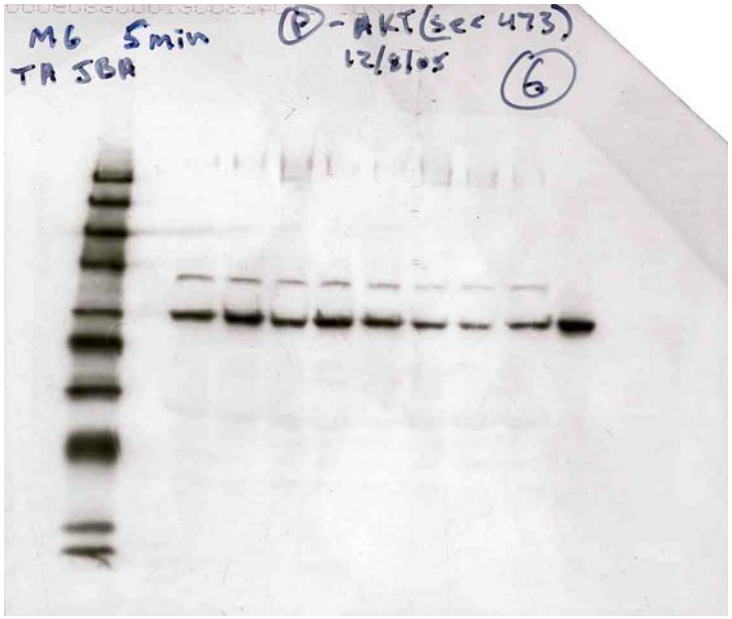
Lane 5: Lean Zucker 1 hour 20 µl

Lane 6: Lean Zucker 3 hour 20 µl

Lane 7: Obese Zucker control 20 µl

Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl



Lane 10: Obese Zucker 3 hour 20 µl

Lane11: Positive Control [L6 + IGF Lysate, 3T3 Cell Extract (untreated) 3T3 cell extract (serum treated)HeLa cell lysate] 16 µl

Lane 12: Mol Wt Marker 8 µl

Subsection B (Raw data)

This section represents the raw data tables produced from spot densitometry of the immunoblot films.

p70S6k data set

Total p70S6k IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	15.2	13.9	12.8	12.4	10.6	13.3	10.1	11.7
%C	15	13.6	13.6	12.3	10.5	13	10.1	11.9
%C	15.6	14.3	13.9	12.8	10.5	11.7	9.9	11.4
%C	15.4	14.7	13.8	12.8	10.2	11.5	10.2	11.5
%C	15.1	13.1	11	13.2	11.5	12.1	11.5	12.5
%C	14.5	13.2	10.8	12.8	11.9	12.5	11.9	12.5
N	6	6	6	6	6	6	6	6
Mean	15.133 33	13.8	12.65	12.716 67	10.866 67	12.35	10.616 67	11.916 67
Strandard Deviation	0.3777 12	0.6260 99	1.4110 28	0.3250 64	0.6713 17	0.7148 43	0.8542 05	0.4833 91
Standard Error of the mean	0.1689 18	0.28	0.6310 31	0.1453 73	0.3002 22	0.3196 87	0.3820 12	0.2161 79

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	0.9118 94	0.8359 03	0.8403 08	0.7180 62	0.8160 79	0.7015 42	0.7874 45
Standard error of the mean	0.0111 62	0.0185 02	0.0416 98	0.0096 06	0.0198 38	0.0211 25	0.0252 43	0.0142 85

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	91.189 43	83.590 31	84.030 84	71.806 17	81.607 93	70.154 19	78.744 49
SE	1.1161 99	1.8502 2	4.1698 08	0.9606 15	1.9838 47	2.1124 71	2.5243 1	1.4284 95

p-p70S6k Thr389 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	13.1	16.2	13.5	13.7	9.5	12.5	9.8	11.6
%C	12.6	16.2	13.6	13.8	9.8	11.8	10	12.2
%C	13.2	15.6	13.2	14.4	9.3	10.7	10.7	13
%C	13.2	15.6	13.2	14.4	9.3	10.7	10.7	13
%C	10.2	17.3	19.1	17.5	8.2	9.3	7.5	10.8
%C	10.9	16.7	18.2	17.4	8	9.4	7.7	11.6
N	6	6	6	6	6	6	6	6
Mean	12.2	16.266 67	15.133 33	15.2	9.0166 67	10.733 33	9.4	12.033 33
Strandard Deviation	1.3160 55	0.6562 52	2.7434 77	1.7674 84	0.7359 8	1.2722 68	1.4422 21	0.8710 15
Standard Error of the mean	0.5885 58	0.2934 85	1.2269 2	0.7904 43	0.3291 4	0.5689 76	0.6449 81	0.3895 3

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	1.3333 33	1.2404 37	1.2459 02	0.7390 71	0.8797 81	0.7704 92	0.9863 39
Standard error of the mean	0.0482 42	0.0240 56	0.1005 67	0.0647 9	0.0269 79	0.0466 37	0.0528 67	0.0319 29

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	133.33 33	124.04 37	124.59 02	73.907 1	87.978 14	77.049 18	98.633 88
SE	4.8242 42	2.4056 13	10.056 72	6.4790 4	2.6978 71	4.6637 35	5.2867 26	3.1928 66

p-p70S6k 421/424 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	4.3	19.2	16.7	9.8	4.4	17.9	13.3	14.2
%C	4.1	19.4	17	9.7	4	18.3	12.9	14.6
%C	5.5	24.8	21.7	8.3	4.2	17.8	7.8	10
%C	5.4	24.3	20.9	8.4	4.1	18.5	8	10.4
%C	4	25.4	20.3	7	5.4	17.5	6.6	13.9
%C	3.8	25.8	20.2	6.5	5.2	17.7	6.9	13.9
N	6	6	6	6	6	6	6	6
Mean	4.516667	23.15	19.466667	8.283333	4.55	17.95	9.25	12.833333
Strandard Deviation	0.741395	3.026384	2.098253	1.352652	0.599166	0.378153	3.031006	2.059773
Standard Error of the mean	0.331562	1.353404	0.938367	0.604924	0.267955	0.169115	1.355507	0.921159

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	5.125461	4.309963	1.833948	1.007388	3.974177	2.047927	2.841328
Standard error of the mean	0.073409	0.299655	0.207757	0.133932	0.059326	0.037443	0.300112	0.203947

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	512.5461	430.9963	183.3948	100.7388	397.4177	204.7927	284.1328
SE	7.340855	29.96546	20.77566	13.39316	5.932588	3.744251	30.01123	20.39466

mTOR data set

Total mTOR IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	11.2	12.8	11.8	16.6	12.3	11.2	9.9	14.2
%C	10.8	12.6	11	17.1	12.6	10.5	10.7	14.7
%C	13	13.7	12.6	14.5	12.3	10	10	13.9
%C	12.8	13.6	12.6	13.8	12.2	11.3	10.6	13.2
%C	10.4	18.2	20.3	12.1	7.8	12.1	10.8	8.2
%C	9.4	17.2	19.7	12.4	9	12.9	11.6	7.7
N	6	6	6	6	6	6	6	6
Mean	11.266 67	14.683 33	14.666 67	14.416 67	11.033 33	11.333 33	10.6	11.983 33
Strandard Deviation	1.4009 52	2.3970 12	4.1778 78	2.0875 02	2.0791 02	1.0519 82	0.6164 41	3.1657 02
Standard Error of the mean	0.6265 25	1.0719 76	1.8684 04	0.9335 59	0.9298 03	0.4704 61	0.2756 81	1.4157 45

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	1.3032 54	1.3017 75	1.2795 86	0.9792 9	1.0059 17	0.9408 28	1.0636 09
Standard error of the mean	0.0556 09	0.0951 46	0.1658 35	0.0828 6	0.0825 27	0.0417 57	0.0244 69	0.1256 58

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	130.32 54	130.17 75	127.95 86	97.928 99	100.59 17	94.082 84	106.36 09
SE	5.5608 71	9.5145 83	16.583 47	8.2860 31	8.2526 88	4.1756 87	2.4468 73	12.565 78

p-mTOR IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	7.1	28.8	19.1	10.2	4.6	15.5	9.7	5.1
%C	7.3	28.3	19.1	10.2	5	15.4	9.2	5.5
%C	10.8	19.7	14.8	14.2	9.5	11.3	9.3	10.4
%C	11.2	19.2	14.6	14.2	9.6	11.4	9.2	10.5
%C	13.2	13.6	10.9	14.5	10.7	9.7	11.6	15.9
%C	13.1	13.6	10.4	15.2	10.1	9.9	11.5	16.3
N	6	6	6	6	6	6	6	6
Mean	10.45	20.533 33	14.816 67	13.083 33	8.25	12.2	10.083 33	10.616 67
Strandard Deviation	2.6987 03	6.7420 08	3.7838 69	2.2631 1	2.7090 59	2.6122 79	1.1513 76	4.8350 46
Standard Error of the mean	1.2068 97	3.0151 17	1.6921 98	1.0120 94	1.2115 28	1.1682 47	0.5149 11	2.1622 98

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	1.9649 12	1.4178 63	1.2519 94	0.7894 74	1.1674 64	0.9649 12	1.0159 49
Standard error of the mean	0.1154 93	0.2885 28	0.1619 33	0.0968 51	0.1159 36	0.1117 94	0.0492 74	0.2069 18

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	196.49 12	141.78 63	125.19 94	78.947 37	116.74 64	96.491 23	101.59 49
SE	11.549 25	28.852 8	16.193 28	9.6851 06	11.593 57	11.179 39	4.9273 78	20.691 85

Akt data set

Total Akt IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	15.2	13	14.1	14.6	11.3	10.6	9.4	11.9
%C	15.1	12.6	14.2	14.5	11.5	10.4	10.1	11.6
%C	15.8	13.5	17.2	15	11.5	9.5	7.8	9.8
%C	14.9	13.3	16.5	15.1	12	9.9	8.2	10.1
%C	15.3	14.2	14.9	15.6	10.4	10.9	9.2	9.5
%C	16.4	14.2	14.4	15.3	10.2	10.8	9.1	9.6
N	6	6	6	6	6	6	6	6
Mean	15.45	13.46667	15.21667	15.01667	11.15	10.35	8.966667	10.41667
Strandard Deviation	0.554076	0.643946	1.313646	0.416733	0.700714	0.546809	0.835863	1.057198
Standard Error of the mean	0.24779	0.287981	0.587488	0.186369	0.313369	0.244544	0.373809	0.472793

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	0.871629	0.984898	0.971953	0.721683	0.669903	0.580367	0.674218
Standard error of the mean	0.016038	0.018644	0.038025	0.012063	0.020282	0.015827	0.024195	0.030601

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	87.16289	98.48975	97.19525	72.16828	66.99029	58.03668	67.42179
SE	1.603802	1.863958	3.802463	1.206271	2.028277	1.582786	2.419477	3.060105

p-Akt 308 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	13.3	22.7	9.3	16.9	11.1	9.8	7.1	9.8
%C	14.1	23.2	8.2	16.4	12.7	9.1	7.7	8.6
%C	12.8	16.5	10	19.1	13	10.6	9.1	8.7
%C	12.2	17.1	10.3	18.6	13.6	10.5	8.5	9.1
%C	15.3	15.3	10.7	19.5	14.5	9.6	5.8	9.3
%C	15.9	15.4	10.2	19.1	14.4	9.7	5.7	9.7
N	6	6	6	6	6	6	6	6
Mean	13.933 33	18.366 67	9.7833 33	18.266 67	13.216 67	9.8833 33	7.3166 67	9.2
Strandard Deviation	1.4459 14	3.6175 5	0.9020 35	1.2940 89	1.2639 88	0.5706 72	1.3920 01	0.4979 96
Standard Error of the mean	0.6466 32	1.6178 17	0.4034 02	0.5787 34	0.5652 73	0.2552 12	0.6225 22	0.2227 11

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	1.3181 82	0.7021 53	1.3110 05	0.9485 65	0.7093 3	0.5251 2	0.6602 87
Standard error of the mean	0.0464 09	0.1161 11	0.0289 52	0.0415 36	0.0405 7	0.0183 17	0.0446 79	0.0159 84

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	131.81 82	70.215 31	131.10 05	94.856 46	70.933 01	52.511 96	66.028 71
SE	4.6409 02	11.611 13	2.8952 31	4.1535 95	4.0569 82	1.8316 67	4.4678 59	1.5984 01

p-Akt 473 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
%C	11.7	16.6	11.7	15.3	14	12.7	8.2	9.8
%C	11.3	17.5	11.6	15.9	13.7	12.1	8.3	9.6
%C	10.9	20.1	11.6	19.9	13	10.4	7	7.2
%C	10.7	19.8	12.7	18.8	12.3	10.9	7.5	7.2
%C	13.5	15.6	13.4	16.9	13.7	10.2	6.4	10.3
%C	14	16.5	12.8	16.8	14.1	9.4	5.9	10.5
N	6	6	6	6	6	6	6	6
Mean	12.016 67	17.683 33	12.3	17.266 67	13.466 67	10.95	7.2166 67	9.1
Strandard Deviation	1.3948 72	1.8584 04	0.7694 15	1.7534 73	0.6889 61	1.2373 36	0.9662 64	1.5073 15
Standard Error of the mean	0.6238 06	0.8311 04	0.3440 93	0.7841 77	0.3081 13	0.5533 53	0.4321 27	0.6740 92

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	1.4715 67	1.0235 78	1.4368 93	1.1206 66	0.9112 34	0.6005 55	0.7572 82
Standard error of the mean	0.0519 12	0.0691 63	0.0286 35	0.0652 57	0.0256 4	0.0460 49	0.0359 61	0.0560 96

	Lean control	Lean 0	Lean 1	Lean 3	Obese conrtol	Obese 0	Obese 1	Obese 3
% RE	100	147.15 67	102.35 78	143.68 93	112.06 66	91.123 44	60.055 48	75.728 16
SE	5.1911 69	6.9162 58	2.8634 65	6.5257 44	2.5640 43	4.6048 83	3.5960 6	5.6096 42

Subsection C (Statistics)

Total p70S6k

t-test

Data source: raw data in TA totals p70paper

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.438)

Group Name	N	Missing	Mean	Std Dev	SEM
p70 lz	6	0	15.133	0.378	0.154
p70 oz	6	0	10.867	0.671	0.274

Difference 4.267

t = 13.568 with 10 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 3.566 to 4.967

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Total mTOR

t-test

Data source: raw data in TA totals p70paper

Normality Test: Failed (P = 0.045)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Data source: raw data in TA totals p70paper

Group	N	Missing	Median	25%	75%
mTOR lz	6	0	11.000	10.400	12.800

mTOR oz 6 0 12.250 9.000 12.300

T = 41.000 n(small)= 6 n(big)= 6 P(est.)= 0.810 P(exact)= 0.818

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.818)

Total Akt

t-test

Data source: raw data in TA totals p70paper

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.627)

Group Name	N	Missing	Mean	Std Dev	SEM
Akt lz	6	0	15.450	0.554	0.226
Akt oz	6	0	11.150	0.701	0.286

Difference 4.300

t = 11.791 with 10 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 3.487 to 5.113

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

p-p70S6k Thr389

Two Way Analysis of Variance

Data source: raw data TA in p70 pathway
Balanced Design

Dependent Variable: p p70 389

Normality Test: Failed (P < 0.050)

Equal Variance Test: Failed (P < 0.050)

Source of Variation	DF	SS	MS	F	P
model	1	41.255	41.255	5.209	0.028
time	3	465.562	155.187	19.595	<0.001
model x time	3	95.996	31.999	4.040	0.013
Residual	40	316.795	7.920		
Total	47	919.608	19.566		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of model depends on what level of time is present. There is a statistically significant interaction between model and time. (P = 0.013)

Power of performed test with alpha = 0.0500: for model : 0.508
Power of performed test with alpha = 0.0500: for time : 1.000
Power of performed test with alpha = 0.0500: for model x time : 0.669

Least square means for model :

Group	Mean
Obese	11.579
lean	13.433

Std Err of LS Mean = 0.574

Least square means for time :

Group	Mean
control	7.717
zero hr	12.308
one hr	13.700
three hr	16.300

Std Err of LS Mean = 0.812

Least square means for model x time :

Group	Mean
Obese x control	8.617
Obese x zero hr	10.483
Obese x one hr	11.000
Obese x three hr	16.217
lean x control	6.817
lean x zero hr	14.133
lean x one hr	16.400
lean x three hr	16.383

Std Err of LS Mean = 1.149

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method) :

Comparisons for factor: **model**

Comparison	Diff of Means	p	q	P	P<0.050
lean vs. Obese	1.854	2	3.228	0.028	Yes

Comparisons for factor: **time**

Comparison	Diff of Means	p	q	P	P<0.050
three hr vs. control	8.583	4	10.565	<0.001	Yes
three hr vs. zero hr	3.992	3	4.913	0.004	Yes
three hr vs. one hr	2.600	2	3.200	0.029	Yes
one hr vs. control	5.983	3	7.365	<0.001	Yes
one hr vs. zero hr	1.392	2	1.713	0.233	No
zero hr vs. control	4.592	2	5.652	<0.001	Yes

Comparisons for factor: **time within Obese**

Comparison	Diff of Means	p	q	P	P<0.05
three hr vs. control	7.600	4	6.615	<0.001	Yes
three hr vs. zero hr	5.733	3	4.990	0.003	Yes
three hr vs. one hr	5.217	2	4.541	0.003	Yes
one hr vs. control	2.383	3	2.074	0.318	No
one hr vs. zero hr	0.517	2	0.450	0.752	Do Not Test
zero hr vs. control	1.867	2	1.625	0.258	Do Not Test

Comparisons for factor: **time within lean**

Comparison	Diff of Means	p	q	P	P<0.05
one hr vs. control	9.583	4	8.341	<0.001	Yes
one hr vs. zero hr	2.267	3	1.973	0.353	No
one hr vs. three hr	0.0167	2	0.0145	0.992	Do Not Test
three hr vs. control	9.567	3	8.327	<0.001	Yes
three hr vs. zero hr	2.250	2	1.958	0.174	Do Not Test

zero hr vs. control	7.317	2	6.368	<0.001	Yes

Comparisons for factor: **model within control**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
Obese vs. lean	1.800	2	1.567	0.275	No

Comparisons for factor: **model within zero hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	3.650	2	3.177	0.030	Yes

Comparisons for factor: **model within one hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	5.400	2	4.700	0.002	Yes

Comparisons for factor: **model within three hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	0.167	2	0.145	0.919	No

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

p-p70S6k 421/424

Two Way Analysis of Variance

Data source: raw data TA in p70 pathway
Balanced Design

Dependent Variable: p p70421-424

Normality Test: Passed (P = 0.097)

Equal Variance Test: Passed (P = 0.220)

Source of Variation	DF	SS	MS	F	P
model	1	88.021	88.021	23.572	<0.001
time	3	1625.925	541.975	145.143	<0.001
model x time	3	368.351	122.784	32.882	<0.001
Residual	40	149.363	3.734		
Total	47	2231.660	47.482		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of model depends on what level of time is present. There is a statistically significant interaction between model and time. (P = <0.001)

Power of performed test with alpha = 0.0500: for model : 0.999
Power of performed test with alpha = 0.0500: for time : 1.000
Power of performed test with alpha = 0.0500: for model x time : 1.000

Least square means for model :

Group	Mean
Obese	11.146
lean	13.854

Std Err of LS Mean = 0.394

Least square means for time :

Group	Mean
control	4.533
zero hr	20.550
one hr	14.358
three hr	10.558

Std Err of LS Mean = 0.558

Least square means for model x time :

Group	Mean
Obese x control	4.550
Obese x zero hr	17.950
Obese x one hr	9.250
Obese x three hr	12.833
lean x control	4.517
lean x zero hr	23.150
lean x one hr	19.467
lean x three hr	8.283

Std Err of LS Mean = 0.789

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method) :

Comparisons for factor: **model**

Comparison	Diff of Means	p	q	P	P<0.050
lean vs. Obese	2.708	2	6.866	<0.001	Yes

Comparisons for factor: **time**

Comparison	Diff of Means	p	q	P	P<0.050
zero hr vs. control	16.017	4	28.712	<0.001	Yes
zero hr vs. three hr	9.992	3	17.912	<0.001	Yes
zero hr vs. one hr	6.192	2	11.100	<0.001	Yes
one hr vs. control	9.825	3	17.613	<0.001	Yes
one hr vs. three hr	3.800	2	6.812	<0.001	Yes
three hr vs. control	6.025	2	10.801	<0.001	Yes

Comparisons for factor: **time within Obese**

Comparison	Diff of Means	p	q	P	P<0.05
zero hr vs. control	13.400	4	16.986	<0.001	Yes
zero hr vs. one hr	8.700	3	11.028	<0.001	Yes
zero hr vs. three hr	5.117	2	6.486	<0.001	Yes
three hr vs. control	8.283	3	10.500	<0.001	Yes
three hr vs. one hr	3.583	2	4.542	0.003	Yes
one hr vs. control	4.700	2	5.958	<0.001	Yes

Comparisons for factor: **time within lean**

Comparison	Diff of Means	p	q	P	P<0.05
zero hr vs. control	18.633	4	23.620	<0.001	Yes
zero hr vs. three hr	14.867	3	18.845	<0.001	Yes
zero hr vs. one hr	3.683	2	4.669	0.002	Yes
one hr vs. control	14.950	3	18.951	<0.001	Yes
one hr vs. three hr	11.183	2	14.176	<0.001	Yes
three hr vs. control	3.767	2	4.775	0.002	Yes

Comparisons for factor: **model within control**

Comparison	Diff of Means	p	q	P	P<0.05
Obese vs. lean	0.0333	2	0.0423	0.976	No

Comparisons for factor: **model within zero hr**

Comparison	Diff of Means	p	q	P	P<0.05
lean vs. Obese	5.200	2	6.592	<0.001	Yes

Comparisons for factor: **model within one hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	10.217	2	12.951	<0.001	Yes

Comparisons for factor: **model within three hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
Obese vs. lean	4.550	2	5.768	<0.001	Yes

p-mTOR

Two Way Analysis of Variance

Data source: raw data TA in p70 pathway
Balanced Design

Dependent Variable: p mTOR

Normality Test: Passed (P = 0.373)

Equal Variance Test: Passed (P = 0.087)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
model	1	235.853	235.853	16.992	<0.001
time	3	303.582	101.194	7.290	<0.001
model x time	3	72.467	24.156	1.740	0.174
Residual	40	555.217	13.880		
Total	47	1167.119	24.832		

The difference in the mean values among the different levels of model is greater than would be expected by chance after allowing for effects of differences in time. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of time is greater than would be expected by chance after allowing for effects of differences in model. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of model does not depend on what level of time is present. There is not a statistically significant interaction between model and time. (P = 0.174)

Power of performed test with alpha = 0.0500: for model : 0.984

Power of performed test with alpha = 0.0500: for time : 0.958
Power of performed test with alpha = 0.0500: for model x time : 0.188

Least square means for model :

Group	**Mean**
Obese	10.288
lean	14.721

Std Err of LS Mean = 0.760

Least square means for time :

Group	**Mean**
control	9.350
zero hr	16.367
one hr	12.450
three hr	11.850

Std Err of LS Mean = 1.076

Least square means for model x time :

Group	**Mean**
Obese x control	8.250
Obese x zero hr	12.200
Obese x one hr	10.083
Obese x three hr	10.617
lean x control	10.450
lean x zero hr	20.533
lean x one hr	14.817
lean x three hr	13.083

Std Err of LS Mean = 1.521

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method) :

Comparisons for factor: **model**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
lean vs. Obese	4.433	2	5.830	<0.001	Yes

Comparisons for factor: **time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
zero hr vs. control	7.017	4	6.524	<0.001	Yes
zero hr vs. three hr	4.517	3	4.200	0.014	Yes
zero hr vs. one hr	3.917	2	3.642	0.014	Yes
one hr vs. control	3.100	3	2.882	0.116	No
one hr vs. three hr	0.600	2	0.558	0.695	Do Not Test
three hr vs. control	2.500	2	2.324	0.108	Do Not Test

Comparisons for factor: **time within Obese**

Comparison	Diff of Means	p	q	P	P<0.05
zero hr vs. control	3.950	4	2.597	0.272	No
zero hr vs. one hr	2.117	3	1.392	0.591	Do Not Test
zero hr vs. three hr	1.583	2	1.041	0.466	Do Not Test
three hr vs. control	2.367	3	1.556	0.520	Do Not Test
three hr vs. one hr	0.533	2	0.351	0.806	Do Not Test
one hr vs. control	1.833	2	1.205	0.399	Do Not Test

Comparisons for factor: **time within lean**

Comparison	Diff of Means	p	q	P	P<0.05
zero hr vs. control	10.083	4	6.629	<0.001	Yes
zero hr vs. three hr	7.450	3	4.898	0.004	Yes
zero hr vs. one hr	5.717	2	3.759	0.011	Yes
one hr vs. control	4.367	3	2.871	0.118	No
one hr vs. three hr	1.733	2	1.140	0.425	Do Not Test
three hr vs. control	2.633	2	1.731	0.228	Do Not Test

Comparisons for factor: **model within control**

Comparison	Diff of Means	p	q	P	P<0.05
lean vs. Obese	2.200	2	1.446	0.313	No

Comparisons for factor: **model within zero hr**

Comparison	Diff of Means	p	q	P	P<0.05
lean vs. Obese	8.333	2	5.479	<0.001	Yes

Comparisons for factor: **model within one hr**

Comparison	Diff of Means	p	q	P	P<0.05
lean vs. Obese	4.733	2	3.112	0.034	Yes

Comparisons for factor: **model within three hr**

Comparison	Diff of Means	p	q	P	P<0.05
lean vs. Obese	2.467	2	1.622	0.258	No

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural

rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

p-Akt 308

Two Way Analysis of Variance

Data source: raw data TA in p70 pathway
Balanced Design

Dependent Variable: p-Akt 308

Normality Test: Passed (P = 0.079)

Equal Variance Test: Passed (P = 0.050)

Source of Variation	DF	SS	MS	F	P
model	1	322.403	322.403	118.451	<0.001
time	3	251.038	83.679	30.744	<0.001
model x time	3	159.905	53.302	19.583	<0.001
Residual	40	108.873	2.722		
Total	47	842.219	17.920		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of model depends on what level of time is present. There is a statistically significant interaction between model and time. (P = <0.001)

Power of performed test with alpha = 0.0500: for model : 1.000
Power of performed test with alpha = 0.0500: for time : 1.000
Power of performed test with alpha = 0.0500: for model x time : 1.000

Least square means for model :

Group	Mean
Obese	9.904
lean	15.088

Std Err of LS Mean = 0.337

Least square means for time :

Group	Mean
control	13.575
zero hr	14.125
one hr	8.550

three hr 13.733
Std Err of LS Mean = 0.476

Least square means for model x time :

Group	**Mean**
Obese x control	13.217
Obese x zero hr	9.883
Obese x one hr	7.317
Obese x three hr	9.200
lean x control	13.933
lean x zero hr	18.367
lean x one hr	9.783
lean x three hr	18.267

Std Err of LS Mean = 0.674

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method) :

Comparisons for factor: **model**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
lean vs. Obese	5.183	2	15.392	<0.001	Yes

Comparisons for factor: **time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
zero hr vs. one hr	5.575	4	11.706	<0.001	Yes
zero hr vs. control	0.550	3	1.155	0.695	No
zero hr vs. three hr	0.392	2	0.822	0.564	Do Not Test
three hr vs. one hr	5.183	3	10.884	<0.001	Yes
three hr vs. control	0.158	2	0.332	0.815	Do Not Test
control vs. one hr	5.025	2	10.551	<0.001	Yes

Comparisons for factor: **time within Obese**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
control vs. one hr	5.900	4	8.760	<0.001	Yes
control vs. three hr	4.017	3	5.964	<0.001	Yes
control vs. zero hr	3.333	2	4.949	0.001	Yes
zero hr vs. one hr	2.567	3	3.811	0.027	Yes
zero hr vs. three hr	0.683	2	1.015	0.477	No
three hr vs. one hr	1.883	2	2.796	0.055	No

Comparisons for factor: **time within lean**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
zero hr vs. one hr	8.583	4	12.744	<0.001	Yes

zero hr vs. control	4.433	3	6.582	<0.001	Yes
zero hr vs. three hr	0.100	2	0.148	0.917	No
three hr vs. one hr	8.483	3	12.595	<0.001	Yes
three hr vs. control	4.333	2	6.434	<0.001	Yes
control vs. one hr	4.150	2	6.162	<0.001	Yes

Comparisons for factor: **model within control**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	0.717	2	1.064	0.456	No

Comparisons for factor: **model within zero hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	8.483	2	12.595	<0.001	Yes

Comparisons for factor: **model within one hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	2.467	2	3.662	0.013	Yes

Comparisons for factor: **model within three hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	9.067	2	13.461	<0.001	Yes

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

p-Akt 473

Two Way Analysis of Variance

Data source: raw data TA in p70 pathway
Balanced Design

Dependent Variable: p akt473

Normality Test: Passed (P = 0.650)

Equal Variance Test: Passed (P = 0.580)

Source of Variation	DF	SS	MS	F	P
model	1	257.613	257.613	144.348	<0.001
time	3	136.108	45.369	25.422	<0.001
model x time	3	162.312	54.104	30.316	<0.001
Residual	40	71.387	1.785		
Total	47	627.420	13.349		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of model depends on what level of time is present. There is a statistically significant interaction between model and time. (P = <0.001)

Power of performed test with alpha = 0.0500: for model : 1.000
Power of performed test with alpha = 0.0500: for time : 1.000
Power of performed test with alpha = 0.0500: for model x time : 1.000

Least square means for model :

Group	Mean
Obese	10.183
lean	14.817

Std Err of LS Mean = 0.273

Least square means for time :

Group	Mean
control	12.742
zero hr	14.317
one hr	9.758
three hr	13.183

Std Err of LS Mean = 0.386

Least square means for model x time :

Group	Mean
Obese x control	13.467
Obese x zero hr	10.950
Obese x one hr	7.217
Obese x three hr	9.100
lean x control	12.017
lean x zero hr	17.683
lean x one hr	12.300
lean x three hr	17.267

Std Err of LS Mean = 0.545

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **model**

Comparison	Diff of Means	p	q	P	P<0.050
lean vs. Obese	4.633	2	16.991	<0.001	Yes

Comparisons for factor: **time**

Comparison	Diff of Means	p	q	P	P<0.050
zero hr vs. one hr	4.558	4	11.820	<0.001	Yes
zero hr vs. control	1.575	3	4.084	0.017	Yes
zero hr vs. three hr	1.133	2	2.939	0.044	Yes
three hr vs. one hr	3.425	3	8.881	<0.001	Yes
three hr vs. control	0.442	2	1.145	0.423	No
control vs. one hr	2.983	2	7.736	<0.001	Yes

Comparisons for factor: **time within Obese**

Comparison	Diff of Means	p	q	P	P<0.05
control vs. one hr	6.250	4	11.460	<0.001	Yes
control vs. three hr	4.367	3	8.007	<0.001	Yes
control vs. zero hr	2.517	2	4.614	0.002	Yes
zero hr vs. one hr	3.733	3	6.845	<0.001	Yes
zero hr vs. three hr	1.850	2	3.392	0.021	Yes
three hr vs. one hr	1.883	2	3.453	0.019	Yes

Comparisons for factor: **time within lean**

Comparison	Diff of Means	p	q	P	P<0.05
zero hr vs. control	5.667	4	10.390	<0.001	Yes
zero hr vs. one hr	5.383	3	9.871	<0.001	Yes
zero hr vs. three hr	0.417	2	0.764	0.592	No
three hr vs. control	5.250	3	9.626	<0.001	Yes
three hr vs. one hr	4.967	2	9.107	<0.001	Yes

one hr vs. control	0.283	2	0.520	0.715	No

Comparisons for factor: **model within control**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
Obese vs. lean	1.450	2	2.659	0.068	No

Comparisons for factor: **model within zero hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	6.733	2	12.346	<0.001	Yes

Comparisons for factor: **model within one hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	5.083	2	9.321	<0.001	Yes

Comparisons for factor: **model within three hr**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
lean vs. Obese	8.167	2	14.974	<0.001	Yes

Chapter 4

Conclusions

1. Type 2 diabetes was found to significantly alter expression of p70S6k and Akt in the tibialis anterior muscle.
2. Type 2 diabetes was found to significantly alter the basal phosphorylation of p70S6k (Thr 389) in the tibialis anterior muscle.
3. Type 2 diabetes was found to significantly alter contraction- induced activation of Akt/mTOR/p70S6k pathway proteins. These diabetes-associated alterations in Akt/mTOR/p70S6k pathway regulation may be due, at least in part, to defects in the ability of diabetic muscle to activate this pathway following a contractile stimulus. The upstream molecules responsible for these alterations remain to be determined.

Future Directions

Future research based on this study should focus on mechanisms associated with differences in contraction induced activation of Akt/mTOR/p70S6k pathway in type 2 diabetic skeletal muscle. In the past several years, considerable progress has been made to elucidate intracellular signaling mechanisms in the regulation of protein synthesis and skeletal muscle mass in contracting skeletal muscle. It is now apparent that the Akt/mTOR/p70S6k pathway plays a critical role in the regulation of protein synthesis.

Our data suggest that the magnitude and time course of the contraction-induced activation of Akt/mTOR/p70S6k pathway proteins are significantly altered with type 2 diabetes. The molecular mechanism(s) responsible for these differences with diabetes are

largely unknown. Additional studies, perhaps employing strategies designed to directly inhibit or activate specific signaling proteins of pathway during HFES may prove to be useful in addressing these possibilities. Further elucidating the molecular mechanisms for differences in the ability of skeletal muscle to activate Akt/mTOR/p70S6k pathway could be of clinical importance and may have implications for the improvement of resistance based programs for the treatment of diabetes mellitus.

Our data suggest that the *in situ* high-frequency electrical stimulation (HFES) induced activation of Akt/mTOR/p70S6k pathway proteins is significantly altered in type 2 diabetes. Nader et al., (2001) using different modes of exercise like high-frequency electrical stimulation, low-frequency electrical stimulation, or running exercise showed activation of p70S6k pathway in the tibialis anterior and soleus muscles of rat [32]. Experiments comparing normal and diabetic animals with different modes of exercise and also using different time points can help provide a better understanding of how diabetes may be associated with differences in how skeletal muscle "sense" and "respond" to different stimuli.

The results of present study have shown that contraction-induced regulation of p70S6k signaling may be altered in the skeletal muscles of the obese Zucker (fa/fa) rat model. Whether similar findings would be observed in other diabetic model or other types of diabetes (e.g. type I) remains to be determined. In future experiments, it will be challenging and informative to determine how p70S6k signaling is regulated in other models.

Koopman et al., (2006) reported that resistance exercise is associated with more pronounced phosphorylation of p70S6k in type II vs. type I muscle fibers [33]. Similarly,

in rats, the phosphorylation of upstream regulators of p70S6k like PKB (or Akt) and mTOR was more pronounced in muscle tissue containing a greater proportion of type II muscle fibers [34, 35]. Oberbach et al., (2006) have recently found that the slow oxidative fiber fraction was reduced by 16% with diabetes, and the fast glycolytic fiber fraction was increased by 49% in vastus lateralis muscles of human subjects with type 2 diabetes [100]. This variation in fiber fraction should invariably result in differences in oxidative and glycolytic enzyme content and activity. This could lead to variations in the ratio of fat and carbohydrate used in muscle during contraction. Taken together, these studies suggest that regulation of p70S6k pathway proteins may differ between fiber types and it is possible that fiber type transitions associated with diabetes may be involved. Further contractile studies using different muscles (the predominantly slow twitch soleus and the predominantly fast twitch EDL) may give better understanding of altered regulation of these proteins with diabetes.

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- ***Effects of Diabetes on pressure induced MAPKs signaling in the inferior vena cava of diabetic and non-diabetic Zucker Rats****;* K. M. Rice[1],D.H.Desai[2],Sunil K.Kakarla[1] , Anjaiah Katta[1] and E. R. Blough[1,2] ; [1] Department of Biological Sciences, Marshall University, [2] Department of

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- ***Aging influences multiple indices of oxidative stress in the heart of the Fischer 344/NNia x Brown Norway/BiNia rat.*** Shinichi Asano[1], Kevin M. Rice[1], Sunil K. Karkarla[1], Anjaiah Katta[1] and Eric R Blough[1,2]: [1]Department of Biological Sciences, Marshall University, [2] Department of Physiology, Marshall University, Joan C. Edwards School of Medicine. Redox report Vol4 issue 12 2007

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