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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

Anjaiah Katta

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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**

By
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A thesis submitted to the
Graduate faculty of the Department of Biology
at
Marshall University
In partial fulfillment of the requirements for the degree
of
Master of Science

Committee:

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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 polypyrimidine 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/ PKB α -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 progression 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% VO₂max 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 afflicted, 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-secretory 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 intramuscular 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]. *Psammomys 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 ($27.9 \pm 3.6\%$ lower) and level of basal p70S6k phosphorylation (Thr 389) ($26.1 \pm 7.5\%$ lower) in the normal and diabetic TA ($P < 0.05$). p70S6k (Thr389) phosphorylation increased $33.3 \pm 7.2\%$, $24.0 \pm 14.9\%$ and $24.6 \pm 11.3\%$ in lean TA at 0-, 1- and 3-hr post-HFES and increased $33.5 \pm 8.0\%$ in obese TA at 3-hr post-HFES ($P < 0.05$). mTOR (Ser 2448) Phosphorylation was elevated in lean TA ($96.5 \pm 40.3\%$, $P < 0.05$) immediately after HFES and remains unaltered in obese TA with HFES. In lean TA, HFES led to increased phosphorylation of Akt (Thr 308) ($31.8 \pm 16.2\%$ and $31.1 \pm 8.8\%$) and Akt (Ser 473) ($47.2 \pm 12.1\%$ and $43.7 \pm 11.7\%$) 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 polypyrimidine 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 $22^{\circ} \pm 2^{\circ}\text{C}$. 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 Thr³⁸⁹ 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 $27.9 \pm 3.6\%$ and $28.2 \pm 10.1\%$ 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 $26.1 \pm 7.5\%$ 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 $33.3 \pm 7.2\%$, $24.0 \pm 14.9\%$ and $24.6 \pm 11.3\%$ 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 $33.5 \pm 8.0\%$ 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 $412.5 \pm 37.2\%$, $331.0 \pm 28.1\%$ and $83.0 \pm 20.7\%$ 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 $294.5 \pm 9.6\%$, $103.3 \pm 35.5\%$ and $182.0 \pm 26.1\%$ 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 $96.5 \pm 40.3\%$ higher than baseline immediately after exercise in lean animals ($P < 0.05$) while it was not different from baseline at 1 and 3 hr post-HFES (Fig. 3). This response differed in obese animals with no alteration in mTOR (Ser 2448) phosphorylation levels at any time point after HFES (Fig. 3). In lean Zucker TA, Akt (Thr 308) phosphorylation was $31.8 \pm 16.2\%$, $29.8 \pm 7.5\%$ and $31.1 \pm 8.8\%$ higher than baseline at 0-, 1- and 3-hr post-HFES, exhibiting a biphasic response ($P < 0.05$; Fig. 4). An altogether opposite response was observed in the obese Zucker TA, where Akt (Thr

308) phosphorylation was $25.3 \pm 6.2\%$, $44.6 \pm 9.0\%$ and $30.4 \pm 5.9\%$ lower than baseline at 0-, 1-, and 3-hours, respectively after HFES ($P < 0.05$). In lean rats, the phosphorylation of Akt (Ser 473) appeared to mirror what was seen at the Thr 308 residue with HFES and was $47.2 \pm 12.1\%$ and $43.7 \pm 11.7\%$ higher than baseline at 0- and 3-hours, respectively ($P < 0.05$; Fig. 4). This pattern of response was essentially opposite in the obese Zucker, where the phosphorylation of Akt (Ser 473) was $18.7 \pm 6.4\%$, $46.3 \pm 5.5\%$ and $32.4 \pm 7.3\%$ lower than baseline at 0-, 1-, and 3-hours post-HFES, respectively ($P < 0.05$; Fig. 4). Taken together these results suggest that the contraction-induced activation of p70S6k signaling may be altered with 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 phosphoinositide-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 phosphorylation. 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

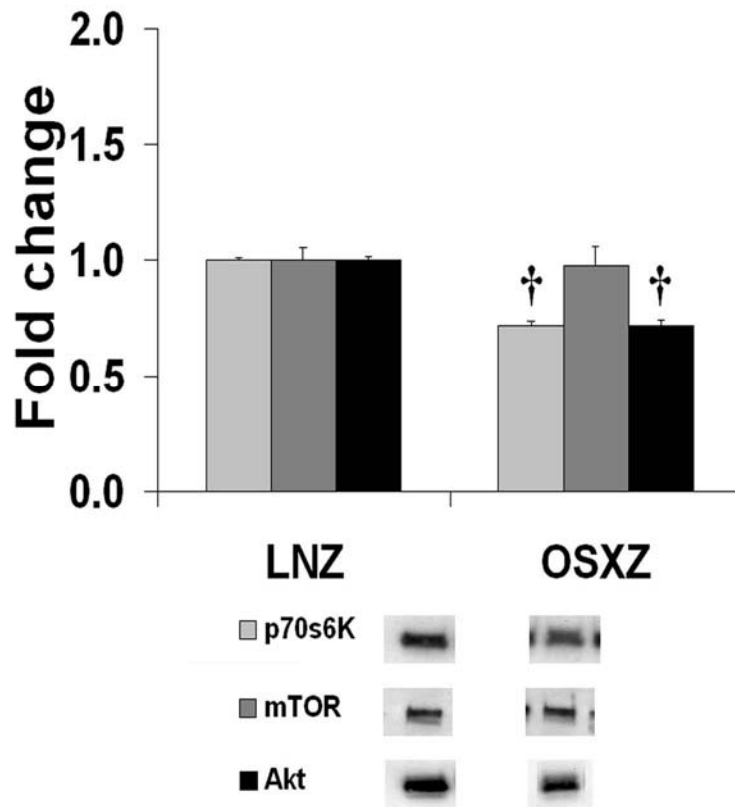


Figure 2

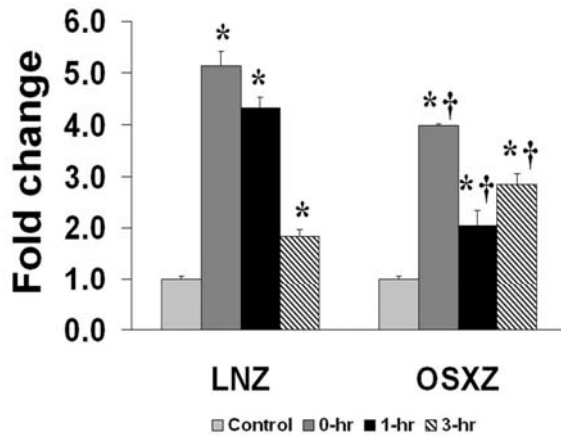
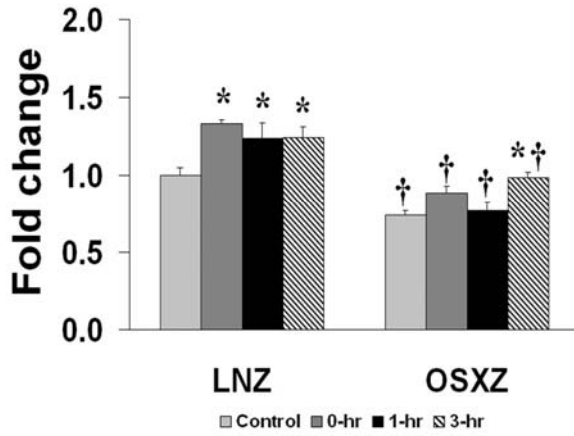


Figure 3

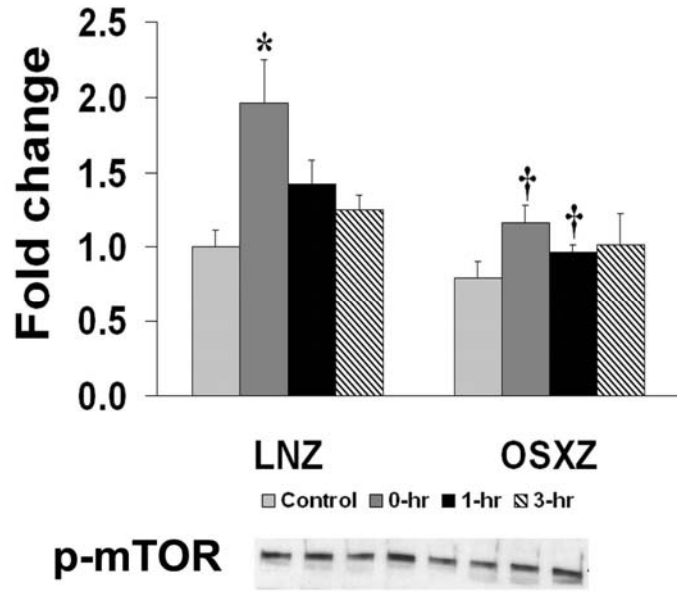
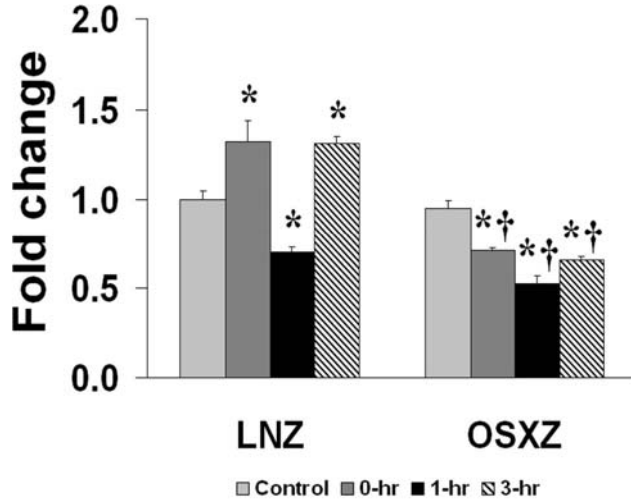
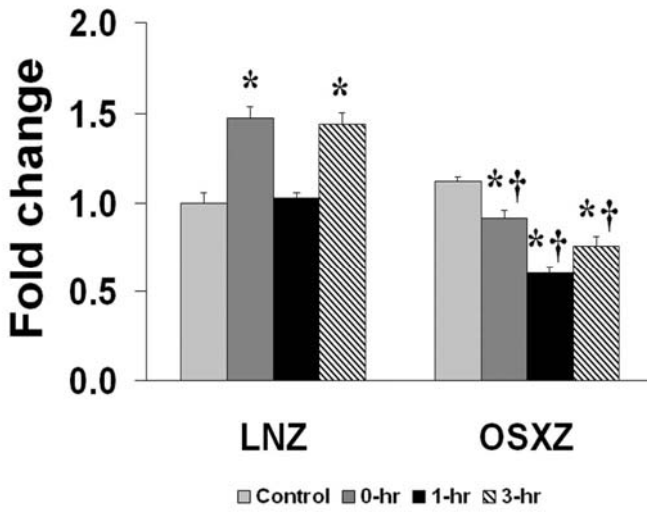


Figure 4



p-Akt 308



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

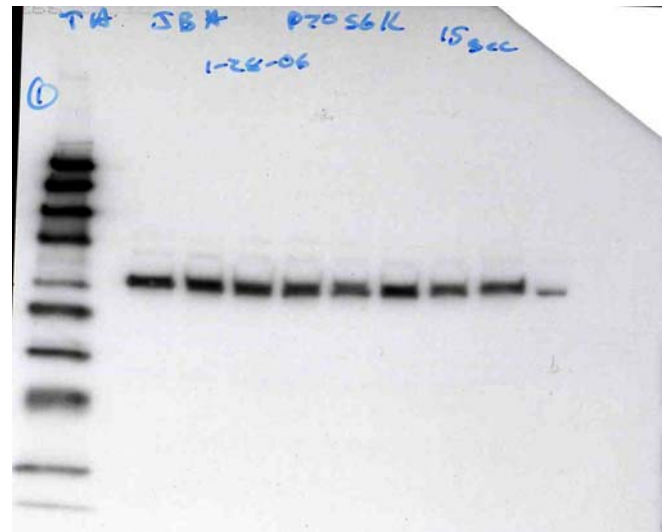
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

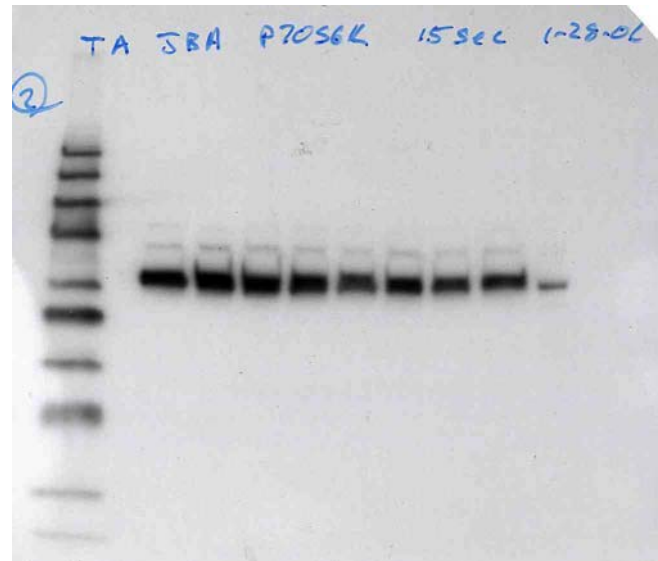
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

Lane 11: 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

Lane 7: Obese Zucker control 20 µl

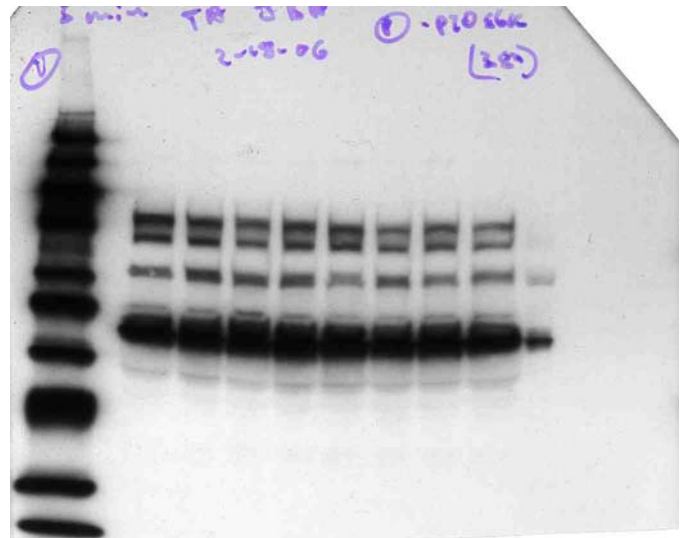
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

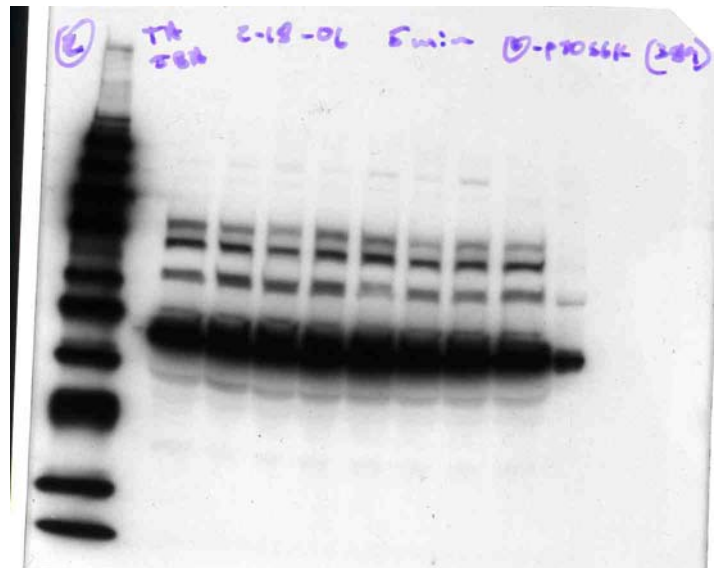
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

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

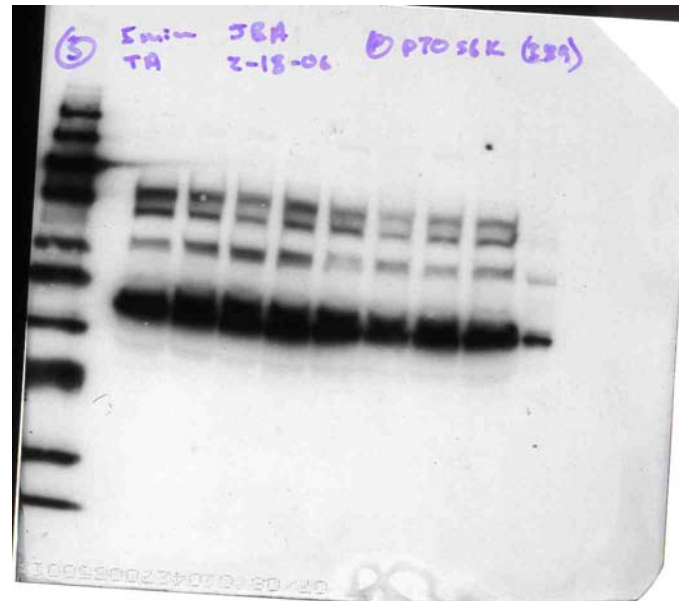
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

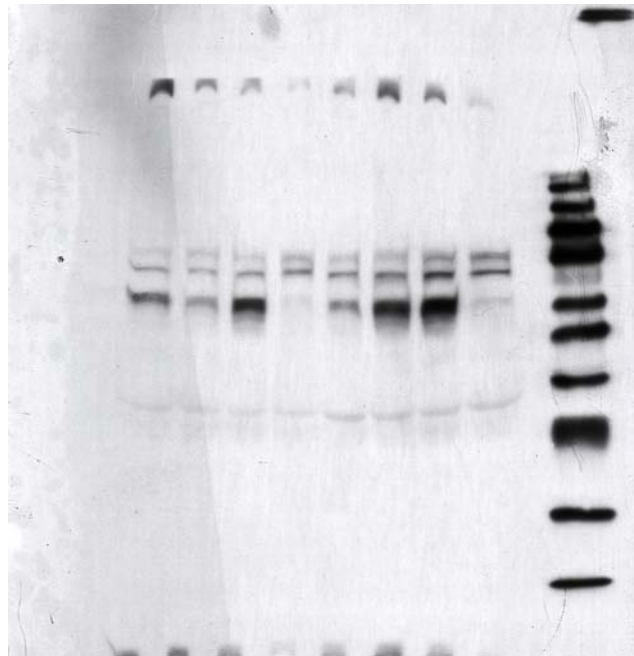
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

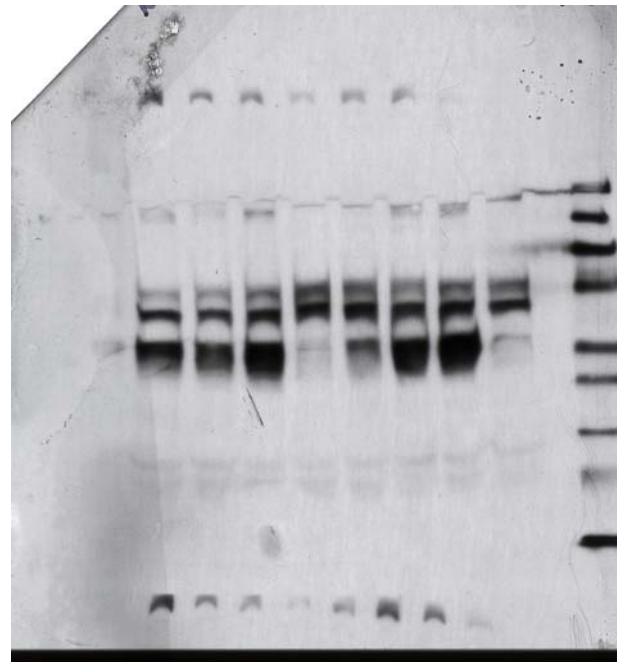
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

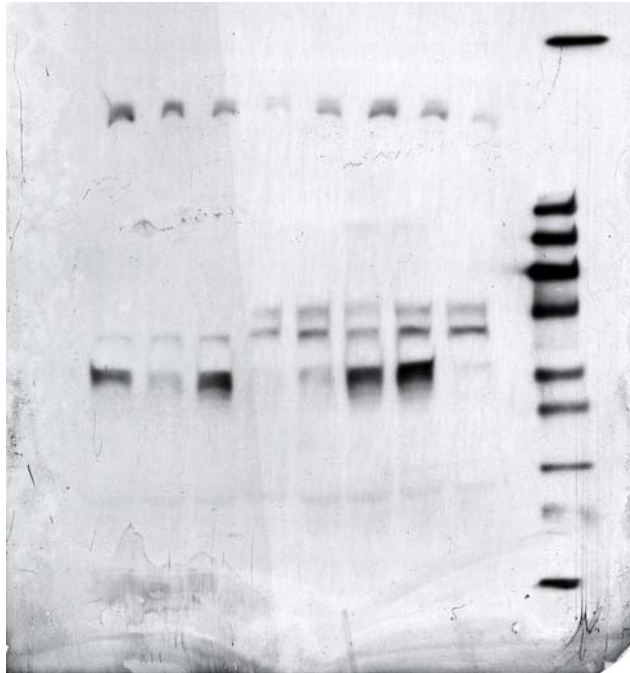
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

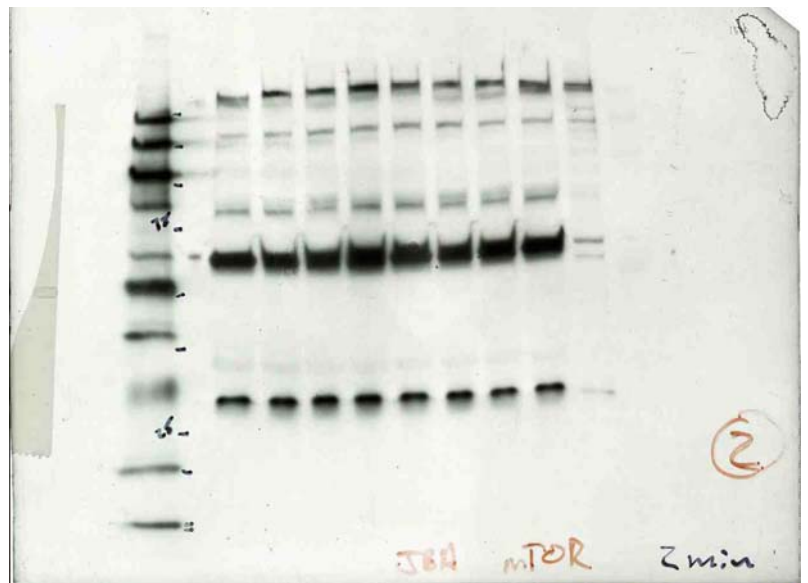
Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

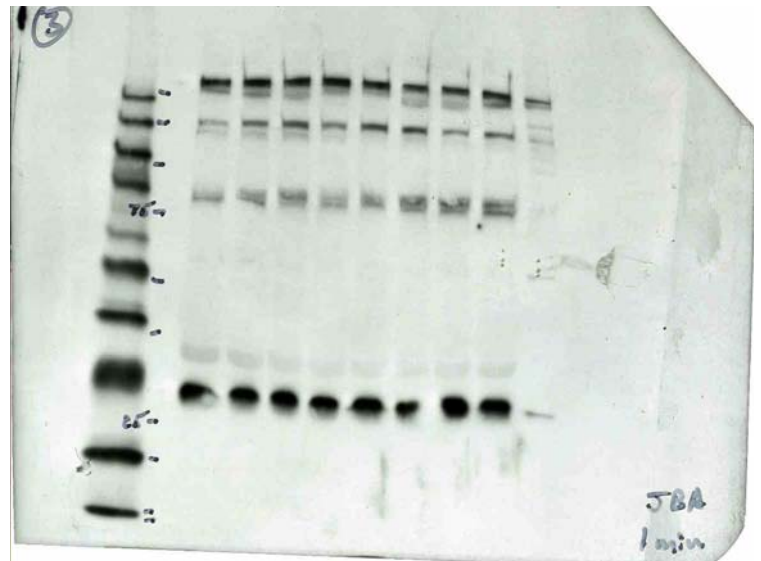
Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

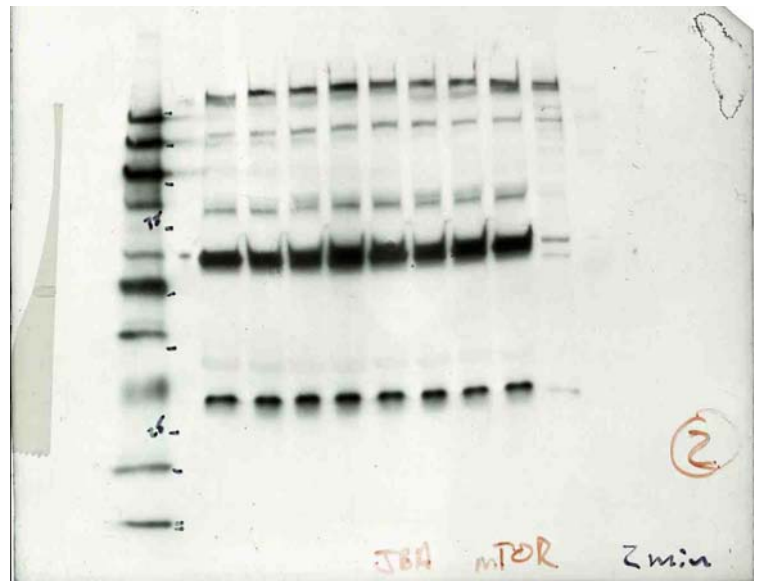
Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

Lane 11: 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

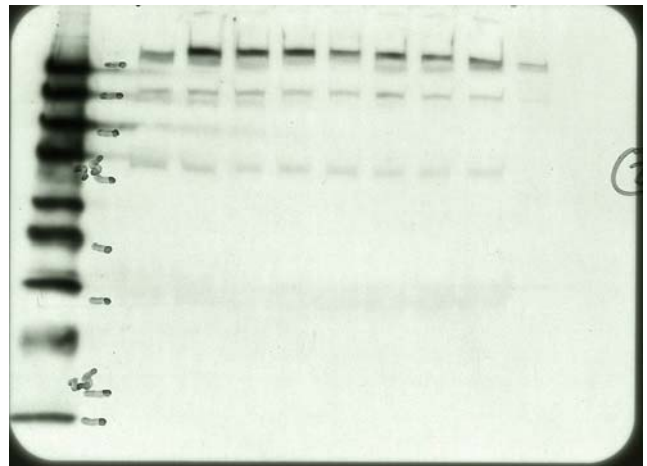
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

Lane 11: 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

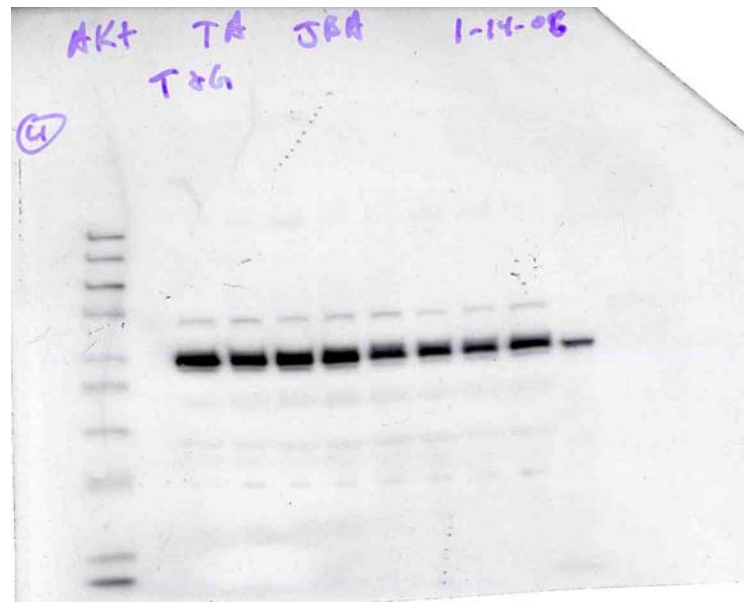
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

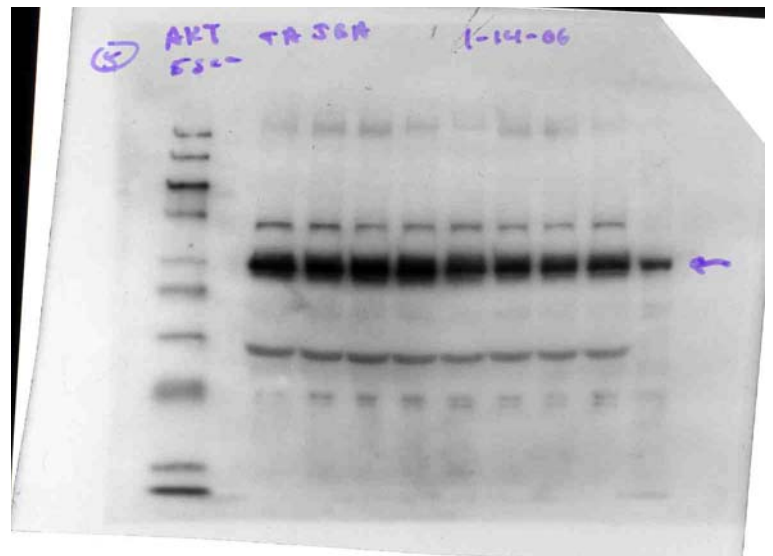
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

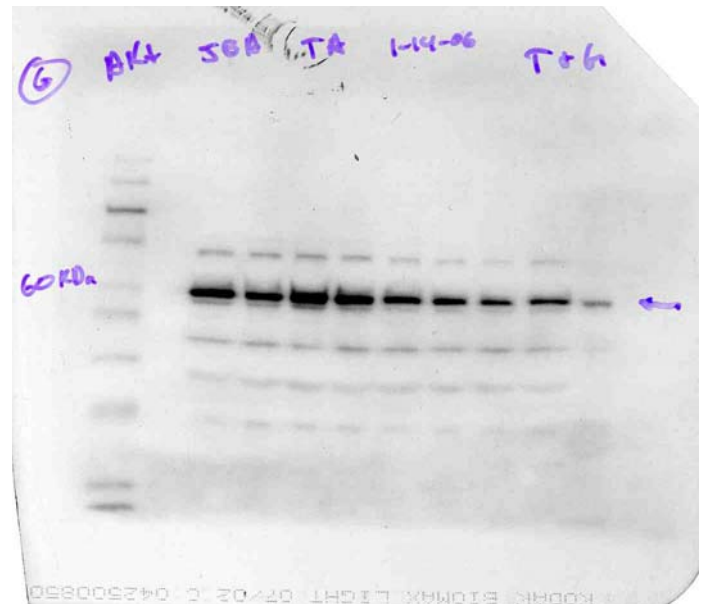
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

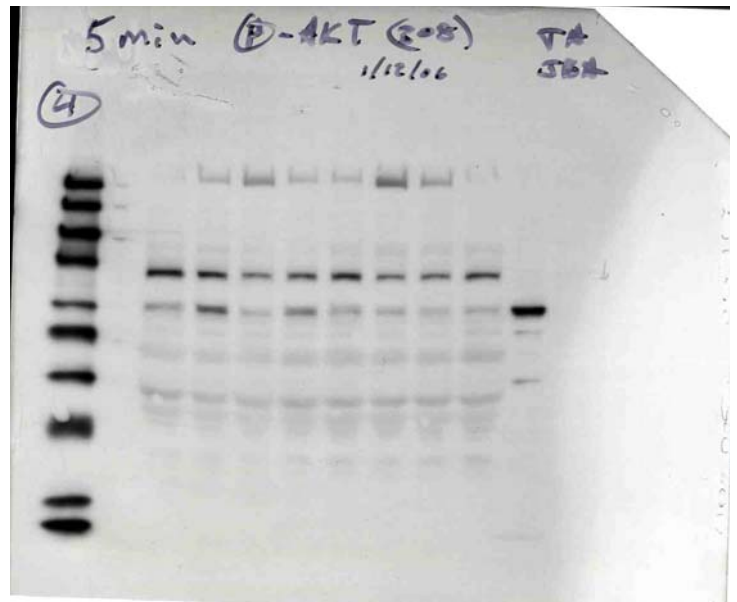
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

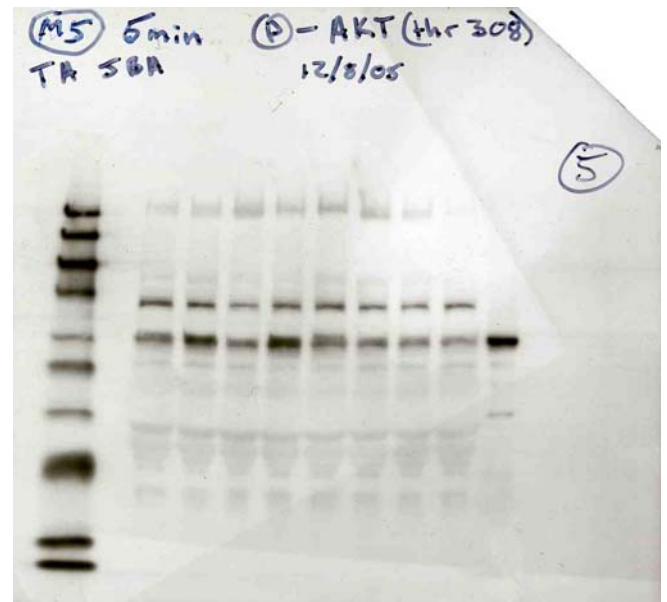
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

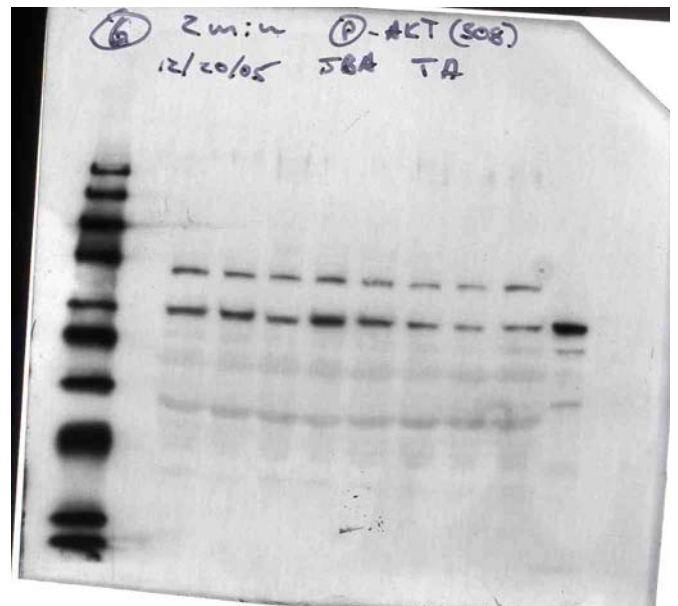
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

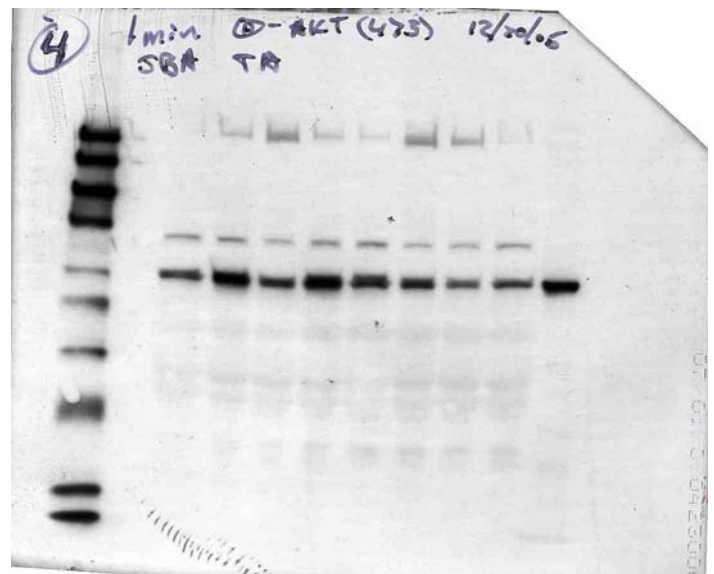
Lane 8: Obese Zucker 0 hour 20 µl

Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

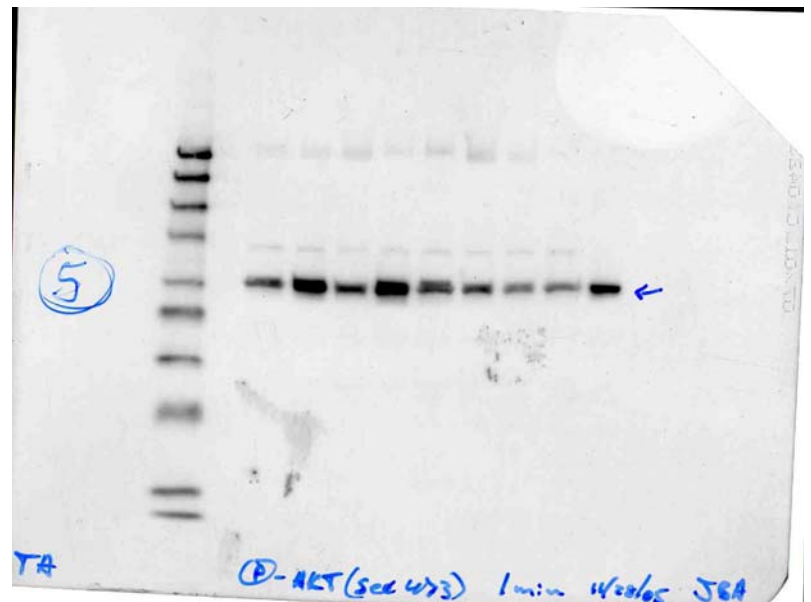
Lane 9: Obese Zucker 1 hour 20 µl

Lane 10: Obese Zucker 3 hour 20 µl

Lane 11: 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

Lane 11: 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 control	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			12.716	10.866		10.616	11.916
Standard Deviation	33	13.8	12.65	67	67	12.35	67	67
Standard Error of the mean	0.3777	0.6260	1.4110	0.3250	0.6713	0.7148	0.8542	0.4833
	12	99	28	64	17	43	05	91
	0.1689		0.6310	0.1453	0.3002	0.3196	0.3820	0.2161
	18	0.28	31	73	22	87	12	79
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
Relative Expression Level		0.9118	0.8359	0.8403	0.7180	0.8160	0.7015	0.7874
Standard error of the mean	1	94	03	08	62	79	42	45
	0.0111	0.0185	0.0416	0.0096	0.0198	0.0211	0.0252	0.0142
	62	02	98	06	38	25	43	85
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
% RE		91.189	83.590	84.030	71.806	81.607	70.154	78.744
SE	100	43	31	84	17	93	19	49
	1.1161	1.8502	4.1698	0.9606	1.9838	2.1124	2.5243	1.4284
	99	2	08	15	47	71	1	95

p-p70S6k Thr389 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese control	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		16.266	15.133		9.0166	10.733		12.033
Standard Deviation	1.3160	0.6562	2.7434	1.7674	0.7359	1.2722	1.4422	0.8710
Standard Error of the mean	0.5885	0.2934	1.2269	0.7904	0.3291	0.5689	0.6449	0.3895
	58	85	2	43	4	76	81	3
Relative Expression Level		1.3333	1.2404	1.2459	0.7390	0.8797	0.7704	0.9863
Standard error of the mean	0.0482	0.0240	0.1005	0.0647	0.0269	0.0466	0.0528	0.0319
	42	56	67	9	79	37	67	29
% RE	100	133.33	124.04	124.59	73.907	87.978	77.049	98.633
SE	4.8242	2.4056	10.056	6.4790	2.6978	4.6637	5.2867	3.1928
	42	13	72	4	71	35	26	66

p-p70S6k 421/424 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese control	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	4.5166		19.466	8.2833				12.833
Standard Deviation	67	23.15	67	33	4.55	17.95	9.25	33
Standard Error of the mean	0.7413	3.0263	2.0982	1.3526	0.5991	0.3781	3.0310	2.0597
	95	84	53	52	66	53	06	73
	0.3315	1.3534	0.9383	0.6049	0.2679	0.1691	1.3555	0.9211
	62	4	67	24	55	15	07	59
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
Relative Expression Level		5.1254	4.3099	1.8339	1.0073	3.9741	2.0479	2.8413
Standard error of the mean	1	61	63	48	8	7	7	28
	0.0734	0.2996	0.2077	0.1339	0.0593	0.0374	0.3001	0.2039
	09	55	57	32	26	43	12	47
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
% RE		512.54	430.99	183.39	100.73	397.41	204.79	284.13
SE	100	61	63	48	8	7	7	28
	7.3408	29.965	20.775	13.393	5.9325	3.7442	30.011	20.394
	55	46	66	16	88	51	23	66

mTOR data set

Total mTOR IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese control	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	14.683	14.666	14.416	11.033	11.333		11.983
Standard Deviation	67	33	67	67	33	33	10.6	33
Standard Error of the mean	1.4009	2.3970	4.1778	2.0875	2.0791	1.0519	0.6164	3.1657
	52	12	78	02	02	82	41	02
	0.6265	1.0719	1.8684	0.9335	0.9298	0.4704	0.2756	1.4157
	25	76	04	59	03	61	81	45
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
Relative Expression Level		1.3032	1.3017	1.2795	0.9792	1.0059	0.9408	1.0636
Standard error of the mean	1	54	75	86	9	17	28	09
	0.0556	0.0951	0.1658	0.0828	0.0825	0.0417	0.0244	0.1256
	09	46	35	6	27	57	69	58
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
% RE		130.32	130.17	127.95	97.928	100.59	94.082	106.36
SE	100	54	75	86	99	17	84	09
	5.5608	9.5145	16.583	8.2860	8.2526	4.1756	2.4468	12.565
	71	83	47	31	88	87	73	78

p-mTOR IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese control	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	14.816	13.083	8.25	12.2	10.083	10.616
Standard Deviation	2.6987	6.7420	3.7838	2.2631	2.7090	2.6122	1.1513	4.8350
Standard Error of the mean	0.03	0.08	0.069	0.01	0.059	0.079	0.076	0.046
	1.2068	3.0151	1.6921	1.0120	1.2115	1.1682	0.5149	2.1622
	0.97	0.17	0.98	0.94	0.28	0.47	0.11	0.98
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	1.9649	1.4178	1.2519	0.7894	1.1674	0.9649	1.0159
Standard error of the mean	0.1154	0.2885	0.1619	0.0968	0.1159	0.1117	0.0492	0.2069
	0.93	0.28	0.33	0.51	0.36	0.94	0.74	0.18
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
% RE	100	196.49	141.78	125.19	78.947	116.74	96.491	101.59
SE	11.549	28.852	16.193	9.6851	11.593	11.179	4.9273	20.691
	25	8	28	06	57	39	78	85

Akt data set

Total Akt IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese control	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	13.466	15.216	15.016	11.15	10.35	8.9666	10.416
Standard Deviation	0.5540	0.6439	1.3136	0.4167	0.7007	0.5468	0.8358	1.0571
Standard Error of the mean	0.2477	0.2879	0.5874	0.1863	0.3133	0.2445	0.3738	0.4727
	9	81	8	69	69	4	09	93
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
Relative Expression Level	1	0.8716	0.9848	0.9719	0.7216	0.6699	0.5803	0.6742
Standard error of the mean	0.0160	0.0186	0.0380	0.0120	0.0202	0.0158	0.0241	0.0306
	38	4	25	63	83	28	95	01
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
% RE	100	87.162	98.489	97.195	72.168	66.990	58.036	67.421
SE	1.6038	1.8639	3.8024	1.2062	2.0282	1.5827	2.4194	3.0601
	2	58	63	71	77	86	77	5

p-Akt 308 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese control	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	18.366	9.7833	18.266	13.216	9.8833	7.3166	9.2
Standard Deviation	1.4459	3.6175	0.9020	1.2940	1.2639	0.5706	1.3920	0.4979
Standard Error of the mean	0.6466	1.6178	0.4034	0.5787	0.5652	0.2552	0.6225	0.2227
	32	17	02	34	73	12	22	11
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
Relative Expression Level		1.3181	0.7021	1.3110	0.9485	0.7093	0.5251	0.6602
Standard error of the mean	1	0.082	0.053	0.0505	0.065	0.033	0.022	0.087
	0.0464	0.1161	0.0289	0.0415	0.0405	0.0183	0.0446	0.0159
	09	11	52	36	7	17	79	84
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
% RE		131.81	70.215	131.10	94.856	70.933	52.511	66.028
	100	82	31	05	46	01	96	71
SE	4.6409	11.611	2.8952	4.1535	4.0569	1.8316	4.4678	1.5984
	02	13	31	95	82	67	59	01

p-Akt 473 IDV Values

	Lean control	Lean 0	Lean 1	Lean 3	Obese control	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	17.683		17.266	13.466		7.2166	
Standard Deviation	67	33	12.3	67	67	10.95	67	9.1
Standard Error of the mean	1.3948	1.8584	0.7694	1.7534	0.6889	1.2373	0.9662	1.5073
	72	04	15	73	61	36	64	15
	0.6238	0.8311	0.3440	0.7841	0.3081	0.5533	0.4321	0.6740
	06	04	93	77	13	53	27	92
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
Relative Expression Level		1.4715	1.0235	1.4368	1.1206	0.9112	0.6005	0.7572
Standard error of the mean	1	67	78	93	66	34	55	82
	0.0519	0.0691	0.0286	0.0652	0.0256	0.0460	0.0359	0.0560
	12	63	35	57	4	49	61	96
	Lean control	Lean 0	Lean 1	Lean 3	Obese control	Obese 0	Obese 1	Obese 3
% RE		147.15	102.35	143.68	112.06	91.123	60.055	75.728
SE	100	67	78	93	66	44	48	16
	5.1911	6.9162	2.8634	6.5257	2.5640	4.6048	3.5960	5.6096
	69	58	65	44	43	83	6	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 oz6 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
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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.

REFERENCES

1. Wild, S., et al., *Global prevalence of diabetes: estimates for the year 2000 and projections for 2030*. *Diabetes Care*, 2004. **27**(5): p. 1047-53.
2. Broderick, T.L., P. Poirier, and M. Gillis, *Exercise training restores abnormal myocardial glucose utilization and cardiac function in diabetes*. *Diabetes Metab Res Rev*, 2005. **21**(1): p. 44-50.
3. Farrell, P.A., et al., *Hypertrophy of skeletal muscle in diabetic rats in response to chronic resistance exercise*. *J Appl Physiol*, 1999. **87**(3): p. 1075-82.
4. Wegner, J.A., et al., *Select cardiovascular and metabolic responses of diabetic rats to moderate exercise training*. *Med Sci Sports Exerc*, 1987. **19**(5): p. 497-503.
5. Baldi, J.C. and N. Snowling, *Resistance training improves glycaemic control in obese type 2 diabetic men*. *Int J Sports Med*, 2003. **24**(6): p. 419-23.
6. Eriksson, J.G., *Exercise and the treatment of type 2 diabetes mellitus. An update*. *Sports Med*, 1999. **27**(6): p. 381-91.
7. Henriksen, E.J., *Invited review: Effects of acute exercise and exercise training on insulin resistance*. *J Appl Physiol*, 2002. **93**(2): p. 788-96.
8. Holten, M.K., et al., *Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes*. *Diabetes*, 2004. **53**(2): p. 294-305.
9. Honkola, A., T. Forsen, and J. Eriksson, *Resistance training improves the metabolic profile in individuals with type 2 diabetes*. *Acta Diabetol*, 1997. **34**(4): p. 245-8.
10. Juel, C., M.K. Holten, and F. Dela, *Effects of strength training on muscle lactate release and MCT1 and MCT4 content in healthy and type 2 diabetic humans*. *J Physiol*, 2004. **556**(Pt 1): p. 297-304.
11. Tudor-Locke, C.E., R.C. Bell, and A.M. Meyers, *Revisiting the role of physical activity and exercise in the treatment of type 2 diabetes*. *Can J Appl Physiol*, 2000. **25**(6): p. 466-92.
12. Willey, K.A. and M.A. Singh, *Battling insulin resistance in elderly obese people with type 2 diabetes: bring on the heavy weights*. *Diabetes Care*, 2003. **26**(5): p. 1580-8.
13. Bruce, C.R., et al., *Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with Type 2 diabetes*. *Diabetologia*, 2004. **47**(1): p. 23-30.
14. Cauza, E., et al., *The relative benefits of endurance and strength training on the metabolic factors and muscle function of people with type 2 diabetes mellitus*. *Arch Phys Med Rehabil*, 2005. **86**(8): p. 1527-33.

15. Tokmakidis, S.P., et al., *The effects of a combined strength and aerobic exercise program on glucose control and insulin action in women with type 2 diabetes*. Eur J Appl Physiol, 2004. **92**(4-5): p. 437-42.
16. Venojarvi, M., et al., *Role of skeletal muscle-fibre type in regulation of glucose metabolism in middle-aged subjects with impaired glucose tolerance during a long-term exercise and dietary intervention*. Diabetes Obes Metab, 2005. **7**(6): p. 745-54.
17. Laurent, G.J. and D.J. Millward, *Protein turnover during skeletal muscle hypertrophy*. Fed Proc, 1980. **39**(1): p. 42-7.
18. Kimball, S.R., R.L. Horetsky, and L.S. Jefferson, *Signal transduction pathways involved in the regulation of protein synthesis by insulin in L6 myoblasts*. Am J Physiol, 1998. **274**(1 Pt 1): p. C221-8.
19. Shah, O.J., S.R. Kimball, and L.S. Jefferson, *Glucocorticoids abate p70(S6k) and eIF4E function in L6 skeletal myoblasts*. Am J Physiol Endocrinol Metab, 2000. **279**(1): p. E74-82.
20. Pullen, N. and G. Thomas, *The modular phosphorylation and activation of p70s6k*. FEBS Lett, 1997. **410**(1): p. 78-82.
21. Bodine, S.C., et al., *Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo*. Nat Cell Biol, 2001. **3**(11): p. 1014-9.
22. Baar, K. and K. Esser, *Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise*. Am J Physiol, 1999. **276**(1 Pt 1): p. C120-7.
23. Hogan, P., T. Dall, and P. Nikolov, *Economic costs of diabetes in the US in 2002*. Diabetes Care, 2003. **26**(3): p. 917-32.
24. Brown, E.J. and S.L. Schreiber, *A signaling pathway to translational control*. Cell, 1996. **86**(4): p. 517-20.
25. Chang, P.Y., et al., *Insulin stimulation of mitogen-activated protein kinase, p90rsk, and p70 S6 kinase in skeletal muscle of normal and insulin-resistant mice. Implications for the regulation of glycogen synthase*. J Biol Chem, 1995. **270**(50): p. 29928-35.
26. Dennis, P.B., et al., *The principal rapamycin-sensitive p70(s6k) phosphorylation sites, T-229 and T-389, are differentially regulated by rapamycin-insensitive kinase kinases*. Mol Cell Biol, 1996. **16**(11): p. 6242-51.
27. Weng, Q.P., et al., *Multiple independent inputs are required for activation of the p70 S6 kinase*. Mol Cell Biol, 1995. **15**(5): p. 2333-40.
28. Pearson, R.B., et al., *The principal target of rapamycin-induced p70s6k inactivation is a novel phosphorylation site within a conserved hydrophobic domain*. Embo J, 1995. **14**(21): p. 5279-87.
29. Ferrari, S., et al., *Activation of p70s6k is associated with phosphorylation of four clustered sites displaying Ser/Thr-Pro motifs*. Proc Natl Acad Sci U S A, 1992. **89**(15): p. 7282-6.
30. Pause, A., et al., *Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function*. Nature, 1994. **371**(6500): p. 762-7.

31. Hernandez, J.M., M.J. Fedele, and P.A. Farrell, *Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats*. J Appl Physiol, 2000. **88**(3): p. 1142-9.
32. Nader, G.A. and K.A. Esser, *Intracellular signaling specificity in skeletal muscle in response to different modes of exercise*. J Appl Physiol, 2001. **90**(5): p. 1936-42.
33. Koopman, R., et al., *Increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers*. Am J Physiol Endocrinol Metab, 2006. **290**(6): p. E1245-52.
34. Parkington, J.D., et al., *Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle*. J Appl Physiol, 2004. **97**(1): p. 243-8.
35. Sakamoto, K., et al., *Akt signaling in skeletal muscle: regulation by exercise and passive stretch*. Am J Physiol Endocrinol Metab, 2003. **285**(5): p. E1081-8.
36. Gingras, A.C., B. Raught, and N. Sonenberg, *Regulation of translation initiation by FRAP/mTOR*. Genes Dev, 2001. **15**(7): p. 807-26.
37. Reynolds, T.H.t., S.C. Bodine, and J.C. Lawrence, Jr., *Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load*. J Biol Chem, 2002. **277**(20): p. 17657-62.
38. Scott, P.H. and J.C. Lawrence, Jr., *Attenuation of mammalian target of rapamycin activity by increased cAMP in 3T3-L1 adipocytes*. J Biol Chem, 1998. **273**(51): p. 34496-501.
39. Sekulic, A., et al., *A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells*. Cancer Res, 2000. **60**(13): p. 3504-13.
40. Bolster, D.R., et al., *Immediate response of mammalian target of rapamycin (mTOR)-mediated signalling following acute resistance exercise in rat skeletal muscle*. J Physiol, 2003. **553**(Pt 1): p. 213-20.
41. Alessi, D.R., et al., *Mechanism of activation of protein kinase B by insulin and IGF-1*. Embo J, 1996. **15**(23): p. 6541-51.
42. Guo, S., et al., *Phosphorylation of serine 256 by protein kinase B disrupts transactivation by FKHR and mediates effects of insulin on insulin-like growth factor-binding protein-1 promoter activity through a conserved insulin response sequence*. J Biol Chem, 1999. **274**(24): p. 17184-92.
43. Proud, C.G. and R.M. Denton, *Molecular mechanisms for the control of translation by insulin*. Biochem J, 1997. **328** (Pt 2): p. 329-41.
44. Chen, W.S., et al., *Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene*. Genes Dev, 2001. **15**(17): p. 2203-8.
45. Walker, K.S., et al., *Activation of protein kinase B beta and gamma isoforms by insulin in vivo and by 3-phosphoinositide-dependent protein kinase-1 in vitro: comparison with protein kinase B alpha*. Biochem J, 1998. **331** (Pt 1): p. 299-308.
46. Williams, M.R., et al., *The role of 3-phosphoinositide-dependent protein kinase 1 in activating AGC kinases defined in embryonic stem cells*. Curr Biol, 2000. **10**(8): p. 439-48.
47. Sakaue, H., et al., *Phosphoinositide 3-kinase is required for insulin-induced but not for growth hormone- or hyperosmolarity-induced glucose uptake in 3T3-L1 adipocytes*. Mol Endocrinol, 1997. **11**(10): p. 1552-62.

48. Sable, C.L., et al., *cAMP stimulates protein kinase B in a Wortmannin-insensitive manner*. FEBS Lett, 1997. **409**(2): p. 253-7.
49. Yano, S., H. Tokumitsu, and T.R. Soderling, *Calcium promotes cell survival through CaM-K kinase activation of the protein-kinase-B pathway*. Nature, 1998. **396**(6711): p. 584-7.
50. Cho, H., et al., *Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice*. J Biol Chem, 2001. **276**(42): p. 38349-52.
51. Cho, H., et al., *Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta)*. Science, 2001. **292**(5522): p. 1728-31.
52. Lund, S., et al., *Evidence against protein kinase B as a mediator of contraction-induced glucose transport and GLUT4 translocation in rat skeletal muscle*. FEBS Lett, 1998. **425**(3): p. 472-4.
53. Sherwood, D.J., et al., *Differential regulation of MAP kinase, p70(S6K), and Akt by contraction and insulin in rat skeletal muscle*. Am J Physiol, 1999. **276**(5 Pt 1): p. E870-8.
54. Widegren, U., et al., *Divergent effects of exercise on metabolic and mitogenic signaling pathways in human skeletal muscle*. Faseb J, 1998. **12**(13): p. 1379-89.
55. Turinsky, J. and A. Damrau-Abney, *Akt kinases and 2-deoxyglucose uptake in rat skeletal muscles in vivo: study with insulin and exercise*. Am J Physiol, 1999. **276**(1 Pt 2): p. R277-82.
56. Berdanier, C.D., *Diet, autoimmunity, and insulin-dependent diabetes mellitus: a controversy*. Proc Soc Exp Biol Med, 1995. **209**(3): p. 223-30.
57. Diamond, J., *The double puzzle of diabetes*. Nature, 2003. **423**(6940): p. 599-602.
58. Zimmet, P., et al., *The metabolic syndrome: a global public health problem and a new definition*. J Atheroscler Thromb, 2005. **12**(6): p. 295-300.
59. Rees, D.A. and J.C. Alcolado, *Animal models of diabetes mellitus*. Diabet Med, 2005. **22**(4): p. 359-70.
60. Dinneen, S., J. Gerich, and R. Rizza, *Carbohydrate metabolism in non-insulin-dependent diabetes mellitus*. N Engl J Med, 1992. **327**(10): p. 707-13.
61. Kahn, B.B., *Type 2 diabetes: when insulin secretion fails to compensate for insulin resistance*. Cell, 1998. **92**(5): p. 593-6.
62. Etgen, G.J., Jr., et al., *Glucose transport and cell surface GLUT-4 protein in skeletal muscle of the obese Zucker rat*. Am J Physiol, 1996. **271**(2 Pt 1): p. E294-301.
63. King, P.A., et al., *Insulin resistance in obese Zucker rat (fa/fa) skeletal muscle is associated with a failure of glucose transporter translocation*. J Clin Invest, 1992. **90**(4): p. 1568-75.
64. Crettaz, M., et al., *Insulin resistance in soleus muscle from obese Zucker rats. Involvement of several defective sites*. Biochem J, 1980. **186**(2): p. 525-34.
65. Henriksen, E.J. and S. Jacob, *Effects of captopril on glucose transport activity in skeletal muscle of obese Zucker rats*. Metabolism, 1995. **44**(2): p. 267-72.
66. Friedman, J.E., et al., *Exercise training increases glucose transporter protein GLUT-4 in skeletal muscle of obese Zucker (fa/fa) rats*. FEBS Lett, 1990. **268**(1): p. 13-6.

67. Jacob, S., et al., *The antioxidant alpha-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle*. *Diabetes*, 1996. **45**(8): p. 1024-9.
68. Ardevol, A., et al., *Lactate-bicarbonate interrelationship during exercise and recovery in lean and obese Zucker rats*. *Int J Obes Relat Metab Disord*, 1997. **21**(5): p. 333-9.
69. Ardevol, A., et al., *Carbohydrate handling in exercising muscle of obese Zucker rats*. *Int J Obes Relat Metab Disord*, 1997. **21**(3): p. 239-49.
70. Cortez, M.Y., et al., *Insulin resistance of obese Zucker rats exercise trained at two different intensities*. *Am J Physiol*, 1991. **261**(5 Pt 1): p. E613-9.
71. Ivy, J.L., et al., *Skeletal muscle glucose transport in obese Zucker rats after exercise training*. *J Appl Physiol*, 1989. **66**(6): p. 2635-41.
72. Brozinick, J.T., Jr., et al., *Effects of exercise training on muscle GLUT-4 protein content and translocation in obese Zucker rats*. *Am J Physiol*, 1993. **265**(3 Pt 1): p. E419-27.
73. Christ, C.Y., et al., *Exercise training improves muscle insulin resistance but not insulin receptor signaling in obese Zucker rats*. *J Appl Physiol*, 2002. **92**(2): p. 736-44.
74. Treadway, J.L., et al., *Effect of exercise on insulin receptor binding and kinase activity in skeletal muscle*. *Am J Physiol*, 1989. **256**(1 Pt 1): p. E138-44.
75. Stickland, N.C., et al., *Inability of muscles in the obese mouse (ob/ob) to respond to changes in body weight and activity*. *J Anat*, 1994. **184** (Pt 3): p. 527-33.
76. Bassel-Duby, R. and E.N. Olson, *Signaling pathways in skeletal muscle remodeling*. *Annu Rev Biochem*, 2006. **75**: p. 19-37.
77. Parkington, J.D., et al., *Differential activation of mTOR signaling by contractile activity in skeletal muscle*. *Am J Physiol Regul Integr Comp Physiol*, 2003. **285**(5): p. R1086-90.
78. Geiger, P.C., et al., *Activation of p38 MAP kinase enhances sensitivity of muscle glucose transport to insulin*. *Am J Physiol Endocrinol Metab*, 2005. **288**(4): p. E782-8.
79. Martineau, L.C. and P.F. Gardiner, *Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension*. *J Appl Physiol*, 2001. **91**(2): p. 693-702.
80. Somwar, R., et al., *Activation of p38 mitogen-activated protein kinase alpha and beta by insulin and contraction in rat skeletal muscle: potential role in the stimulation of glucose transport*. *Diabetes*, 2000. **49**(11): p. 1794-800.
81. Wretman, C., et al., *Effects of concentric and eccentric contractions on phosphorylation of MAPK(erk1/2) and MAPK(p38) in isolated rat skeletal muscle*. *J Physiol*, 2001. **535**(Pt 1): p. 155-64.
82. Mylabathula, D.B., et al., *Age-associated changes in MAPK activation in fast- and slow-twitch skeletal muscle of the F344/NNiaHSD X Brown Norway/BiNia rat model*. *Exp Gerontol*, 2006. **41**(2): p. 205-14.
83. Ionescu, E., J.F. Sauter, and B. Jeanrenaud, *Abnormal oral glucose tolerance in genetically obese (fa/fa) rats*. *Am J Physiol*, 1985. **248**(5 Pt 1): p. E500-6.
84. Pearson, R.B. and G. Thomas, *Regulation of p70s6k/p85s6k and its role in the cell cycle*. *Prog Cell Cycle Res*, 1995. **1**: p. 21-32.

85. Dufner, A. and G. Thomas, *Ribosomal S6 kinase signaling and the control of translation*. Exp Cell Res, 1999. **253**(1): p. 100-9.
86. Jefferies, H.B., et al., *Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k*. Embo J, 1997. **16**(12): p. 3693-704.
87. Coutant, A., et al., *PI3K-FRAP/mTOR pathway is critical for hepatocyte proliferation whereas MEK/ERK supports both proliferation and survival*. Hepatology, 2002. **36**(5): p. 1079-88.
88. Mourani, P.M., et al., *Unique, highly proliferative growth phenotype expressed by embryonic and neointimal smooth muscle cells is driven by constitutive Akt, mTOR, and p70S6K signaling and is actively repressed by PTEN*. Circulation, 2004. **109**(10): p. 1299-306.
89. Wu, X., et al., *Insulin promotes rat retinal neuronal cell survival in a p70S6K-dependent manner*. J Biol Chem, 2004. **279**(10): p. 9167-75.
90. Hei, Y.J., et al., *Skeletal muscle mitogen-activated protein kinases and ribosomal S6 kinases. Suppression in chronic diabetic rats and reversal by vanadium*. Diabetes, 1995. **44**(10): p. 1147-55.
91. Fluckey, J.D., et al., *Insulin stimulation of muscle protein synthesis in obese Zucker rats is not via a rapamycin-sensitive pathway*. Am J Physiol Endocrinol Metab, 2000. **279**(1): p. E182-7.
92. Markuns, J.F., et al., *Effects of streptozocin-induced diabetes and islet cell transplantation on insulin signaling in rat skeletal muscle*. Endocrinology, 1999. **140**(1): p. 106-11.
93. Vanhaesebroeck, B. and D.R. Alessi, *The PI3K-PDK1 connection: more than just a road to PKB*. Biochem J, 2000. **346 Pt 3**: p. 561-76.
94. Sakamoto, K., et al., *Contraction regulation of Akt in rat skeletal muscle*. J Biol Chem, 2002. **277**(14): p. 11910-7.
95. Shaw, M., P. Cohen, and D.R. Alessi, *The activation of protein kinase B by H2O2 or heat shock is mediated by phosphoinositide 3-kinase and not by mitogen-activated protein kinase-activated protein kinase-2*. Biochem J, 1998. **336 (Pt 1)**: p. 241-6.
96. Gosmanov, A.R., et al., *Impaired expression and insulin-stimulated phosphorylation of Akt-2 in muscle of obese patients with atypical diabetes*. Am J Physiol Endocrinol Metab, 2004. **287**(1): p. E8-E15.
97. Oku, A., et al., *Inhibitory effect of hyperglycemia on insulin-induced Akt/protein kinase B activation in skeletal muscle*. Am J Physiol Endocrinol Metab, 2001. **280**(5): p. E816-24.
98. Melian, E., et al., *Tissue-specific response of IGF-I mRNA expression to obesity-associated GH decline in the male Zucker fatty rat*. J Endocrinol, 1999. **160**(1): p. 49-56.
99. Valerio, A., et al., *TNF-alpha downregulates eNOS expression and mitochondrial biogenesis in fat and muscle of obese rodents*. J Clin Invest, 2006. **116**(10): p. 2791-8.
100. Oberbach, A., et al., *Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes*. Diabetes Care, 2006. **29**(4): p. 895-900.

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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. Rice¹,D.H.Desai²,Sunil K.Kakarla¹ , Anjaiah Katta¹ 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/BNia rat.*** Shinichi Asano¹, Kevin M. Rice¹, Sunil K. Karkarla¹, Anjaiah Katta¹ and Eric R Blough^{1,2}: ¹Department 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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- ***High force contraction-induced mitogen activated protein kinase (MAPK) signaling in the rat skeletal muscle is altered in diabetes.*** Anjaiah Katta¹, Sunil K. Kakarla¹, and ^{1,2}Eric R Blough: ¹ Department of Biological Sciences, Marshall University, ² Department of Physiology, Marshall University, Joan C. Edwards School of Medicine (In review).
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- ***Altered dystrophin-glycoprotein complex composition in the aging rat heart.*** Sunil K. Karkarla¹, Anjaiah Katta¹, Kevin M. Rice¹, Devashich H. Desai², and Eric R Blough^{1,2}: Department of Biological Sciences, Marshall University, ² Department of Physiology, Marshall University, Joan C. Edwards School of Medicine (In preparation)
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