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Effect of Empagliflozin on insulin sensitivity in the lean and obese Zucker rat: a model of metabolic syndrome

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EFFECT OF EMPAGLIFLOZIN ON INSULIN SENSITIVITY IN THE LEAN AND OBESE ZUCKER RAT
A MODEL OF METABOLIC SYNDROME

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APPROVAL OF THESIS

We, the faculty supervising the work of Veda Gayatri Sushma Penta, affirm that the thesis *Effect of Empagliflozin on insulin sensitivity in the lean and obese Zucker rat – A model of metabolic syndrome*, meets the high academic standards for original scholarship and creative work established by the masters in Biological Sciences and the college of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

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ABSTRACT

Metabolic syndrome is one of the fastest growing health problems in the world. The medical costs associated with treating this disorder are staggering. Allowed to proceed untreated, metabolic syndrome can lead to a markedly decreased quality of life and a variety of medical conditions including heart and kidney failure. Whether the sodium glucose co-transporter-2 (SGLT-2) inhibitor Empagliflozin can be used to prevent the development of metabolic syndrome is not well understood. This proposal is specifically designed to address this gap in our knowledge. The expected outcomes of this work will identify the time course and degree of interrelatedness between changes in insulin sensitivity / obesity, alterations in expression of the mitogen activated protein kinases (MAPK), and the effects of Empagliflozin treatment on these parameters in the fast twitch extensor digitorium longus (EDL) and the slow-twitch soleus muscles in lean and obese Zucker rats. Male five-week-old lean and obese Zucker rats were randomly assigned to one of the four groups- lean control, lean treated, obese control, and obese treated. Animals were treated with either Empagliflozin (10 mg/kg BW / day) or placebo for 25 weeks. Compared to that seen in the obese controls, Empagliflozin treatment in the obese animals was associated with decreased body weight and improvements in glucose tolerance. Empagliflozin treatment did not appear to affect EDL or soleus muscle weight or the expression of ERK1/2-, p38- or JNK-MAPK. Taken together, these data suggest that the long-term use of Empagliflozin in diabetic obese Zucker rats does not appear to affect the expression / activation of MAPK proteins in the EDL and soleus.

CHAPTER 1

INTRODUCTION

The major health problems worldwide, obesity and Type - 2 diabetes mellitus, are considered to be closely related [1-6]. Type - 2 diabetes is one component within a group of disorders called the metabolic syndrome. More than 171 million people worldwide are estimated to have diabetes and this number is expected to increase to 366 million by 2030 [7]. High levels of blood glucose resulting from defects in insulin production, insulin action, or both are characteristics of a serious lifelong condition known as diabetes mellitus (DM) which is a complication of metabolic syndrome [8].

Metabolic syndrome, also known as Syndrome X or insulin resistance syndrome, is one of the fastest growing health problems in the United States. The incidence of metabolic syndrome is rapidly growing worldwide, and it is estimated that more than one out of every three US adults suffers from this disorder [9, 10]. The price of treating this disorder is staggering (>$150 billion per year) and is compounded by the fact that metabolic syndrome is a primary risk factor for the development of cardiovascular disease [11, 12]. The molecular mechanism(s) by which metabolic syndrome increases the risk of developing cardiovascular disease is not well understood.

The obese Zucker rat (fa/fa) exhibits hyperlipidemia, hyperglycemia and hyperinsulinemia with abdominal adiposity. The genetically obese Zucker (fa/fa) rat is usually considered the most appropriate model for early diabetes related studies as individuals that suffer from Type - 2 diabetes usually exhibit attributes of metabolic syndrome [13]. Mitogen activated protein kinases (MAPKs) are involved in a myriad of protein signaling cascades, which are initiated in direct cellular response to changes in various stimuli. These cascades help the cell regulate various cellular functions including, but not limited to cell differentiation, mitosis, proliferation, gene expression and apoptosis. Several articles have suggested that diabetic and normal rat muscle differ in the

expression and regulation of MAPK proteins [14-19]. These differences in muscle response between diabetic and non-diabetic muscle are not fully understood but may provide insight into why glucose regulation may differ with diabetes. The changes in the phenotype of diabetic muscle due to Empagliflozin treatment have not been studied. Whether treatment of Type - 2 diabetes pharmacologically alters signal transduction processes in muscle is unknown, but the existence of differences, if present, may help to provide new targets for drug therapies to better regulate blood glucose levels in diabetic populations.

Purpose

Jardiance is a highly potent and specific oral antihyperglycemic drug that inhibits the sodium glucose co-transporter-2 (SGLT-2). This molecule has been shown to be an effective inhibitor of renal glucose reabsorption as it promotes urinary glucose excretion. Empagliflozin has been shown to be quite effective in lowering blood glucose in animals and humans and is currently undergoing phase III clinical trials for the treatment of Type - 2 diabetes [20].

Our long – term goal is to investigate whether Empagliflozin can be used as a therapeutic agent for the prevention of metabolic syndrome. The purpose of this study was to determine whether prolonged Empagliflozin treatment in obese Zucker rats is associated with alterations in insulin sensitivity and glucose related signaling in skeletal muscle. We hypothesized that Empagliflozin treatment would be associated with differences in the regulation of the MAPK in the skeletal muscles. To test this hypothesis, insulin resistance and the expression of MAPK proteins from 10, 20 and 30-week-old muscle tissue of obese Zucker rats were assessed.

Specific Aims

The economic burden of metabolic syndrome is estimated to be >$150 billion [21]. Empagliflozin is effective in lowering blood glucose in animals [22]. However, there have been no

studies which have examined the therapeutic effect of Empagliflozin for the prevention of metabolic syndrome.

The objectives of this study were to: (1.) Determine the effects of Empagliflozin on glucose regulation in the genetically obese Zucker rat (Leprfa/fa), and (2.) Determine the effects of Empagliflozin treatment on the expression of proteins that are involved in regulation of glucose uptake into muscle. To test this hypothesis and accomplish the objective of this study, the following specific aims are proposed:

Specific Aim #1: 1. To determine the effect of Empagliflozin treatment on the time course change in insulin sensitivity and glucose metabolism in the obese Zucker rat. Untreated and Empagliflozin treated obese Zucker rats will be subjected to glucose tolerance tests (conducted at 5, 10, 20, and 30 weeks of age) to determine how Empagliflozin intervention might affect the regulation of blood glucose levels during the early, middle and late stages of metabolic syndrome progression.

Hypothesis: Empagliflozin treatment will be associated with improvements in insulin sensitivity and glucose metabolism.

Specific Aim # 2: 2. To investigate the effects of Empagliflozin treatment on the expression of muscle mitogen activated protein kinase expression. To determine whether Empagliflozin treatment can affect the expression of the MAPKs, skeletal muscles from 30-week-old lean and obese Zucker rats will be subjected to SDS-PAGE and immunoblotting.

Hypothesis: Metabolic syndrome will be associated with alterations in the expression and basal phosphorylation level of MAPKs in the extensor digitalis and soleus muscles.

The expected outcomes of this work will: Identify the time course and degree of interrelatedness between treatment-associated changes in insulin sensitivity and alterations in the expression / regulation of muscle MAPK proteins during the progression of metabolic syndrome in the obese Zucker rat.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

This chapter frames on the review of literature related to this study. The following topics pertaining to this study will be discussed: 1) Type - 1 vs. Type - 2 diabetes vs metabolic syndrome, 2) animal models of diabetes, 3) role of skeletal muscles in diabetes, 4) molecular regulators of insulin signaling, and 5) SGLT2 drugs used to treat diabetes.

Diabetes And Metabolic Syndrome

Diabetes mellitus (DM) is a chronic disease that is prevalent in the United States (US) and throughout the World. DM is associated with elevated glucose levels and impaired insulin regulation. The prevalence of diabetes globally rose from 4.7% in 1980 to 8.5 % in 2014 [23]. The rising number of adults affected by DM is a pressing concern. According to World Health Organization, about 2.2 million deaths were caused in 2012 due to high blood glucose. Worldwide, nearly 422 million people had diabetes in 2014 [23]. In the United States alone, 9.1% of the population in 2016 suffered from diabetes, making diabetes the seventh most prevalent disease in the US [24].

There are two forms of diabetes: Type - 1 and Type - 2. Type - 1 diabetes is an autoimmune disease in which the β-cells in the islets of Langerhans are destroyed resulting in a deficient or absent insulin production. There is evidence to support the hypothesis that Type - 1 diabetes may be hereditary [25]. This disorder is normally present in early childhood, although there is data to support the possibility that an autoimmune response can initiate the development of Type - 1 diabetes in adults. This form of diabetes is also called juvenile/ childhood-onset diabetes or insulin dependent DM. About 5-10% of all the diabetic population are Type - 1 DM. Immunomodulation

and insulin therapy are the primary management strategies used for the treatment of Type - 1 DM [26].

Type – 2 diabetes was formerly called “adult-onset diabetes” and is usually seen in obese and physically inactive people. It is the most common type of diabetes [27-29]. Type-2 DM is a long-term metabolic disorder where the body is ineffective in insulin usage for energy production. In this disorder, the pancreas secretes insulin however; the cells in the body become insensitive to insulin leading to an increase in insulin secretion by the pancreas. Over time, the body’s cells become more resistant to insulin, which leads to hyperglycemia and the hypersecretion of insulin which can lead to pancreatic dysfunction [30].

A pathophysiological condition known as metabolic syndrome has emerged as a risk factor for the development of Type - 2 diabetes. Metabolic syndrome, also called syndrome X, is a cluster of symptoms that increase the risk of cardiovascular diseases and diabetes [31]. Metabolic syndrome is not really a disease, but rather a constellation of clinical findings mainly characterized by insulin resistance and obesity [32]. The prevalence of metabolic syndrome in the adults of the United States was 34.7% in 2011-2012 [33]. The causes of metabolic syndrome are not fully understood but believed to be the result of an underlying disorder of energy storage and utilization [34]. Metabolic syndrome conveys an increased risk of mortality among susceptible populations. Alleviating insulin resistance may be a prophylactic measure to combat the symptoms associated with metabolic syndrome [35].

Animal Models of Diabetes

For diabetic drug discovery, rats or mice are the most widely used models. Humans and rats are omnivores, have a similar neuroanatomy and share many of the different molecular pathways

which regulate food intake and energy homeostasis [36]. In addition, the lifespan of rats is rather short making them good models to examine the long-term effects of intervention.

Within Type - 1 diabetic research, there are four induction methods to produce a Type - 1 diabetic rodent model: chemical induction, spontaneous autoimmunity, genetically induced, and virally induced [36].

The chemical induction mechanism is achieved by administering a high dose of streptozotocin [37, 38], a dose of alloxan [39], or multiple low doses of streptozotocin [40]. These drugs destroy a high percentage of the endogenous beta cells resulting in little endogenous insulin production. One disadvantage to the model is the potential for chemical toxicity in other organs of the body [41].

The spontaneous autoimmunity models are currently limited to three rodent models: the non-diabetic (NOD) mouse, the Biobreeding (BB) rat and the LEW.1AR1/Ztm-iddm rat. There are several limitations regarding these models. The onset of diabetes in the NOD mouse model usually occurs at 10-14 weeks but in some instances, diabetes is not seen until week 30. In addition, the females have higher incidence of diabetes than the males [42-51]. The BB rat autoimmune model develops lymphopenia, which is not characteristic of human Type - 1 diabetes [52-56]. The LEW.1AR1/iddm rats, unlike the NOD and BB model, do not exhibit other autoimmune diseases, however limited research had been conducted on this model [57-63].

The genetically induced Type - 1 diabetic models consist of one mouse strain, the AKITA mouse. This mouse has a spontaneous mutation in the insulin 2 gene resulting in misfolded pro-insulin and endoplasmic reticulum stress. This model requires insulin treatment starting at 3-4 weeks of age and if untreated will rarely survive longer than 12 weeks [64-67].

Virus induced models of Type - 1 diabetes have been investigated in a number of studies [68-78]. However, the induction of Type - 1 diabetes through viral exposure is complicated and

the outcome is dependent on a number of variables including timing of infection and viral replication levels [79, 80].

Within the Type - 2 diabetic rodent models, there are four induction mechanisms which have been employed to produce rodent models of Type - 2 diabetes [36]: monogenic obese models [36], polygenic obese models [81], induced obesity [82, 83], and non-obese models [84].

Type - 2 diabetic monogenic obese models include a number of rodent strains, including: the $Lep^{ob/ob}$ mouse [85], the $Lepr^{db/db}$ mouse [86], the Zucker fatty rats and Zucker diabetic fatty (ZDF) rats [87]. Among these models, the ZDF rat is a robust model for Type – 2 diabetes [88]. As seen in humans, diabetic progression in ZDF rat presents with typical signs of insulin resistance including hyperinsulinemia, hypertension, and dyslipidemia [89]. These rats also exhibit impaired glucose oxidation, glycogen synthesis and glycolysis similar to that seen in human [90]. ZDF rats are genetically prone to obesity because of recessive mutation in leptin receptor gene [90]. These rats are characterized by modifications to the insulin-signaling pathway, especially in the skeletal muscles and have reduced expression of the insulin receptor substrate (IRS). IRS expression is required for insulin-stimulated tyrosine phosphorylation and Phosphatidylinositol (PI) 2 kinase activity. In obese rats, insulin-mediated AKT and extracellular signal-regulated kinase (ERK 1/2) - MAPK activity are reduced [91].

The rodent polygenic obese models of Type - 2 diabetes include the KK mice [92], the Otsuka Long-Evans Tokushima Fat rat (OLETF) [93], the New Zealand Obese (NZO) mice [94], the TallyHo/Jng mice [95], and the NoncNZO10/LtJ mice [81]. Of these models, many have not been fully characterized [81, 95-104]. In addition, it should also be noted that the male sex bias is greater in these models, as far more females than males display the Type-2 diabetes phenotype [81].

The Type - 2 diabetic rodent models for induced obesity involves feeding the rodents a high fat diet [82, 83]. This induction method requires constant monitoring of feed intake to insure the

rodents do not reduce their food intake to compensate for increase caloric intake. It is also important to note that there is a heterogeneity to the response of the high fat diet even among pure-breed strains indicating that the variance in response may not be purely genetic [105-109].

The Type - 2 diabetic rodent non-obese models consist of the Goto–Kakizaki (GK) rats [110] and the human islet amyloid polypeptide expressing (hIAPP) mice [111]. These models do not develop obesity but are potential models for beta cell inadequacies [84].

Role Of Skeletal Muscles In Diabetes

Skeletal muscle plays a fundamental role in the homeostasis and regulation of glucose and carbohydrate metabolism for the whole-body. Muscular activity is thought to regulate, at least in part, glucose transport in skeletal muscle [112]. The primary tissue for glucose disposal and utilization is the skeletal muscle, making it the major site for peripheral insulin resistance [113]. In human skeletal muscle, glucose transport is mediated by the insulin-sensitive glucose transporter 4 (GLUT4) in a rate limiting manner. It is thought that two distinct and separate signaling pathways mediate the rate limited uptake of glucose, one stimulated by muscle contraction and the second stimulated by insulin [113].

Skeletal muscle mitochondrial activity is the primary site of glucose metabolism and disposal. In Type - 2 diabetes, findings of reduced oxidative enzymes levels have been reported in skeletal muscle suggesting evidence of reduced oxidative capacity [114-117]. Additional studies have also reported oxidative enzyme and glycolytic enzyme activity mismatches [116, 118]. The earliest hallmark of Type - 2 diabetes is skeletal muscle insulin resistance [119]. Electron microscopy has also shown that Type - 2 diabetic patients have altered skeletal muscle mitochondrial morphology and reduced mitochondrial size. These mitochondria also show a 40% reduction in citrate synthase and rotenone-sensitive $NADH_2$ activity [115, 120]. Type - 2 diabetic

patients are reported to have a decrease in the expression of genes involved in oxidative phosphorylation [121-123]. Magnetic resonance studies have demonstrated a reduction in inorganic phosphate/phosphocreatine ratio and fatty acid oxidation and excess intramuscular lipid accumulation which may lead to mitochondrial dysfunction in Type - 2 diabetes [120]. It may be suggested from these studies that Type - 2 diabetes is associated with mitochondrial dysfunction. Nonetheless, to date, no direct measurements in intact cells from Type - 2 diabetic humans have indicated a change in mitochondrial O_2 flux capacity [124].

In addition to changes associated with Type - 2 diabetes, obesity can cause changes in skeletal muscles that increase the risk of developing Type – 2 diabetes. These changes include mitochondrial dysfunction, muscular atrophy and slow to fast fiber transformation [125]. Increased fatty acid levels have been shown to be due to decreased fatty acid oxidation, which can lead to insulin resistance in skeletal muscles. Obesity can also cause impaired glucose uptake [126].

Muscle atrophy is a physiological condition associated with an imbalance in protein synthesis and degradation. The pathways leading to these changes are still an area of great research with recent studies suggesting that several different signaling molecules may be involved including: MAPK, I/IGF-1, myostatin, leptin, IL-6, IL-10, TNF-alpha, AGE/RAGE, glucocorticoids, angiotensin II, growth hormone, testosterone and estrogen just to name a few [127]. Decrease in the mass of the muscle, may lead to further complications as diabetes advances [128].

Fast And Slow Twitch Muscles

It is thought that skeletal muscle is the primary site of glucose disposal. Skeletal muscle has two primary functions: movement and maintenance of posture. Energetic and mechanical properties of muscle are defined by a muscle's fiber type. Slow twitch muscles are involved primarily in the maintenance of posture, therefore, have a relatively low rate of ATP consumption during isometric

contraction and low rates of unloaded shortening, relaxation, and force development [129]. In contrast, fast twitch muscle fibers consume ATP at a rate seven fold higher than postural muscles during isometric contraction, exhibit rapid contraction kinetics, and are primarily involved in dynamic activity [129]. Many molecular and anatomical differences exist between fast and slow muscle fiber types including citrate synthase (CS) activity capillary density (CD), and myosin heavy chain (MHC) isoform composition [130].

Within the rodent models, the extensor digitorum longus (EDL) is a predominantly fast-twitch glycolytic muscle of hind limb. The EDL is made of oxidative as well as glycolytic muscle fibers [128]. The EDL contains 53% Type - IIa, 42% Type - IIb and 5% Type - I fibers. The soleus is a slow-twitch oxidative muscle, which contains predominately of 77% of Type - I fibers, 18 % of Type - IIa fibers and 5% of IIb fibers [131].

Type - 2 diabetes has been shown to cause a decreased fiber area in both fast (EDL) and slow twitch (soleus) muscles, as well as, a decrease in muscle weights [130]. This atrophy may be due to excess reactive oxygen species (ROS) that can cause increased muscle membrane fragility. Increased fiber damage, reduced amino acid uptake, and diminished protein synthesis rates have also been seen in the muscles obtained from diabetic models [132]. Insulin resistance promotes protein catabolism due to impaired insulin signaling and leads to decreased muscle mass. The obese Zucker (ZDF) rat shows lesser muscle mass compared to the lean rat, making it an ideal research model to investigate Type - 2 diabetic changes in skeletal muscle [133].

Molecular Regulators: MAPK Proteins: ERK 1/2, p38, JNK

Alterations in the MAPK pathway are thought to occur early in the diabetic progression with insulin resistance [134]. When microarray data of the diabetic myocytes was compared with

control gene sets, there was an upregulation in inflammatory gene expression. How diabetes may affect MAPK pathway signaling is not well understood.

It is thought that ERK1/2 phosphorylation levels are similar in the muscle tissues of Type - 2 diabetes versus that seen in control patients both in the presence and absence of insulin. The ERK1/2- MAPK signaling controls the pathophysiological conditions responsible for muscle wasting such as muscle degeneration, aging and obesity [135]. ERK1/2 is a mitogen-activated protein kinase that is activated by interferon regulatory factor (IRF-1) in high glucose levels [136]. Besides IRF-1, increased intracellular ROS and high glucose levels also stimulate activation of ERK1/2 [137].

The p38-MAPK is thought to play a functional role in myogenic differentiation. The activation of p38 increases during muscle contractions and is also thought to act as the key factor regulating cytokine gene expression [138, 139]. Supporting this contention, the basal phosphorylation level of p38-MAPK is increased in muscles of Type-2 diabetes and obese patients when compared to controls. Conversely, the insulin-stimulated p38 activity is elevated in controls but decreased in T2DM patients [134].

Insulin also phosphorylates and activates the stress-activated kinase JNK-MAPK. The phosphorylation level of JNK1 is increased in muscle with Type-2 diabetes when compared to control. [134].

SGLT2 Inhibitors Used To Treat Diabetes

The use of pharmacological interventions in the treatment of diabetes has been a focus of pharmaceutical companies for decades [140]. One emerging class of drugs is the inhibitors of the sodium-glucose co-transporter Type - 2 (iSGLT-2), consisting of Canagliflozin, Dapagliflozin and Empagliflozin. The mechanism of SGLT2 inhibitors is unique to this class of drugs. This inhibition

occurs at the proximal convoluted tubule and involves the reversible and selective inhibition of the sodium-glucose co-transporter Type – 2 [141]. At the renal level, a reduction in blood glucose and an increase in glucose elimination in the urine occurs due to a reduction in the reabsorption of glucose. Caloric loss and osmotic diuresis occur in response to the increased elimination of renal glucose. This reduction in blood glucose and corresponding caloric loss results in a decrease in weight [142].

SGLT2 inhibitors act in a β-cell independent manner. One advantage of using SGLT2 inhibitor drugs is the fact it can be used with other glucose-lowering drugs, as it does not convey a risk of hypoglycemia when used except when combined with insulin or its secretagogue. Currently, Empagliflozin is one of the three SGLT2 inhibitors proved to be effective in phase 3 trials in United States. In addition Empagliflozin has proven to lower cardiovascular risk, lower blood pressure and reduce body weight [143].

Summary

Diabetes mellitus is a complex disease that is of concern to the whole world. Understanding the pathophysiology and progression of diabetes, as well as, potential risk factors such as metabolic syndrome will provide insight into early intervention and treatment for this debilitating and deadly disease. The use of animal models such as the obese Zucker fatty (fa/fa) rat provides an appropriate tool to study metabolic syndrome and the progression of Type - 2 diabetes from pre-diabetes, early onset, to full blown Type - 2 diabetes. Utilizing this model, we will investigate the use of Empagliflozin, a newer class of SGLT2 inhibitor drugs, on the progression of metabolic syndrome in the obese Zucker fatty (fa/fa) rat.

CHAPTER 3

MATERIALS AND METHODS

Animals

Four-week-old male lean Zucker (strain code 186) and obese Zucker rats (strain code 185) were purchased from the Charles River Laboratories (Wilmington, MA, USA) and housed two per cage in an AAALAC approved vivarium. Housing conditions consisted of a 12H: 12H dark-light cycle and the temperature were maintained at 22 ±2˚C. Animals were provided with food and water *ad libitum* and allowed to acclimatize for one week before any experiments were conducted. Animals were assigned at random to one of four different groups- lean control (LC), lean treated (LT), obese control (OC), and obese treated (OT) accordingly. The treated animals received Empagliflozin (10 mg/kg body weight/day) that was kindly supplied by Boehringer Ingelheim (KG, Germany) in drinking water for 25 weeks. Food consumption and body weights were measured once per week throughout the study duration. All procedures were performed in accordance with Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and Institutional Animal Care and Use Committee (IACUC) of Marshall University.

Materials

p38, phosphorylated Thr 182/ Tyr 180 p38, ERK1/2, phosphorylated Thr202/Tyr204 ERK1/2, pJNK, phosphorylated Thr183/ Tyr 185 pJNK 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).

Intra-Peritoneal Glucose Tolerance Test (IPGTT)

Animals were fasted overnight before performing the IPGTT test. In addition, Empagliflozin was removed from drinking water ~28-32 hours before performing the test to accurately determine the long-term effects of the drug on glucose handling. The time period for fasting was chosen based on the previous studies that have reported the half-life of Empagliflozin in rodents to be approximately 12 hours [144].

IPGTT was performed by single intraperitoneal injection of glucose at the rate of 1.5g / kg body weight. Blood glucose levels were measured using Bayer Contour Next Ez blood glucose monitoring system (Ascensia, NJ, USA) at baseline and after 15, 30, 60, 90 and 120 minutes of glucose administration.

Serum Biochemical Analysis And Multiplex Assay

Animals were humanely sacrificed using inhalant isoflurane anesthesia at the chosen time point and blood was collected through the cardiac puncture into a BD Vacutainer® tube. Serum was collected by centrifugation of the tubes at 1000 x g for 10 minutes. Biochemical parameters were determined by using Abaxis VetScan® analyzer (Abaxis, Union City, CA, USA) as described previously [145]. Milliplex® multiplex assays were performed to evaluate the changes in inflammatory markers in serum and urine samples according to manufacturer's instructions (EMD Millipore, MA, USA).

Blood And Skeletal Muscle Collection

EDL and soleus muscles were harvested from both the hind limbs. Once excised, muscles were blotted dry, trimmed of visible fat and tendon projections, weighed, immediately frozen in liquid nitrogen, and stored at -80° C.

Skeletal Muscle Sectioning And Staining

The frozen EDL and soleus muscles were sectioned (10 μm) using a cryo-microtome. The sections were preserved at -80 ^{0} C until used and stained using Hematoxylin & Eosin kit according to manufacturer's instructions. The sections were viewed using an Evos® microscope (Thermo Fisher Scientific, Massachusetts, USA) at 20 X magnification and digital images were recorded. The muscle fiber size was compared between normal and Empagliflozin treated muscle (N = 6 animals per group, 3 images per animal).

Preparation Of Protein Isolates

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 2000x g to pellet particulate matter. This process was repeated twice, and the supernatants combined for protein concentration determination using the Bradford method (Pierce, Rockford, IL). Samples were diluted to a concentration of 3 μg/ μl in SDS loading buffer and boiled for 5 minutes.

SDS-PAGE And Immunoblotting

Samples (60mg of protein) were equally diluted with 4x Laemilli buffer and loaded onto 10% PAGEr Gold Precast gels (Lonza, Rockland, ME), subjected to SDS-PAGE, and then transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in TBST for 1 h and later probed with respective primary antibodies (Cell Signaling Technology, Danvers, MA). Membranes were washed with TBST (3 x 5 min) and incubated with secondary anti-rabbit (Cell Signaling Technology, Danvers, MA) for 1 h. Immunoreactivity was obtained using Supersignal West Pico Chemiluminescent substrate (Pierce, Rockford, IL, USA). Exposure time was adjusted 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

Data were analyzed using the SigmaPlot v. 12 program (Systat Software Inc., San Jose, CA) and the results are presented as mean ± SEM. The required sample size was calculated using the resource equation method. A one-way analysis of variance (ANOVA) or two-way repeated measures ANOVA was performed for overall comparisons followed by the appropriate *post hoc* test to determine significant differences between groups. For non-normally distributed samples, a Kruskal Wallis h test was performed. A p-value of ≤ 0.05 was considered to be statistically significant.

CHAPTER 4

RESULTS

Effect Of Long-Term Empagliflozin Treatment On Body Weight And Food Intake In The Obese Zucker Rat

Compared to the lean control animals, there was no significant change in the body weight of the lean animals that had been treated for 5, 10 and 15 weeks. However, lean treated animals had a significantly lower body weight when compared to their counterparts at 20, 25, and 30 weeks of treatment (Figure 1). Similarly, there was no significant change in the body weight of obese control animals that have been treated at 5, 10, and 30 weeks compared to their treated counterparts. There was a decrease ($p<0.05$) in body weight in obese treated animals at 15, 20, and 25 weeks of treatment (Figure 1). Compared to their control counterparts, the lean treated groups did not show a significant change in food consumption (Figure 2). Similarly, the obese treated group did not show a significant change in food consumption when compared to control animals except at the 30-week time point (Figure 2).

Long-Term Empagliflozin Treatment Attenuates Circulating Levels Of Glucose And Other Biochemical Parameters In The Obese Zucker Rat

Compared to their control counterparts, the lean and obese treated groups did not show a significant change in levels of albumin, globulin, ALP, BUN, calcium, phosphorus, sodium potassium, creatinine, and TBIL. However, the lean treated animals showed a significant decrease in levels of total protein while obese treated animals showed a significant decrease in amylase and glucose levels when compared to their control counterparts (Table 1).

Effects Of Empagliflozin On Glucose Tolerance In Obese Zucker Rats

IPGTT was conducted to investigate the effect of Empagliflozin on long-term glucose handling in Zucker rats. Compared to the lean control animals, lean treated animals did not show any significant difference in glucose levels at either 0, 15, 30, 60, 90, and 120 minutes after glucose administration. Between the obese groups, glucose levels were similar at 0, 15, and 30 minutes while at 60 and 90 minutes after glucose injection, the treated groups showed a significant decrease in circulating glucose levels when compared to the controls (Figure 3).

Long-Term Empagliflozin Treatment Does Not Alter Weight Of Muscles In The Hind Limbs.

The effects of Empagliflozin on weight of soleus and EDL muscles in the hind limbs are shown in Figures 4 – 5. Compared to the lean control animals, there was no significant change in soleus muscle mass of the lean animals that had been treated for 5, 10, 20, and 30 weeks (Figure 4). Similarly, there was no significant change in the weight of soleus muscle in obese animals that had been treated for 5, 10, 20, and 30 weeks compared to their counterparts (Figure 4).

Compared to their controls, the lean treated groups did not show a significant change in EDL mass (Figure 5). Similarly, the obese treated groups did not show a significant decrease in EDL muscle weight when compared to controls except at the 30-week time point (Figure 5).

Effects Of Long Term Empagliflozin Treatment On Muscle Fiber Size In Obese Zucker Rats.

Compared to the lean control animals, there was no significant change in EDL muscle fiber size in 30-week-old lean treated muscle (Figure 6 A, B). Similarly, there was no significant change in the muscle fiber size in obese animals that had been treated compared to their counterparts (Figure 6 C, D).

Compared to their control counterparts, the lean treated group did not show a significant change in muscle fiber size in soleus muscle (Figure 7 A, B). Similarly, the obese treated group did not show a significant change in muscle fiber size when compared to its lean counterpart at 30 weeks of age (Figure 7 C, D).

Long-Term Empagliflozin Treatment Does Not Alter P38- MAPK Phosphorylation In The Soleus Muscle Of Obese Zucker Rat.

In the soleus muscle, there was no statistical difference in p38 phosphorylation (Thr182/ Tyr180) between the lean control group and lean treated groups or the obese control when compared to obese treated group. There was also no difference between the lean and obese groups (Figure 8A).

Compared between the groups, the lean and obese control group exhibited a statistical difference ($p<0.05$) in total p38 from that observed in the treated group. In addition, there was statistical difference between obese control and treated group when compared to the lean control group (Figure 8 B). When the phosphorylation of p38 over total p38 was compared, there was no statistical difference ($p<0.05$) between the lean or obese groups in soleus muscle (Figure 8 C).

Effect Of Long-Term Empagliflozin Treatment Decreases P44/42- MAPK Phosphorylation In The Soleus Muscle Of Obese Zucker Rat.

In the soleus muscle, there was a statistical difference ($p<0.05$) in ERK1/2 phosphorylation (Thr202/Tyr204) between the lean control group and the lean treated group and the obese control when compared to obese treated group. There was also a difference seen between the lean control when compared to obese control and obese treated group. The lean treated group showed a significant difference when compared to obese control and obese treated groups (Figure 9A).

Compared between the groups, only the lean control group exhibited a statistical difference ($p<0.05$) in total ERK1/2 with the treated group. The obese control was not different from the obese treated group. In addition, there was statistical difference between the obese control and the obese treated groups when compared to the lean controls and the lean treated group (Figure 9 B).

When the phosphorylation of pERK1/2 over total pERK1/2 was compared there was no statistical difference ($p<0.05$) between lean groups in the soleus muscle. There was significant decrease between the obese control and the obese treated group. In addition, there was a statistically significant difference between the obese control and the obese treated group when compared to the lean control and the lean treated group (Figure 9 C).

Long-Term Empagliflozin Treatment Does Not Alter P-JNK MAPK Phosphorylation In The Soleus Muscle Of Obese Zucker Rat.

In the soleus muscle, there was significant statistical difference ($p<0.05$) in pJNK phosphorylation (Thr183/ Tyr185) between the lean control group when compared to the lean treated group and the obese controls when compared to the obese treated group. Differences

were also seen between the lean control when compared with the obese control and the obese treated groups. In addition, there was difference in the obese treated when compared to the lean treated group (Figure 10 A).

Compared between the groups, the obese control group had a statistical difference ($p<0.05$) in total pJNK when compared with the treated group. There was no difference in the lean control group when compared to the lean treated group. In addition, there was statistical difference between the obese control and the obese treated group when compared to the lean control and lean treated animals (Figure 10 B).

When the phosphorylation of pJNK over total pJNK was compared in the soleus muscle of obese Zucker rat there was statistical difference ($p<0.05$) between the lean control group when compared to lean treated group. There was no difference between the obese control group and obese treated soleus muscle. In addition, there was significant difference between the lean control group when compared to the obese control and the obese treated groups. (Figure 10 C).

Long-Term Empagliflozin Treatment Alters P38- MAPK Phosphorylation In The EDL Muscle Of Obese Zucker Rat.

In the EDL muscle, there was no statistical difference ($p<0.05$) in p38 phosphorylation (Thr182/ Tyr180) between the lean and obese control group and the lean and obese treated groups (Figure 11A). The obese control group had statistical difference ($p<0.05$) in total p38 when compared with the obese treated group. However, there was no difference in lean control and lean treated groups. In addition, there was statistical difference between the obese control and when compared to the lean control group. Differences were

also seen between the obese treated and the obese control groups when compared to the lean treated animals (Figure 11 B).

When phosphorylation of p38 over total p38 was compared there is increased statistical difference ($p<0.05$) between the obese control and obese treated groups in the EDL muscle. There was no difference seen between lean groups. In addition, there was significant difference in obese control group when compared to the lean treated and the lean control groups (Figure 11 C).

Effect Of Long-Term Empagliflozin Treatment On P44/42- MAPK Phosphorylation In The EDL Muscle Of Obese Zucker Rat.

In the EDL muscle, there was a statistical difference ($p<0.05$) in ERK1/2 phosphorylation (Thr202/Tyr204) between the lean control group when compared to lean treated group and the obese controls when compared to the obese treated group. There were also differences seen between the lean control when compared to obese group control and obese treated group. The lean treated group showed a significant difference when compared to obese control and obese treated groups (Figure 12 A).

Compared between the groups, both the lean and obese control groups exhibited significant differences ($p<0.05$) in total ERK1/2 with the treated groups. In addition, there was statistical difference between obese control when compared to lean controls (Figure 12 B).

When the phosphorylation of pERK1/2 over total pERK1/2 was compared, there was a statistical difference ($p<0.05$) between the lean and obese groups in the EDL muscle. In addition, there was statistical difference between the obese control and the obese treated

group when compared to lean control group. Differences were also seen between lean treated and obese control groups (Figure 12 C).

Long-Term Empagliflozin Treatment Doesn't Alter P-JNK MAPK Phosphorylation In The EDL Muscle Of Obese Zucker Rat.

In the EDL muscle, there was a significant statistical difference ($p<0.05$) in pJNK phosphorylation (Thr183/ Tyr185) between the lean control group and lean treated group. There were no differences between the obese groups. Differences were also seen between the lean controls when compared with obese control and obese treated groups (Figure 13 A).

The lean control group had statistical difference ($p<0.05$) in total pJNK with the treated group. There was no difference in obese control group when compared to obese treated group. In addition, there was statistical difference between obese treated group when compared to lean treated animals (Figure 13 B).

When phosphorylation of pJNK over total pJNK was compared in the EDL muscle of obese Zucker rat there was no statistical difference ($p<0.05$) between the lean control group when compared to lean treated. Similarly, there was no difference within the obese control group when compared to obese treated group. Conversely, there was difference between obese treated group when compared to lean control and lean treated groups. (Figure 13 C).

CHAPTER 5

DISCUSSION

The obese Zucker rat is an accepted animal model of metabolic syndrome [146]. Metabolic syndrome is also known as insulin resistance syndrome as increased insulin resistance is a defining characteristic of this disorder [147]. The effects of Empagliflozin treatment for the prevention of metabolic syndrome have not been fully studied. In this study, lean and obese Zucker rats were treated for 25 weeks to investigate the long-term therapeutic effects of the drug. We found that the long treatment of these rats was not associated with a significant decrease in the body weight when compared to the control group (Figure 1). A previous study also reported that body weight and heart/body ratios were not significantly changed with Empagliflozin treatment in the Zucker diabetic rat [148]. Surprisingly, there was no significant difference in the food consumption between control and treated animals except in the 30-week obese treated animals (Figure 2).

Next, we examined the effect of Empagliflozin treatment on circulating blood glucose levels and other biochemical parameters. Although there were not many differences in the tested biochemical parameters between treated and control groups, we did find a significant decrease in total protein in the lean treated animals when compared to lean control animals and a significant decrease in circulating glucose levels in obese treated compared to that seen in the obese control animals (Table 1). To extend upon these findings, we next conducted the IPGTT test to examine the effect of Empagliflozin on glucose tolerance. Similar to previous work, we observed that there was significant difference at 60 and 90 minutes which suggests

treatment with Empagliflozin was helpful in reducing the blood glucose levels (Figure 3) [149].

We next compared muscle mass and fiber size in the lean and Obese Zucker rats with and without Empagliflozin treatment. Similar to previous work describing a decrease in muscle weight between lean and obese female Zucker rats, we saw decreased muscle weight in obese rats when compared to lean rats [150]. However, we failed to detect any appreciable change in muscle weight in control versus the treated groups (Figure 4 and 5) As expected, based on these findings, we also failed to see any alterations in muscle fiber size in either the EDL or soleus muscles with treatment (Figure 6 and 7).

Skeletal muscle plays a major role in the regulation of blood glucose levels [52]. As such, decrease in muscle insulin sensitivity can lead to an increased dependence on fatty acids for energy production. This elevated reliance on fatty acids is thought to lead to increased secretion of inflammatory cytokines and a further decrease in insulin sensitivity of skeletal muscle [48, 51]. Increased phosphorylation of the MAPK proteins leads to reduced downstream signaling and glucose disposal (Figure 4 and 5) [48, 53-55]. We found that treatment associated improvements in glucose disposal were characterized by decreased phosphorylation (activation) of the ERK1/2 and increased phosphorylation of p38 in the soleus and EDL muscles, respectively (Figures 9 and 12) [147, 150-154]. The physiological significance of these alterations remain unknown; however, it is interesting to note that previous reports have suggested that insulin-stimulated phosphorylation of the three major MAPK proteins (ERK1/2, p38, and JNK) may be altered in metabolic syndrome [155-158]. In addition, other work has demonstrated that Empagliflozin treatment was associated with reduced phosphorylation in ob/ob mice [159]. Why differences may exist between the current study and previous work is not yet known but could be due, at least in part, to

differences in the animal model of investigation used. Additional research is needed to explore further.

The p38 is thought to play an important role in cell differentiation and in modulating the production of key inflammatory mediators in the cell [160, 161]. In skeletal muscle, the phosphorylation of p38 is elevated with increased insulin resistance [162]. Why treatment was found to increase p38 phosphorylation in the obese Zucker EDL muscle is currently unclear and it is likely that future studies perhaps looking at other molecules or other models of metabolic syndrome will be needed to further understand the mechanism(s) of Empagliflozin on the regulation insulin sensitivity in metabolic syndrome.

In a similar fashion, the physiological significance of these alterations remain unclear; however, it is interesting to note that previous reports have suggested that insulin-stimulated phosphorylation of p38 may be altered in metabolic syndrome [155, 161, 162]. In addition to p38, it is thought that JNK and ERK1/2 may also play roles in the progression of insulin resistance [156]. Other work has shown that the phosphorylation of JNK inhibits insulin signaling in the rat soleus muscle by enhancing phosphorylation of IRS-1 at Ser636 [157]. The phosphorylation of ERK1/2, JNK, and p38 was greatly reduced by treatment of Empagliflozin when compared to lean control levels in ob/ob mice [158]. In this study, we observed that there was no alteration in the amount of phosphorylated p44/42-, JNK- and p38 – MAPK in the skeletal muscle of obese Zucker rat at 30 weeks of age (Figures 9-13).

Our data suggest that MAPK regulation in skeletal muscle is not altered when the obese Zucker rat is treated with the Empagliflozin. Given these data, it is likely that future studies perhaps looking at other molecules or other models of metabolic syndrome might be needed to further understand the mechanistic effect of drug Empagliflozin on regulation of insulin sensitivity in metabolic syndrome.

CHAPTER 6

CONCLUSIONS

Metabolic syndrome is characterized by insulin resistance which if allowed to proceed unchecked can lead to Type-2 diabetes and cardiovascular dysfunction. According to the CDC, more than one third adult population of the United States is categorized as having metabolic syndrome [33]. The main intent of this study was to investigate if Empagliflozin treatment would be effective on alleviating the signs of metabolic syndrome in the fast and slow twitch muscles of the lean and obese Zucker rat model. The following conclusions were drawn from the present study.

1. Empagliflozin treatment did not alter serum glucose levels, feed consumption, or muscle mass but did improve glucose sensitivity.
2. Empagliflozin treatment-associated changes in glucose sensitivity were accompanied by decreased phosphorylation of ERK in obese soleus and increased phosphorylation of p38 in obese EDL muscles.
3. Empagliflozin treatment associated changes in glucose sensitivity were not accompanied by alterations in the phosphorylation of JNK and p38 in the soleus and the phosphorylation of ERK and JNK in the EDL muscles.

FUTURE DIRECTIONS

Future research based on this study should focus on studying other pathways like GLUT-2, AKT or IRS that might have caused changes in insulin sensitivity in skeletal muscle of the obese Zucker rat. The results of present study have shown that MAPK related signaling

is for the most part, unaltered in the skeletal muscle of the obese Zucker rat (fa/fa) following Empagliflozin treatment. Whether similar findings would be observed in other metabolic syndrome models or with other drugs remains to be determined.

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APPENDICES

APPENDIX A

MARSHALL UNIVERSITY
www.marshall.edu

Office of Research Integrity

November 28, 2018

Sushma Penta
530 21st Street
Huntington, WV 25703

Dear Ms. Penta:

This letter is in response to the submitted thesis abstract entitled *"Effect of Empagliflozin on Insulin Sensitivity in the Lean and Obese Zucker Rat Model of Metabolic Syndrome."* After assessing the abstract it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #590. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director

APPENDIX B

Table 1:

Empagliflozin attenuates diabetes-induced alterations in serum biochemical parameters. Results are expressed as mean ± S.E.M. *$p < 0.05$ compared with lean control group, $^{\$}p < 0.05$ compared with lean treated group, and $^{\#}p < 0.05$ compared with obese control group (n=6-8/group).

Analyte	30 WK LC	30 WK LT	30 WK OC	30 WK OT
Albumin (g/dL)	3.1 ± 0.15	3.03 ± 0.17	3.02 ± 0.04	2.96 ± 0.07
Globulin (g/dL)	2.07 ± 0.19	1.84 ± 0.15	2.82 ± 0.09*$	2.74 ± 0.13
Tot. Protein (g/dL)	5.17 ± 0.08	4.89 ± 0.08*	5.78 ± 0.05*$	5.67 ± 0.10*$
ALT (U/L)	40.50 ± 2.74	64.50 ± 6.28*	37.00 ± 2.62$	43.00 ± 3.91$
ALP (U/L)	106.33±10.91	111.75±7.42	106.83±8.2	108.86±8.65
Amylase (U/L)	698.17±22.75	645.75±20.89	872.5±16.02*$	803.14±22.05*$#
BUN (mg/dL)	18.33±0.71	17.75±0.86	35.83±9.12	35.71±9.93
Calcium (mg/dL)	10.25±0.06	9.86±0.10	11.33±0.16$	11.54±0.27$
Phosphorus (mg/dL)	5.65±0.26	5.71±0.27	7.08±0.78	6.97±0.91
Sodium (mmol/L)	135.00±0.58	135.88±0.67	138.00±1.06*	138.43±0.75*
Potassium (mmol/L)	4.62±0.14	4.68±0.19	5.82±0.22*$	5.30±0.24*
Creatinine (mg/dL)	0.43±0.06	0.51±0.04	0.73±0.15	0.71±0.20
Glucose (mg/dL)	424.50±10.05	380.38±9.03	427.67±25.64	348.43±9.10*#
TBIL (mg/dL)	0.33±0.02	0.31±0.02	0.37±0.02	0.47±0.03*$

Figures

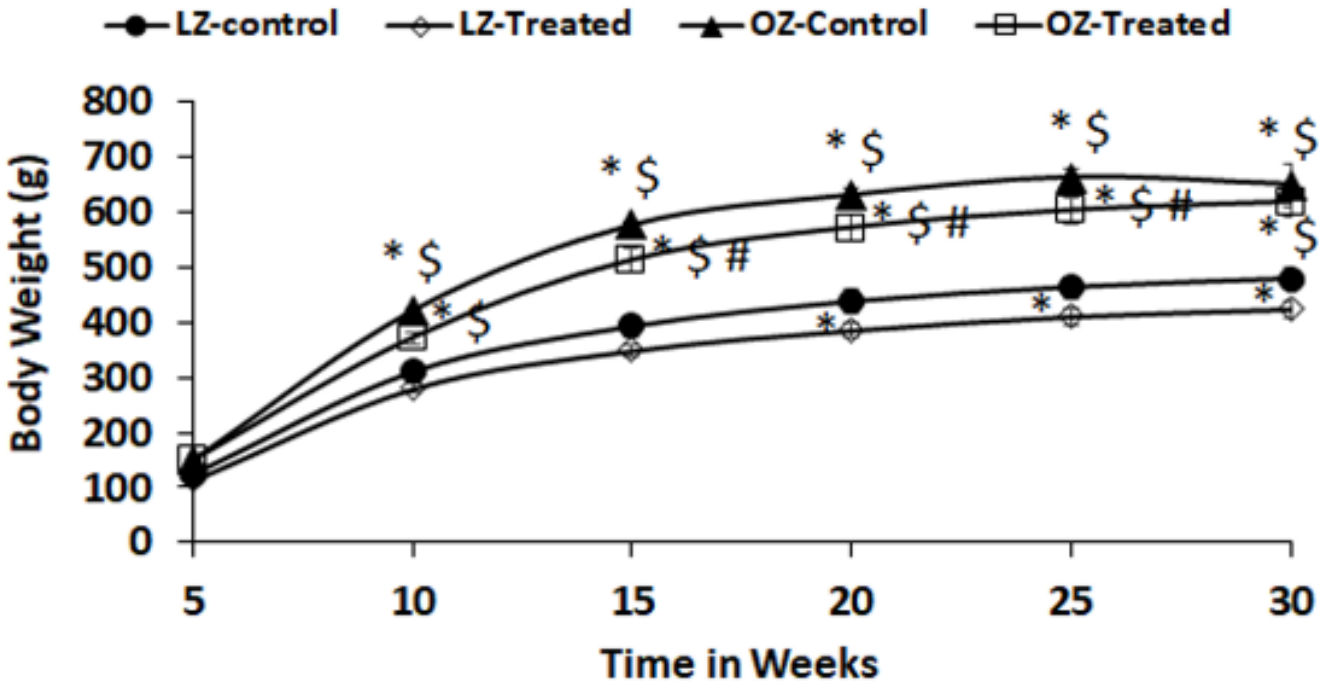


Figure 1

Long-term treatment with Empagliflozin attenuates body weight gain in Zucker rats. Results are expressed as mean ± S.E.M. *$p < 0.05$ compared with lean control group, $^{\$}p < 0.05$ compared with lean treated group, and $^{\#}p < 0.05$ compared with obese control group (n=6-8/group). This figure was provided by Dr. Manne.

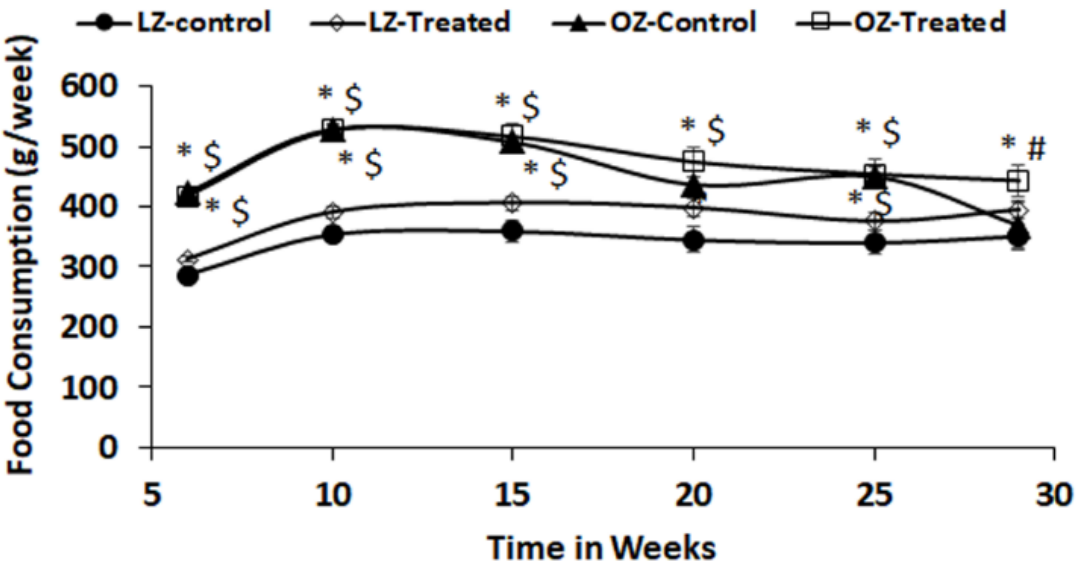


Figure 2

Food consumption in Zucker rats treated with Empagliflozin. Results are expressed as mean ± S.E.M. *$p < 0.05$ compared with lean control group, $^{\$}p < 0.05$ compared with lean treated group, and $^{\#}p < 0.05$ compared with obese control group (n=6-8/group). This figure was provided by Dr. Manne.

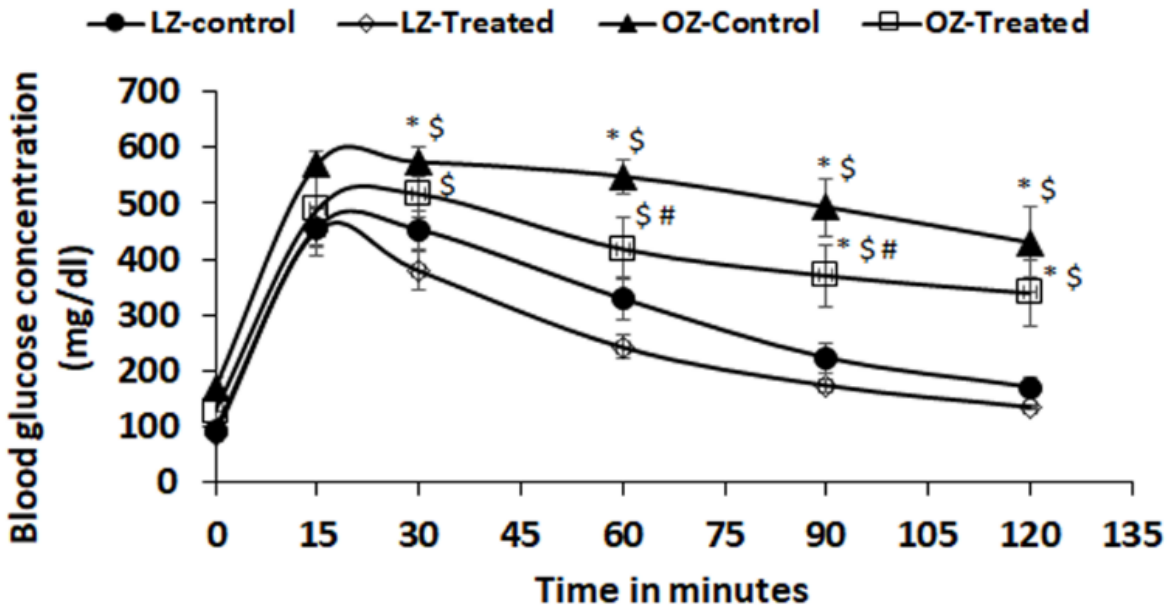


Figure 3

Long-term treatment with Empagliflozin improves glucose tolerance in obese Zucker animals. Intra-Peritoneal Glucose Tolerance Test (IPGTT) Blood glucose concentration in 30-week-old Zucker rats. Results are expressed as mean ± S.E.M. *$p < 0.05$ compared with lean control group, $^{\$}p < 0.05$ compared with lean treated group, and $^{\#}p < 0.05$ compared with obese control group (n=5-9/group). This figure was provided by Dr. Manne.

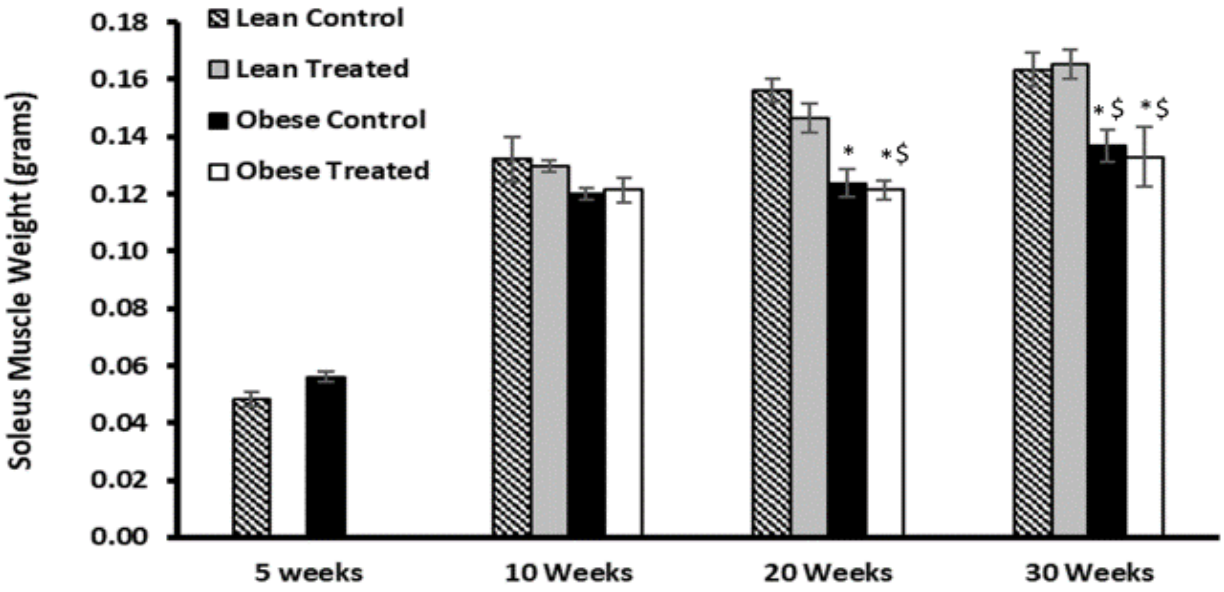


Figure 4

Long-term treatment with Empagliflozin does not appear to alter soleus muscle weight. Results are expressed as mean ± S.E.M. *$p < 0.05$ compared with lean control group and $^{\$}p < 0.05$ compared with lean treated group (n=6-8/group).

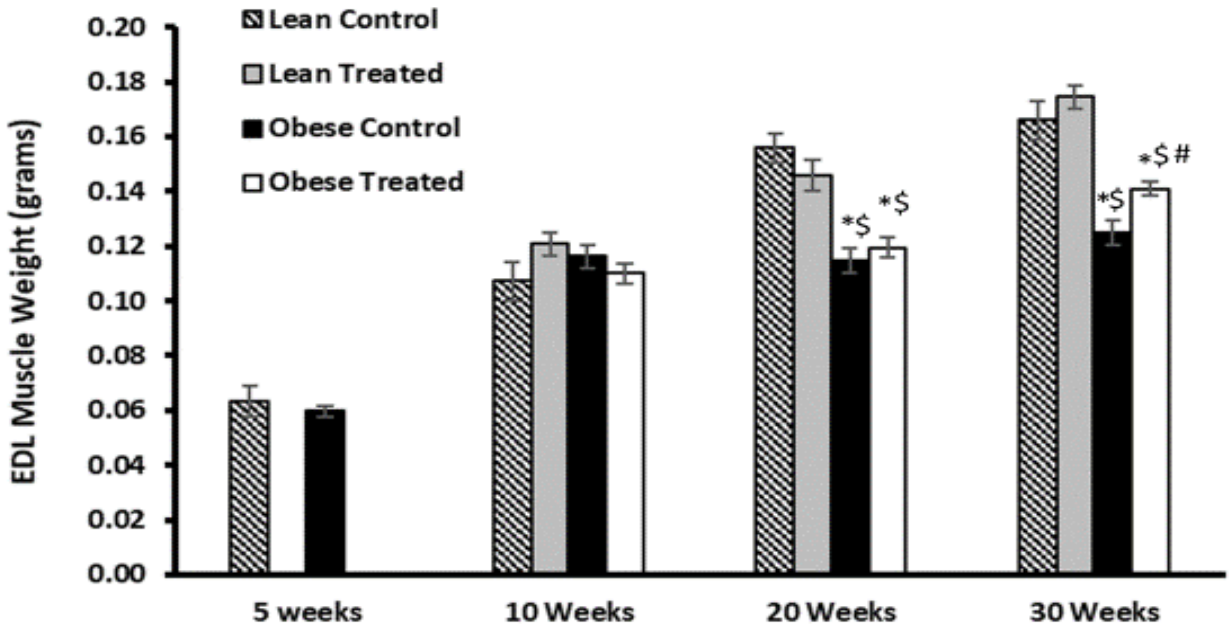


Figure 5

Long-term treatment with Empagliflozin does not appear to alter EDL muscle weight. Results are expressed as mean ± S.E.M. *$p < 0.05$ compared with lean control group, $^{\$}p < 0.05$ compared with lean treated group, and $^{\#}p < 0.05$ compared with obese control group (n=6-8/group).

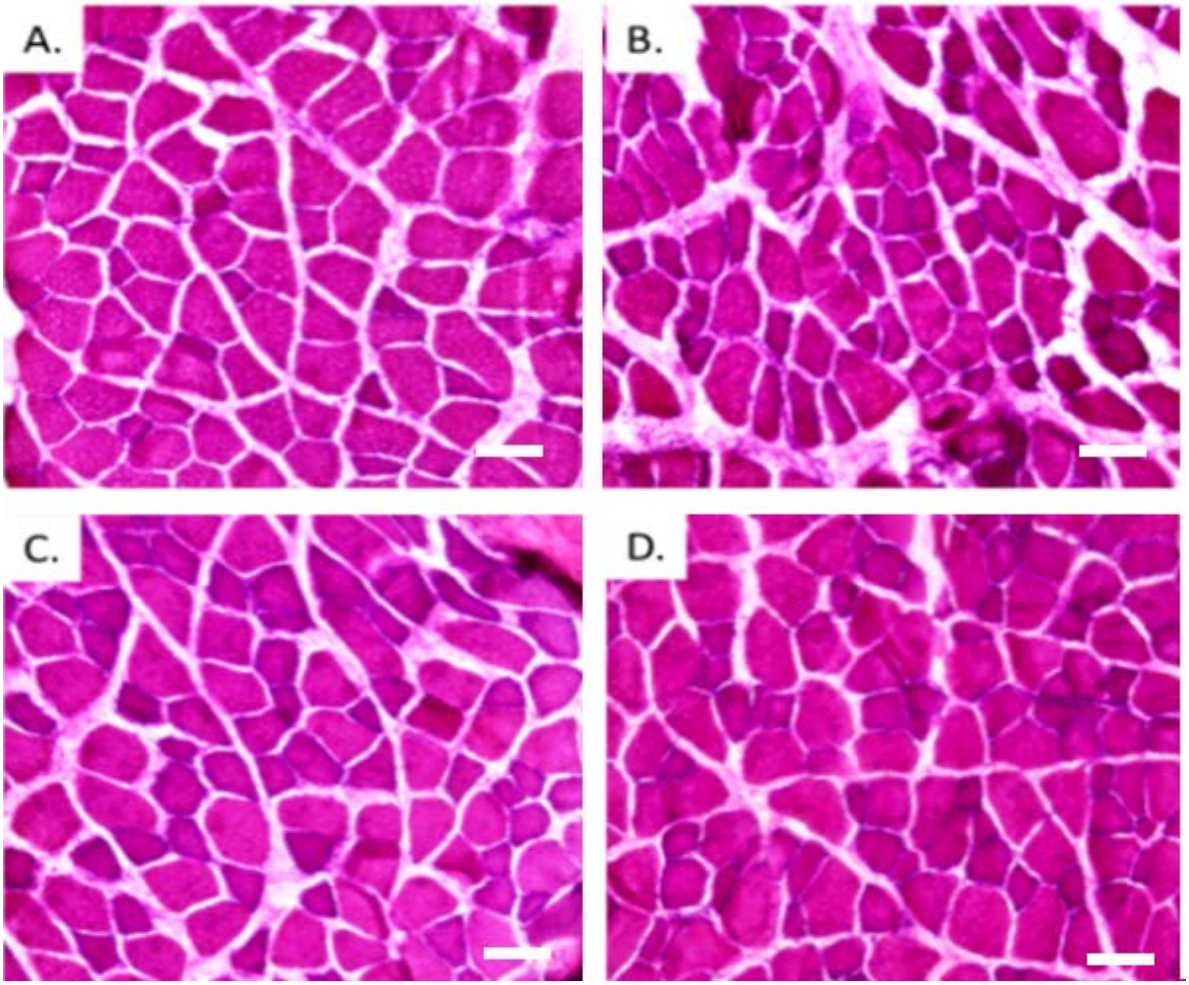


Figure 6

Long term treatment with Empagliflozin does not appear to alter EDL muscle fiber size. Lean control (A), Lean treated (B), Obese Control (C), and Obese treated (D). Scale bar = 100 µm.

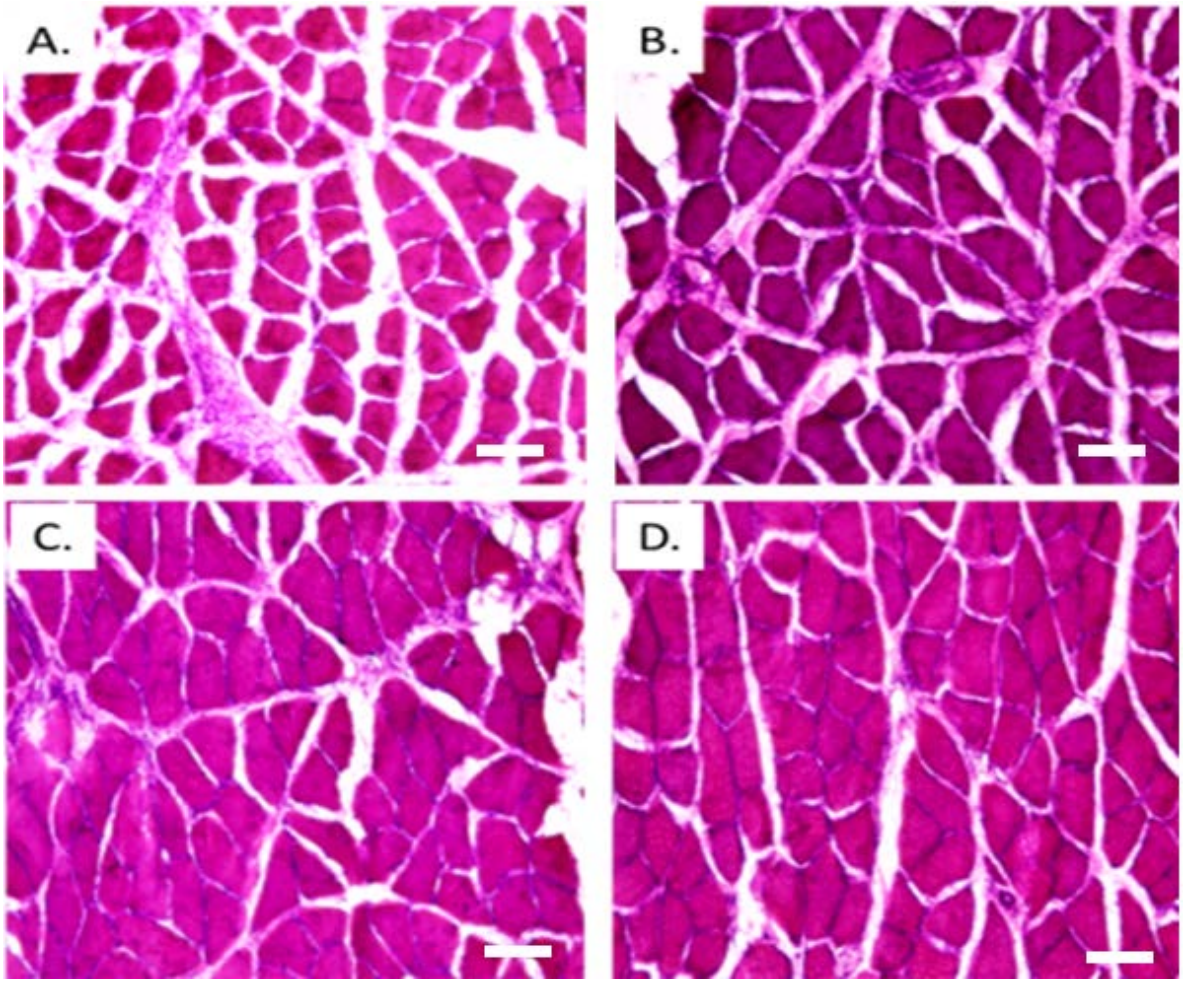


Figure 7

Long term treatment with Empagliflozin does not appear to alter soleus muscle fiber size. Lean control (A), Lean treated (B), Obese Control (C), and Obese treated (D). Scale bar = 100 µm.

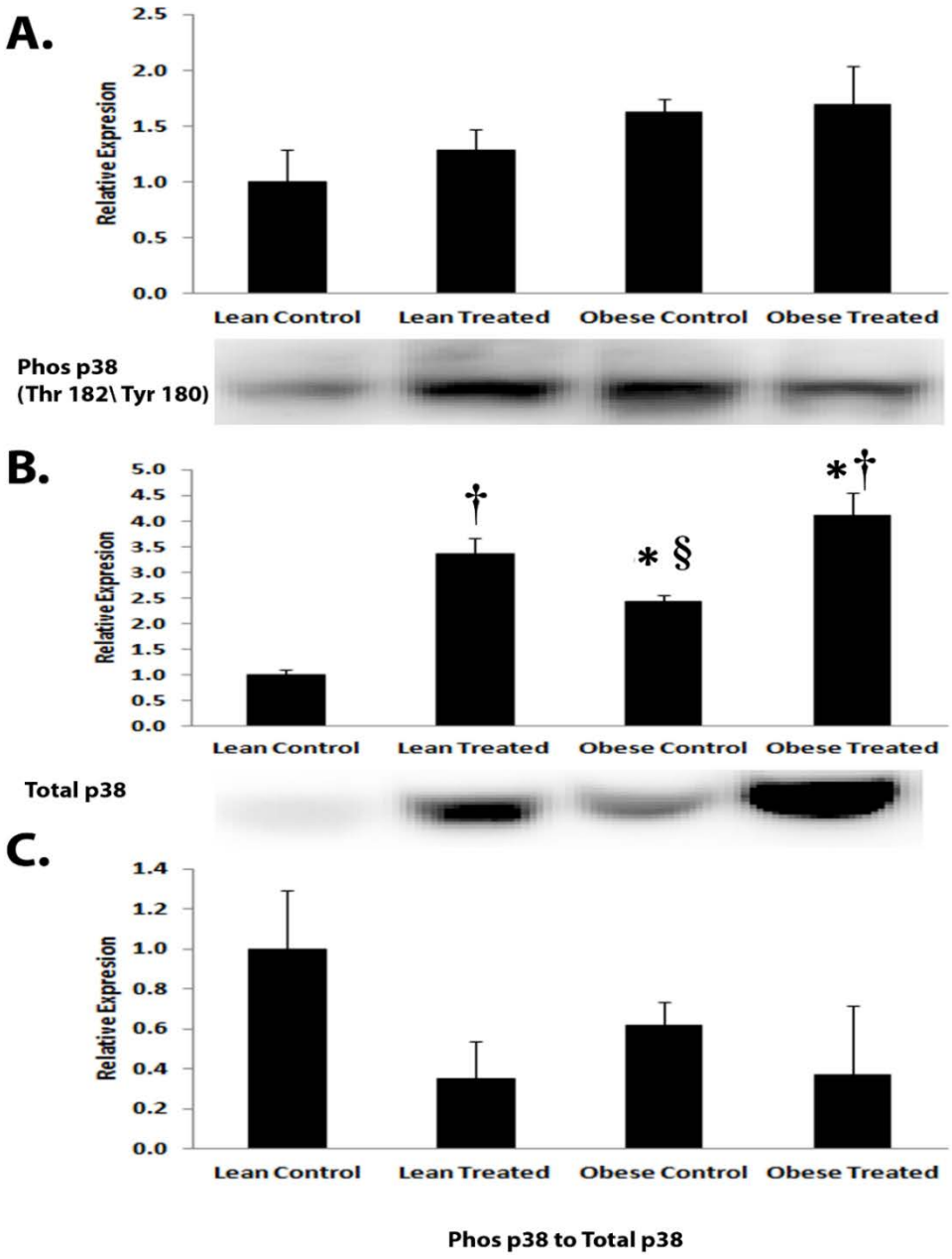


Figure 8

Effect of Empagliflozin treatment on p38-MAPK phosphorylation in soleus muscle. Comparison between the groups is shown in the graph. *$p<0.05$ denotes comparison with lean control, $^{\dagger}p<0.05$ comparison within groups and $^{\S}p<0.05$ with lean treated(n=6-8/group).

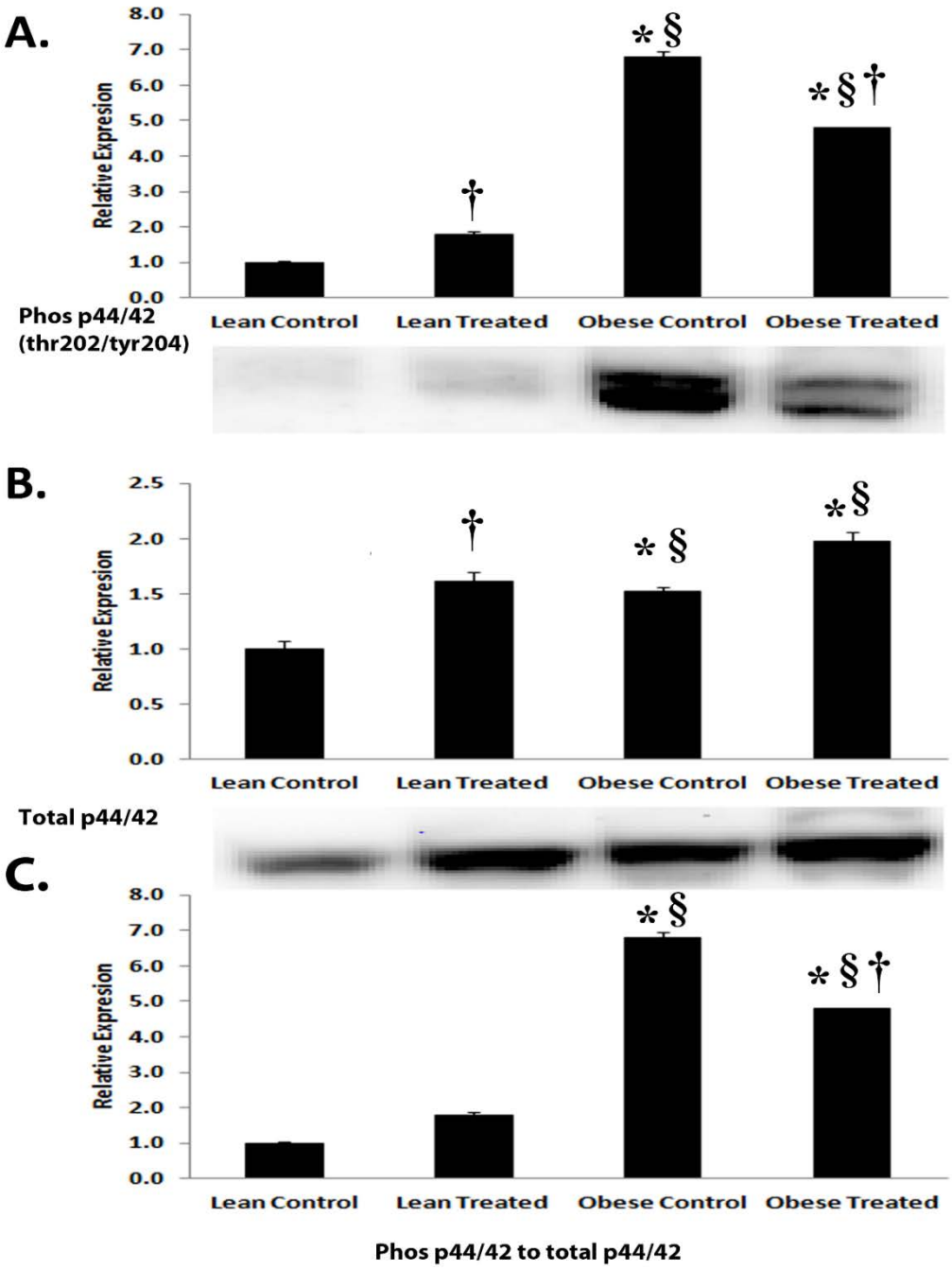


Figure 9

Effect of Empagliflozin treatment on p42-44/ERK1/2-MAPK phosphorylation in soleus muscle. Symbols denote comparison between the columns in the graph. *p <0.05 with lean control, §p <0.05 with lean treated and †p <0.05 within groups (n=6-8/group)

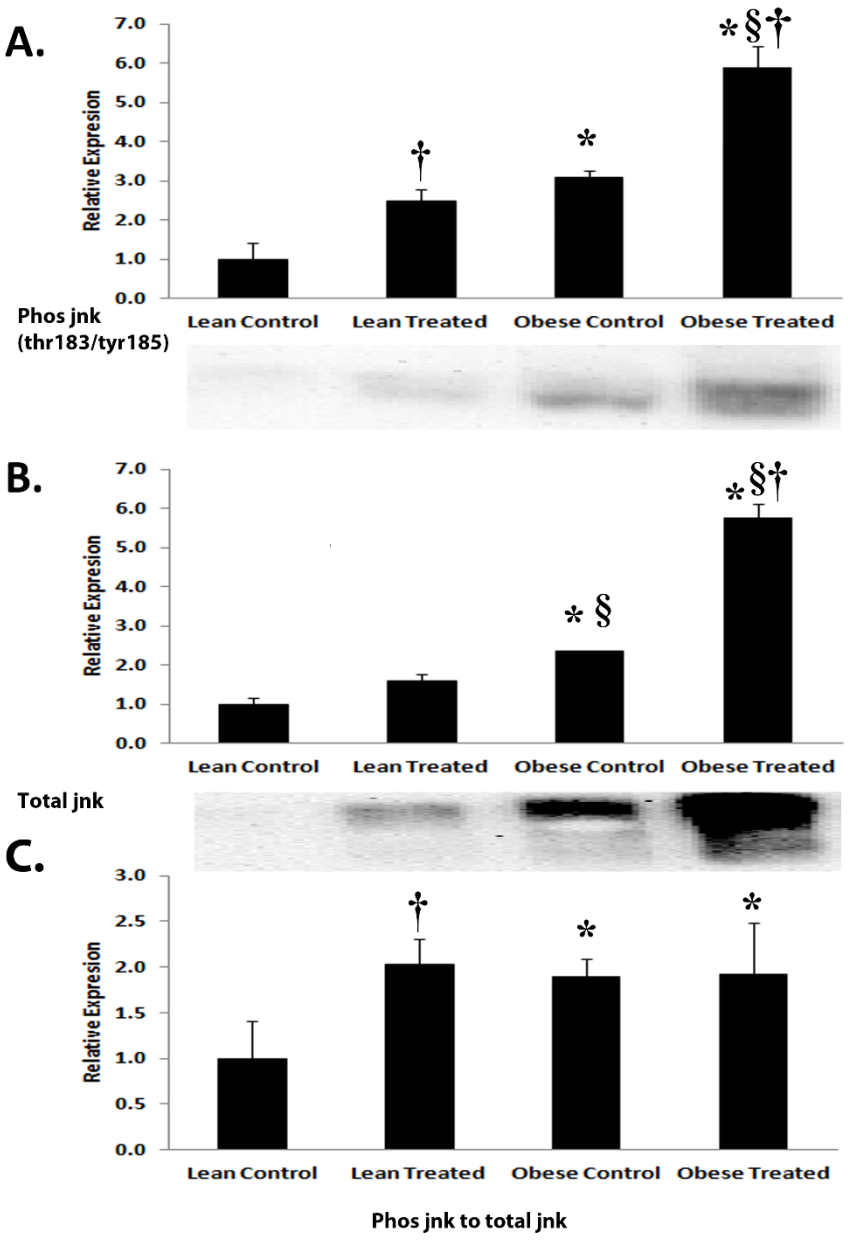


Figure 10

Effect of Empagliflozin treatment on JNK -MAPK phosphorylation in soleus muscle. Symbols denote comparison between the columns in the graph. *p <0.05 with lean control, $^{\S}p < 0.05$ with lean treated and $^{\dagger}p < 0.05$ within groups (n=6-8/group).

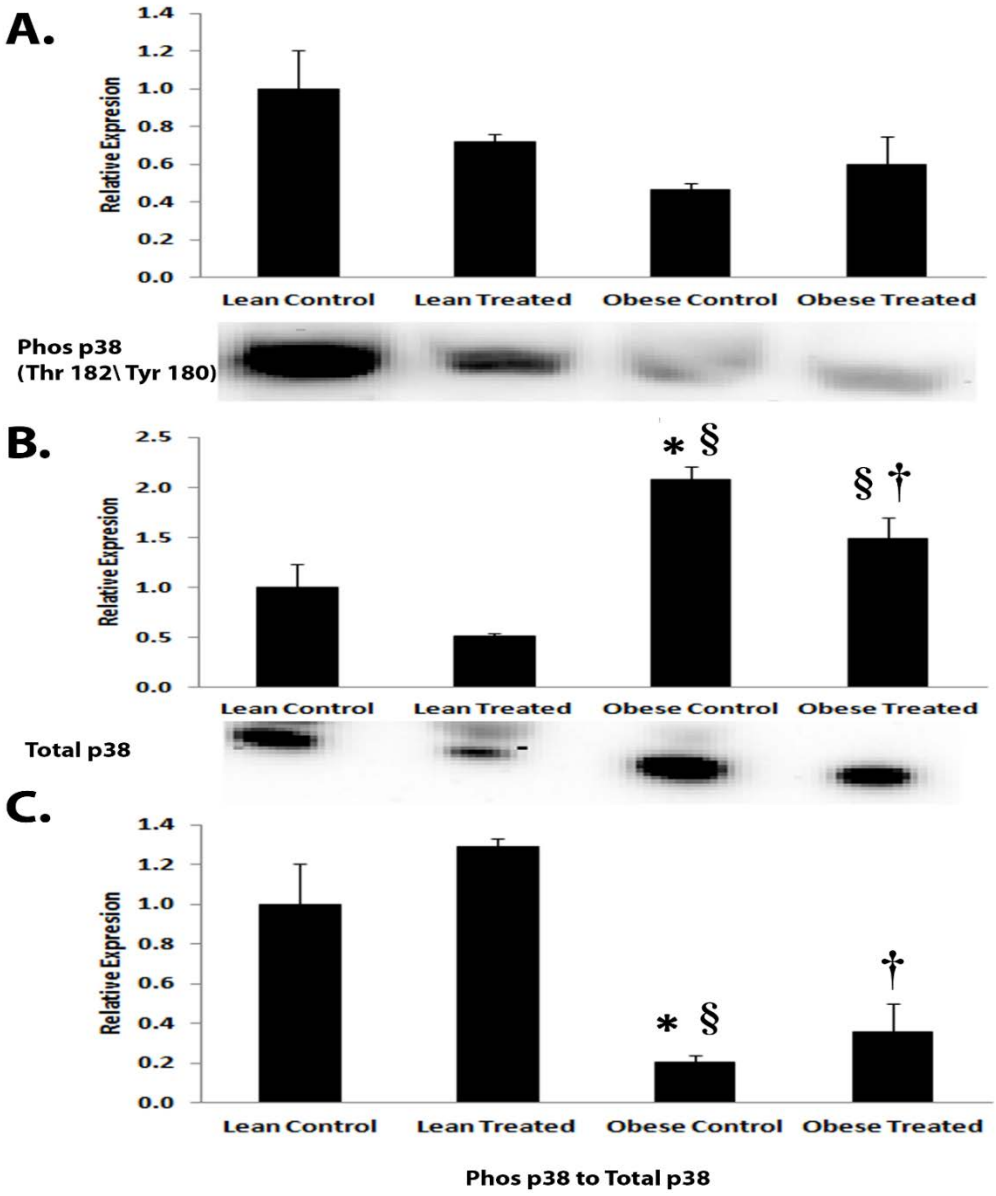


Figure 11

Effect of Empagliflozin treatment on p38-MAPK phosphorylation in EDL muscle. Symbols denote comparison between the columns in the graph. *$p<0.05$ with lean control, $^{\S}p<0.05$ with lean treated and $^{\dagger}p<0.05$ within groups (n=6-8/group).

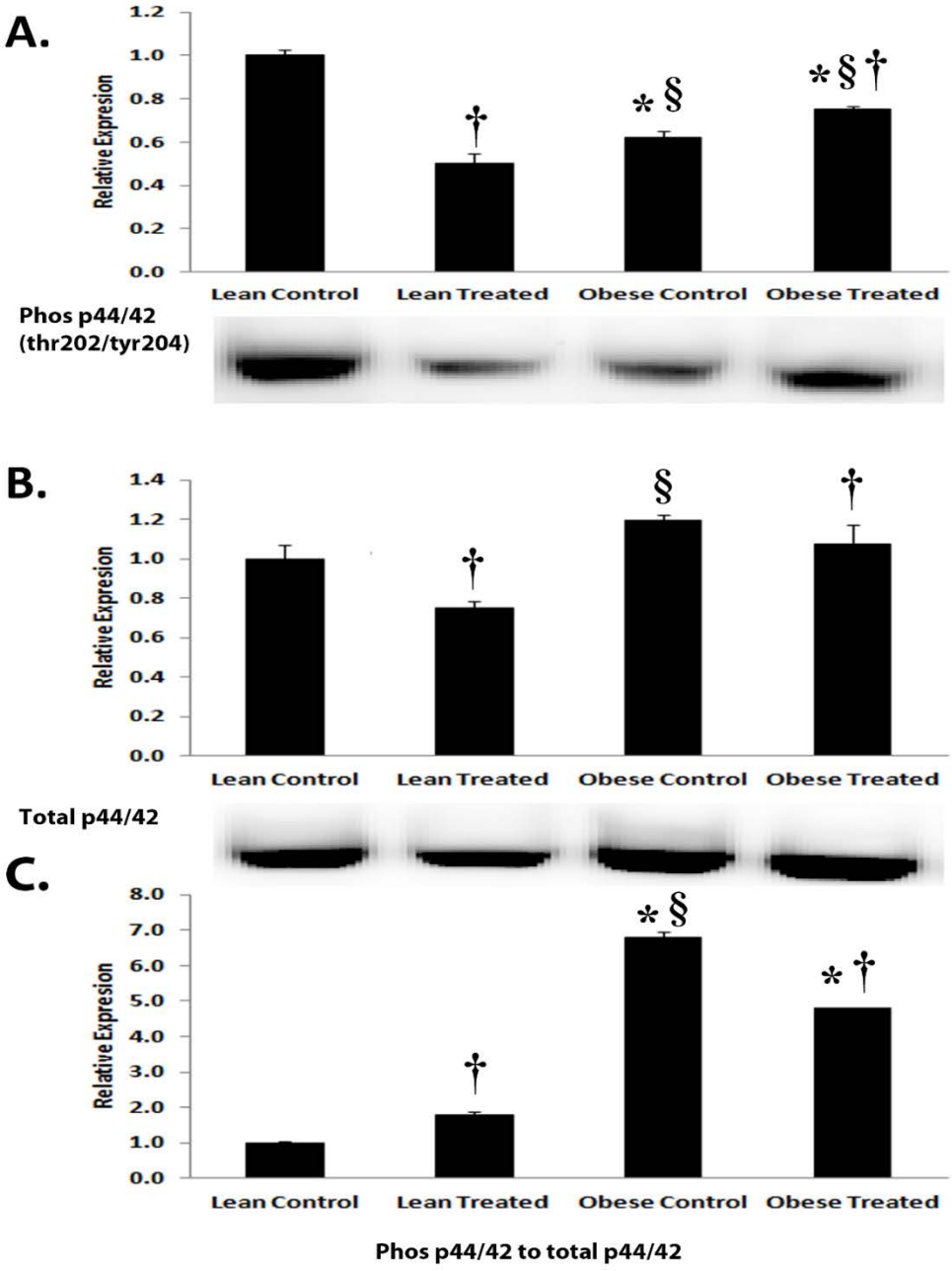


Figure 12

Effect of Empagliflozin treatment on p42-44/ ERK1/2-MAPK phosphorylation in EDL muscle. Symbols denote comparison between the columns in the graph. *p <0.05 with lean control, §p <0.05 with lean treated and †p<0.05 within groups (n=6-8/group).

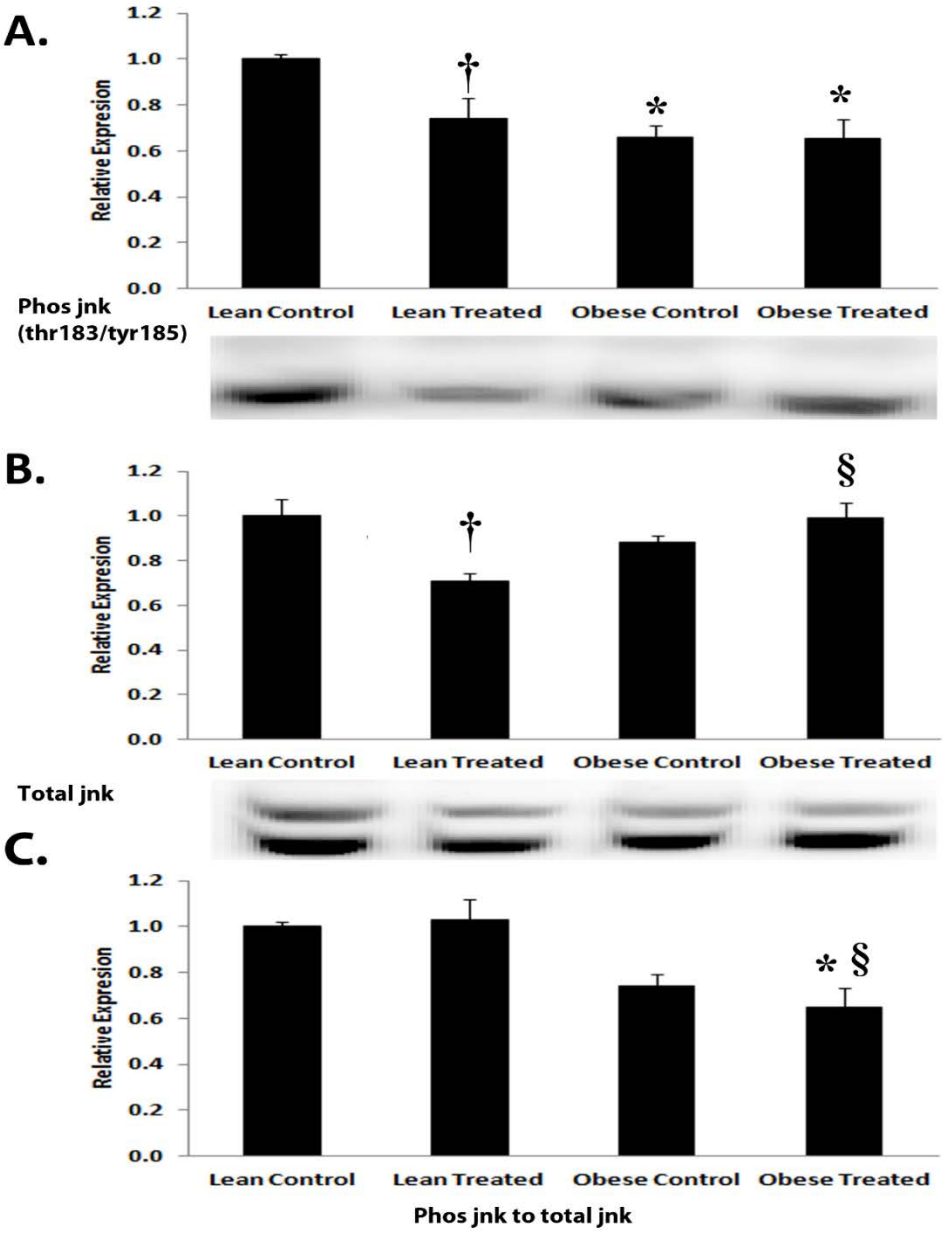


Figure 13

Effect of Empagliflozin treatment on JNK -MAPK phosphorylation phosphorylation in EDL muscle. Symbols denote comparison between the columns in the graph. *p<0.05 with lean control and §p <0.05 with lean treated (n=6-8/group).

APPENDIX C

Soleus

Film Properties Report for p38

Experimenter: Sushma Penta

Muscle / Tissue: Soleus Species: Rat (Zucker)

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

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

Primary Antibody: p38 (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 minutes Molecular weight: 43 kDa

Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative controlLane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Soleus

Film Properties Report for p-p38

Experimenter: Sushma Penta

Muscle / Tissue: Soleus Species: Rat (Zucker)

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

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

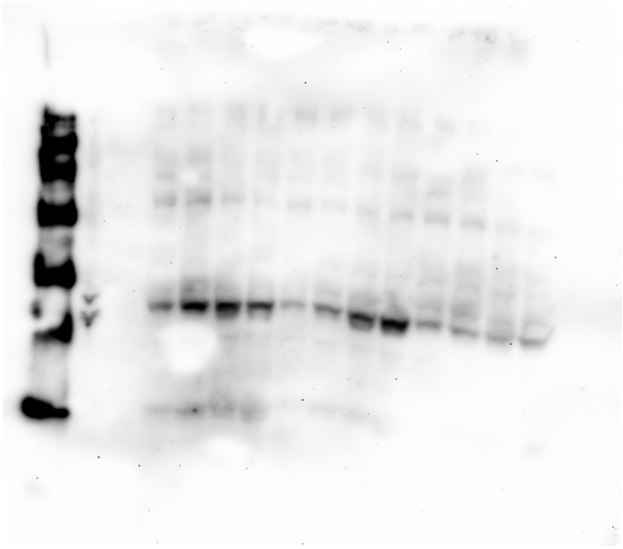
Primary Antibody: p-p38 (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 43 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Soleus

Film Properties Report for ERK1/2

Experimenter: Sushma Penta

Muscle / Tissue: Soleus Species: Rat (Zucker)

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

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

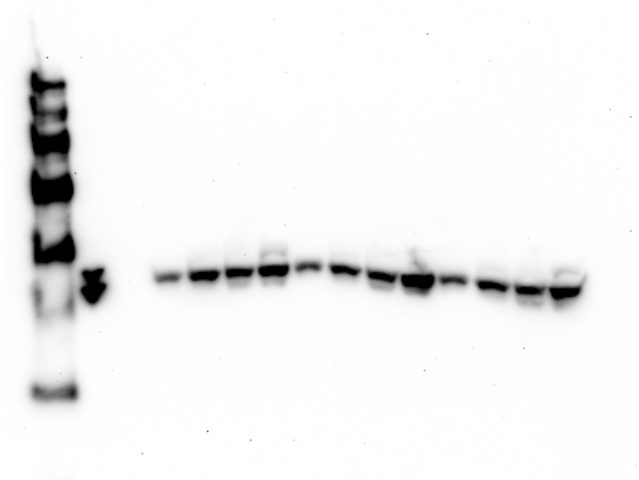
Primary Antibody: ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 42,44 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Soleus

Film Properties Report for p-ERK1/2

Experimenter: Sushma Penta

Muscle / Tissue: Soleus 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

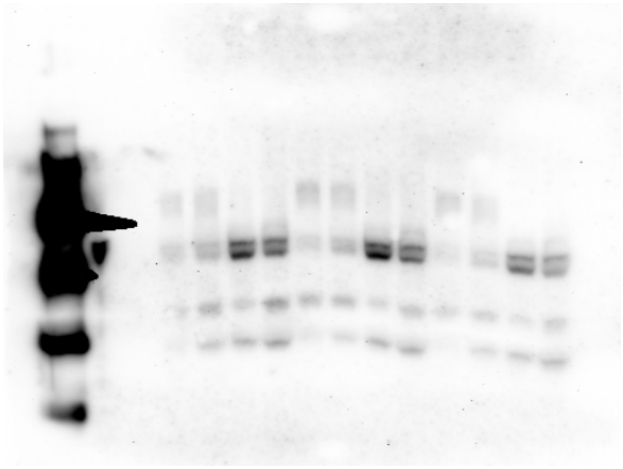
Primary Antibody: p-ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 42,44 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Soleus

Film Properties Report for JNK

Experimenter: Sushma Penta

Muscle / Tissue: Soleus Species: Rat (Zucker)

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

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

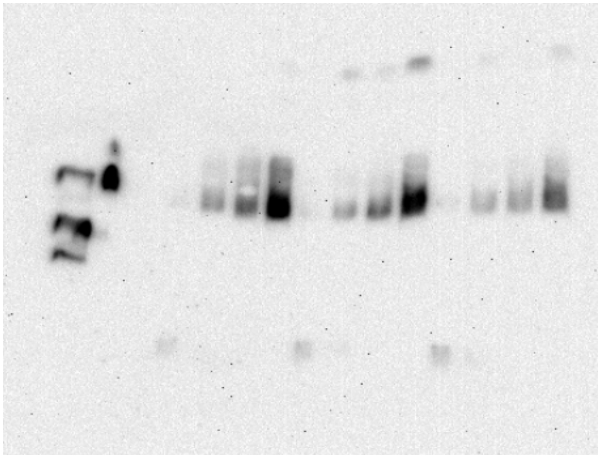
Primary Antibody: JNK (Cell Signaling) Primary Antibody Dilution: 1:1000

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: 2.4 minutes Molecular weight: 46,54 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Soleus

Film Properties Report for p-JNK

Experimenter: Sushma Penta

Muscle / Tissue: Soleus Species: Rat (Zucker)

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

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

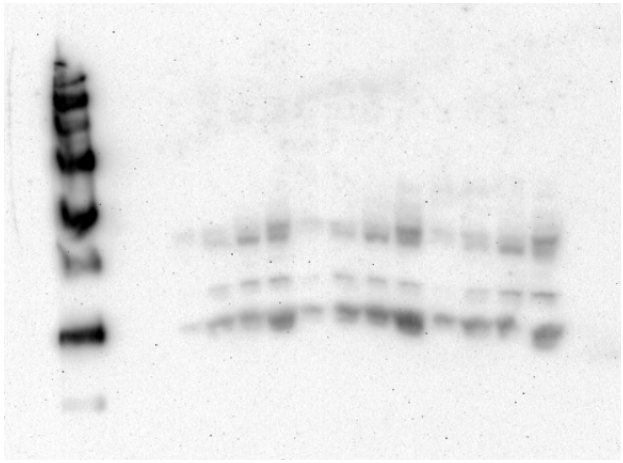
Primary Antibody: p-JNK (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 46,54 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Extensor digitorum longus (EDL)

Film Properties Report for p38

Experimenter: Sushma Penta

Muscle / Tissue: EDL Species: Rat (Zucker)

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

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

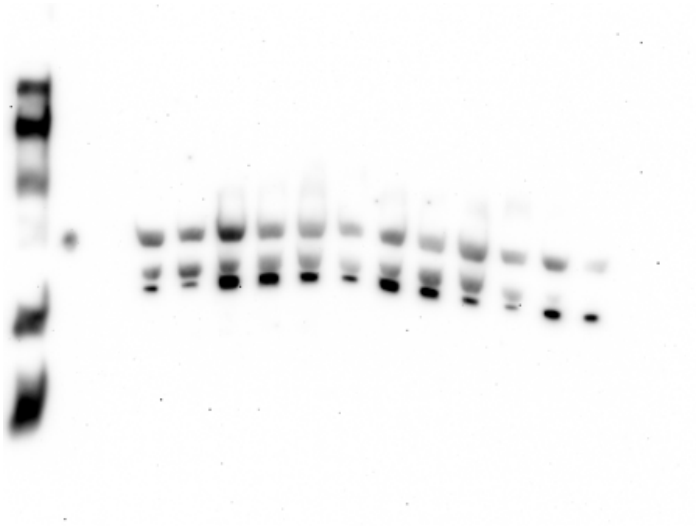
Primary Antibody: p38 (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 43 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Extensor digitorum longus (EDL)

Film Properties Report for p-p38

Experimenter: Sushma Penta

Muscle / Tissue: EDL Species: Rat (Zucker)

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

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

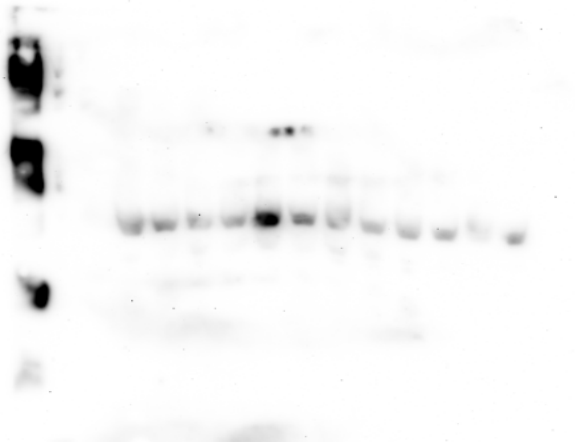
Primary Antibody: p-p38 (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 43 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Extensor digitorum longus (EDL)

Film Properties Report for ERK1/2

Experimenter: Sushma Penta

Muscle / Tissue: EDL Species: Rat (Zucker)

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

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

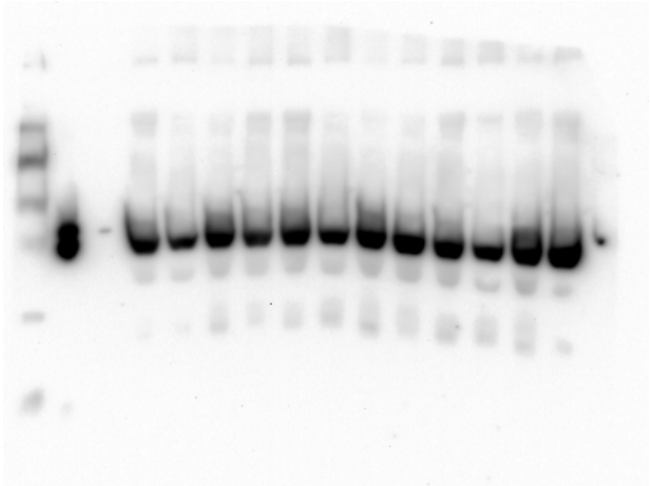
Primary Antibody: ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1:1000

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: 1-minute Molecular weight: 42,44 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Extensor digitorum longus (EDL)

Film Properties Report for p-ERK1/2

Experimenter: Sushma Penta

Muscle / Tissue: EDL Species: Rat (Zucker)

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

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

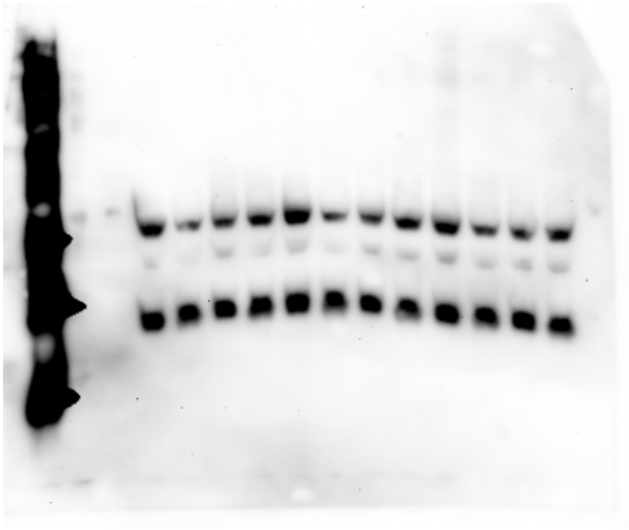
Primary Antibody: p-ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 42,44 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week 16 µl Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Extensor digitorum longus (EDL)

Film Properties Report for JNK

Experimenter: Sushma Penta

Muscle / Tissue: EDL Species: Rat (Zucker)

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

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

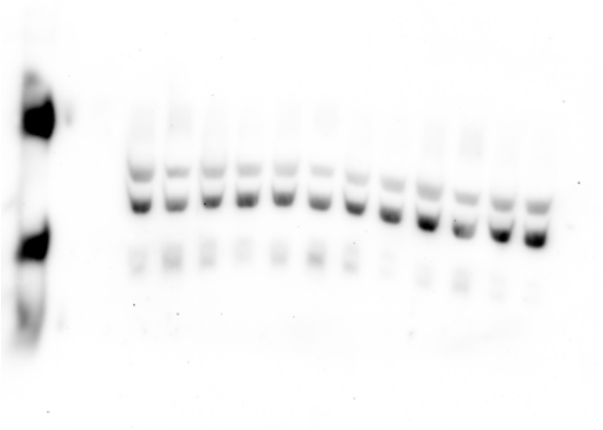
Primary Antibody: JNK (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 46,54 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

Extensor digitorum longus (EDL)

Film Properties Report for p-JNK

Experimenter: Sushma Penta

Muscle / Tissue: EDL Species: Rat (Zucker)

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

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

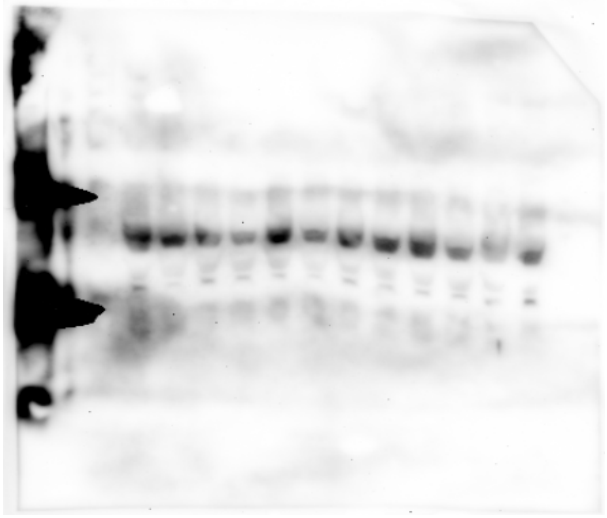
Primary Antibody: p-JNK (Cell Signaling) Primary Antibody Dilution: 1:1000

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 minutes Molecular weight: 46,54 kDa



Lane 1: Biotinylated Ladder

Lane 2: Mol Wt Marker

Lane 3: Negative control

Lane 4: Lean Zucker control 30 week

Lane 5: Lean Zucker treated 30 week

Lane 6: Obese Zucker control 30 week

Lane 7: Obese Zucker treated 30 week

Lane 8: Lean Zucker control 30 week

Lane 9: Lean Zucker treated 30 week

Lane 10: Obese Zucker control 30 week

Lane 11: Obese Zucker treated 30 week

Lane 12: Lean Zucker control 30 week

Lane 13: Lean Zucker treated 30 week

Lane 14: Obese Zucker control 30 week

Lane 15: Obese Zucker treated 30 week

Lane16: Negative Control

APPENDIX D

Statistics

Total p38(Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus total p38 paper

Normality Test (Shapiro-Wilk) Passed (P = 0.443)

Equal Variance Test: Passed (P = 0.601)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	9.164	1.718	0.992
Lean Treated	3	0	30.846	4.7	2.714
Obese Control	3	0	22.254	1.995	1.152
Obese Treated	3	0	37.736	6.767	3.907

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1364.136	454.712	24.311	<0.001
Residual	8	149.632	18.704		
Total	11	1513.768			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

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

Comparison	Diff of Means	p	q	P	P<0.050
Obese Treated vs. Lean Control	28.572	4	11.443	<0.001	Yes
Obese Treated vs. Obese Control	15.482	3	6.2	0.006	Yes
Obese Treated vs. Lean Treated	6.89	2	2.759	0.087	No
Lean Treated vs. Lean Control	21.682	3	8.684	<0.001	Yes
Lean Treated vs. Obese Control	8.592	2	3.441	0.041	Yes
Obese Control vs. Lean Control	13.09	2	5.243	0.006	Yes

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

Phos p38(Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus phos p38 paper

Normality Test (Shapiro-Wilk) Passed (P = 0.696)

Equal Variance Test: Passed (P = 0.778)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	17.853	9.097	5.252
Lean Treated	3	0	22.95	5.795	3.346
Obese Control	3	0	29.052	3.537	2.042
Obese Treated	3	0	30.144	10.781	6.225

Source of Variation	DF	SS	MS	F	P
Between Groups	3	294.46	98.153	1.602	0.264
Residual	8	490.191	61.274		
Total	11	784.65			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.264).

Power of performed test with alpha = 0.050: 0.125

The power of the performed test (0.125) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Phos/Total p38(Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus phos/Total p38 paper

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks
Data source: Data 1 in Notebook1

Group	N	Missing	Median		25%	75%
Lean Control	3	0	1.969	0.787		3.622
Lean Treated	3	0	0.68	0.628		0.921
Obese Control	3	0	1.204		1.173	1.563
Obese Treated	3	0	0.712		0.702	0.936

H = 6.897 with 3 degrees of freedom. (P = 0.075)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.075)

Total ERK1/2(Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus total p44/42 pathway

Normality Test (Shapiro-Wilk) Passed (P = 0.828)

Equal Variance Test: Passed (P = 0.639)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	34.575	4.193	2.421
Lean Treated	3	0	15.425	1.794	1.036
Obese Control	3	0	24.655	3.162	1.826
Obese Treated	3	0	25.346	0.829	0.478

Source of Variation	DF	SS	MS	F	P
Between Groups	3	550.829	183.61	23.328	<0.001
Residual	8	62.967	7.871		
Total	11	613.796			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Lean Control vs. Lean Treated	19.15	4	11.823	<0.001	Yes
Lean Control vs. Obese Control	9.921	3	6.125	0.006	Yes
Lean Control vs. Obese Treated	9.229	2	5.698	0.004	Yes
Obese Treated vs. Lean Treated	9.921	3	6.125	0.006	Yes
Obese Treated vs. Obese Control	0.691	2	0.427	0.771	No
Obese Control vs. Lean Treated	9.23	2	5.698	0.004	Yes

Phos ERK ½(Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus phos ERK ½ paper

Normality Test (Shapiro-Wilk) Passed (P = 0.935)

Equal Variance Test: Passed (P = 0.122)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	6.979	0.684	0.395
Lean Treated	3	0	12.339	1.209	0.698
Obese Control	3	0	47.28	2.296	1.326
Obese Treated	3	0	33.402	0.455	0.263

Source of Variation	DF	SS	MS	F	P
Between Groups	3	3156.262	1052.087	567.964	<0.001
Residual	8	14.819	1.852		
Total	11	3171.081			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

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

Comparisons for factor:

Comparison	Diff of Means	p	q	p	P<0.050
Obese Control vs. Lean Control	40.302	4	51.288	<0.001	Yes
Obese Control vs. Lean Treated	34.942	3	44.467	<0.001	Yes
Obese Contro vs. Obese Treate	13.878	2	17.662	<0.001	Yes
Obese Treated vs. Lean Control	26.423	3	33.627	<0.001	Yes
Obese Treated vs. Lean Treated	21.064	2	26.806	<0.001	Yes
Lean Treated vs. Lean Control	5.36	2	6.821	0.001	Yes

Phos/Total ERK1/2 (Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus Phos/ total ERK1/2 paper

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in Notebook3

Group	N	Missing	Median	25%	75%
Lean Control	3	0	0.479	0.331	0.492
Lean Treated	3	0	0.5	0.395	0.52
Obese Control	3	0	1.912	1.793	1.981
Obese Treated	3	0	1.081	0.935	1.099

H = 9.667 with 3 degrees of freedom. (P = 0.022)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.022)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

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

Comparison	Diff of Ranks	q	P<0.05
Obese Control vs Lean Control	25	4.003	Yes
Obese Control vs Lean Treated	20	4.216	Yes
Obese Control vs Obese Treated	9	2.777	Yes
Obese Treated vs Lean Control	16	3.373	Yes
Obese Treated vs Lean Treated	11	3.395	Yes
Lean Treated vs Lean Control	5	1.543	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Total JNK (Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus total JNK paper

Normality Test (Shapiro-Wilk) Passed (P = 0.897)

Equal Variance Test: Passed (P = 0.284)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Lean Control	3	0	9.36	2.867	1.655
Lean Treated	3	0	14.817	3.212	1.854
Obese Control	3	0	21.982	0.0971	0.056
Obese Treated	3	0	53.841	5.922	3.419

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	3	3567.709	1189.236	88.732	<0.001
Residual	8	107.221	13.403		
Total	11	3674.93			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

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

Comparisons for factor:

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
Obese Treated vs. Lean Control	44.481	4	21.045	<0.001	Yes
Obese Treated vs. Lean Treated	39.024	3	18.463	<0.001	Yes
Obese Treate vs. Obese Contro	31.86	2	15.073	<0.001	Yes
Obese Control vs. Lean Control	12.622	3	5.972	0.007	Yes
Obese Control vs. Lean Treated	7.165	2	3.39	0.044	Yes
Lean Treated vs. Lean Control	5.457	2	2.582	0.105	No

Phos JNK (Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus phos JNK paper

Normality Test (Shapiro-Wilk) Passed (P = 0.675)

Equal Variance Test: Passed (P = 0.585)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	8.047	5.679	3.279
Lean Treated	3	0	20.01	3.983	2.299
Obese Control	3	0	24.706	2.65	1.53
Obese Treated	3	0	47.236	7.869	4.543

Source of Variation	DF	SS	MS	F	P
Between Groups	3	2420.538	806.846	27.571	<0.001
Residual	8	234.114	29.264		
Total	11	2654.653			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Obese Treated vs. Lean Control	39.189	4	12.548	<0.001	Yes
Obese Treated vs. Lean Treated	27.226	3	8.717	<0.001	Yes
Obese Treated vs. Obese Control	22.53	2	7.214	0.001	Yes
Obese Control vs. Lean Control	16.659	3	5.334	0.014	Yes
Obese Control vs. Lean Treated	4.696	2	1.504	0.319	No
Lean Treated vs. Lean Control	11.964	2	3.83	0.027	Yes

Phos/Total JNK (Soleus)

One Way Analysis of Variance

Data source: Raw data in Soleus phos/total JNK paper

Normality Test (Shapiro-Wilk) Passed (P = 0.438)

Equal Variance Test: Passed (P = 0.462)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	0.548	0.277	0.16
Lean Treated	3	0	1.112	0.0721	0.0416
Obese Control	3	0	1.039	0.108	0.0622
Obese Treated	3	0	1.055	0.0563	0.0325

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.618	0.206	8.528	0.007
Residual	8	0.193	0.0242		
Total	11	0.812			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.007).

Power of performed test with alpha = 0.050: 0.890

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Lean Treated vs. Lean Control	0.564	4	6.282	0.009	Yes
Lean Treated vs. Obese Control	0.0726	3	0.809	0.838	No
Lean Treated vs. Obese Treated	0.0574	2	0.639	0.663	Do Not Test
Obese Treated vs. Lean Control	0.506	3	5.642	0.01	Yes
Obese Treated vs. Obese Control	0.0152	2	0.17	0.908	Do Not Test
Obese Control vs. Lean Control	0.491	2	5.473	0.005	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.

Total p38(EDL)

One Way Analysis of Variance

Data source: Raw data in EDL total p38 paper

Normality Test (Shapiro-Wilk) Passed (P = 0.720)

Equal Variance Test: Passed (P = 0.645)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	19.69	8.088	4.669
Lean Treated	3	0	10.003	1.281	0.739
Obese Control	3	0	40.919	4.394	2.537
Obese Treated	3	0	29.388	6.945	4.01

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1577.299	525.766	15.626	0.001
Residual	8	269.182	33.648		
Total	11	1846.482			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.001).

Power of performed test with alpha = 0.050: 0.995

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Obese Control vs. Lean Treated	30.916	4	9.231	<0.001	Yes
Obese Control vs. Lean Control	21.228	3	6.339	0.005	Yes
Obese Control vs. Obese Treated	11.53	2	3.443	0.041	Yes
Obese Treated vs. Lean Treated	19.385	3	5.788	0.009	Yes
Obese Treated vs. Lean Control	9.698	2	2.896	0.075	No
Lean Control vs. Lean Treated	9.687	2	2.893	0.075	No

Phos p38(EDL)

One Way Analysis of Variance

Data source: Raw data in EDL phos p38 paper

Normality Test (Shapiro-Wilk) Passed (P = 0.843)

Equal Variance Test: Passed (P = 0.624)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Lean Control	3	0	35.931	12.528	7.233
Lean Treated	3	0	25.73	2.595	1.498
Obese Control	3	0	16.776	1.935	1.117
Obese Treated	3	0	21.563	9.007	5.2

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	3	598.424	199.475	3.21	0.083
Residual	8	497.122	62.14		
Total	11	1095.546			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.083).

Power of performed test with alpha = 0.050: 0.367

The power of the performed test (0.367) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Phos/ Total p38 (EDL)

One Way Analysis of Variance

Data source: Raw data in EDL phos/ total p38 paper

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
Lean Control	3	0	2.005	1.209	2.576
Lean Treated	3	0	2.417	2.33	2.572
Obese Control	3	0	0.449	0.424	0.457
Obese Treated	3	0	0.749	0.517	0.859

H = 9.462 with 3 degrees of freedom. (P = 0.024)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.024)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

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

Comparison	Diff of Ranks	q	P<0.05
Lean Treated vs Obese Control	24	3.843	Yes
Lean Treated vs Obese Treated	15	3.162	No
Lean Treated vs Lean Control	3	0.926	Do Not Test
Lean Control vs Obese Control	21	4.427	Yes
Lean Control vs Obese Treated	12	3.703	Do Not Test
Obese Treated vs Obese Control	9	2.777	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison. For example, if you had four rank sums sorted in order, and found no significant difference between rank sums 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 rank sums is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the rank sums, even though one may appear to exist.

Total ERK1/2 (EDL)

One Way Analysis of Variance

Data source: Raw data in EDL total ERK1/2 paper

Normality Test (Shapiro-Wilk) Passed (P = 0.931)

Equal Variance Test: Passed (P = 0.105)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	24.845	3.047	1.759
Lean Treated	3	0	18.694	1.296	0.748
Obese Control	3	0	29.729	1.113	0.643
Obese Treated	3	0	26.731	4.204	2.427

Source of Variation	DF	SS	MS	F	P
Between Groups	3	195.447	65.149	8.723	0.007
Residual	8	59.747	7.468		
Total	11	255.194			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.007).

Power of performed test with alpha = 0.050: 0.898

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Obese Control vs. Lean Treated	11.035	4	6.994	0.005	Yes
Obese Control vs. Lean Control	4.884	3	3.096	0.133	No
Obese Control vs. Obese Treated	2.998	2	1.9	0.216	Do Not Test
Obese Treated vs. Lean Treated	8.037	3	5.094	0.017	Yes
Obese Treated vs. Lean Control	1.886	2	1.195	0.423	Do Not Test
Lean Control vs. Lean Treated	6.151	2	3.898	0.025	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.

Phos ERK1/2 (EDL)

One Way Analysis of Variance

Data source: Raw data in Soleus phos ERK1/2 paper

Normality Test (Shapiro-Wilk) Passed (P = 0.119)

Equal Variance Test: Passed (P = 0.859)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Lean Control	3	0	34.811	1.542	0.89
Lean Treated	3	0	17.542	2.573	1.486
Obese Control	3	0	21.567	1.781	1.028
Obese Treated	3	0	26.081	1.054	0.608

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	3	494.493	164.83	149.645	<0.001
Residual	8	26.561	3.32		
Total	11	521.054			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

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

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
Lean Control vs. Lean Treated	17.269	4	16.415	<0.001	Yes
Lean Control vs. Obese Control	13.244	3	12.589	<0.001	Yes
Lean Control vs. Obese Treated	8.73	2	8.298	<0.001	Yes
Obese Treated vs. Lean Treated	8.539	3	8.117	0.001	Yes
Obese Treated vs. Obese Control	4.514	2	4.29	0.016	Yes
Obese Control vs. Lean Treated	4.026	2	3.826	0.027	Yes

Phos/Total ERK1/2 (EDL)

One Way Analysis of Variance

Data source: Raw data in EDL phos/total ERK1/2 paper

Normality Test (Shapiro-Wilk) Passed (P = 0.882)

Equal Variance Test: Passed (P = 0.246)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	1.41	0.107	0.062
Lean Treated	3	0	0.935	0.0745	0.043
Obese Control	3	0	0.725	0.0324	0.0187
Obese Treated	3	0	0.989	0.128	0.0737

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.742	0.247	28.71	<0.001
Residual	8	0.0689	0.00861		
Total	11	0.811			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Lean Control vs. Obese Control	0.685	4	12.789	<0.001	Yes
Lean Control vs. Lean Treated	0.475	3	8.857	<0.001	Yes
Lean Control vs. Obese Treated	0.421	2	7.855	<0.001	Yes
Obese Treated vs. Obese Control	0.264	3	4.934	0.02	Yes
Obese Treated vs. Lean Treated	0.0536	2	1.001	0.499	No
Lean Treated vs. Obese Control	0.211	2	3.932	0.024	Yes

Total JNK (EDL)

One Way Analysis of Variance

Data source: Raw data in EDL total JNK paper

Normality Test (Shapiro-Wilk) Passed (P = 0.939)

Equal Variance Test: Passed (P = 0.834)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	27.94	3.757	2.169
Lean Treated	3	0	19.765	1.639	0.946
Obese Control	3	0	24.594	1.428	0.824
Obese Treated	3	0	27.701	3.3	1.905

Source of Variation	DF	SS	MS	F	P
Between Groups	3	130.523	43.508	5.854	0.02
Residual	8	59.453 7.432			
Total	11	189.976			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.020).

Power of performed test with alpha = 0.050: 0.708

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Lean Control vs. Lean Treated	8.175	4	5.194	0.026	Yes
Lean Control vs. Obese Control	3.347	3	2.126	0.339	No
Lean Control vs. Obese Treated	0.24	2	0.152	0.917	Do Not Test
Obese Treated vs. Lean Treated	7.935	3	5.042	0.018	Yes
Obese Treated vs. Obese Control	3.107	2	1.974	0.2	Do Not Test
Obese Control vs. Lean Treated	4.828	2	3.068	0.062	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.

Phos JNK (EDL)

One Way Analysis of Variance
Data source: Raw data in EDL phos JNK paper

Normality Test (Shapiro-Wilk) Passed (P = 1.000)

Equal Variance Test: Passed (P = 0.523)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	32.739	1.046	0.604
Lean Treated	3	0	24.188	5.215	3.011
Obese Control	3	0	21.631	2.913	1.682
Obese Treated	3	0	21.442	4.776	2.757

Source of Variation	DF	SS	MS	F	P
Between Groups	3	253.651	84.55	5.676	0.022
Residual	8	119.166	14.896		
Total	11	372.817			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.022).

Power of performed test with alpha = 0.050: 0.690

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Lean Control vs. Obese Treated	11.296	4	5.069	0.029	Yes
Lean Control vs. Obese Control	11.107	3	4.985	0.019	Yes
Lean Control vs. Lean Treated	8.551	2	3.837	0.027	Yes
Lean Treated vs. Obese Treated	2.746	3	1.232	0.672	No
Lean Treated vs. Obese Control	2.557	2	1.147	0.441	Do Not Test
Obese Control vs. Obese Treated	0.189	2	0.0848	0.954	Do Not Test

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.

Phos/ Total JNK (EDL)

One Way Analysis of Variance
Data source: Raw data in EDL phos/total JNK paper

Normality Test (Shapiro-Wilk) Passed (P = 0.853)

Equal Variance Test: Passed (P = 0.769)

Group Name	N	Missing	Mean	Std Dev	SEM
Lean Control	3	0	1.187	0.169	0.0977
Lean Treated	3	0	1.221	0.213	0.123
Obese Control	3	0	0.877	0.0697	0.0403
Obese Treated	3	0	0.768	0.0915	0.0528

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.456	0.152	6.962	0.013
Residual	8	0.175	0.0218		
Total	11	0.63			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.013).

Power of performed test with alpha = 0.050: 0.801

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

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Lean Treated vs. Obese Treated	0.453	4	5.31	0.023	Yes
Lean Treated vs. Obese Control	0.344	3	4.028	0.051	No
Lean Treated vs. Lean Control	0.0339	2	0.397	0.786	Do Not Test
Lean Control vs. Obese Treated	0.419	3	4.913	0.021	Yes
Lean Control vs. Obese Control	0.31	2	3.631	0.033	Do Not Test
Obese Control vs. Obese Treated	0.109	2	1.282	0.391	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.

APPENDIX E

Abbreviations

AAALAC	Association for assessment and accreditation of laboratory animal care
ADA	American Diabetes Association
ANOVA	One-way analysis of variance on ranks
BSA	Bovine serum albumin
DM	Diabetes Mellitus
ECL	Enhanced chemiluminescence
ERK1/2	Extracellular related kinase ½
IOD	Integrated Optical Densities
i.p.	Intra peritoneal
i.v.	Intravenous
LZ	Lean Zucker
JNK	Jun-N-terminal kinase
KRB	Krebs-Ringers Buffer Solution
MAPK	Mitogen activated protein kinase
NHANES	National Health and Nutrition Examination Survey
NHIS	National Health Interview Survey
OZ	Obese Zucker
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with 0.5% tween
PKB	Protein kinase B

PRT	Progressive Resistance Training p70S6k70 kDa ribosomal S6 kinase ROS Reactive oxygen species
s.c.	Subcutaneous
SEM	Standard Error Mean
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
TBS	Tris buffered saline
TBST	Tris buffered saline with 0.5% tween
T 2 DM	Type - 2 diabetes mellitus

CURRICULUM VITAE

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EDUCATION

Bachelor of Veterinary Sciences and Animal Husbandr Sept 2009 – Apr 2014
Sri Venkateshwara Veterinary University, India

Masters of biological sciences: Expected graduation: Fall 2018
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Concentrations: **Molecular biology**
Thesis title: **Effect of Empagliflozin treatment on insulin sensitivity in the lean and obese Zucker rat model of metabolic syndrome.**
Advisor**: Dr. Eric Blough**

CAREER SUMMARY

Apr 2014 – Nov 2014 Rotational internship, Andhra Pradesh

- Worked as an intern in government animal hospital. Underwent clinical training of consultation of medicinal, surgical and gynecological cases of small animals which include prognosis, treatment, preventive and management.
- Learned various management procedures and learned the duties of a veterinarian in a local governmental slaughterhouse.
- Field visits to various poultry, swine, cattle and sheep farms to learn about management and breeding protocols.
- Visited a zoo park to know about housing, feeding, and management of various wild animals.
- Worked as an intern in a private pet clinic where assisted the chief veterinarian with daily routine clinical procedures.

Nov 2014 – July 2016 Associate Veterinarian, Visakha pet clinic

- Worked as an outpatient physician; diagnosed the health status of the pets based on clinical examination, history of vaccination, illness and lab reports.
- Developed individual treatment plans and dispensed drugs accordingly.
- Performed minor surgical procedures and assisted with major surgeries.
- Record keeping for the protocols, vaccinations, and medicines.
- Monitored the progress of the patients and followed – up for each individual case after the course of treatment.

Oct 2015 – July 2016 Assistant Veterinarian, Indira Gandhi Zoological Park.

- Daily monitoring of health status of animals housed in the Zoo Park.
- Inspection of feed, vaccination status, and management of the animals.
- Record keeping of animals housed, medical history and drug repository.
- Post-mortem examinations and screening of live animal samples for diagnosis.
- Dispensing drugs for diseased animals and as prophylactic measures.
- Teaching internship students about the daily activities, feed inspection, various management techniques and basic restraining of wild animals.

Aug 2016 – June 2018 Research Assistant, Marshall University

- Working under RII PAR grant. Studying *Caenorhabditis elegans* for aquatic toxicity bioassays under fluorescent microscopy.
- Research on Study of the effect of drug Empagliflozin on skeletal muscles of Zucker rat.
- Worked on BI project for detecting efficacy of Empagliflozin in metabolic syndrome Zucker rat.

PRESENTATIONS

Institute of water security and science, spring symposium 2017 – Poster presentation: *Study of the interrelationship between heavy metals in effluents in Caenorhabditis elegans under a fluorescent microscope.*

Ohio River Basin Consortium for Research & Education (ORBCRE) and the Ohio River Basin Alliance (ORBA), 2017 – oral presentation: *Assessing toxicants in effluents using fluorescent microscopy in C.elegans.*

CERTIFICATIONS

National Cadet Corps – India

SKILLS

- Immunoblotting
- Handling of laboratory animals
- Surgery
- Various staining procedures
- Protein purification
- Operating imaging equipment
- Management and husbandry of various species of animals
- Small animal medicine
- Record keeping