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Effects of aging and gender on regulators of muscle adaptation in F344/BN rat model

By

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A thesis submitted to the

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At

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In partial fulfillment of the requirements for the degree

Of

Master of Science

Committee:

Dr. Eric Blough (Thesis adviser) …….

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ABSTRACT

Sarcopenia is the loss of muscle mass and strength that occurs with aging. Here we examine the effects of aging and gender on the regulation of molecules believed to regulate muscle growth and adaptation in the F344/BN rat. In male animals, soleus and EDL muscle/body weight ratio declined continuously with aging while muscle atrophy in female animals plateaued at 26-months and remained constant thereafter. Aging increased the phosphorylation of protein kinase-B (Akt) and the mammalian target of rapamycin (mTOR) in the female but not male soleus muscle. This finding was associated with the attenuation of muscle atrophy observed in female animals. Male and female soleus muscles exhibited higher p70S6k phosphorylation with aging. Irrespective of muscle type or gender, aging was associated with increased calcineurin expression. Taken together, these data suggest that indices of protein synthesis and muscle adaptation are regulated differently with aging in different muscle types and gender.

(Keywords: Aging, sarcopenia, F344/BN, muscle, gender, soleus, EDL, diaphragm)

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LIST OF SYMBOLS

AAALAC	Association for assessment and accreditation of laboratory animal care
CSA	Cross sectional area
EDL	Extensor digitorum longus
eIF-4	Eukaryotic initiation factor - 4
F344/BN	Fisher 344 Brown Norway hybrid
GSK-3ß	Glycogen synthase kinase 3 beta
IGF-1	Insulin like growth factor-1
IOD	Integrated optical densities
KRB	Krebs-Ringers Buffer solution
MAPK	Mitogen activated protein kinase
mTOR	Mammalian target of rapamycin
NF-AT	Nuclear factor activating T cells
p70S6K	p70 ribosomal s6 kinase
ROS	Reactive oxygen species
4E-BP1	Eukaryotic initiation factor 4E binding protein-1

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CHAPTER-I

INTRODUCTION

Sarcopenia is the loss of muscle mass that occurs as a result of the aging process. The number of aged (>60 years) increased from 31.2 million people in 1990 to 35.0 million in 2000 in the United States (WHO Brasilia declaration on healthy aging, 1996). It has been estimated that the direct healthcare costs of sarcopenia were $18 billon in the United States in 2000 [1] with this number expected to rise as the number of aged increase. Sarcopenia is a consequence of normal aging that is characterized by decreased muscle strength, reduced performance and diminished quality of life [2, 3], Indeed, by the seventh and eighth decade of life, maximal voluntary contractile strength is decreased, on average, by 20–40% for both men and women. The etiology of sarcopenia includes physical inactivity, motor-unit remodeling, decreased protein synthesis, increased cytokine activity, oxidative stress, and decreased hormone levels[4]. How gender differences may affect the cause or progression of sarcopenia is not well understood [5].

Rat models and substrains exhibit different life expectancies [6] and biological characteristics [7]. Probability of survival curves generated by the National Institute of Aging (NIA) were employed to identify F1 generation hybrid of Fischer 344/NNiaHSd X Brown Norway/BiNia (F344/BN), male and female rats ages so as to correspond roughly to humans in their third, seventh, and eighth decade of life, respectively [8]. This latter time point was chosen because the World Health Organization defines this age

group as ‘‘elderly’’, a time where muscle atrophy and dysfunction are present and accelerating in humans [9]. The relatively new F344/BN hybrid model appears to be more resistant to diseases associated with aging [10] than the Fischer 344 (F344) rat strain. Average survival age is higher in the F344/N X BN than the F344, suggesting perhaps that they are a better model of age-related muscle loss since they appear to survive long enough to develop sarcopenia [10]. How aging affects skeletal muscle in the aging F344/BN is not well understood. Indeed, to our knowledge, there are no studies that have compared aging male and female F344/BN rats together within the same study.

Aging in the rodents is accompanied by a progressive loss of skeletal muscle fibers. The muscle twitch also becomes slower, probably as a result of fiber-type conversion from fast-twitch glycolytic to slow-twitch oxidative fibers [11]. Recent data has demonstrated that fast twitch and slow twitch muscles in male animals respond differently during aging [11, 12]. Whether similar differences between muscle types are observed in the aging female F344/BN rat has not been investigated.

Although age-associated decreases in strength per unit muscle mass, or muscle quality, may play a role, the majority of strength loss can be accounted for by decreased muscle mass [13]. It is thought that the ability of skeletal muscle to adapt to an increased contractile stimulus diminishes with aging [14]. The mechanism(s) responsible for this decreased adaptive potential are not clear. Studies investigating striated muscle hypertrophy have established that the p70 ribosomal protein S6 kinase (p70S6k) plays a critical role in regulating protein synthesis and muscle adaptation following an exercise stimulus [15-17]. In addition to p70S6k, protein kinase B (Akt),

glycogen synthase kinase-3 beta(GSK - 3β), the mammalian target of rapamycin (mTOR) and calcineurin (CnA), have also been identified as key signaling molecules involved in the regulation of muscle adaptation to increased contractile loading [18]. How the regulation of these molecules is altered with aging or with genders is not well understood.

HYPOTHESIS/PURPOSE OF STUDY

Our long term goal is to identify the cellular and molecular mechanisms of age-related skeletal muscle loss. The present study was designed to determine whether aging alters the regulation of proteins thought to be involved in regulating muscle adaptation. Here we examine the effects of aging and gender on the expression and phosphorylation of Akt, mTOR, p70S6k, and calcineurin in the fast-twitch extensor digitorum longus (EDL), the slow-twitch soleus and the continuously active diaphragm. We hypothesized that aging will alter the concentration of signal transduction proteins in EDL, soleus and diaphragm differently and that the regulation of these molecules may differ across genders.

Specific Aim: To determine how aging effects the regulation of p70S6k, Akt, mTOR and calcineurin in the male and female F344/BN EDL, soleus and diaphragm muscles.

Hypothesis: Aging will alter the regulation of p70S6k, Akt, mTOR and calcineurin differently in male and female F344/BN EDL, soleus, and diaphragm muscles.

SIGNIFICANCE OF STUDY:

Sarcopenia is associated with increased health care costs and negatively impacts quality of life for many of the United States aging population. A greater understanding of the factors that cause and contribute to the progression of sarcopenia is needed. Further studies on the mechanisms leading to sarcopenia could provide the basis for prevention and the establishment of therapeutic methods that will contribute to an increase in the standard of living among elderly people [19]. This study will determine how aging alters the regulation of proteins thought to govern muscle adaptation. The identification of age-related changes in the regulation of these proteins could lead to a greater understanding of the mechanisms leading to sarcopenia and could provide the basis for the establishment of therapeutic interventions that could be used to target sarcopenia.

CHAPTER-II

REVIEW OF LITERATURE

INTRODUCTION

A review of the pertinent literature concerning the present study will be presented in the following chapter. The following areas will be addressed: 1) Age associated alterations in skeletal muscle, 2) Regulators of muscle plasticity and protein synthesis, and 3) Effects of gender on age-related changes in muscle mass and quality.

Age associated alterations in skeletal muscle

Isometric and dynamic strength increases up to the third decade, remains almost constant to the fifth decade, and then decreases with increasing age [20]. The loss of muscle mass and strength with aging, also referred to as sarcopenia, is highly prevalent and predicts several adverse outcomes, including disability, institutionalization and mortality. Although the exact mechanisms underlying sarcopenia are unknown, accumulating evidence suggests that an age-related acceleration of myocyte loss via apoptosis might represent a key mechanism driving the onset and progression of muscle loss [21]. A number of physiological factors have been suggested to be involved in sarcopenia, including an age-related reduction of growth hormone [22], thyroxine [23], and, in women and men, estrogen and testosterone [24], respectively. A number of mechanisms proposed include alterations in motor unit organization [25-28], contraction-induced injuries [29, 30], deficient satellite cell recruitment [31], increases in

free radicals and oxidative stress [32, 33], and age-related accumulation of mitochondrial abnormalities. Young *et al*., (1984, 1985), used ultrasonography and found 25-35% reductions in the cross sectional area of the quadriceps muscle in men and women over the age of 65 years when compared to young [34, 35]. The computed tomography scanning technique, performed by several researchers, has shown similar age-related reductions in cross sectional area of the psoas major and sacrospinalis muscles,[36] the quadriceps muscle [37], the brachial biceps and triceps muscles[38, 39] and the plantar flexors [38, 39] of men over the age of 65.

A great deal of research on aging has been performed on the aged rats because of the difficulties associated with aging studies in humans such as ethical, cross sectional design and inability to control life time activity pattern of the subjects. As with human beings, aging in animals also appears to affect muscle function. In rodents, several investigators have noted a similar, preferential reduction in type II fiber cross sectional area (CSA) and little or no decline in type I fiber CSA [40, 41]. Taken together, these data suggest that age-associated changes in fiber cross sectional area, muscle mass, may vary across different muscle fiber types and muscles depending on whether the muscle is used for weight bearing or non-weight bearing activity [42].

Skeletal muscle exhibits a great deal of plasticity, which is specific to the stimulus it receives [43, 44]. Muscle plasticity or the ability of muscle to adapt to an altered contractile stimulus decreases with aging in both humans and animals [14, 45-49]. Hypertrophy of muscle cells in response to increased functional demand is a well established phenomenon. It has been shown that resistance training in humans leads to muscle hypertrophy which is mainly the result of an enhanced cross-sectional area of

individual muscle fibers [23, 50, 51]. Similar adaptations have been observed in animals after ablation or denervation of synergistic muscles [50, 52]. The mechanisms underlying age-associated decreases in muscle plasticity are unknown. Faulkner *et al*., 2007 concluded that, for both humans and rats, the timing and magnitude of the loss of motor units is similar to that for muscle fibers which suggests that the mechanism responsible for the loss of fibers and for the loss of whole motor units is the same. It is thought that the degree of atrophy of the fibers that remain is largely dependent on the habitual level of physical activity of the individual [53].

Summary:

The age associated deterioration in size, mass, and function of skeletal muscle was observed in both human and animal skeletal muscle and can be or at least partly be reversed by resistance training. These age associated changes may be due to changes in hormonal activity, muscle type (weight bearing or non weight bearing), or due to increased oxidative stress and several others, but the exact molecular mechanisms underlying the sarcopenia is still unknown.

Regulators of muscle plasticity and protein synthesis

A decrease in the production of anabolic hormones such as testosterone, growth hormone and insulin-like growth factor-1(IGF-1) impairs the capacity of skeletal muscle to incorporate amino acids and synthesize proteins. An increase in the release of catabolic agents, specifically interleukin-6, amplifies the rate of muscle wasting among the elderly [54]. IGF-1 belongs to the insulin family of peptides and acts as a growth factor in any tissues and tumors. Locally acting IGF-1 enhances muscle growth and differentiation, prevents age related muscle atrophy, and potentiates regeneration after injury [55, 56]. IGF-1 also increases skeletal muscle anabolic processes and attenuates the ubiquitin – proteasome pathway and the formation of oxidative products. Similarly, IGF-1 decreases oxidative damage in the myocardium with aging [57]. Entela *et al*., in 2001, examined mitochondrial abnormalities in muscles undergoing sarcopenia and concluded that different muscles accumulate different levels of electron transport system abnormalities during normal aging and also that these electron transport system abnormalities contribute to senescent muscle atrophy [58]. The exact role of the mitochondria in muscle sarcopenia is not well understood.

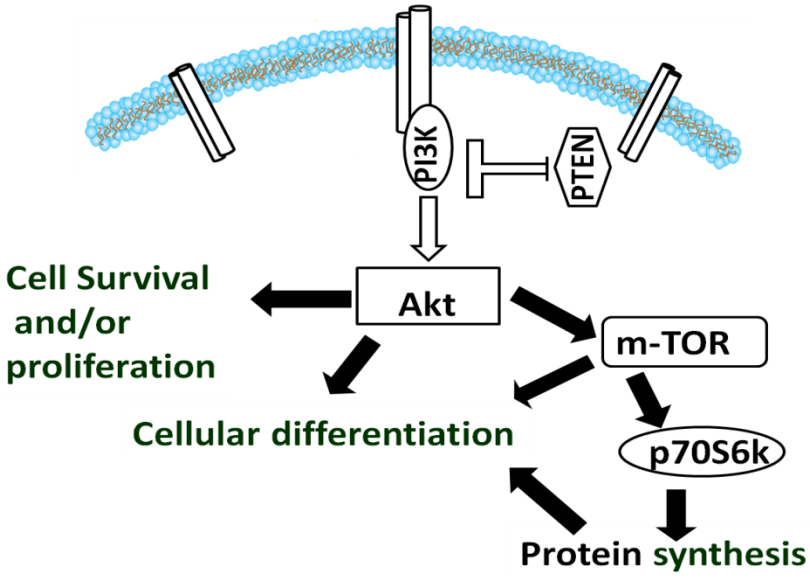
Tumor necrosis factor-alpha (TNF- α) is elevated in the serum as a result of aging and it promotes pro-apoptotic signaling upon binding to the type I TNF receptor [59]. It is not known if activation of this apoptotic pathway contributes to the well documented age-associated decline in muscle mass. Skeletal muscles containing type II fibers are more susceptible to muscle mass losses via the extrinsic apoptotic pathway [60]. There is an overall increase in calpain activities associated with muscle aging,

suggesting that the calcium-dependent proteolytic system is indeed involved in sarcopenia [61]. Calcineurin (CnA), a calcium-sensitive phosphatase, plays a critical role in transduction of calcium(Ca^{2+}) signals in different types of cells [62]. The main substrate of calcineurin is the nuclear factor of activated T cells (NFAT), a family of transcription factors (NFATC1–C4). During periods of sustained elevations of calcium, calcineurin dephosphorylates NFATC1–C4, allowing NFAT to translocate to the nucleus. A Ca^{2+}-dependent CnA signaling pathway has been implicated both in the hypertrophic response and in the neurally dependent transition of skeletal muscle from fast-twitch to the slow-twitch phenotype [63, 64]. How CnA is regulated with aging in skeletal muscle has not been fully elucidated.

Human sarcopenia may be linked to a reduction in the activity or sensitivity of anabolic signaling proteins such as growth hormone receptor (GHR), IGF-1, and protein kinase B (Akt). In addition, TNF- α, suppressor of cytokine signaling-3 (SOCS-3), and myostatin are other potential candidates that may be involved in regulating age-associated muscle loss [65]. A lack of myostatin appears to reduce age-related sarcopenia and loss of muscle regenerative capacity [66]. Akt plays a number of roles that may be important in sarcopenia including suppression of apoptosis and the modulation of muscle-specific protein degradation via the inhibition of the expression of the ubiquitin E3 ligases atrogin-1 and muscle ring factor-1 (MuRF-1). Akt activity also promotes protein translation via the inhibition of glycogen synthase kinase-3 β and the activation of mammalian target of rapamycin (mTOR). The activation of Akt is known to be sensitive to insulin and IGF-I levels [67]. The phosphatidyl inositol 3-kinase(PI3K) signaling substrate mTOR has been implicated in skeletal muscle hypertrophy during

overload [68]. After an acute bout of contractile activity and during chronic periods of overload, phosphorylation of mTOR and its downstream target 70-kDa ribosomal protein S6 kinase (p70S6K) are increased [68-71], and the degree of p70S6K phosphorylation after a single bout of contractile activity is strongly associated with increase in muscle weight. Correspondingly, pharmacological inhibition of mTOR prevents overload-induced hypertrophy in both type I and type II fibers [68]. Bodine *et al*., in 2001, concluded that activation of the Akt/mTOR pathway and its downstream targets, p70S6K and PHAS-1/4E-BP1, is requisitely involved in regulating skeletal muscle fiber size, and that activation of the Akt/mTOR pathway can oppose muscle atrophy induced by disuse [68]. How aging may affect the ability of skeletal muscle to regulate Akt →mTOR→p70S6k signaling is not well understood.

Figure 1



Schematic representation of molecules thought to be involved in Akt-mTOR pathway

Although extracellular regulated kinase 1/2 (ERK1/2) activity does not appear to be sufficient for myogenesis *in vitro* or for muscle hypertrophy *in vivo* [72], ERK1/2 signaling is required for proliferation of satellite cells and may play a permissive role in the network of signals required for regeneration and hypertrophy of skeletal muscle. Emerging evidence suggests that nuclear factor-kappa B (NF-kappa B) is one of the most important signaling pathways linked to the loss of skeletal muscle mass in various physiological and patho-physiological conditions. Activation of NF-kappa B in skeletal muscle leads to degradation of specific muscle proteins, induces inflammation and fibrosis, and blocks the regeneration of myofibers after injury / atrophy [73]. How aging may alter the regulation of ERK1/2 and NF-kappa B in skeletal muscle has not been established.

Summary

The protein content in a skeletal muscle is dependent on the rate of protein synthesis and the rate of protein degradation. The anabolic hormones like growth hormone, insulin, testosterone in males, and estrogen in females initiates protein synthesis *via* several signaling pathways. Akt activity promotes protein translation via the inhibition of glycogen synthase kinase-3 β and the activation of mammalian target of rapamycin (mTOR) and it's down stream protein, s6 ribosomal protein kinase (p70S6k). An increase in the release of catabolic agents like interleukin-6, TNF- α and activation of molecules like nuclear factor-kappa B(Nf-KB), B-cell CLL/lymphoma 3(BCL3) amplifies the rate of protein degradation in skeletal muscle among the elderly. How skeletal

muscle is able regulate all these anabolic and catabolic molecules with aging is not well understood.

Effects of gender on age-related changes in muscle mass and quality

In the developed world, most women live one-third of their lives in a state of profound estrogenic deprivation [74]. In addition, with advancing age of women there is a decrease in the serum levels of testosterone and androgens [75]. Moreover, elderly males have altered local levels of bioactive estrogens, secondary to reduced secretion of adrenal sex-steroid precursors to estrogen by aromatization [76]. Age-related strength losses are secondary to decline in skeletal muscle mass in men and women. While women may experience earlier strength losses than men, overall, age associated decreases in strength are similar. Although men may experience greater losses of total muscle mass, recent evidence points toward greater declines in muscle quality in older women [77]. Men are stronger than women at all ages [78] and in both sexes the average values for maximum voluntary strength of the dorsi flexors and plantar flexors begin to decline in the 6th decade [78]. Body mass index (BMI) is a strong predictor of skeletal muscle mass in women and men. The relative rate of skeletal muscle loss in men substantially exceeds the relative rate of bone mineral loss. In women, the relative losses of skeletal muscle and bone proceed at similar rates [79]. Aging men and women would therefore be anticipated to develop very different musculoskeletal relationships in old age. Falls, weakness, frailty, and ultimately fractures can potentially arise from changes in bone composition and quality, a loss of supporting and protective skeletal muscle, or a combination of the two [13, 79]. Several studies have shown differences in the prevalence of sarcopenia between men and women, with men being more susceptible [80-82], again suggesting that hormones may play a role in age-related muscle loss. Sex-related differences exist at the whole muscle and single fiber levels

with regarding to the strength and fiber cross-sectional area [83]. There is epidemiological data suggesting that there is a relationship with reduced testosterone levels and the decline in muscle mass, strength and functional status [84-86]. Testosterone replacement has resulted in increased muscle mass and strength in hypogonadal populations, elderly men and increased strength and protein synthesis in elderly women [87].

Age-related sarcopenia is largely confined to type II muscle fibers when compared to type I muscle fibers [88]. Rice KM and Blough ER, in 2006, worked on sarcopenia related apoptosis in different muscle fiber types and concluded that the apoptotic regulatory events differ between fiber types in the aging male F344/BN rats and that mitochondrial-dependent apoptosis pathways may not play a primary role in the loss of muscle nuclei with aging [89]. Whether similar findings are observed in the aging female F344/BN rats is not known.

Satellite cells are small mononuclear progenitor cells with virtually no cytoplasm found in mature muscle. They are found sandwiched between the basement membrane and sarcolemma (cell membrane) of individual muscle fibers, and can be difficult to distinguish from the sub-sarcolemmal nuclei of the fibers. This cell type was discovered in 1961 by Mauro in muscles of anuran amphibians and given the term satellite cells. Although satellite cells are found in all muscle fibers their distribution is imbalanced. Increased numbers of satellite cells are found within muscles composed of type I, slow oxidative fibers such as the soleus compared with muscles composed of type II, fast glycolytic fiber types such as EDL [90]. How aging affects the regulation of muscle satellite cells is not well understood.

Summary:

Sarcopenia is observed in both males and females but the molecular mechanism underlying the incidence of sarcopenia is not well understood. The sarcopenia involves more of the type II, fast glycolytic fibers than the type I, slow oxidative fibers in aging males but whether similar findings are observed in the aging females is not known. The molecular signaling mechanisms occurring in different muscle types and genders should be looked at to investigate the differences in signaling with gender, age, and fiber type.

CHAPTER-III

Effects of aging and gender on regulators of muscle adaptation in F344/BN rat model

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ABSTRACT

Sarcopenia is the loss of muscle mass and strength that occurs with aging that if allowed to proceed unchecked can lead to disability. Here we compare the total content and phosphorylation levels of several molecules believed to regulate muscle growth and adaptation in the slow twitch muscle-soleus, the fast twitch muscle-extensor digitorum longus (EDL), and the continuously active diaphragm muscles of 6-(adult), 30-(aged), and 36-month(very aged) male and 6-(adult), 26-(aged), and 30-month(very aged) female Fischer 344XBrown Norway rats. In male animals, soleus and EDL muscle/ body weight ratio was lower in the 30-month (6- vs. 30-month soleus: 20%,($P<0.05$); 6- vs. 30-month EDL: 25.58%,($P<0.05$)) animals and decreased further at 36-months (30- vs. 36-month soleus: 18.75%,($P<0.05$) ;30- vs. 36-month: EDL 15.63%($P<0.05$)). Conversely, muscle atrophy in the aging female animals plateaued at 26-months and remained constant thereafter (6- vs. 26-and 30-month soleus: 25%($P<0.05$) and 25%); 6- vs. 26- and 30-month EDL: 23.52%($P<0.05$) and 27.45%). Aging increased the phosphorylation (activation) of protein kinase B (Akt) and the mammalian target of rapamycin (mTOR) in the female soleus. Both the male and female soleus exhibited higher p70S6k phosphorylation with aging while the aged EDL and diaphragm muscles failed to exhibit increased phosphorylation of Akt, mTOR or p70S6k with aging. Irrespective of muscle type, aging in both the genders was associated with increased calcineurin expression. Taken together, these data suggest that indices of protein synthesis and muscle adaptation are regulated differently with aging in different muscle types and gender.

INTRODUCTION

Sarcopenia is the loss of muscle mass that occurs as a result of the aging process. It has been estimated that the direct healthcare costs of sarcopenia are in excess of $18 billon in the United States [1] and this number is expected to rise as the number of aged increase. Age-related atrophy is thought to be a consequence of normal aging and it is characterized by decreased muscle strength, reduced performance and diminished quality of life [2, 3]. Indeed, by the seventh and eighth decade of life, maximal voluntary contractile strength is decreased, on average, by 20–40% for both men and women. The etiology of sarcopenia is not fully understood but likely includes changes in physical inactivity, motor-unit remodeling, decreased protein synthesis, increased cytokine activity, oxidative stress, and decreased hormone levels [4]. How gender differences may affect the cause or progression of sarcopenia is not well understood [5]. Similarly, recent data has also demonstrated that fast twitch and slow twitch muscles in male animals respond differently during aging [11, 12]. Whether similar differences between muscle types are observed in the aging female rats has not, to our knowledge, been investigated.

The molecular mechanism(s) underlying age-associated muscle loss remain to be elucidated. Studies investigating striated muscle hypertrophy have established that the p70 ribosomal protein S6 kinase (p70S6k) plays a critical role in regulating protein synthesis and muscle adaptation following an exercise stimulus [15-17]. In addition to p70S6k, several other molecules including protein kinase B (Akt), mammalian target of rapamycin (mTOR) and calcineurin (CnA) have also been identified as key signaling molecules involved in the regulation of muscle adaptation to increased contractile

loading [18]. How the regulation of these molecules is altered with aging or gender is not well understood. The purpose of this investigation was to examine the regulation of Akt, mTOR, p70S6k and calcineurin with aging in male and female Fischer 344/NNiaHSd X Brown Norway/BiNia (F344/BN) rats. Given the fact that muscle mass and adaptation decrease with increasing age, we hypothesized that the regulation of molecules involved in governing this process may also be altered with aging. Using probability of survival curves generated by the National Institute of Aging (NIA) we selected differently aged male and female F344/BN rats corresponding roughly to humans in their third, seventh, and eighth decades of life [8]. This latter time point was chosen because the World Health Organization defines this age group as ‘‘elderly’’, a time where muscle atrophy and dysfunction are present and accelerating in humans [9]. Our findings suggest that indices of muscle adaptation are regulated differently with aging in different muscle types and gender.

MATERIAL AND METHODS

Animals

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals as approved by the Council of the American Physiological Society and the Animal Use Review Board of The Marshall University. All procedures were conducted in strict accordance with Public Health Service animal welfare policy. Adult (6 months), aged (30 months) and very aged (36 months) male and Adult (6 months), aged (26 months) and very aged (30 months) female F1 Fischer 344/NNiaHSd X Brown Norway/BiNia rats were obtained from the National Institute on Aging. Rats were barrier housed two per cage in an AAALAC approved vivarium. Housing conditions consisted of a 12 Hour: 12 Hour dark-light cycle and temperature was maintained at 22 ± 2 °C. Animals were provided food and water *ad libitum*. Rats were allowed to recover from shipment for at least two weeks before experimentation began, and during this time the animals were carefully observed and weighed weekly. None of the older animals exhibited signs of failure to thrive, such as precipitous weight loss, disinterest in the environment, or unexpected gait alterations. Systolic blood pressure was determined with the animal un-anaesthetized using a programmed electro sphygmomanometer with pneumatic tail cuff (Narco - Biosystems, Houston, TX). Animals were acclimatized to the procedure for a minimum of 3 days prior to obtaining blood pressure.

Materials

Total and phosphorylated forms of AKT (catalog # 9272, 9271, 9275), m-TOR (catalog # 2972, 2971), P70S6K (Catalog # 9202, 9204, 9234) and mouse IgG and rabbit IgG antibodies were purchased from Cell Signaling Technology (Beverly, MA). Calcineurin (catalog # C1956) antibody was purchased from Sigma-Aldrich (St. Louis, MO). Precast 10% SDS-PAGE gels were procured from Cambrex Biosciences (Baltimore, MD), and enhanced chemiluminiscence (ECL) western blot 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-Aldrich (St. Louis, MO).

Tissue isolation

Animals were anesthetized with a ketamine-xylazine (4:1) cocktail (50 mg/kg intra peritoneal injection) and supplemented as necessary for reflexive response. Soleus, EDL, and diaphragm muscles were quickly removed, blotted dry, weighed, and immediately frozen in liquid nitrogen. Tissues were stored at -80° C until use.

Western blotting

Soleus, EDL, and diaphragm muscles were homogenized on ice, 2 times for 30 seconds in T-PER (2mL/100mg tissue weight) supplemented with 1mM PMSF, 1mM

Na_3VO_4, and 1mM NaF. After homogenization and centrifugation (9000 X g X 60 min. at 4ºC), the supernatant was separated from the pellet and stored in aliquots at -80ºC until use. Protein concentrations of the supernatant were determined in triplicate using bovine serum albumin (BSA) as a standard and the Bradford method (Pierce, Rockford, IL). Samples were diluted to a concentration of 2.5mg/ml in SDS-loading buffer and after boiling for 5 minutes, 50 µg of total protein for each age or time point were separated on 10% SDS-PAGE precast gels. Western blot transfer of protein onto nitrocellulose membranes was performed using standard conditions. To verify transfer of proteins and equal loading of lanes the membranes were stained with Ponceau S. For immune detection, membranes were blocked in 5% Milk in TBS-T for 1 hour at room temperature and then incubated with the appropriate primary antibody overnight at 4^0C. After washing in TBS-T, the membranes were exposed to horseradish peroxidase-labeled IgG secondary antibody for 1 hour at room temperature. Protein bands were visualized with ECL (Amersham Biosciences). 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 (Alpha Ease FC). 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 between expression levels of different signaling molecules, immunoblots were stripped with Restore western blot stripping buffer as detailed by the manufacturer (Pierce, Rockford, IL) and re-probed. After verifying the absence of residual HRP activity on the membrane by reaction with the ECL reagent, membranes were washed and re-probed.

Data Analysis

Results are presented as mean ± SEM. Multiple group comparisons were performed by two-way ANOVA followed by post-hoc testing where appropriate. For all comparisons, the alpha level was set at $P < 0.05$

RESULTS

Body mass, muscle mass and morphology

In male animals, soleus and EDL muscle / body weight ratio was lower in the 30-month (6-month soleus vs. 30-month: 20%, ($P<0.05$); 6-month EDL vs. 30-month: 25.58% ($P<0.05$)) animals and decreased further at 36-months (30-month soleus vs. 36-month soleus: 18.8%, ($P<0.05$) ;30-month EDL vs. 36-month: EDL 15.6% ($P<0.05$)) (Figure 1). Conversely, muscle atrophy in the aging female animals plateaued at 26-months and remained constant thereafter (6 month soleus vs. 26-and 30-month: 25% ($P<0.05$) and 25%); 6 month EDL vs. 26- and 30-month: 23.5% ($P<0.05$) and 27.5%).

Aging effects on AKT-mTOR-p70S6K pathway related protein expression and phosphorylation

To investigate whether aging affected the total and phosphorylated amounts of AKT, mTOR, and p70S6k expression in the skeletal muscle, we performed protein gel electrophoresis and immunoblotting using antibodies which recognize both the unphosphorylated and phosphorylated forms of these molecules. Immunoreactive bands of ~60kDa, ~289kDa, and ~70kDa, corresponded to the predicted molecular mass of the AKT, mTOR, and p70S6k respectively.

Soleus

Aging did not alter the amount of p-Akt (Ser 308) or p-Akt (Ser 473) in the male soleus. Compared to 6-month animals the amount of p-mTOR was 27.1% lower in the 36-month soleus (Fig 4A). Relative to 6-month animals, p-p70S6k (Ser 389) was 86% higher in 36-month old animals while calcineurin levels were 118.1% and 279.9% higher in the soleus muscles of 30- and 36-month animals (Figs. 5A and 6A). Opposite of what we found in the male animals, the amounts of p-Akt (Ser 308), p-Akt (Ser 473) and p-p70S6k (ser 389) were 141.1%, 182.1%, and 96 % higher in the soleus of 30-month female animals. Compared to 6-month animals, the amount of p-mTOR was 53.9% and 104.9% higher in 26- and 30-month female animals (Fig. 4A). Calcineurin levels were 17.4% and 63.3% higher in the soleus muscles of 26- and 30-month animals (Fig 6A).

EDL

Compared to the 6-month animals the amount of p-Akt (ser 473) was 29% higher in the EDL muscles of 36-month male and the amount of p-mTOR and p-p70S6k (ser389) were 27.1% and 79.7% lower in the 36-month male EDL(Fig 3B, 4B and 5B). Relative to the 6-month animals, the calcineurin levels were 183.7% and 259.3% higher in 30- and 36-month animals (Fig 6B). In female rats, aging did not alter the levels of p-Akt (Ser 473), p-mTOR, and p-p70S6k (Ser 389). Relative to the 6-month animals, the p-Akt (Ser 308) levels were 24.5% lower in 30-month (Fig 3B) and calcineurin levels were 69.2% and 116.1% higher in 26- and 30-month animals(Fig 6B).

Diaphragm

Aging did not alter the levels of p-Akt (Ser 308), p-Akt (Ser 473), and p-mTOR in the diaphragm of both male and female animals. Compared to the 6-month male animals the amount of p-p70S6k (Ser 389) was 42.6% higher in 36-month and did not change in female animals (Fig 5C). Relative to the 6-month male animals the amount of calcineurin was 76.7% and 351.9% higher in 30- and 36-month animals(Fig 6C). In female animals the calcineurin levels were 34.1% and 25.3% higher in 26- and 30-month animals (Fig 6C).

DISCUSSION

The intent of this study was to examine the effects of age on the intramuscular concentration of proteins thought to regulate protein synthesis and muscle plasticity in the fast-twitch EDL, the slow-twitch soleus, and diaphragm. Probability of survival curves generated by the NIA were employed to identify animal ages so as to correspond roughly to humans in their third, seventh, and eighth decade of life, respectively. The major finding of the present study is that the extent and potential mechanisms of age-related muscle atrophy may differ between muscles and with gender. Whether the changes we observe here in aging rats are also applicable to aging humans will require further studies.

Effects of aging on indicators of muscle adaptation

Similar to previous reports examining the effects of age on muscle mass in aging F344/BN male rats[10, 89] we found skeletal muscle mass continued to decrease with increasing age (Fig 1). Conversely, in the aging females, muscle atrophy expressed relative to body weight decreased in the aged (26-month animals) and then remained constant thereafter (Fig 1). This finding is supported by Iannuzzi-Sucich, (2002) who examined appendicular skeletal mass by dual x-ray absorptiometry in men and women and concluded that muscle atrophy varies with aging across gender in humans [81]. Why the degree of muscle loss with aging might vary across gender is not known. To our knowledge this finding in the F344/BN rat strain has not be reported before. In an

effort to understand these phenomena better, we examined how aging may affect the regulation of molecules previously found to be involved mediating muscle adaptation.

Protein kinase B (Akt) plays a number of roles that may be important in sarcopenia [67, 91, 92] including the suppression of apoptosis and modulation of muscle-specific protein degradation via the inhibition of the expression of the ubiquitin E3 ligases atrogin- 1 and MuRF-1. In addition, increased Akt phosphorylation (activity) also promotes protein translation via the inhibition of glycogen synthase kinase-3 and the activation of mTOR[67]. The factors and pathways that regulate Akt are not totally understood but it has been shown that Akt activity is increased with the phosphorylation of Thr308 and Ser473 [92]. With aging we found that Akt phosphorylation (Ser 308 and Ser 473) was increased in the female soleus at 26- and 30-months and in the aging female EDL at 26-months. Conversely, Akt phosphorylation was not increased with aging in the male or in the diaphragm irrespective of gender. Why aging tended to increase Akt phosphorylation in the female but not in the male animals is not known. In addition, why Akt phosphorylation was not altered in the aging diaphragm is also not clear. Nonetheless, these data suggest that Akt is regulated with aging differently between muscle types and genders.

To confirm these data, we next examined how aging may affect the regulation of mTOR. The mammalian target of rapamycin, mTOR is a Ser/Thr protein kinase[93-95], that is thought to lie downstream of Akt [96] and thought to act as a sensor for ATP and amino acids [97]. Under anabolic conditions, mTOR functions to increase translational activity by its ability to activate p70S6K and relieve the repression of 4E-BP1 [98]. Similar to our findings for Akt, we found an increased phosphorylation (activation) of

mTOR in the aging female soleus (Fig 4A). Conversely, mTOR phosphorylation was not increased in the aging male or in the diaphragm muscles of either gender.

Similar to our findings for Akt and mTOR, the phosphorylation of p70S6k also appears to be regulated differently with aging and gender. Here we observe that the amount of phosphorylated p70S6k increases in the soleus of aging male and female animals (Fig 5A.) while it also appears to be elevated in the diaphragm of aging male animals (Fig. 5C). It has been shown that p70S6k plays a critical role in the translation of transcripts involved in the cell cycle progression and the translational machinery [99]. We suggest that this increased phosphorylation observed in the aged soleus indicates that the protein anabolism mechanism is intact up to and including p70S6k. As such, we believe that our data indicates that the aged soleus muscle is fully capable of elevated protein synthesis. Importantly, these data may also suggest that the differences in the loss of muscle mass with age in female animals may be attenuated compared to those observed in male animals. Taken further, these data collectively suggest that the elevation of protein synthetic potential in the face of sarcopenia observed in the present study indicates that a deficiency in the signals used to initiate protein anabolism is not the primary cause of sarcopenia in these animals. Whether other physiological mechanisms may be participating in age-associated muscle loss in this model is actively being pursued in our laboratory.

Aging increases the muscle content of calcineurin

Besides factors that may be directly involved in the regulation of protein synthesis, we also investigated the age-associated expression of calcineurin (CnA).

Calcineurin is a Ca2+/calmodulin-dependent protein serine/threonine phosphatase, is a mediator of Ca2+ signaling in different cell systems [100]. CnA has been implicated in the regulation of satellite cell fusion and in the control of myosin heavy chain expression [64, 101-103]. The function of calcineurin in skeletal muscle is not entirely understood, however, previous data have demonstrated that this calcium-sensitive phosphatase acts to dephosphorylate a family of transcription factors called NFAT. This dephosphorylation in turn is thought to activate NFAT by unmasking a nuclear localization signal allowing the movement of NFAT into the nucleus [62]. Calcineurin signaling has been implicated in a broad spectrum of cellular processes including cell-cycle regulation and apoptosis while it has been shown that calcineurin is required for proper cardiovascular and skeletal muscle development [104, 105]. In skeletal muscle, calcineurin is thought to regulate the expression of slow myosin heavy chain [106]. Compared to adult (6-month) animals, we observed age-associated increases in the amount of calcineurin levels in each of the muscles examined irrespective of gender (Fig 6). Why aging may increase calcineurin levels is not known, however recent data suggests that aging in humans and the F344/BN rat model is associated with an increase in the amount of slow myosin heavy chain expression [97]. Although not measured in the present study, it is likely that the age-associated increase in calcineurin we observe here may be related to changes in the amount of slow myosin heavy chain expression. Future studies designed to directly test this assertion are currently ongoing.

In summary, we demonstrate that aging has a profound effect on the mass of the F344/BN EDL and soleus muscles. Because the ages of the oldest animals used in the current study correspond roughly to humans in their eighth decade, these data are

consistent with the hypothesis that the F344/BN strain is an excellent model for the study of age associated changes in human muscle and for the study of sarcopenia. In addition, we also show that the expression of the muscle plasticity regulators, Akt, mTOR, $p70^{S6k}$, and calcineurin are regulated differently between muscle types and across gender during aging. In addition, our data also suggest that the activation of these molecules occurs during the progression of muscle atrophy. Taken together, these data suggest that alterations in the expression and / or activation of these molecules are, by themselves, not sufficient to explain why skeletal muscle wasting occurs with aging. Future research aimed at examining other pathways or molecules will no doubt be of interest in helping to increase our understanding of age-associated muscle atrophy.

ACKNOWLEDGEMENTS

This study was supported by National Institute on Aging grant to Eric Blough, Marshall University and by NSF EPSCoR grant to Marshall University.

FIGURE AND FIGURE LEGENDS

This section presents all the figures used in this thesis document and the legends explaining the figures.

Figure 2

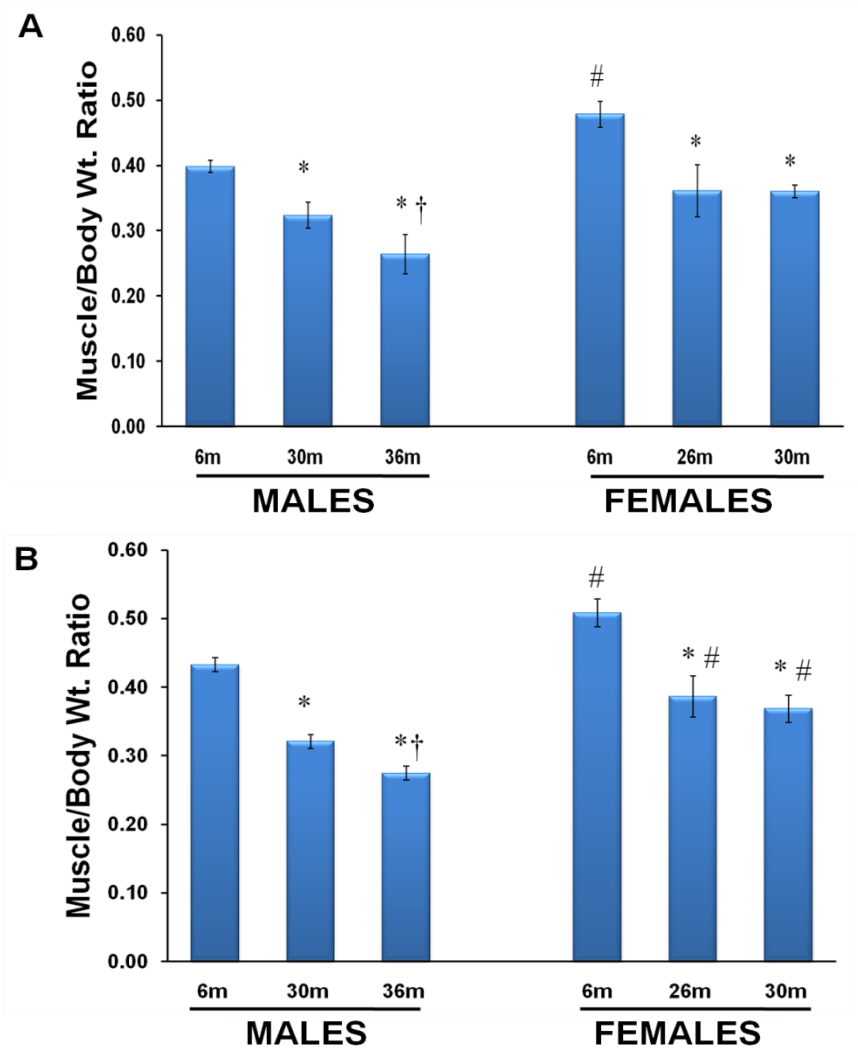

A
Muscle/Body Wt. Ratio
0.60
0.50
0.40
0.30
0.20
0.10
0.00
*
* †
#
*
*
6m
30m
36m
6m
26m
30m
MALES
FEMALES
B
Muscle/Body Wt. Ratio
0.60
0.50
0.40
0.30
0.20
0.10
0.00
*
*†
#
* #
* #
6m
30m
36m
6m
26m
30m
MALES
FEMALES

Figure 2. Muscle to body weight ratio of: A) Soleus B) EDL of 6- (young adult), 30- (aged) and 36-month (very aged) male and 6- (young adult), 26- (aged) and 30-month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. The data are presented as percent of the 6 month value (n = 4). * indicates significant difference from the corresponding 6 month value, † significantly different with 26- or 30-month animals within the same gender and # significantly different from age-matched males, ($p < 0.05$).

Figure 3

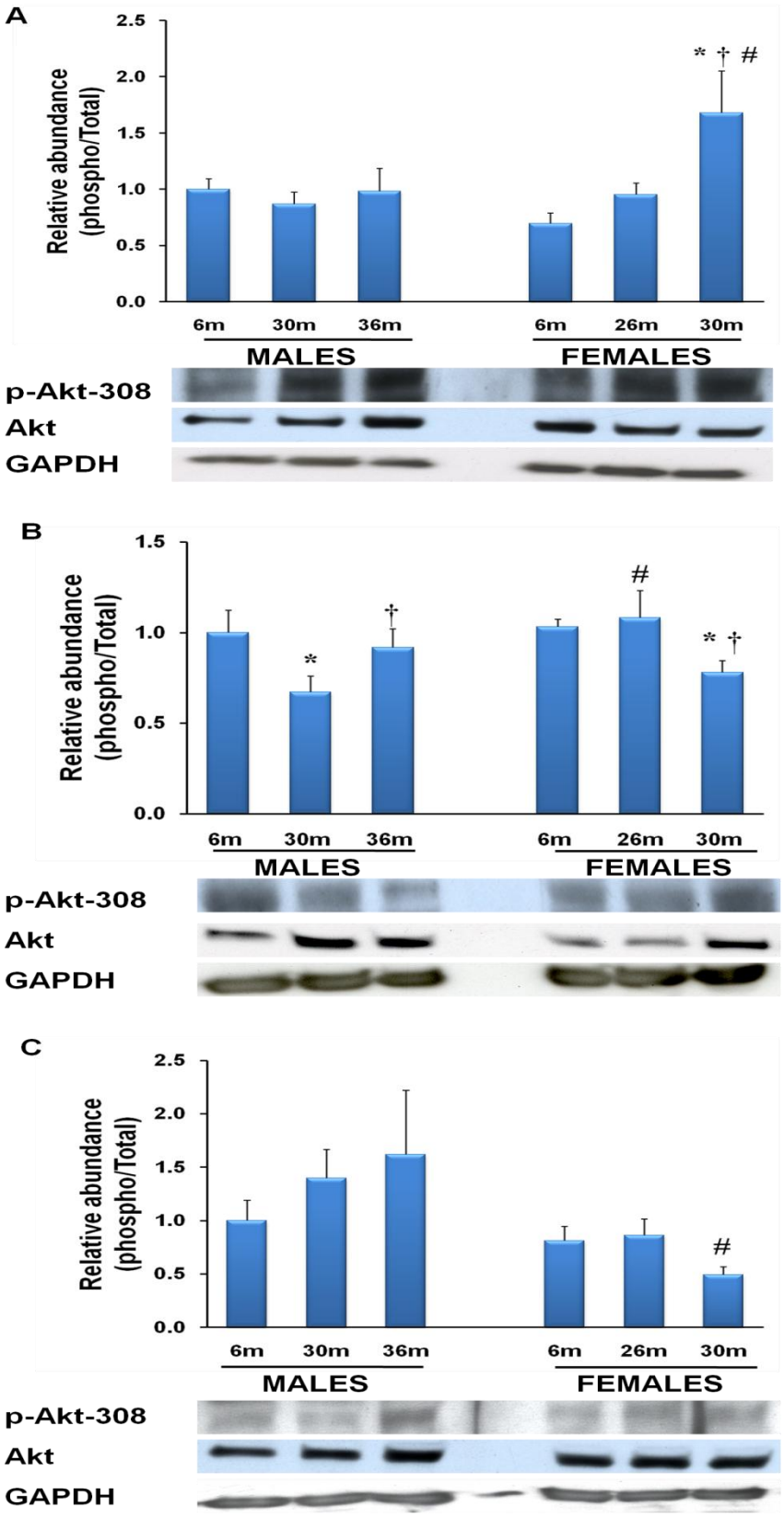

A
Relative abundance (phospho/Total)
2.5
2.0
1.5
1.0
0.5
0.0
* † #
6m 30m 36m 6m 26m 30m
MALES FEMALES
p-Akt-308
Akt
GAPDH
B
Relative abundance (phospho/Total)
1.5
1.0
0.5
0.0
* † # * †
6m 30m 36m 6m 26m 30m
MALES FEMALES
p-Akt-308
Akt
GAPDH
C
Relative abundance (phospho/Total)
2.5
2.0
1.5
1.0
0.5
0.0
#
6m 30m 36m 6m 26m 30m
MALES FEMALES
p-Akt-308
Akt
GAPDH

Figure 3. Tissue content of phospho Akt (Ser308)/ Total Akt/ GAPDH in: A) Soleus B) EDL and, C) Diaphragm in 6 month (young adult), 30 month (aged) and 36 month (very aged) male and 6 month (young adult), 26 month (aged) and 30 month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. Relative changes in protein levels were determined by Western blot analysis. The data are presented as percent of the 6 month value (n = 4). An asterisk indicates significant difference from the corresponding 6 month value, a dagger symbol indicates a difference with 30- or 26-month animals with in the gender and a number symbol indicates a significant difference across the gender, ($p < 0.05$).

Figure 4

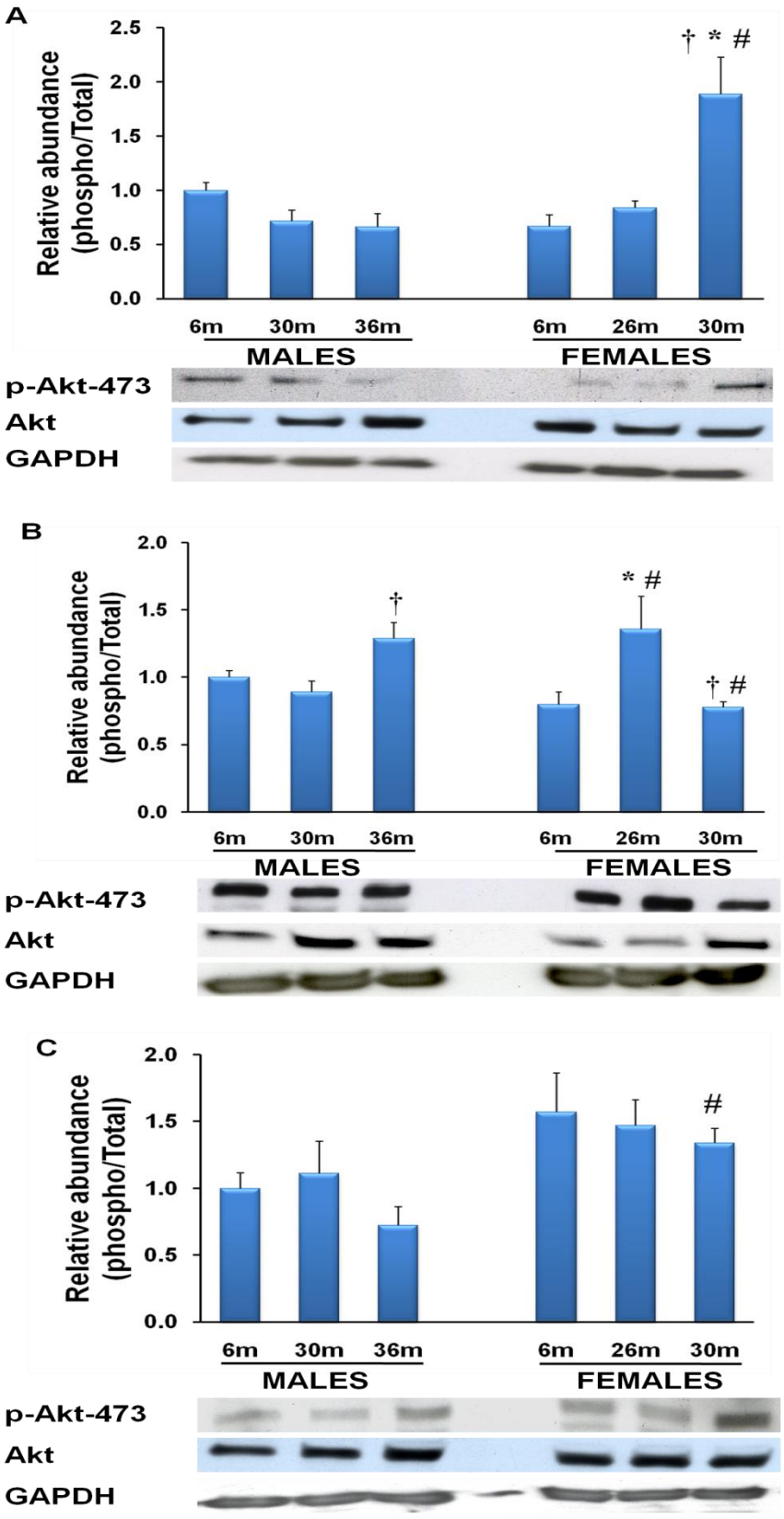

A
Relative abundance (phospho/Total)
2.5
2.0
1.5
1.0
0.5
0.0
† * #
6m 30m 36m
6m 26m 30m
MALES
FEMALES
p-Akt-473
Akt
GAPDH
B
Relative abundance (phospho/Total)
2.0
1.5
1.0
0.5
0.0
†
* #
† #
6m 30m 36m
6m 26m 30m
MALES
FEMALES
p-Akt-473
Akt
GAPDH
C
Relative abundance (phospho/Total)
2.0
1.5
1.0
0.5
0.0
#
6m 30m 36m
6m 26m 30m
MALES
FEMALES
p-Akt-473
Akt
GAPDH

Figure 4. Tissue content of phospho Akt (Ser473)/ Total Akt/ GAPDH in: A) Soleus B) EDL and, C) Diaphragm in 6 month (young adult), 30 month (aged) and 36 month (very aged) male and 6 month (young adult), 26 month (aged) and 30 month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. Relative changes in protein levels were determined by Western blot analysis. The data are presented as percent of the 6 month value (n = 4). An asterisk indicates significant difference from the corresponding 6 month value, a dagger symbol indicates a difference with 30- or 26-month animals with in the gender and a number symbol indicates a significant difference across the gender, ($p < 0.05$).

Figure 5

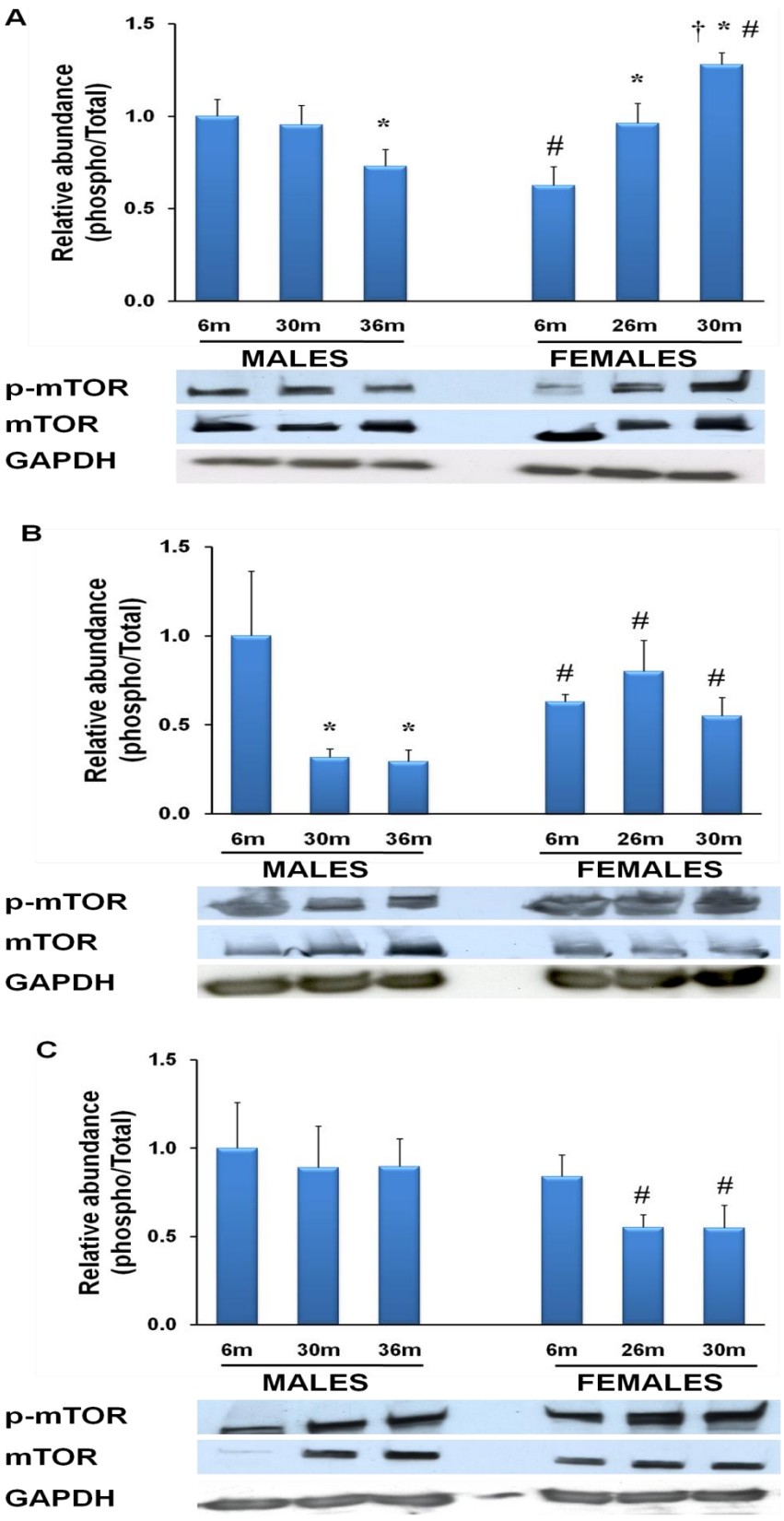

A
Relative abundance (phospho/Total)
1.5
1.0
0.5
0.0
† * #
*
#
*
6m
30m
36m
6m
26m
30m
MALES
FEMALES
p-mTOR
mTOR
GAPDH
B
Relative abundance (phospho/Total)
1.5
1.0
0.5
0.0
*
*
#
#
#
6m
30m
36m
6m
26m
30m
MALES
FEMALES
p-mTOR
mTOR
GAPDH
C
Relative abundance (phospho/Total)
1.5
1.0
0.5
0.0
#
#
6m
30m
36m
6m
26m
30m
MALES
FEMALES
p-mTOR
mTOR
GAPDH

Figure 5. Tissue content of phospho mTOR/ Total mTOR/ GAPDH in: A) Soleus B) EDL and, C) Diaphragm in 6 month (young adult), 30 month (aged) and 36 month (very aged) male and 6 month (young adult), 26 month (aged) and 30 month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. Relative changes in protein levels were determined by Western blot analysis. The data are presented as percent of the 6 month value (n = 4). An asterisk indicates significant difference from the corresponding 6 month value, a dagger symbol indicates a difference with 30- or 26-month animals with in the gender and a number symbol indicates a significant difference across the gender, ($p < 0.05$).

Figure 6

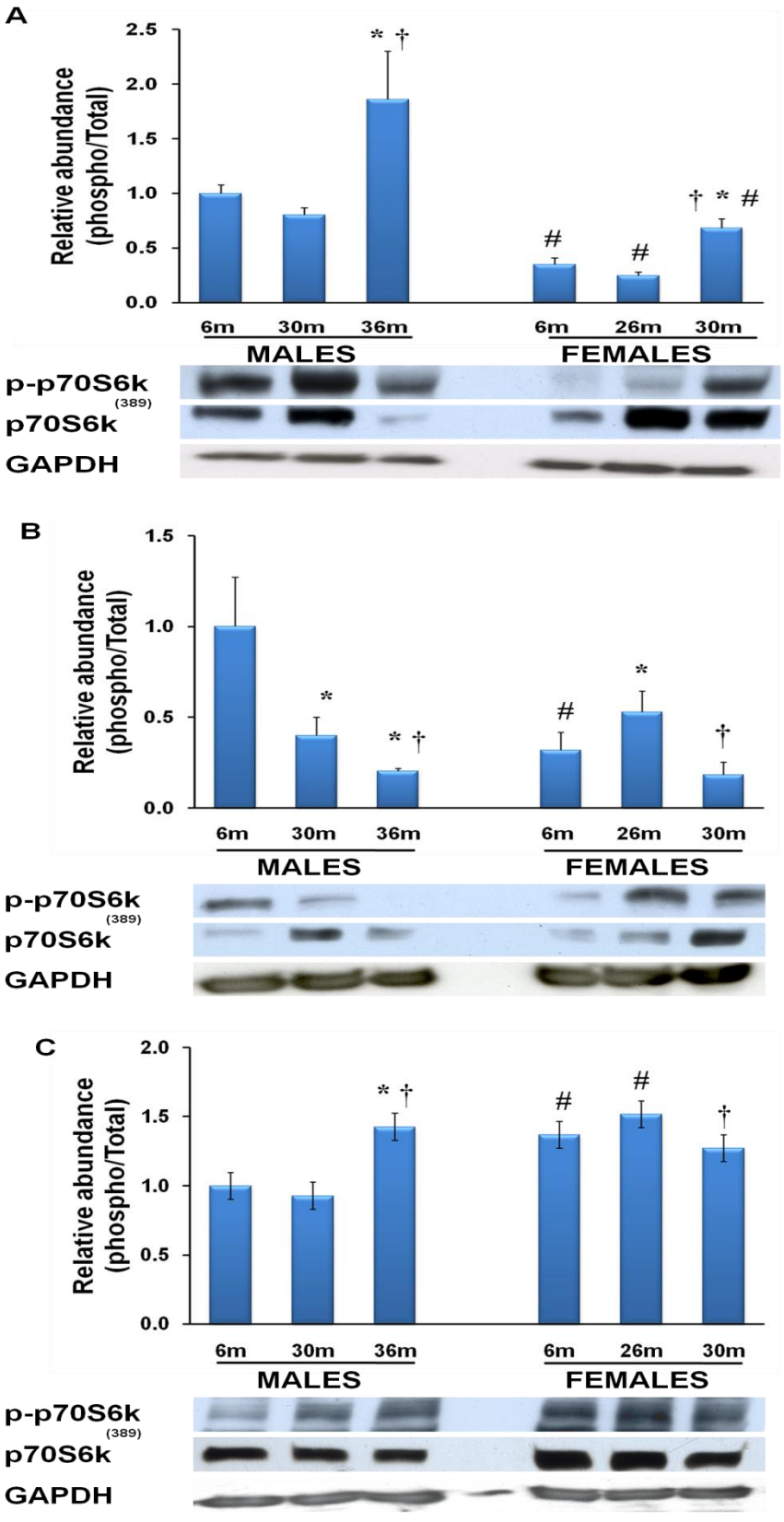
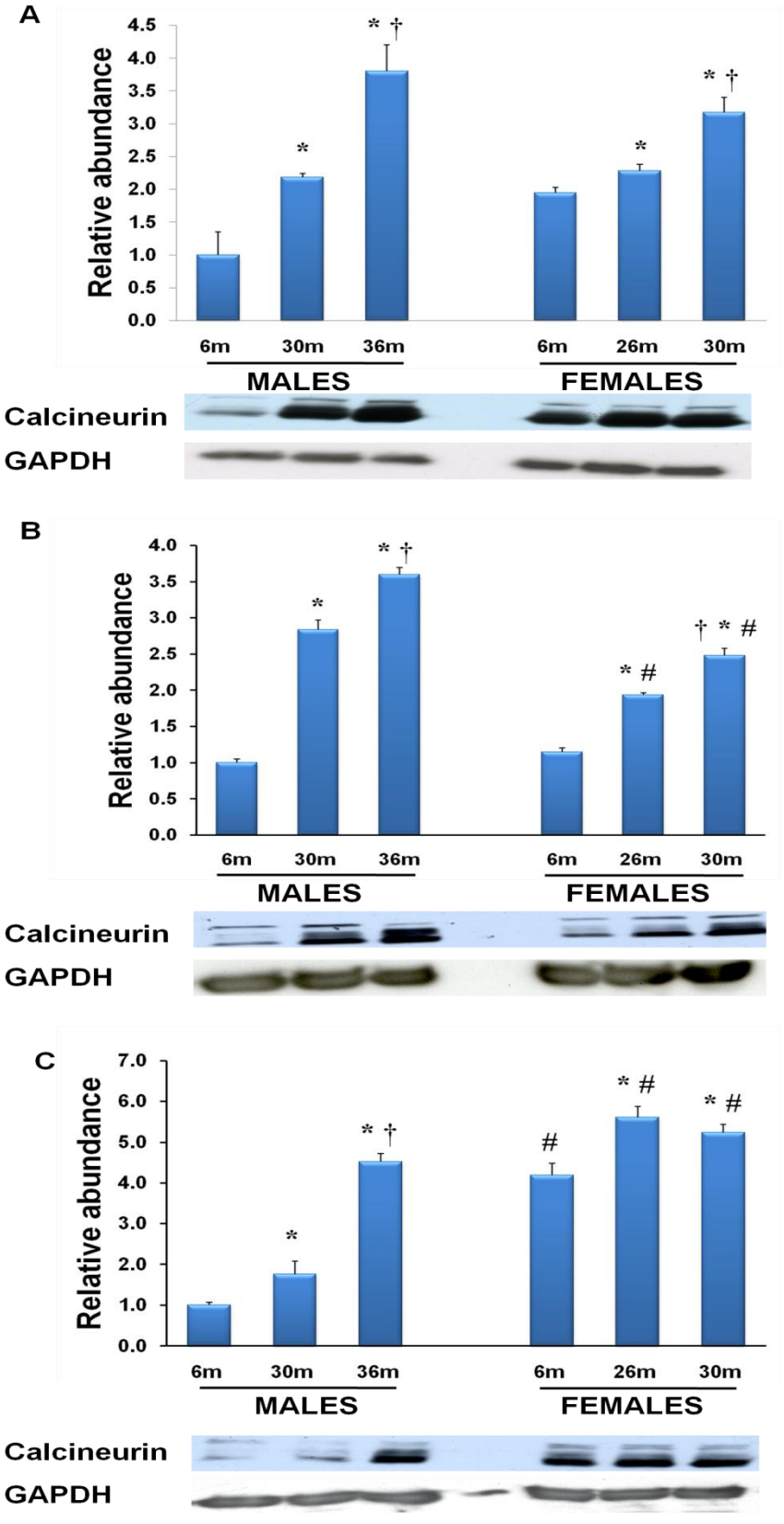

A
Relative abundance (phospho/Total)
2.5
2.0
1.5
1.0
0.5
0.0
* †
# #
† * #
6m 30m 36m 6m 26m 30m
MALES FEMALES
p-p70S6k (389)
p70S6k
GAPDH
B
Relative abundance (phospho/Total)
1.5
1.0
0.5
0.0
* * †
* †
6m 30m 36m 6m 26m 30m
MALES FEMALES
p-p70S6k (389)
p70S6k
GAPDH
C
Relative abundance (phospho/Total)
2.0
1.5
1.0
0.5
0.0
* †
# # †
6m 30m 36m 6m 26m 30m
MALES FEMALES
p-p70S6k (389)
p70S6k
GAPDH

Figure 6. Tissue content of phospho p70S6k (Ser389)/ Total p70S6k/ GAPDH in: A) Soleus B) EDL and, C) Diaphragm in 6 month (young adult), 30 month (aged) and 36 month (very aged) male and 6 month (young adult), 26 month (aged) and 30 month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. Relative changes in protein levels were determined by Western blot analysis. The data are presented as percent of the 6 month value (n = 4). An asterisk indicates significant difference from the corresponding 6 month value, a dagger symbol indicates a difference with 30- or 26-month animals with in the gender and a number symbol indicates a significant difference across the gender, ($p < 0.05$).

Figure 7


A
Relative abundance
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4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* †
*
* †
*
6m
30m
36m
6m
26m
30m
MALES
FEMALES
Calcineurin
GAPDH
B
Relative abundance
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* †
*
† * #
* #
6m
30m
36m
6m
26m
30m
MALES
FEMALES
Calcineurin
GAPDH
C
Relative abundance
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
* †
*
* #
#
* #
6m
30m
36m
6m
26m
30m
MALES
FEMALES
Calcineurin
GAPDH

Figure 7. Tissue content of calcineurin/ GAPDH in: A) Soleus B) EDL and, C) Diaphragm in 6 month (young adult), 30 month (aged) and 36 month (very aged) male and 6 month (young adult), 26 month (aged) and 30 month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. Relative changes in protein levels were determined by Western blot analysis. The data are presented as percent of the 6 month value (n = 4). An asterisk indicates significant difference from the corresponding 6 month value, a dagger symbol indicates a difference with 30- or 26-month animals with in the gender and a number symbol indicates a significant difference across the gender, ($p < 0.05$).

Figure 8

	Soleus		EDL		Diaphragm	
	Male	Female	Male	Female	Male	Female
p-Akt(308)	-	↑	-	↓	-	-
p-Akt(473)	-	↑	-	-	-	-
p-mTOR	↓	↑	↓	-	-	-
p-p70S6k(389)	↑	↑	↓	-	↑	-
Calcineurin	↑	↑	↑	↑	↑	↑

Figure 8. Summary of the results for all the molecules in : A) Soleus B) EDL and, C) Diaphragm in 6-month (adult), 30-month (aged) and 36-month (very aged) male and 6-month (adult), 26-month (aged) and 30-month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. The very aged group (36- and 30-month males and females respectively) data are presented as a comparison with the 6 month value. '-' indicates no significant difference from the corresponding 6-month value. '↑' indicates a significant increase with age while ↓ indicates a significant decrease with age.

CHAPTER IV

CONCLUSIONS

1. Aging affects the regulation of molecules thought to be involved in affecting muscle plasticity differently in different muscles and across genders. Specifically, we observed different alterations across genders in the amount and basal phosphorylation of Akt, mTOR, and p70S6k in the slow twitch-soleus, fast twitch-EDL, and continuously active diaphragm.
2. Differences in the regulation of the molecules thought to govern protein synthesis between aging male and female animals may help to explain why the extent of muscle atrophy differs with gender in the F344/BN rats.
3. The alterations in Akt, mTOR and p70S6k observed with aging in the skeletal muscles of F344/BN rats suggest that a decrease in the signal to initiate protein synthesis is not the cause of sarcopenia.
4. The increase in the expression levels of calcineurin suggests that there may be a phenotypic transformation from fast twitch-type II to slow twitch-type I with age in the soleus, EDL and diaphragm of male and female F344/BN rats.

FUTURE DIRECTIONS

Future directions for research based on this study should focus on the mechanisms involved in the protein degradation, factors causing apoptosis, and the effects of reactive oxygen species on the initiation and progression of muscle atrophy with aging.

We observed an increase or no change in the expression of anabolic biomarkers of protein synthesis with aging in F344/BN male and female rats. This suggests that a decrease in protein synthesis may not be a cause for induction and progression of sarcopenia. To further investigate this possibility, it may be useful to examine other upstream molecules that may be involved in regulating protein synthesis in muscle. Examples here include phosphatase and tensin homolog 10 (PTEN), insulin like growth factor-1 (IGF-1) or the mitogen activated protein kinases (MAPK). Further, it is well known that the total protein content of a tissue is regulated by a balance between the protein synthesis and protein degradation[107, 108]. As such, examining the regulation of protein degradation pathways may also reveal new and interesting information for understanding how aging affects skeletal muscle. Looking into the expression of the biomarkers involved in protein degradation mechanisms may be valuable to understand the incidence of sarcopenia.

The free radical theory of aging states that there is an increase in the concentration of reactive oxygen species (ROS) with age[109]. It is thought that increased ROS may be positively associated with protein degradation[110]. To address this possibility, the effects of these reactive oxygen species with aging in the skeletal muscle could be analyzed by oxyblot analysis and/or by some immunohistochemical methods such as

hydroethidium staining. Additional data regarding how aging affects ROS levels in the skeletal muscles of aging male and female F344/BN rats will no doubt be useful in increasing our understanding of muscle atrophy in these animals.

With aging we observed an increase in the expression of calcineurin in all the muscles we examined. Given the positive correlation between calcineurin levels and slow myosin heavy chain expression, future efforts could examine the effects of aging on myosin heavy chain expression [111]. The finding of an increased slow myosin at time points exhibiting augmented calcineurin expression may be useful in determining if this molecule plays a role in modulating age-associated changes in muscle phenotype.

APPENDIX

This section includes table showing body weights, muscle weights and muscle to body weight ratio, and film properties reports, raw data tables, and statistics of various molecules in soleus, EDL, and diaphragm muscles used this study.

Table 1

	Body Wt(g)	Soleus(mg)	Avg.Soleus to Body wt ratio (mg/g)	EDL(mg)	Avg.EDL to Body wt ratio (mg/g)
MALE					
6m	422.50±42.34	168.38±12.66	0.40±0.01	182.88±12.29	0.43±0.01
30m	551.00±21.32	178.38±14.23	0.32±0.02 *	176.88±1.93	0.32±0.01 *
36m	465.00±17.09	122.88±19.55	0.26±0.03 * †	127.75±6.96	0.27±0.01 * †
FEMALE					
6m	228.00±15.06	109.13±12.92	0.48±0.02 #	115.88±11.34	0.51±0.02 #
26m	322.25±44.80	116.38±16.01	0.36±0.04 *	124.63±1.84	0.39±0.03 * #
30m	310.00±19.87	111.75±3.86	0.36±0.01 *	114.25±19.21	0.37±0.02 * #

Table 1. Muscle to body weight ratio of: A) Soleus B) EDL of 6- (young adult), 30- (aged) and 36-month (very aged) male and 6- (young adult), 26- (aged) and 30-month (very aged) female Fischer 344/Brown Norway F1 hybrid rats. The data are presented as percent of the 6 month value (n = 4). * indicates significant difference from the corresponding 6 month value, † significantly different with 26- or 30-month animals within the same gender and # significantly different from age-matched males, ($p < 0.05$).

SOLEUS

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/06/30	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45 minutes

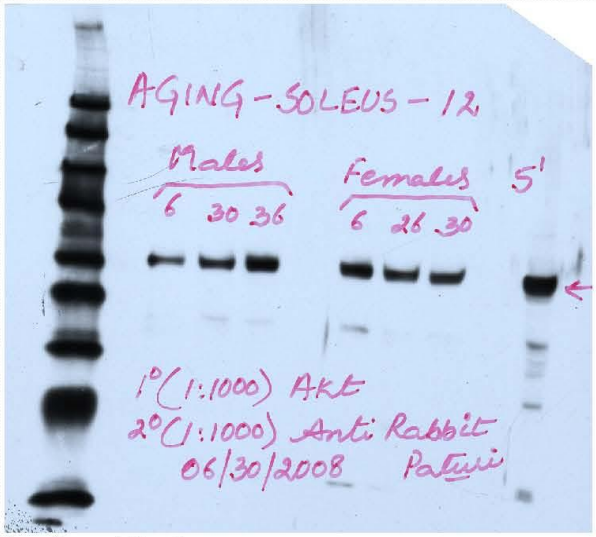
Primary Antibody

Name (Do Not Abbreviate)	Protein kinase - B (AKT)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

5'

Molecular Wieght

60

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS\Akt

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

email Report

Signature

Laboratory of Molecular Physiology, Marshall University, Science Bldg. Suite 311, 1 John Marshall Drive, Huntington, WV, 25755 * Phone: 304-696-3267 Fax: 304-696-7136
http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	17.00	18.80	12.20	15.60	20.00	16.50
	16.80	18.80	12.20	15.80	19.80	16.60
	18.60	19.30	15.00	14.10	15.40	17.50
	18.70	19.50	15.00	13.90	15.10	17.80
	16.80	18.80	12.20	15.80	19.80	16.60
	18.70	19.50	15.00	13.90	15.10	17.80
N	6	6	6	6	6	6
Mean	17.77	19.12	13.60	14.85	17.53	17.13
STDEV	0.99	0.35	1.53	0.97	2.56	0.63
SEM	0.44	0.16	0.69	0.44	1.14	0.28

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	5.517	3	9.824	<0.001	Yes
30.000 vs. 6.000	1.350	2	2.404	0.100	No
6.000 vs. 36.000	4.167	2	7.420	<0.001	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 6.000	2.683	3	4.778	0.006	Yes
30.000 vs. 36.000	0.400	2	0.712	0.618	No
36.000 vs. 6.000	2.283	2	4.066	0.008	Yes

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	2.917	2	5.194	0.001	Yes

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	1.583	2	2.820	0.055	No

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	3.533	2	6.292	<0.001	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/06/30	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45 minutes

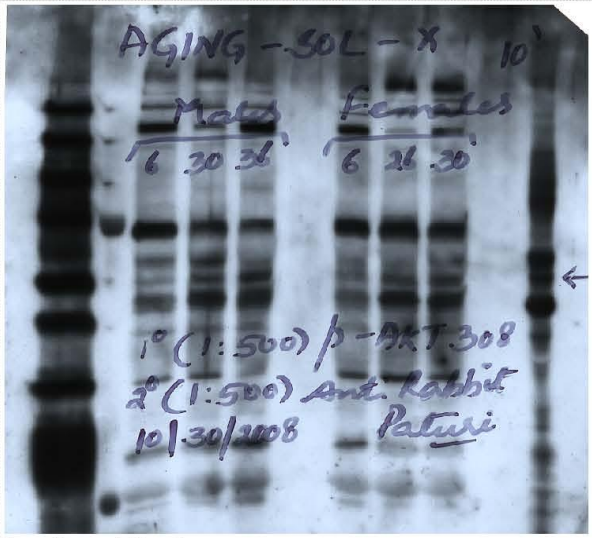
Primary Antibody

Name (Do Not Abbreviate)	phospho protein kinase - B (p-Akt-308)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:500	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

10'

Molecular Wieght

60

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS\p-Akt-473

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	0.94	0.55	0.46	0.44	0.87	2.65
	0.89	0.53	0.47	0.45	0.86	2.71
	1.00	1.11	1.43	0.67	0.87	0.89
	0.61	0.96	1.49	0.64	1.37	1.00
	1.16	0.93	0.92	0.94	0.77	1.22
	1.17	0.93	0.93	0.89	0.77	1.23
N	6	6	6	6	6	6
Mean	0.96	0.84	0.95	0.67	0.92	1.62
STDEV	0.21	0.24	0.45	0.21	0.23	0.84
SEM	0.09	0.11	0.20	0.09	0.10	0.37

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.292	2	1.680	0.244	No

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.0822	2	0.472	0.741	No

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.669	2	3.842	0.011	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 30.000	0.126	3	0.724	0.866	No
6.000 vs. 36.000	0.0146	2	0.0839	0.953	Do Not Test
36.000 vs. 30.000	0.111	2	0.640	0.654	Do Not Test

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	0.947	3	5.438	0.002	Yes
36.000 vs. 30.000	0.698	2	4.010	0.008	Yes
30.000 vs. 6.000	0.249	2	1.428	0.321	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/06/30	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction
Gel type	10%	Electrophoresis Voltage	124
Transfer Voltage	25		
Protien Load per Well	50 ug	Duration	01 : 30 hrs
Duration	45 minutes		

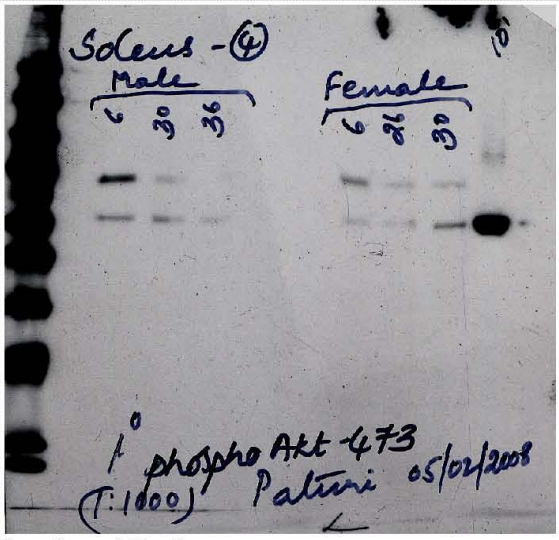
Primary Antibody

Name (Do Not Abbreviate)	phospho protein kinase - B (p-Akt-473)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

10'

Molecular Wieght

60

☑ Used For Analysis

Location of Scan	\\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS\p-Akt-473
Location of Excel Data	\\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

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Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	0.94	0.55	0.46	0.44	0.87	2.65
	0.89	0.53	0.47	0.45	0.86	2.71
	1.18	0.94	0.93	0.88	0.76	1.25
	0.80	0.51	0.38	0.51	1.13	2.53
	1.16	0.93	0.92	0.94	0.77	1.22
	1.17	0.93	0.93	0.89	0.77	1.23
N	6	6	6	6	6	6
Mean	1.02	0.73	0.68	0.68	0.86	1.93
SD	0.17	0.22	0.27	0.24	0.14	0.77
SEM	0.07	0.10	0.12	0.11	0.06	0.34

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.339	2	2.254	0.122	No

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.128	2	0.848	0.553	No

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	1.252	2	8.319	<0.001	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 36.000	0.344	3	2.287	0.254	No
6.000 vs. 30.000	0.290	2	1.929	0.183	Do Not Test
30.000 vs. 36.000	0.0538	2	0.357	0.802	Do Not Test

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	1.248	3	8.286	<0.001	Yes
36.000 vs. 30.000	1.071	2	7.113	<0.001	Yes
30.000 vs. 6.000	0.177	2	1.173	0.414	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45 minutes

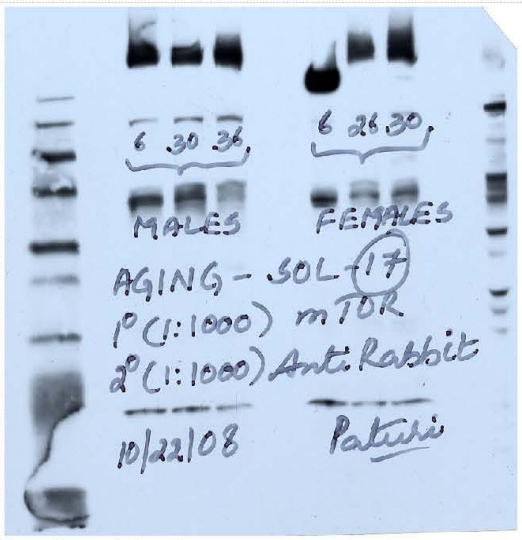
Primary Antibody

Name (Do Not Abbreviate)	mammalian target of rapamycin (mTOR)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

3'

Molecular Wieght

289

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS\p-mTOR

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	18.60	12.90	16.30	20.90	12.40	18.90
	18.70	12.80	16.40	20.80	12.60	18.70
	15.30	11.70	16.90	23.80	15.60	16.70
	15.20	11.50	17.10	23.80	15.70	16.70
	16.10	12.70	15.70	21.10	14.80	19.60
	16.10	12.50	15.70	21.30	14.90	19.50
N	6	6	6	6	6	6
Mean	16.67	12.35	16.35	21.95	14.33	18.35
SD	1.58	0.60	0.59	1.44	1.47	1.32
SEM	0.71	0.27	0.26	0.65	0.66	0.59

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 30.000	4.317	3	8.542	<0.001	Yes
6.000 vs. 36.000	0.317	2	0.627	0.661	No
36.000 vs. 30.000	4.000	2	7.915	<0.001	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 30.000	7.617	3	15.072	<0.001	Yes
6.000 vs. 36.000	3.600	2	7.124	<0.001	Yes
36.000 vs. 30.000	4.017	2	7.948	<0.001	Yes

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	5.283	2	10.455	<0.001	Yes

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	1.983	2	3.925	0.010	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	2.000	2	3.958	0.009	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45 minutes

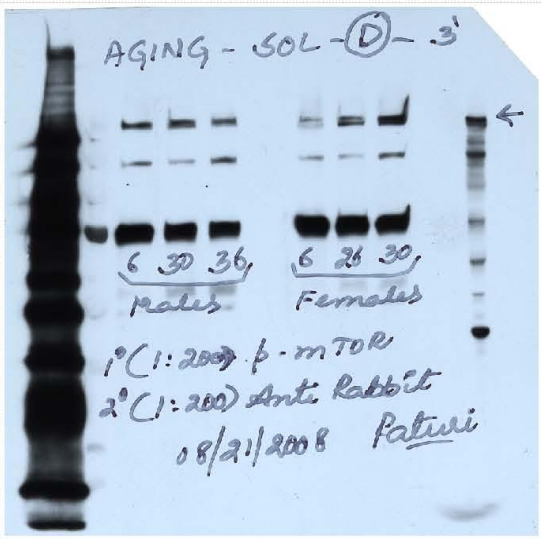
Primary Antibody

Name (Do Not Abbreviate)	phospho mammalian target of rapamycin (p-mTOR)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

3'

Molecular Wieght

289

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS\p-mTOR

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

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Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	0.85	1.28	0.76	0.54	1.28	1.49
	0.89	1.30	0.77	0.49	1.28	1.50
	1.25	1.06	0.79	0.58	1.06	1.49
	1.24	1.10	0.76	0.58	1.04	1.51
	1.34	0.70	0.57	0.96	1.07	1.26
	1.04	0.87	1.18	0.99	0.62	1.21
N	6	6	6	6	6	6
Mean	1.10	1.05	0.80	0.69	1.06	1.41
SD	0.20	0.23	0.20	0.22	0.24	0.14
SEM	0.09	0.10	0.09	0.10	0.11	0.06

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.414	2	4.839	0.002	Yes

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.00825	2	0.0965	0.946	No

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.606	2	7.088	<0.001	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 36.000	0.299	3	3.490	0.050	Yes
6.000 vs. 30.000	0.0515	2	0.602	0.674	No
30.000 vs. 36.000	0.247	2	2.888	0.050	No

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	0.722	3	8.436	<0.001	Yes
36.000 vs. 30.000	0.351	2	4.103	0.007	Yes
30.000 vs. 6.000	0.371	2	4.333	0.005	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction
Gel type	10%	Electrophoresis Voltage	124
Protien Load per Well	50 ug	Duration	01 : 30 hrs
Transfer Voltage	25		
Duration	45 minutes		

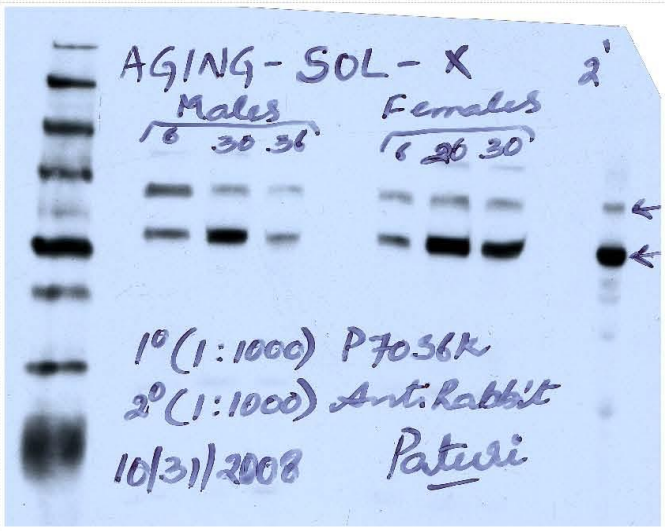
Primary Antibody

Name (Do Not Abbreviate)	S6 Ribosomal Protein(p70S6k)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Positive Control	Sample Buffer	Sample Buffer



Place Scan of Film Here

Exposure time

2minute

Molecular Wieght

70

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	14.90	21.50	4.20	8.90	27.50	23.00
	14.80	21.70	4.00	8.70	27.60	23.10
	12.10	20.90	7.80	9.60	27.70	21.90
	12.20	20.90	7.70	10.00	27.60	21.60
	16.20	26.70	6.90	5.80	27.10	17.30
	16.30	26.40	7.00	5.80	27.10	17.40
N	6	6	6	6	6	6
Mean	14.42	23.02	6.27	8.13	27.43	20.72
SD	1.86	2.76	1.72	1.87	0.27	2.67
SEM	0.83	1.23	0.77	0.84	0.12	1.20

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	16.750	3	20.207	<0.001	Yes
30.000 vs. 6.000	8.600	2	10.375	<0.001	Yes
6.000 vs. 36.000	8.150	2	9.832	<0.001	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 6.000	19.300	3	23.283	<0.001	Yes
30.000 vs. 36.000	6.717	2	8.103	<0.001	Yes
36.000 vs. 6.000	12.583	2	15.180	<0.001	Yes

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	6.283	2	7.580	<0.001	Yes

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	4.417	2	5.328	<0.001	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	14.450	2	17.432	<0.001	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45 minutes

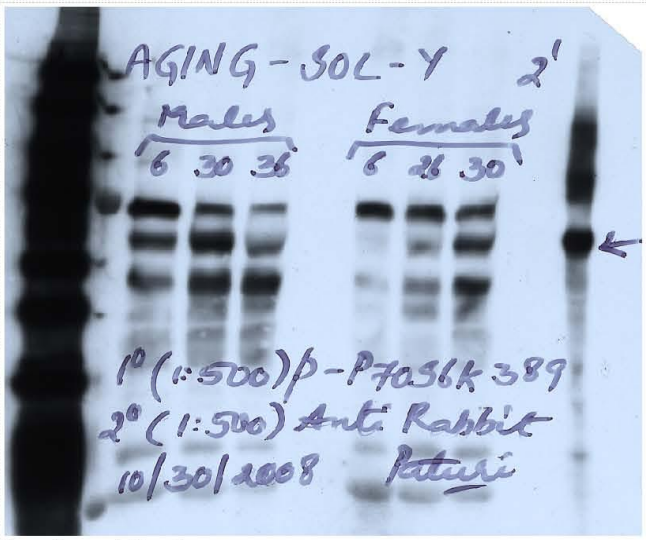
Primary Antibody

Name (Do Not Abbreviate)	phospho S6 Ribosomal Protein(p-P70S6k389)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:500	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Positive Control	Sample Buffer	Sample Buffer



Place Scan of Film Here

Exposure time

2minute

Molecular Wieght

70

☐ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	1.59	1.33	3.93	0.49	0.29	0.83
	1.59	1.30	4.15	0.52	0.29	0.82
	1.62	1.28	2.01	0.39	0.43	1.02
	1.57	1.29	2.05	0.38	0.42	1.06
	1.43	1.02	2.30	0.71	0.37	1.12
	1.18	1.02	2.26	0.66	0.42	1.31
N	6	6	6	6	6	6
Mean	1.50	1.21	2.78	0.52	0.37	1.03
SD	0.17	0.14	0.98	0.14	0.07	0.19
SEM	0.08	0.06	0.44	0.06	0.03	0.08

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.974	2	5.650	<0.001	Yes

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.835	2	4.843	0.002	Yes

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	1.759	2	10.204	<0.001	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 30.000	1.578	3	9.157	<0.001	Yes
36.000 vs. 6.000	1.287	2	7.468	<0.001	Yes
6.000 vs. 30.000	0.291	2	1.689	0.242	No

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 30.000	0.654	3	3.796	0.031	Yes
36.000 vs. 6.000	0.502	2	2.914	0.048	Yes
6.000 vs. 30.000	0.152	2	0.881	0.538	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/10/16	Project	Aging and Aortic Stiffness
Report Number	ASMSOL-01	Tissue/ cell line/ etc.	F1 SOLEUS - Cytosolic Fraction
Gel type	10%	Electrophoresis Voltage	124
Protien Load per Well	50 ug	Duration	01 : 30 hrs
Transfer Voltage	25		
Duration	45 minutes		

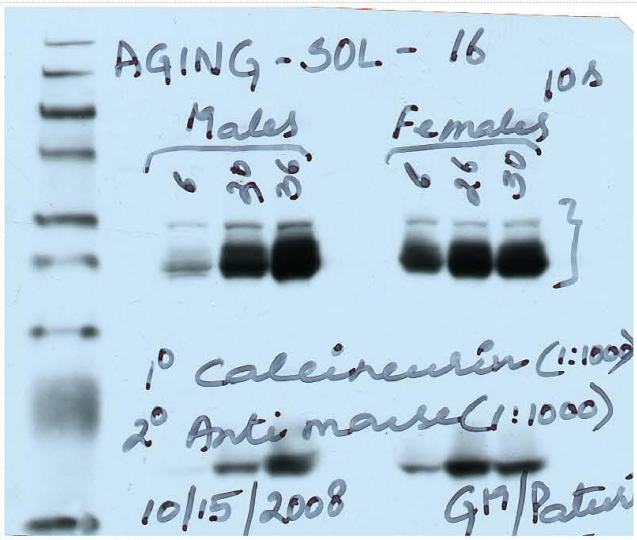
Primary Antibody

Name (Do Not Abbreviate)	Calcineurin A (CnA)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% milk in TBST
Incubation time	1:00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

10 sec

Molecular Wieght

61

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\SOLEUS\Calcineurin

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\SOLEUS

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	2.40	16.70	28.80	10.70	20.30	21.10
	2.80	16.50	28.70	10.70	20.30	21.10
	5.90	18.80	24.10	13.50	18.80	19.00
	5.80	18.90	24.20	13.40	18.30	19.40
	16.60	16.60	12.60	10.10	16.10	28.10
	16.60	16.60	12.60	10.10	16.10	28.10
N	6	6	6	6	6	6
Mean	8.35	17.35	21.83	11.42	18.32	22.80
SD	6.55	1.16	7.44	1.60	1.89	4.19
SEM	2.93	0.52	3.33	0.71	0.85	1.88

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	13.483	3	7.281	<0.001	Yes
36.000 vs. 30.000	4.483	2	2.421	0.097	No
30.000 vs. 6.000	9.000	2	4.860	0.002	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	11.383	3	6.147	<0.001	Yes
36.000 vs. 30.000	4.483	2	2.421	0.097	No
30.000 vs. 6.000	6.900	2	3.726	0.013	Yes

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	3.067	2	1.656	0.251	No

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.967	2	0.522	0.715	No

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.967	2	0.522	0.715	No

EDL

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/10	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	12%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

Primary Antibody

Name (Do Not Abbreviate): Protein Kinase - B (Akt)

Dilution: 1 : 1000 Medium: 5 % BSA in TBST

Incubation time: Overnight

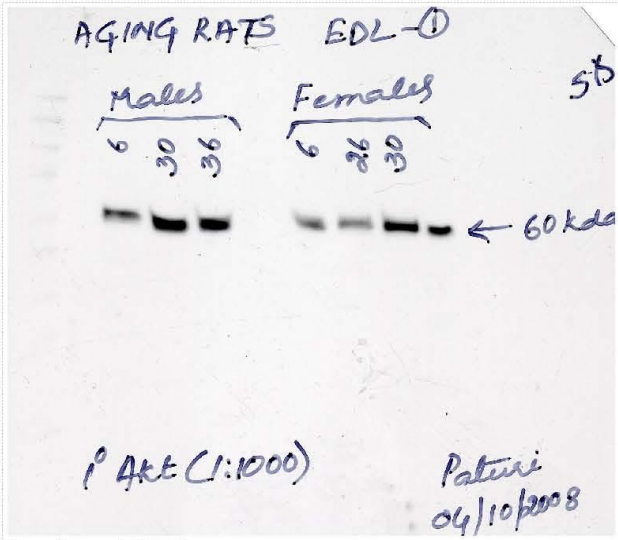
Secondary Antibody

Name: Anti Rabbit

Dilution: 1 : 1000 Medium: 5 % Milk in TBST

Incubation time: 1 hr

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Positive Control	Sample Buffer	Sample Buffer



Place Scan of Film Here

Exposure time: 5 sec

Molecular Wieght: 60 kda

☑ Used For Analysis

Location of Scan: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\Akt\Good Films

Location of Excel Data: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\EDL

Notes

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Signature

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Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	13.60	24.80	21.40	9.90	9.70	20.50
	13.90	24.60	21.50	10.00	9.80	20.30
	12.10	15.30	13.40	15.20	21.40	22.60
	12.20	15.30	13.40	15.30	21.30	22.60
	12.70	21.40	18.20	16.40	13.30	18.00
	12.60	21.80	18.30	16.30	13.00	17.90
N	6	6	6	6	6	6
Mean	12.85	20.53	17.70	13.85	14.75	20.32
STDEV	0.74	4.29	3.63	3.06	5.33	2.08
SEM	0.33	1.92	1.62	1.37	2.39	0.93

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 6.000	7.683	3	5.351	0.002	Yes
30.000 vs. 36.000	2.833	2	1.973	0.173	No
36.000 vs. 6.000	4.850	2	3.378	0.024	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	6.467	3	4.503	0.009	Yes
36.000 vs. 30.000	5.567	2	3.877	0.010	Yes
30.000 vs. 6.000	0.900	2	0.627	0.661	No

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	1.000	2	0.696	0.626	No

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	5.783	2	4.028	0.008	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	2.617	2	1.822	0.208	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/07/23	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

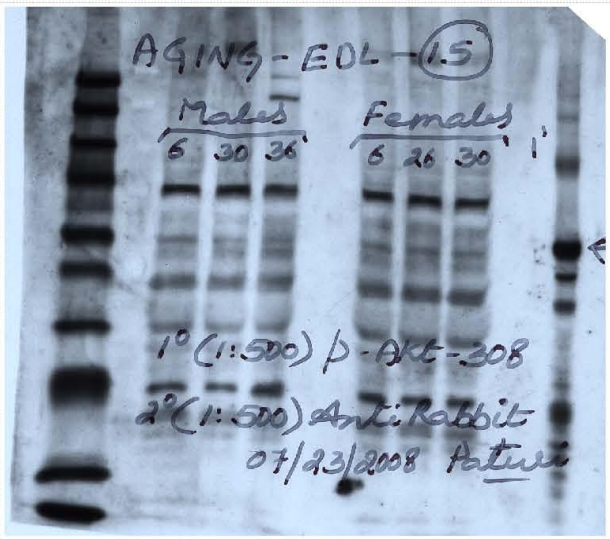
Primary Antibody

Name (Do Not Abbreviate)	phospho Protein Kinase - 308 (p - AKT - 308)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% Milk in TBST
Incubation time	1:00 hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

1'

Molecular Wieght

60

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\p-Akt-308

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\EDL

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	1.41	0.75	0.87	1.31	1.58	0.74
	1.42	0.77	0.89	1.23	1.51	0.74
	0.86	1.00	1.35	1.20	0.82	0.90
	0.78	1.00	1.35	1.23	0.84	0.90
	1.20	0.55	0.96	1.10	1.38	1.07
	1.25	0.58	0.95	1.07	1.37	1.06
N	6	6	6	6	6	6
Mean	1.16	0.78	1.06	1.19	1.25	0.90
SD	0.28	0.19	0.23	0.09	0.33	0.14
SEM	0.12	0.09	0.10	0.04	0.15	0.06

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.0367	2	0.399	0.780	No

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.473	2	5.146	0.001	Yes

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.162	2	1.759	0.223	No

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 30.000	0.379	3	4.127	0.018	Yes
6.000 vs. 36.000	0.0939	2	1.021	0.476	No
36.000 vs. 30.000	0.286	2	3.105	0.036	Yes

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	0.349	3	3.799	0.031	Yes
30.000 vs. 6.000	0.0571	2	0.621	0.664	No
6.000 vs. 36.000	0.292	2	3.179	0.032	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/14	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

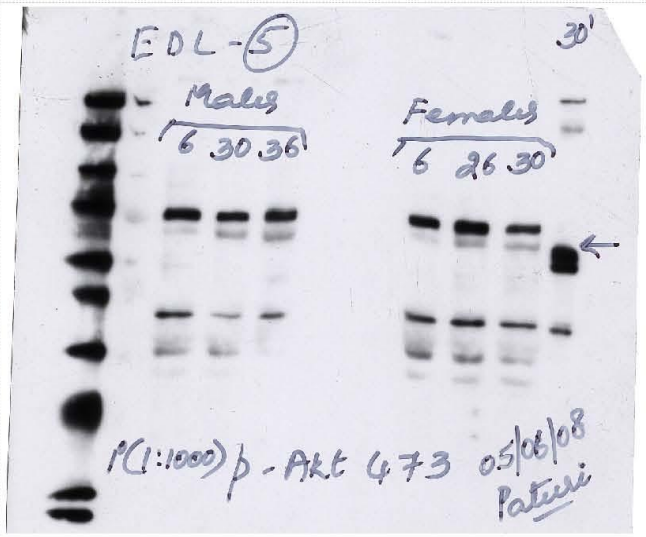
Primary Antibody

Name (Do Not Abbreviate)	phospho Protein kinase- B- 473		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% Milk in TBST
Incubation time	One hour		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Positive Control	Sample Buffer	Sample Buffer



Place Scan of Film Here

Exposure time

5 Minute

Molecular Wieght

60

☑ Used For Analysis

Location of Scan	\\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\p-Akt-473
Location of Excel Data	\\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\EDL\p-Akt-473

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	1.02	0.77	1.13	1.10	1.62	0.79
	0.90	0.71	0.99	1.08	2.27	0.78
	1.03	1.10	1.58	0.73	1.08	0.68
	1.02	1.17	1.60	0.68	1.00	0.73
	0.98	0.84	1.18	0.63	1.61	0.91
	1.22	0.92	1.48	0.69	0.78	0.89
N	6	6	6	6	6	6
Mean	1.03	0.92	1.33	0.82	1.39	0.80
SD	0.11	0.18	0.26	0.21	0.55	0.09
SEM	0.05	0.08	0.12	0.09	0.24	0.04

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.210	2	1.845	0.202	No

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.478	2	4.206	0.006	Yes

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.528	2	4.646	0.003	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 30.000	0.409	3	3.601	0.042	Yes
36.000 vs. 6.000	0.298	2	2.621	0.074	No
6.000 vs. 30.000	0.111	2	0.980	0.494	No

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	0.596	3	5.251	0.002	Yes
30.000 vs. 6.000	0.576	2	5.071	0.001	Yes
6.000 vs. 36.000	0.0205	2	0.180	0.900	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/08/18	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

Primary Antibody

Name (Do Not Abbreviate): mammalian target of rapamycin(mTOR)

Dilution: 1:1000 (p-PTEN) Medium: 5% BSA in TBST

Incubation time: Overnight

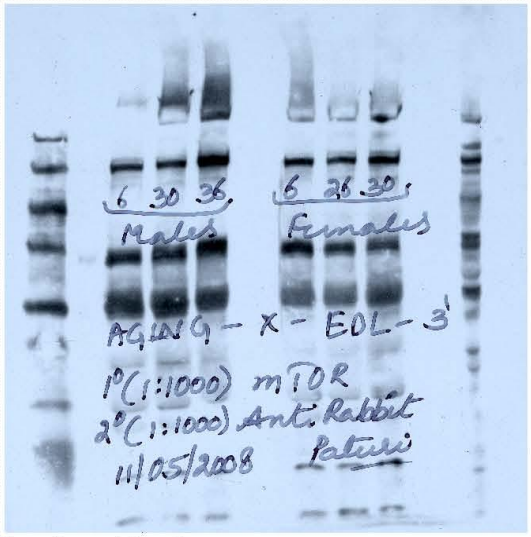
Secondary Antibody

Name: Anti-Rabbit

Dilution: 1:1000 Medium: 5% Milk in TBST

Incubation time: 1:00 hr

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time: 1.5 '

Molecular Wieght: 61

☑ Used For Analysis

Location of Scan: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\Calcineurin

Location of Excel Data: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\EXCEL\EDL

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	13.00	19.30	23.50	17.20	13.00	13.90
	13.30	19.10	23.10	17.30	13.30	13.80
	13.15	19.20	23.30	17.25	13.15	13.85
	7.00	21.40	33.00	15.80	8.40	14.40
	6.80	21.50	33.30	15.50	8.70	14.20
	6.90	21.45	33.15	15.65	8.55	14.30
N	6	6	6	6	6	6
Mean	10.03	20.33	28.23	16.45	10.85	14.08
SD	3.43	1.23	5.40	0.88	2.52	0.26
SEM	1.53	0.55	2.41	0.39	1.13	0.11

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	23.232	3	17.817	<0.001	Yes
36.000 vs. 30.000	9.922	2	7.609	<0.001	Yes
30.000 vs. 6.000	13.310	2	10.208	<0.001	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 30.000	7.499	3	5.751	0.001	Yes
6.000 vs. 36.000	6.009	2	4.609	0.003	Yes
36.000 vs. 30.000	1.489	2	1.142	0.426	No

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	8.037	2	6.164	<0.001	Yes

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	12.772	2	9.795	<0.001	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	21.205	2	16.262	<0.001	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/08/18	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

Primary Antibody

Name (Do Not Abbreviate): phospho mammalian target of rapamycin (p-mTOR)

Dilution: 1:1000 (p-PTEN) Medium: 5% BSA in TBST

Incubation time: Overnight

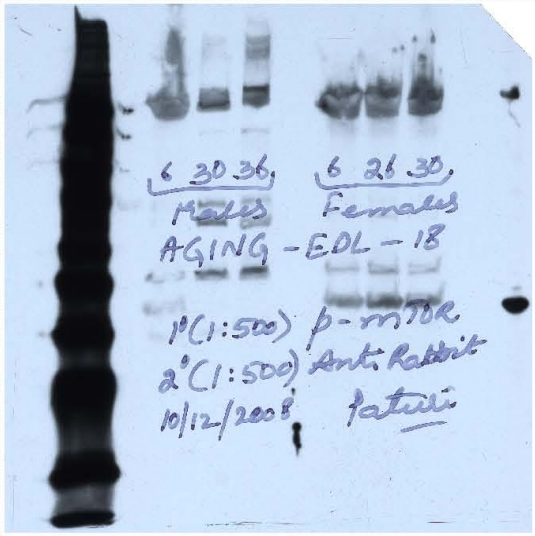
Secondary Antibody

Name: Anti-Rabbit

Dilution: 1:500 Medium: 5% Milk in TBST

Incubation time: 1:00 hr

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time: 1.5 '

Molecular Wieght: 61

☑ Used For Analysis

Location of Scan: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\p-mTOR

Location of Excel Data: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\EXCEL\EDL

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	1.30	0.73	0.72	1.31	1.30	0.91
	1.27	0.73	0.73	1.35	1.23	0.93
	1.29	0.73	0.73	1.33	1.26	0.92
	2.80	0.50	0.45	1.14	2.00	1.39
	2.85	0.52	0.45	1.15	1.95	1.39
	2.60	0.60	0.49	1.34	1.96	1.09
N	6	6	6	6	6	6
Mean	2.02	0.64	0.59	1.27	1.62	1.11
SD	0.81	0.11	0.15	0.10	0.39	0.23
SEM	0.36	0.05	0.07	0.04	0.17	0.10

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.751	2	4.751	0.002	Yes

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.980	2	6.202	<0.001	Yes

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.513	2	3.249	0.029	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 36.000	1.425	3	9.022	<0.001	Yes
6.000 vs. 30.000	1.382	2	8.746	<0.001	Yes
30.000 vs. 36.000	0.0437	2	0.276	0.846	No

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	0.510	3	3.229	0.074	No
30.000 vs. 6.000	0.349	2	2.207	0.129	Do Not Test
6.000 vs. 36.000	0.161	2	1.022	0.476	Do Not Test

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/14	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

Primary Antibody

Name (Do Not Abbreviate): Ribosomal protein S6 kinase (p70S6K)

Dilution: 1:1000 Medium: 5% BSA in TBST

Incubation time: Overnight

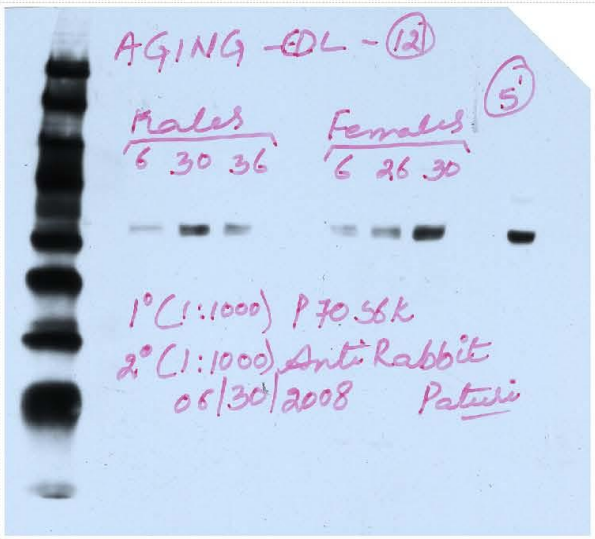
Secondary Antibody

Name: Anti-Rabbit

Dilution: 1:1000 Medium: 5% Milk in TBST

Incubation time: One hour

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Positive Control	Sample Buffer	Sample Buffer



Place Scan of Film Here

Exposure time: 5 Minute

Molecular Wieght: 70

☑ Used For Analysis

Location of Scan: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\p70s6k

Location of Excel Data: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\EDL\p70s6k

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	8.9	22.5	12.4	8.9	14.2	33.1
	7.8	23.3	12.1	8.3	14.1	34.5
	13.5	21.7	13.9	14.2	14.9	21.7
	13.8	21.1	14.2	14.2	15	21.5
	8.35	22.9	12.25	8.6	14.15	33.8
	13.65	21.4	14.05	14.2	14.95	21.6
N	6	6	6	6	6	6
Mean	11.00	22.15	13.15	11.40	14.55	27.70
SD	2.93	0.88	0.99	3.07	0.44	6.70
SEM	1.31	0.39	0.44	1.37	0.20	3.00

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	7.383	3	3.686	0.037	Yes
30.000 vs. 6.000	5.167	2	2.579	0.078	No
6.000 vs. 36.000	2.217	2	1.107	0.440	No

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	16.917	3	8.445	<0.001	Yes
36.000 vs. 30.000	11.633	2	5.808	<0.001	Yes
30.000 vs. 6.000	5.283	2	2.638	0.072	No

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.267	2	0.133	0.926	No

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.150	2	0.0749	0.958	No

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	18.867	2	9.419	<0.001	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/05/08	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

Primary Antibody

Name (Do Not Abbreviate): phospho-Ribosomal proteinS6 kinase (p-p70S6K389)

Dilution: 1:1000 Medium: 5% BSA in TBST

Incubation time: Overnight

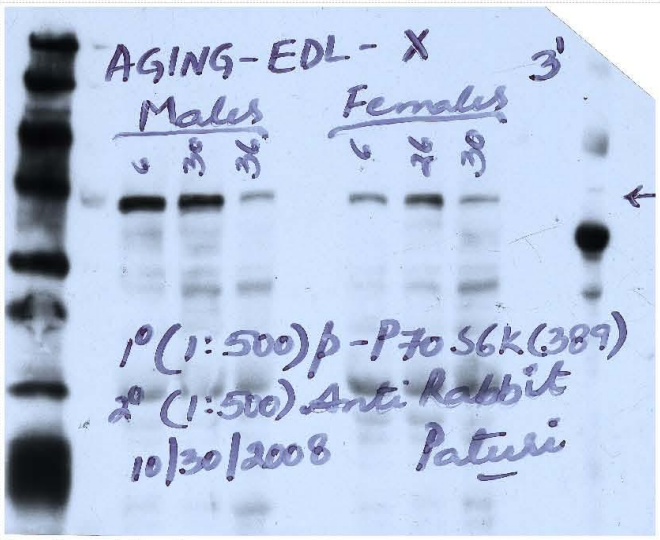
Secondary Antibody

Name: Anti-Rabbit

Dilution: 1:500 Medium: 5% Milk in TBST

Incubation time: One hour

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Positive Control	Sample Buffer	Sample Buffer



Place Scan of Film Here

Exposure time: 3 Minute

Molecular Wieght: 70

☑ Used For Analysis

Location of Scan: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\phospho p70s6k389

Location of Excel Data: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\EDL\phospho p70s6k

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	2.85	0.87	0.53	0.99	1.62	0.50
	3.25	0.84	0.54	1.06	1.63	0.48
	2.26	1.31	0.54	0.65	1.08	0.38
	2.20	1.35	0.54	0.64	1.08	0.38
	3.34	1.05	0.58	1.05	1.39	0.37
	1.86	0.91	0.47	0.62	1.54	0.77
N	6	6	6	6	6	6
Mean	2.63	1.05	0.53	0.84	1.39	0.48
SD	0.61	0.22	0.04	0.22	0.26	0.15
SEM	0.27	0.10	0.02	0.10	0.11	0.07

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	1.793	2	14.364	<0.001	Yes

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.338	2	2.711	0.065	No

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.0517	2	0.414	0.772	No

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 36.000	2.094	3	16.778	<0.001	Yes
6.000 vs. 30.000	1.575	2	12.621	<0.001	Yes
30.000 vs. 36.000	0.519	2	4.157	0.006	Yes

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	0.909	3	7.282	<0.001	Yes
30.000 vs. 6.000	0.556	2	4.453	0.004	Yes
6.000 vs. 36.000	0.353	2	2.828	0.055	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/08/18	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 EDL - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

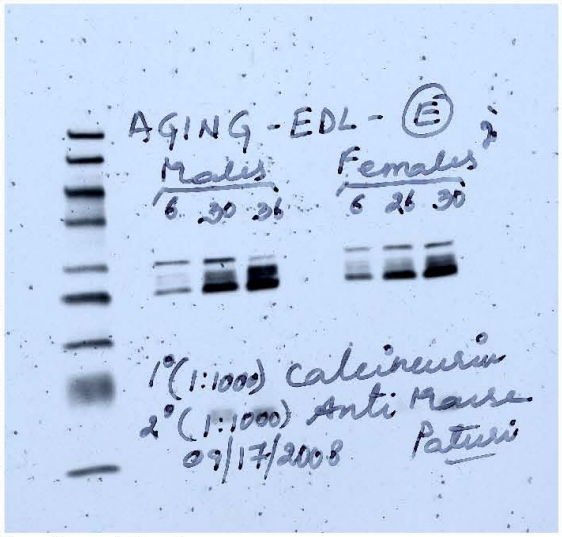
Primary Antibody

Name (Do Not Abbreviate)	Calcineurin A (CnA)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:1000	Medium	5% Milk in TBST
Incubation time	1:00 hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

1.5 '

Molecular Wieght

61

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\EDL\Calcineurin

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\EXCEL\EDL

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	8.40	22.60	24.20	8.20	15.50	21.20
	7.90	22.70	24.60	7.90	15.60	21.30
	9.00	17.50	22.60	9.70	15.90	25.30
	9.00	17.50	22.60	9.70	16.00	25.20
	7.10	19.50	26.60	7.40	16.10	23.20
	6.80	19.30	26.40	7.40	16.90	23.20
N	6	6	6	6	6	6
Mean	8.03	19.85	24.50	8.38	16.00	23.23
SD	0.94	2.33	1.75	1.06	0.50	1.79
SEM	0.42	1.04	0.78	0.48	0.22	0.80

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	16.467	3	26.438	<0.001	Yes
36.000 vs. 30.000	4.650	2	7.466	<0.001	Yes
30.000 vs. 6.000	11.817	2	18.972	<0.001	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	14.850	3	23.842	<0.001	Yes
36.000 vs. 30.000	7.233	2	11.613	<0.001	Yes
30.000 vs. 6.000	7.617	2	12.229	<0.001	Yes

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.350	2	0.562	0.694	No

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	3.850	2	6.181	<0.001	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	1.267	2	2.034	0.161	No

DIAPHRAGM

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

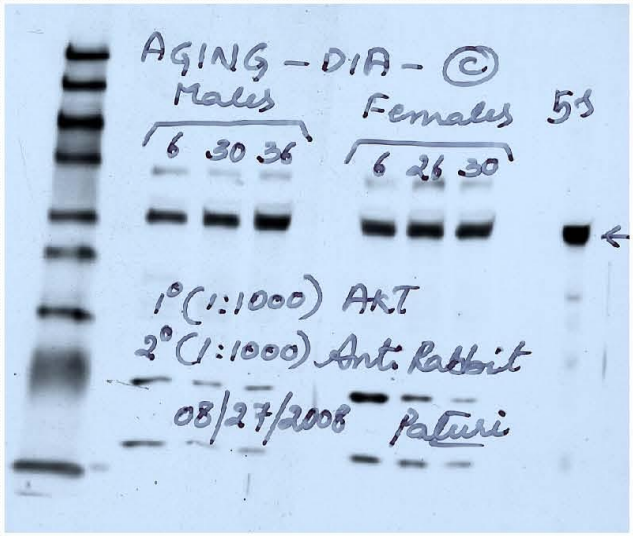
Primary Antibody

Name (Do Not Abbreviate)	Protein kinase - B (AKT)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti Rabbit		
Dilution	1:1000	Medium	5% Milk in TBST
Incubation time	1 : 00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

5 sec

Molecular Wieght

60

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\AKT

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	14.4	15.7	19.2	16	18.2	16.5
	14.3	15.9	19.2	15.9	18.1	16.5
	13.6	15.5	20.6	15.1	17.7	17.5
	13.6	15.6	20.7	14.9	17.7	17.5
	14.2	17.8	17.9	17.3	17.1	15.8
	14	17.8	17.9	17.1	17.3	15.8
N	6	6	6	6	6	6
Mean	14.02	16.38	19.25	16.05	17.68	16.60
SD	0.35	1.11	1.23	0.99	0.43	0.76
SEM	0.16	0.49	0.55	0.44	0.19	0.34

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	5.233	3	14.623	<0.001	Yes
36.000 vs. 30.000	2.867	2	8.010	<0.001	Yes
30.000 vs. 6.000	2.367	2	6.613	<0.001	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 6.000	1.633	3	4.564	0.008	Yes
30.000 vs. 36.000	1.083	2	3.027	0.041	Yes
36.000 vs. 6.000	0.550	2	1.537	0.286	No

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	2.033	2	5.681	<0.001	Yes

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	1.300	2	3.632	0.016	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	2.650	2	7.405	<0.001	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction
Gel type	10%	Electrophoresis Voltage	124
Protien Load per Well	50 ug	Duration	01 : 30 hrs
Transfer Voltage	25		
Duration	45minutes		

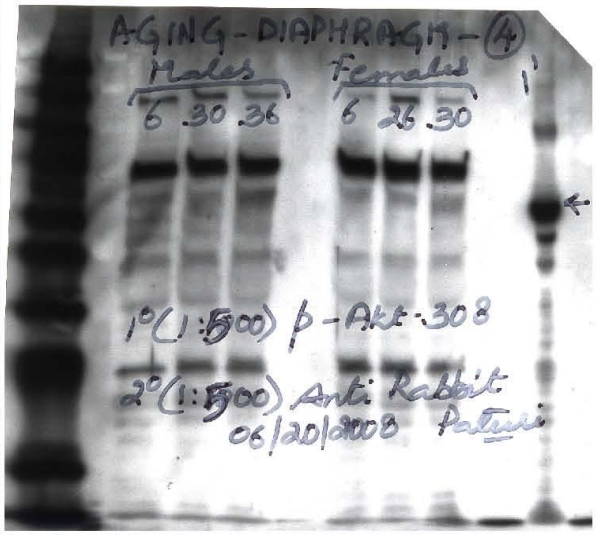
Primary Antibody

Name (Do Not Abbreviate)	Phospho protein Kinase-B-308 (p-AKT-308)		
Dilution	1:500	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:500	Medium	5% Milk in TBST
Incubation time	1 : 00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

1 minute

Molecular Wieght

60

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\p-Akt-308

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	1.66	2.20	3.76	0.82	0.71	0.43
	1.76	2.19	3.74	0.88	0.73	0.43
	0.78	0.97	1.07	1.42	1.45	0.79
	0.77	0.98	1.05	1.38	1.47	0.79
	1.29	2.00	1.23	0.74	1.15	0.62
	1.25	2.15	1.29	0.83	0.97	0.65
N	6	6	6	6	6	6
Mean	1.25	1.75	2.02	1.01	1.08	0.62
STDEV	0.42	0.60	1.34	0.30	0.34	0.16
SEM	0.19	0.27	0.60	0.14	0.15	0.07

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.238	2	0.891	0.534	No

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.668	2	2.500	0.087	No

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	1.405	2	5.257	<0.001	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	0.772	3	2.888	0.120	No
36.000 vs. 30.000	0.273	2	1.021	0.476	Do Not Test
30.000 vs. 6.000	0.499	2	1.867	0.197	Do Not Test

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	0.464	3	1.736	0.447	No
30.000 vs. 6.000	0.0687	2	0.257	0.857	Do Not Test
6.000 vs. 36.000	0.395	2	1.479	0.304	Do Not Test

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction
Gel type	10%	Electrophoresis Voltage	124
Protien Load per Well	50 ug	Duration	01 : 30 hrs
Transfer Voltage	25		
Duration	45minutes		

Primary Antibody

Name (Do Not Abbreviate): phospho - Protein kinase - B (p-AKt-473)

Dilution: 1:1000 Medium: 5% BSA in TBST

Incubation time: Overnight

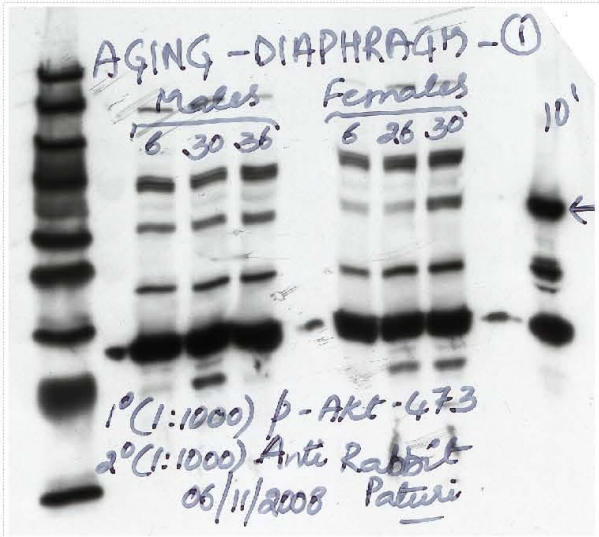
Secondary Antibody

Name: Anti-Rabbit

Dilution: 1:2500 Medium: 5% Milk in TBST

Incubation time: 1 : 00hr

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time: 15'

Molecular Wieght: 60

☑ Used For Analysis

Location of Scan: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\GAPDH

Location of Excel Data: \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

email Report

Signature

Laboratory of Molecular Physiology, Marshall University, Science Bldg. Suite 311, 1 John Marshall Drive, Huntington, WV, 25755 * Phone: 304-696-3267 Fax: 304-696-7136
http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	1.00	1.00	1.00	1.00	1.00	1.00
	1.00	1.00	1.00	1.00	1.00	1.00
	0.48	0.35	0.30	2.53	1.87	1.55
	0.71	0.52	0.41	1.80	1.82	1.41
	1.25	1.88	0.69	0.82	0.96	0.98
	0.84	1.15	0.41	1.15	1.14	1.14
N	6	6	6	6	6	6
Mean	0.88	0.98	0.64	1.38	1.30	1.18
SD	0.26	0.54	0.31	0.66	0.43	0.24
SEM	0.12	0.24	0.14	0.29	0.19	0.11

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.502	2	2.836	0.054	No

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.316	2	1.784	0.217	No

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.545	2	3.075	0.038	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	0.345	3	1.946	0.366	No
30.000 vs. 6.000	0.101	2	0.569	0.690	Do Not Test
6.000 vs. 36.000	0.244	2	1.377	0.338	Do Not Test

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 36.000	0.201	3	1.137	0.703	No
6.000 vs. 30.000	0.0855	2	0.482	0.736	Do Not Test
30.000 vs. 36.000	0.116	2	0.655	0.647	Do Not Test

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

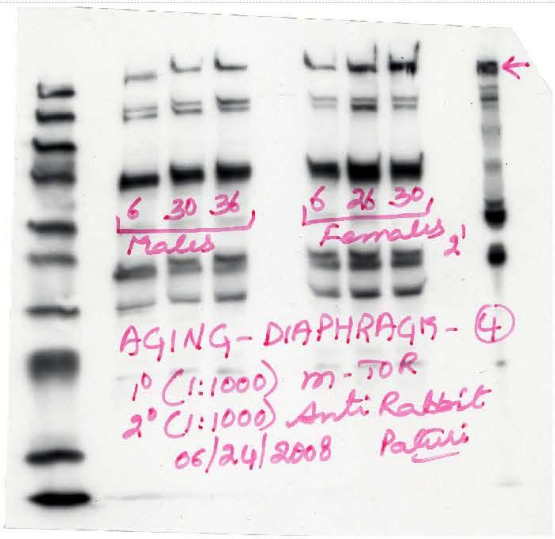
Primary Antibody

Name (Do Not Abbreviate)	Mammalian Target of rapamycin (m-TOR)		
Dilution	1 : 1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti Rabbit		
Dilution	1 : 1000	Medium	5% Milk in TBST
Incubation time	1 : 00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

5 minute

Molecular Wieght

289

☐ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\mTOR

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	10.6	10.1	13	12.1	24.2	30
	10.8	9.7	13.3	11.8	24.6	29.7
	11.8	15.8	13.2	14.5	21.1	23.7
	10.7	17.3	13.3	16	20	22.7
	3.8	20	23.8	14.1	19.5	18.9
	3.6	20	23.6	15.2	19.4	18.2
N	6	6	6	6	6	6
Mean	8.55	15.48	16.70	13.95	21.47	23.87
SD	3.78	4.62	5.42	1.68	2.35	5.10
SEM	1.69	2.07	2.43	0.75	1.05	2.28

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
36.000 vs. 6.000	8.150	3.469	0.002	0.017	Yes
30.000 vs. 6.000	6.933	2.951	0.006	0.025	Yes
36.000 vs. 30.000	1.217	0.518	0.608	0.050	No

Comparisons for factor: **Age within F**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
36.000 vs. 6.000	9.917	4.220	0.000	0.017	Yes
30.000 vs. 6.000	7.517	3.199	0.003	0.025	Yes
36.000 vs. 30.000	2.400	1.021	0.315	0.050	No

Comparisons for factor: **Sex within 6**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
F vs. M	5.400	2.298	0.029	0.050	Yes

Comparisons for factor: **Sex within 30**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
F vs. M	5.983	2.546	0.016	0.050	Yes

Comparisons for factor: **Sex within 36**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
F vs. M	7.167	3.050	0.005	0.050	Yes

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

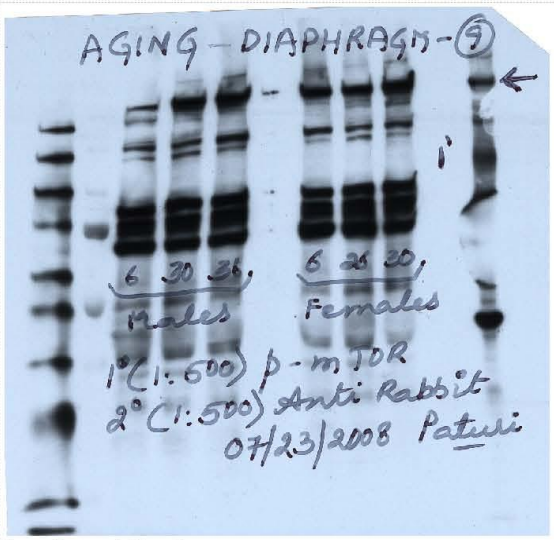
Primary Antibody

Name (Do Not Abbreviate)	phospho-mammalian Target of rapamycin (p-m-TOR)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti Rabbit		
Dilution	1:1000	Medium	5% Milk in TBST
Incubation time	1 : 00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

1'

Molecular Wieght

289

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\p-mTOR

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

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Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	1.09	1.92	1.68	1.49	0.67	0.43
	1.12	2.00	1.62	1.53	0.62	0.45
	1.01	1.00	1.39	0.96	0.95	0.84
	1.08	0.97	1.33	0.86	1.02	0.88
	2.16	0.94	0.87	1.26	0.76	1.05
	2.22	0.91	0.89	1.19	0.77	1.09
N	6	6	6	6	6	6
Mean	1.45	1.29	1.30	1.21	0.80	0.79
SD	0.58	0.52	0.35	0.27	0.16	0.29
SEM	0.26	0.23	0.16	0.12	0.07	0.13

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.233	2	1.466	0.308	No

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.491	2	3.089	0.037	Yes

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.505	2	3.176	0.032	Yes

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 30.000	0.159	3	1.002	0.760	No
6.000 vs. 36.000	0.152	2	0.955	0.505	Do Not Test
36.000 vs. 30.000	0.00750	2	0.0472	0.974	Do Not Test

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 36.000	0.423	3	2.664	0.161	No
6.000 vs. 30.000	0.417	2	2.625	0.073	Do Not Test
30.000 vs. 36.000	0.00628	2	0.0395	0.978	Do Not Test

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

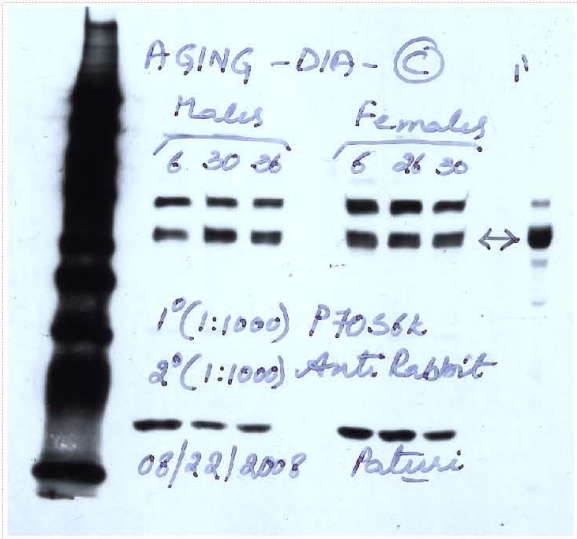
Primary Antibody

Name (Do Not Abbreviate)	Ribosomal protein S6 kinase (P70S6K)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti Rabbit		
Dilution	1:1000	Medium	5% Milk in TBST
Incubation time	1 : 00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

1'

Molecular Wieght

70

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\P70S6k

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	13.6	18.4	14	19.1	18.6	16.2
	13.6	18.3	13.9	19.4	18.6	16.2
	8.5	22.1	17.4	16.7	17.8	17.4
	8.1	22.7	17.7	16.5	18	17.1
	11.9	18.9	17	17.7	16.8	17.7
	11.8	19	16.9	17.7	16.9	17.7
N	6	6	6	6	6	6
Mean	11.25	19.90	16.15	17.85	17.78	17.05
SD	2.42	1.96	1.73	1.20	0.79	0.69
SEM	1.08	0.88	0.77	0.53	0.35	0.31

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 6.000	8.650	3	13.296	<0.001	Yes
30.000 vs. 36.000	3.750	2	5.764	<0.001	Yes
36.000 vs. 6.000	4.900	2	7.532	<0.001	Yes

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
6.000 vs. 36.000	0.800	3	1.230	0.663	No
6.000 vs. 30.000	0.0667	2	0.102	0.943	Do Not Test
30.000 vs. 36.000	0.733	2	1.127	0.432	Do Not Test

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	6.600	2	10.145	<0.001	Yes

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	2.117	2	3.253	0.029	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.900	2	1.383	0.336	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/11/13	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

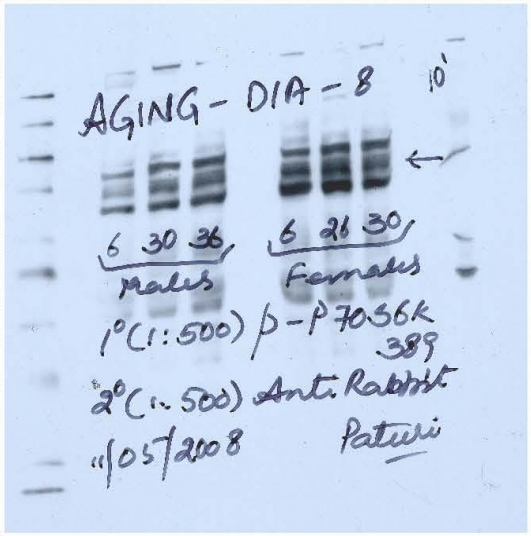
Primary Antibody

Name (Do Not Abbreviate)	phospho-Ribosomal proteinS6 kinase(p-p70S6K-389)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti-Rabbit		
Dilution	1:500	Medium	5% Milk in TBST
Incubation time	1 : 00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

10'

Molecular Wieght

70, 85

☑ Used For Analysis

Location of Scan	\\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\p-p70S6k-389
Location of Excel Data	\\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

email Report

Signature

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http://www.science.marshall.edu/blough

Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	0.60	0.78	1.30	1.02	1.17	1.11
	0.57	0.80	1.33	1.00	1.18	1.09
	1.18	0.73	1.11	1.22	1.24	0.99
	1.21	0.73	1.07	1.24	1.23	1.02
	0.66	0.75	1.08	1.11	1.32	1.01
	0.68	0.75	1.09	1.10	1.30	1.01
N	6	6	6	6	6	6
Mean	0.82	0.76	1.16	1.12	1.24	1.04
SD	0.29	0.03	0.12	0.10	0.06	0.05
SEM	0.13	0.01	0.05	0.04	0.03	0.02

Statistics

Two Way Analysis of Variance

Comparisons for factor: **SEX within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.301	2	5.251	<0.001	Yes

Comparisons for factor: **SEX within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.481	2	8.404	<0.001	Yes

Comparisons for factor: **SEX within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
M vs. F	0.125	2	2.191	0.132	No

Comparisons for factor: **AGE within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 30.000	0.407	3	7.106	<0.001	Yes
36.000 vs. 6.000	0.348	2	6.081	<0.001	Yes
6.000 vs. 30.000	0.0586	2	1.024	0.475	No

Comparisons for factor: **AGE within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 36.000	0.200	3	3.489	0.050	Yes
30.000 vs. 6.000	0.122	2	2.129	0.143	No
6.000 vs. 36.000	0.0779	2	1.361	0.344	No

Laboratory of Molecular Physiology
Western Blot Film Record

Print Form

Experimenter	Satyanarayana Paturi		
Date	2008/04/24	Project	Aging and Aortic Stiffness
Report Number	ASMEDL-01	Tissue/ cell line/ etc.	F1 DIAPHRAGM - Cytosolic Fraction

Gel type	10%	Electrophoresis Voltage	124	Transfer Voltage	25
Protien Load per Well	50 ug	Duration	01 : 30 hrs	Duration	45minutes

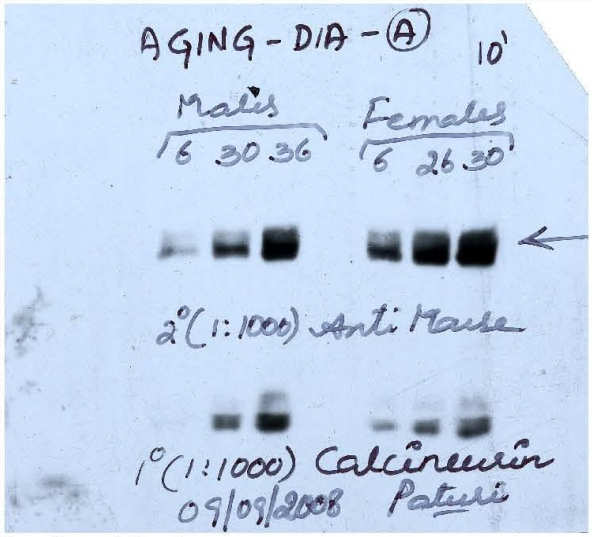
Primary Antibody

Name (Do Not Abbreviate)	Calcineurin A (CnA)		
Dilution	1:1000	Medium	5% BSA in TBST
Incubation time	Overnight		

Secondary Antibody

Name	Anti Rabbit		
Dilution	1:1000	Medium	5% Milk in TBST
Incubation time	1 : 00hr		

Lane # 1	Lane # 2	Lane # 3	Lane # 4	Lane # 5	Lane # 6	Lane # 7	Lane # 8	Lane # 9	Lane # 10	Lane # 11	Lane # 12
BL	MW	6m Male	30m Male	36m Male	Sample Buffer	6m Female	26m Female	30m Female	Sample Buffer	Positive Control	Sample Buffer



Place Scan of Film Here

Exposure time

10'

Molecular Wieght

61 Kda

☑ Used For Analysis

Location of Scan \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\FILMS\DIAPHRAGM\Calcineurin

Location of Excel Data \\Blough-raid\exp-data\Project folder\Aging & aortic stiffness\Excel\DIAPHRAGM

Notes

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Raw data

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

	6m male	30m male	36m male	6m female	26m female	30m female
	5.70	12.30	21.30	13.20	21.30	26.10
	5.50	12.20	21.40	13.20	21.60	26.10
	3.80	4.70	17.90	19.20	27.60	26.80
	4.40	5.10	17.60	18.60	27.80	26.50
	4.50	7.50	21.00	20.10	24.20	22.60
	4.30	7.40	22.60	19.10	24.00	22.60
N	6	6	6	6	6	6
Mean	4.70	8.20	20.30	17.23	24.42	25.12
SD	0.74	3.34	2.05	3.16	2.81	1.97
SEM	0.33	1.49	0.92	1.41	1.26	0.88

Statistics

Two Way Analysis of Variance

Comparisons for factor: **Age within M**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
36.000 vs. 6.000	0.961	3	10.690	<0.001	Yes
36.000 vs. 30.000	0.732	2	8.138	<0.001	Yes
30.000 vs. 6.000	0.229	2	2.552	0.081	No

Comparisons for factor: **Age within F**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
30.000 vs. 6.000	0.347	3	3.864	0.028	Yes
30.000 vs. 36.000	0.106	2	1.184	0.409	No
36.000 vs. 6.000	0.241	2	2.680	0.068	No

Comparisons for factor: **Sex within 6**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.906	2	10.074	<0.001	Yes

Comparisons for factor: **Sex within 30**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	1.024	2	11.386	<0.001	Yes

Comparisons for factor: **Sex within 36**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
F vs. M	0.186	2	2.064	0.155	No

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