1-1-2005

Effects of Aging on Pressure-Induced Mapk Activation in the Rat Aorta

Kevin M. Rice
rice9@marshall.edu

Marshall University

College of Science

Department of Biological Science

Thesis for Master of Science (M.S.) in Biology

Kevin M. Rice

EFFECTS OF AGING ON PRESSURE-INDUCED MAPK ACTIVATION IN THE RAT AORTA

Committee Members: Dr. Eric Blough, Dr David Mallory, and Dr. Nicki Locascio

- Place of Publication -- Huntington, WV
- Year of Publication (year submitted for graduation) 2005

Key words; aorta, vascular smooth muscle, MAPK, mechanotransduction, aging

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS / NOMENCLATURE

CAD .....Coronary Artery Disease

CHD ……..…….Coronary Heart Disease

CHF ……Congestive Heart Failure

ERK …….. Extracellular Regulating Kinase

JNK ……. c-Jun NH_2 -Terminal Kinase

KRB ……..……Krebs-Ringers Buffer

NIA ……... National Institute of Aging

NIH ……..…… National Institute of Health

MAPK ……...Mitogen Activated Protein Kinase

SAPK …… Stress-Activated Protein Kinase

SMC ……Smooth Muscle Cells

USDA ……United States Department of Agriculture

VSMC …….Vascular Smooth Muscle Cells

WHO ……World Health Organization

Abstract

With age, the cardiovascular system experiences substantial alterations in cellular morphology and function. The factors regulating these changes are unknown; however, the mitogen activated protein kinase (MAPK) pathways have emerged as critical components for mediating numerous cellular responses including control of cell growth, differentiation and adaptation. Here we compare the expression, basal activation and the ability of increased pressure to activate the MAPK pathways in adult (6 month old), aged (30 month old) and very aged (36 month old) Fischer 344 x Brown Norway F1 Hybrid rats. Histochemical analysis demonstrated an age-related increase in tunica media thickness of approximately 11% and 21% in aortae from aged and very aged animals, respectively. Western blot analysis of the MAPK family extracellular signal-regulated kinase (ERK 1/2), p38, and c-Jun NH_2 -terminal kinase (JNK) MAPKs showed differential expression and activation among these proteins with age. Expression of ERK 1/2, p38, and JNK were unchanged, slightly increased (10 ± 17.5%) or significantly increased (72.3 ± 27%), respectively, in very aged aortae. By comparison, basal activation levels of these proteins were reduced (-26.2 ± 7.4%), markedly increased (97.0 ± 16.8%) and slightly increased (14.4 ± 4.5%), respectively, in very aged versus 6-month rat aortae. An acute increase of aortic intraluminal pressure (200 mm Hg) indicated that ERK 1/2 regulation differed from p38 or JNK. Pressure loading-induced phosphorylation of ERK1/2 was unchanged or increased with aging while p38 and JNK phosphorylation was attenuated ($P<0.01$). These observations confirm previous conclusions that MAPK proteins are mechanically regulated and expand these studies to suggest that MAPK expression and the control of activation are changed with aging.

Chapter 1

INTRODUCTION

By the year 2030, the number of older Americans will double from the current 35 million people over the age of 65. The risk of coronary artery disease, hypertension, congestive heart failure, and stroke greatly increase with age, and with advanced aging the incidence and prevalence of these diseases steeply raise. Through the many improvements in health care and modern medical science the elderly now comprise the fastest growing segment of the population in the United States (Census, 1990). Cardiovascular diseases (CVD) have reached epidemic proportions on a global dimension, with hypertension, cardiovascular diseases, and heart related illnesses a major health concern throughout the United States and the world. More than 30% of all deaths worldwide are due to cardiovascular diseases. Cardiovascular diseases are expected to be the leading cause of mortality and disability by the year 2020, surpassing even infectious diseases. (66)

Little is known about the normal aging process involved in vascular smooth muscle. This is due primarily to the emphasis on vascular smooth muscle diseases within symptomatic patients. Understanding true age related changes may lead us to a better understanding of the true nature of CVD. To determine the threshold at which these disease mechanisms manifest themselves into pathological illness we must consider age-associated changes in cardiovascular structure and function along with pathophysiologic disease mechanisms. Gerontological investigations can be compromised when the difference observed between young and old animals are actually different between normal and diseased states. Animals chosen for investigation of age-related phenomena

should have certain characteristics (136). Mortality rates should increase exponentially with chronological age indicating a population aging naturally without perturbation by infectious disease and poor diet (59) Thus far, several strains and lines of rats have been used in gerontological investigations, including he Wistar, Brown Norway, Sprague-Dawley, and with the greatest frequency in literature the Fisher 344 (F344) (81). However, recent reports detailing an absence of age associated changes in F344 muscle morphology (23) along with an increased degree of lesion formation in the aging F344 model and other rat strain models have suggested that the F1 hybrid rat strain may be more suitable for gerontological investigation (81, 119). Recently, on the basis of these pathological assessments the National Institute of Aging (NIA) has recommended the F1 hybrid as the superior1 animal model for aging research and has selected the F1 hybrid model to be the standard rat strain to be used by the NIA to conduct gerontological research (119).

To date only two published reports, (104) (131) have investigated age associated alteration in smooth muscle in the F1 model. However, only one addressed vascular smooth muscle changes with age (131). These researchers demonstrated marked increases in vitamin A in aged aortic vessel walls (131). No further research has been conducted to characterize other age associated alterations in the vasculature of these animals. Furthermore, no research has examined the ability of vascular smooth muscle (VSM) from these animals of different ages to adapt to alteration in pressure.

The fundamental mechanism, whereby mechanical stress acts upon a cell to initiate intracellular signaling, is known as mechanotrasduction. This mechanism is implemented in many cell types. Processes governing tissue architecture (18, 112, 132,

145), metabolic response (48), and cellular growth and survival (26) (105), all utilize mechanotransdution. Sensitivity to mechanical forces appear in all adhesion-dependents cells (105, 134) no where is this more evident than in mechanocytes or cells routinely subjected to mechanical forces, such as skeletal muscle sells (31, 132), osteocytes (95), chondrocytes (47, 140), airway smooth muscle cells (114, 115), cardiomyocytes (107, 112, 142, 143), vascular endothelial (85)and smooth muscle cells (99).

Mitogen-activated protein kinase (MAPK) cascades involvement in mechanically induced signaling remains consistent across various cell types (2, 36, 47, 49, 62, 79, 107, 109-111, 142, 143, 147). MAPK activation has been linked to many extra-cellular mechanisms. MAPKs may act as points of convergence for various cell signaling cascades triggering gene expression (29).

Recently, three parallel cascades of MAPKs have been described in mammalian cells: extracellular signal-regulated kinase p42/44 (ERK 1/2), p38, and c-Jun NH-terminal kinase (JNK)/stress-activated protein kinase (SAPK). ERK1/2 and p38 MAPK pathways have been proposed as the most likely modulators of vascular smooth muscle contraction (21, 65, 91, 94, 135, 144). Within the large conduit vessel, in which intraluminal pressure induced spontaneous tone is not developed, MAPK have been shown to be phosphorylated and activated by mechanical stretch (4, 82, 98) However, how this mechanical stretch induced phosphorylation is altered with age is unknown. Yet another area which has been poorly addressed is if stretch induced phosphorylation is a true physiological response for VSMC. The concern raised by this line of thought leads us to the question, does mechanical stretch truly model force perception or transduction experienced by VSMC under hypertensive pressures?

Although not well understood, the increased risk of CVD with aging is thought, in part, to be due to age-associated changes in cardiovascular structure and / or function. How aging may affect the ability of the vascular to initiate and respond to physiological stimuli has not been well studied. Recent studies have demonstrated that increased mechanical stretch is, by itself, able to influence smooth muscle cell proliferation, the production of extracellular matrix protein, actin synthesis, and smooth muscle cell size. These data suggest that mechanical stimuli are important in regulating vascular structure and function. The precise pathways regulating stretch-induced alterations in vascular phenotype are not known however vessel stretch leads to the activation of protein kinase C and the mitogen activated protein kinase (MAPK) pathways (111, 133). Although the effects of mechanical stress could be mediated in part, by activation of mechanosensitive ion channels or by locally and systemically released growth factors, the seminal studies of Ingber and others (50-57) have demonstrated that mechanical input itself is able to trigger cellular signaling mechanisms through the process of mechanotransduction. How mechanotransduction occurs in fully differentiated vascular tissues and how aging affects these processes is not known.

PURPOSE

The purposes of this research project are to examine: 1) whether alterations in age change the basal levels of extracellular signal-regulated kinase p42/44 (ERK 1/2), p38, and c-Jun NH-terminal kinase (JNK)/stress-activated protein kinase (SAPK) protein expression 2) and whether aging affects the way in which the aorta activates these MAPKs pathways. Alteration in basal levels of protein expression and changes in pathway signaling may point toward a key in understanding age-associated pathology. Determining these alterations may lead to steps for early intervention in pathological onset.

SPECIFIC AIMS

The marked increase in vessel wall thickness and increased stiffening (arteriosclerosis) is one of the major problems in aging. -The long-term goals of this study are to understand the cellular mechanisms that might regulate adaptations to increased vessel wall thickness. The goals of this study are to firstly, determine the comparative extent of the aging alteration on vascular smooth muscle basal MAPKs expression in the F1 rat model and secondly to determine if the activation of these pathways are altered with age.

Specific Aim #1

To determine if the basal levels of MAPKs are altered with age.

Hypothesis: Aging will alter aortic MAPK content and basal phosphorylation.

Specific Aim #2

To determine if the pressure induced phosphorylation of the MAPK pathway is altered with age.

Hypothesis: Aging will alter the extent of aortic MAPK phosphorylation following an increase in intraluminal pressure.

Figures and Legends

Figure 1-1

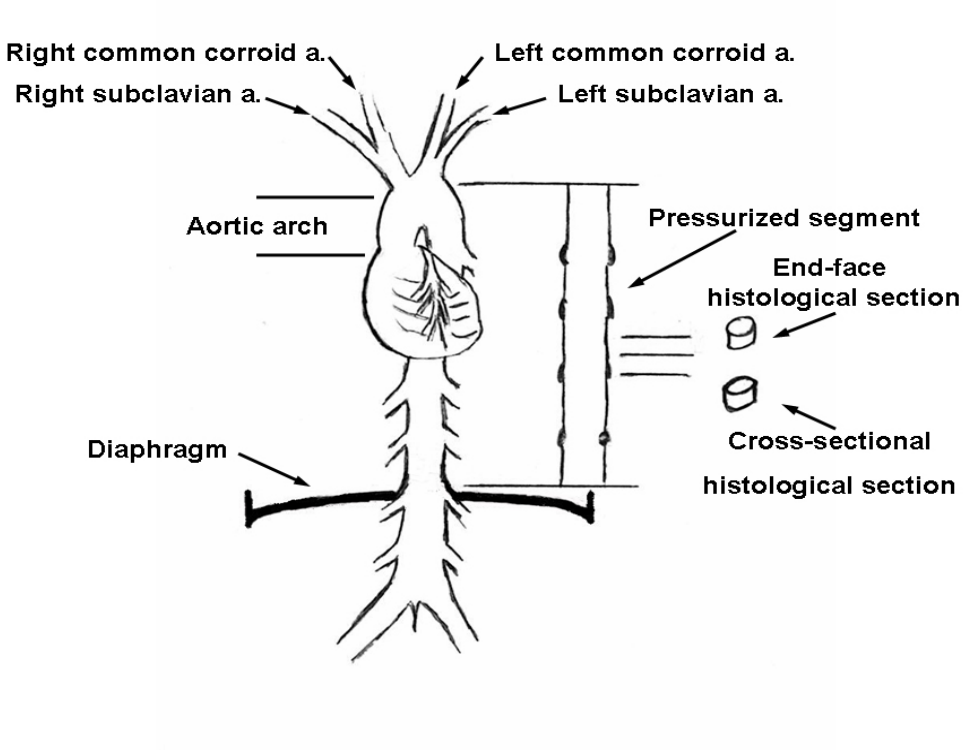


Figure 1 – 1 This is a representation of the anatomy from which the vessel sections were taken.

Figure 1-2

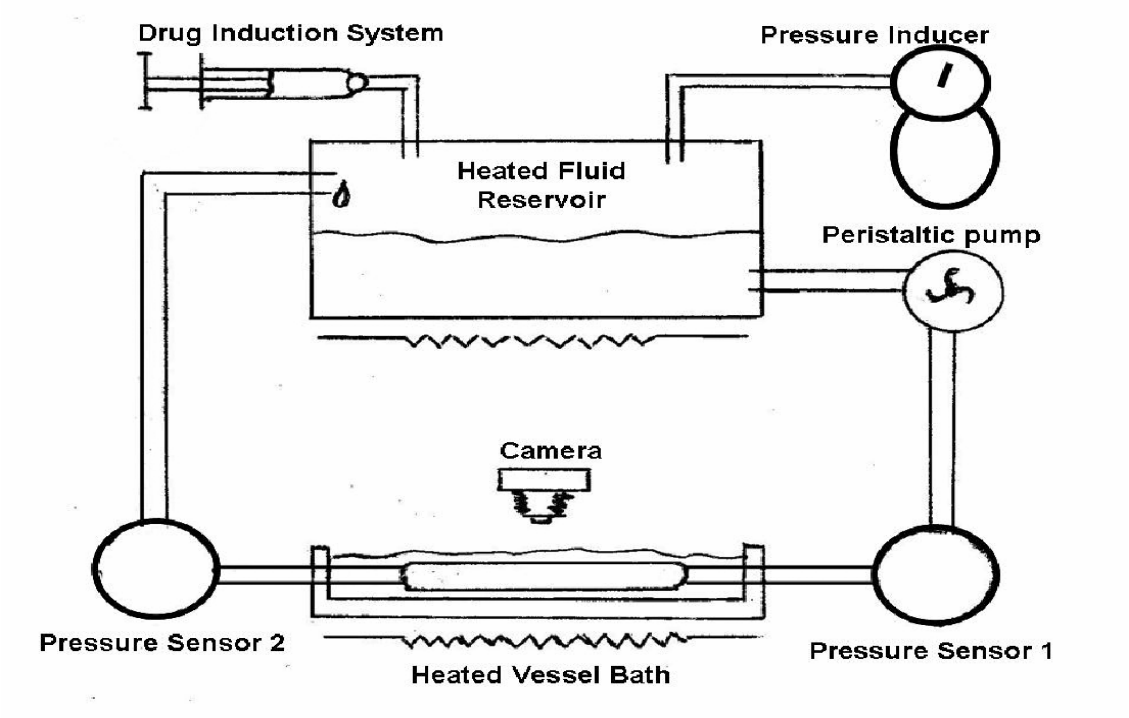


Figure 1 – 2 This is a representative model of the pressurization system designed by the author.

Chapter 2

Review of Literature

INTRODUCTION

The following chapter presents a review of the pertinent literature concerning the present study. Specifically, the following areas will be addressed: 1.) Mitogen activated kinases and the regulation of MAPK activity by mechanical stimuli and 2.) the F344XBN strain as an aging model for cardiovascular investigation.

Age associated alterations in vascular smooth muscle

Vascular aging contributes to the age-dependent rise in hypertension and atherosclerotic disease and the chronic heart failure or stroke that result from these diseases. Recent studies examining large cohorts have demonstrated that aging, by itself, confers a greater risk for cardiovascular diseases (CVD) than do the other major risk factors such as plasma lipid levels, smoking, diabetes, or sedentary life style (19). Although not well understood, the increased risk of CVD with aging is thought, in part, to be due to age-associated changes in cardiovascular structure and / or function. Age-associated remodeling of the wall of large arteries of rodents and nonhuman primates is quite similar to that observed in humans and includes luminal dilation, intimal and medial thickening, and endothelial dysfunction (68). The mechanisms and etiology responsible for the age-related changes in vascular smooth muscle physiology and function are quite complex and not well understood.

Mitogen activated kinases and the regulation of MAPK activity by mechanical stimuli

Recent studies have demonstrated that increased mechanical stretch is, by itself, able to influence smooth muscle cell proliferation, the production of extracellular matrix protein, actin synthesis, and smooth muscle cell size. These data suggest that mechanical stimuli are important in regulating vascular structure and function. The precise pathways regulating stretch-induced alterations in vascular phenotype are not known. Although the effects of mechanical stress could be mediated in part, by activation of mechanosensitive ion channels or by locally and systemically released growth factors, the seminal studies of Ingber and others (50-57) have demonstrated that mechanical input itself is able to trigger cellular signaling mechanisms through the process of mechanotransduction. How mechanotransduction occurs in fully differentiated vascular tissues and how aging affects these processes is not known. Processes governing tissue architecture (18, 112, 132, 145), metabolic response (48), and cellular growth and survival (26) (105), all utilize mechanotransdution. Sensitivity to mechanical forces appears in all adhesion-dependents cells (105, 134) with this property being particularly evident in the mechanocytes or cells routinely subjected to mechanical forces, such as skeletal muscle sells (31, 132), osteocytes (95), chondrocytes (47, 140), airway smooth muscle cells (114, 115), cardiomyocytes (107, 112, 142, 143), vascular endothelial (85)and smooth muscle cells (99).

The involvement of the mitogen-activated protein kinase (MAPK) cascades in mechanically induced signaling remains consistent across various cell types (2, 36, 47, 49, 62, 79, 107, 109-111, 142, 143, 147). MAPKs may act as points of convergence for

various cell signaling cascades triggering gene expression (29). The MAPK pathway is one of the most significant signaling systems used by an organism to elicit a variety of responses at the cellular level.

Recently, three parallel cascades of MAPKs have been described in mammalian cells: extracellular signal-regulated kinase p42/44 (ERK), p38, and cJun NH-terminal kinase (JNK). The MAPK response to chemical and mechanical stresses is regulated through substrate-level phosphorylation of three-tiered cascades composed of a MAPK, a MAPK kinase (MEK), and a MEK kinase (MEKK) (24). MAPKs are activated through dual phosphorylation on threonine and tyrosine residues. Multiple factors have been shown to activate substrate level phosphorylation of MAPKs including hormones, growth factors, reactive oxygen species, decreases in pH, and mechanical stress (138). Of the currently identified MAPKs the ERK1/2 and p38 MAPK pathways have been proposed as the most likely modulators of vascular smooth muscle contraction (21, 65, 91, 94, 135, 144). Within the large conduit vessel, in which intraluminal pressure induced spontaneous tone is not developed, MAPK proteins have been shown to be phosphorylated and activated by mechanical stretch (4, 82, 98) However, how this mechanical stretch induced phosphorylation is altered with age is unknown.

Summary

Since it is unclear whether aging affects the ability of vascular smooth muscle to respond to mechanical stimulation, it remains possible that both aging or age related changes in tissue structure and function may contribute to the observed age associated changes in MAPK mechanotransduction. This age-associated change of vascular smooth

muscle structure leads to an altered ability to respond to force and eventually, a decreased functional capacity.

The F344XBN strain as an aging model for cardiovascular investigation.

Age-associated remodeling of the wall of large arteries of rodents and nonhuman primates is quite similar to that observed in humans and includes luminal dilation, intimal and medial thickening, and endothelial dysfunction(15, 16, 20, 23, 30). In an attempt to provide a better rodent aging model, the National Institute on Aging has developed the Fischer 344/Brown Norway F1 Hybrid (F33XBN). The generation of this model is important because studies have demonstrated that these animals age with minimal disease(81, 119) while often living longer, presumably because of their better health. Despite a number of studies examining the Fischer 344 X Brown Norway (F1) rat strain as a model of human skeletal muscle atrophy (3, 7, 10-14, 17, 22, 25, 32, 34, 35, 40-42, 72-74, 83, 89, 90, 92, 96, 97, 101, 106, 116, 122, 125, 129, 130), the use of the F344XBN (F1) rat model for vascular smooth muscle research has been limited. –Spinetti *et al*, addressed MCP-1 and its receptor CCR2 in aortic VSMCs in the F344XBN. They found that MCP-1 and CCR2 mRNAs and proteins increased with age. They concluded that MCP-1/CCR2 signaling my play a role in age-associated arterial remodeling. Smith *et al* addressed vascular endothelial dysfunction in aging aortas. Based on research from their lab, they indicate a decrease in eNOS phosphorylation with age; they attribute this loss to deceases in AKT basil levels of phosphorylation. And Gaballa *et al* examined large artery remodeling in aortas during aging: biaxial passive and active stiffness (28, 113, 118). They document increases in media thickness, collagen content, and collagen/elastin

ratio in the carotid arteries with age. Also of interest is their finding of decrease in elastin density, and the number of smooth muscle cell nuclei. Taken together, these data suggest that multiple factors are involved in age associated changes in vascular smooth muscle phenotypic properties, and that the structural changes that occur with age are associated with changes in active and passive stiffness, as well as changes in protein expression and activation levels.

Due of this lack of study, our present research utilizes this model selected by the NIH as a model for human aging, and addresses the use of the F344XBN rat strain as a model for cardiovascular aging in humans.

Summary

The declines in functional capacity with advancing age in humans are well documented. Much of the evidence in the literature to date suggests that the loss of physiologic function observed with advancing age is reversible, perhaps even in the frail elderly. Due to the methodological shortcomings associated with performing invasive measures in aged humans, little is known about how aging influences cellular signal transduction. Research concerning cardiovascular aging research in rodent models is almost nonexistent. The need for the development of a rodent model that effectively mimics human vascular aging and morphology is extremely important due to the detrimental effects seen in cardiovascular pathology in humans.

Chapter 3

EFFECTS OF AGING ON PRESSURE-INDUCED MAPK ACTIVATION IN THE RAT AORTA

K. M. Rice[1], R. S. Kinnard[1], R. Harris[2, 3],

G. L. Wright[3] and E. R. Blough[1, 3]

[1] Department of Biological Sciences, Marshall University

[2] Department of Biological Sciences, West Virginia State University

[3] Department of Physiology, Joan C. Edwards School of Medicine,

Marshall University

Author for correspondence:

Eric Blough, PhD.

Laboratory of Molecular Physiology

Suite 311, Science Bldg

Department of Biological Sciences

1 John Marshall Drive

Marshall University

Huntington, WV 25755-1090

E-mail: blough@marshall.edu

Running Title: Pressure-induced aortic MAPK activation with aging.

Abstract

With age, the cardiovascular system experiences substantial alterations in cellular morphology and function. The factors regulating these changes are unknown; however, the mitogen activated protein kinase (MAPK) pathways have emerged as critical components for mediating numerous cellular responses including control of cell growth, differentiation and adaptation. Here we compare the expression, basal activation and the ability of increased pressure to activate the MAPK pathways in adult (6 month old), aged (30 month old) and very aged (36 month old) Fischer 344 x Brown Norway F1 Hybrid rats. Histochemical analysis demonstrated an age-related increase in tunica media thickness of approximately 11% and 21% in aortae from aged and very aged animals, respectively. Western blot analysis of the MAPK family extracellular signal-regulated kinase (ERK 1/2), p38, and c-Jun NH_2 -terminal kinase (JNK) MAPKs showed differential expression and activation among these proteins with age. Expression of ERK 1/2, p38, and JNK were unchanged, slightly increased (10 ± 17.5%) or significantly increased (72.3 ± 27%), respectively, in very aged aortae. By comparison, basal activation levels of these proteins were reduced (-26.2 ± 7.4%), markedly increased (97.0 ± 16.8%) and slightly increased (14.4 ± 4.5%), respectively, in very aged versus 6-month rat aortae. An acute increase of aortic intraluminal pressure (200 mm Hg) indicated that ERK 1/2 regulation differed from p38 or JNK. Pressure loading-induced phosphorylation of ERK1/2 was unchanged or increased with aging while p38 and JNK phosphorylation was attenuated ($P<0.01$). These observations confirm previous conclusions that MAPK proteins are mechanically regulated and expand these studies to suggest that MAPK expression and the control of activation are changed with aging.

Key words; aorta, vascular smooth muscle, MAPK, mechanotransduction, aging

Introduction

Cardiovascular disease (CVD), which is responsible for more than 30% of all deaths worldwide, is expected to be the leading cause of mortality and disability by the year 2020, surpassing even infectious diseases (66, 67, 69-71). Aging is the single largest risk factor for CVD, making heart failure the leading cause of death of individuals over the age of 65 years. It is thought that the impact of age on the risk of the occurrence, severity, and prognosis of cardiovascular disease is due, in part, to age-associated changes in cardiovascular structure and/or function. However this hypothesis has not been well studied. The lack of information concerning the effects of aging on the vasculature is not surprising when one considers the difficulties associated with human aging studies, e.g. cross-sectional design and an inability to control for lifetime activity patterns. Because of these methodological difficulties, many aging vascular studies have been performed using the rat. However, many rat strains such as the Fisher 344, Sprague-Dawley, Long-Evans, and Wistar fail to exhibit a similar degree of age-associated muscle atrophy or muscle impairment when compared to humans. (23, 43) The reasons for differences between the aging human and rat are not entirely known. However, inconsistencies may in part be explained by the use of rat strains prone to premature death, age related disease, or variations in the specific muscles evaluated. In an attempt to minimize these confounding variables, the National Institute on Aging has developed the Fischer 344/Brown Norway F1 Hybrid as a rodent model for age-related physiological studies. The generation of this model is important because studies have demonstrated that these animals age with minimal disease (81, 119) while often living longer, presumably because of their better health. For example, male rats of the Fischer

344 and Brown Norway strains are reported to have median life spans of 103 and 129 weeks, respectively; whereas, their F1 hybrid has a median life span of 145 weeks when fed *ad libitum*. To our knowledge: however, the F1 hybrid model has not yet been utilized for the investigation of aging effects on the physiological signaling of vascular smooth muscle. In the present study, we employed probability of survival curves generated by the NIA for the F1 hybrid strain to ensure that the rats used in this study corresponded roughly to humans in their third, sixth, and eighth decade of life. This latter time point was chosen because cardiovascular dysfunction in humans accelerates during the eighth decade of life, and because this age represents one of the fastest growing segments of the aging population in the United States.

Blood pressure induces mechanical stress on vascular smooth muscle that, if excessive, leads to adaptive remodeling in the form of smooth muscle hypertrophy and hyperplasia. (58, 88, 93) In addition to adaptive remodeling, excessive wall strain causes vascular inflammation, which has been implicated in the pathogenesis of atherosclerosis.(38) The factors regulating these changes are unknown; however, the mitogen activated protein kinase (MAPK) pathways have been identified as important signaling proteins involved in the control of cell growth, differentiation and adaptation. Three parallel cascade pathways of MAPK intracellular signaling have been described: the extracellular signal-regulated kinases (ERK1/2), c-Jun NH_2 -terminal kinase (JNK), and p38 kinase. These pathways require duel phosphorylation on threonine and tyrosine residues by specific upstream proteins to initiate their signaling mechanism. (8, 9, 16, 39, 63, 84, 103, 123, 146) Studies in rats and humans have implicated the MAPK signaling network in the regulation of protein synthesis, mRNA stability, as mediators of apoptosis

and the inflammatory response, and as key players in the control of load-induced alterations in protein expression.(30, 46, 93, 141) While these data together suggest that MAPK signaling plays an important role in the regulation of gene expression, it is still not known how MAPK activity is regulated with aging.

We hypothesized that aging will alter the ability of vessels to appropriately initiate intracellular signaling to mechanical load (stretch). Hence, we propose that altered mechanotransduction may contribute to age-related changes in vascular smooth muscle morphology and function. In the present study, we compared resting levels and the MAPK phosphorylation after increases in intraluminal pressure in isolated aortae from different age groups of Fisher 344/Brown Norway F1 hybrid rats. The results suggest significant alteration in MAPK expression and stress-induced activation with aging.

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 F1 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 12H: 12H 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 unanesthetized using a programmed electrosphygmomanometer with pneumatic tail cuff (Narco-Biosystems, Houston, TX). Animals were acclimated to the procedure for a minimum of 3 days prior to obtaining blood pressure.

Materials

Anti-p38 MAPK, JNK, and p44/42 MAPK (ERK1/2) mouse IgG and rabbit IgG antibodies were purchased from Cell Signaling Technology (Beverly, MA). Precast 10% SDS-PAGE gels were procured from Cambrex Biosciences (Baltimore, MD), and enhanced chemiluminescence (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 (St. Louis, MO).

Isolated Vessel Protocol

Rats were anesthetized with a ketamine-xylazine (1:4) cocktail (50 mg/kg ip) and supplemented as necessary for reflexive response. In a sterile aseptic environment, the ventral surface of the thorax was shaved and the superficial musculature was exposed by means of a transverse incision through the skin distal to the thoracic cavity. After midline

laparotomy and perforation of the heart, the aorta was isolated and the *in situ* length taken as the distance from the subclavian artery to the diaphragm. Aortas were removed from the left ventricle to the renal arch and placed in Krebs-Ringer bicarbonate buffer (KRB) maintained at 37°C.

Isolated aortas were cleaned of connective tissue, transected at the subclavian artery and the diaphragmatic insertion, and secured with silk suture onto polystyrene tubing (outside diameter 3.0 mm; inner diameter 2.6 mm) with the aid of a dissection microscope. After mounting, all micro vessels were cauterized to prevent leakage and vessel length was adjusted with the aid of a micromanipulator to coincide to the *in situ* resting length. All dissection and mounting procedures were performed rapidly and with care to prevent stretching or tearing of the aorta with the vessels incubated in oxygenated (95 O_2:5 CO_2) KRB maintained at 37ºC throughout the procedure. After mounting, aortas were allowed to equilibrate in the vessel chamber for at least 1 hr. before the pressure loading experiment. To examine the effect of increased loading on aortic signal transduction, mounted vessels were subjected to 200 mm Hg of pressure for 15 min. This pressure was selected based on a previous report detailing an increase in c-fos expression following loading at "hypertensive levels" (204 ±5 mm Hg) (87) and to minimize the effect of potential differences in vessel compliance. To minimize the possibility of hypoxia in thickened vessels, the aortae were perfused with oxygenated (95% O_2, 5% CO_2) KRB maintained at 37º C during the incubation, using a peristaltic pump with the flow rate set at 11.1 ml/min., resulting in a shear stress of ~0.5 dynes/cm^2. The intraluminal pressure was controlled by adjusting the air pressure introduced into a fluid reservoir (Fig 3-1A). The system was calibrated before all experiments. System pressure

was monitored using pressure transducers (Gould model P23ID) situated before entry into and exit from the mounted vessel. During the loading procedure, the pressure in vessels was raised in a stepwise fashion (10 mm Hg / min) to a mean arterial pressure of 200 mm Hg which was then maintained for an additional 15 minutes. Control vessels were pressurized just to the point where pressure was recorded (zero pressure) and maintained at this condition for 35 minutes. Vessel diameter was obtained by the aid of a video camera (Logitech Quick Cam Pro 4000) mounted on an adjustable stand. External vessel diameter in μm was determined from still video images using Adobe Photoshop and a calibrated computer software program (Measure it Right, Ver. 1.0). Twenty-four (6 pre-, 6 post-pressurization and 12 during pressurization) still images were taken for each experiment. The mean of four width measurements, taken perpendicular to the long axis of the vessel, was obtained from each still frame to calculate vessel distention with increased loading.

Western Blot Analysis

At the end of each experiment, two segments were cut from each vessel for biochemical and histological analysis, and immediately snap-frozen in liquid nitrogen. Samples were homogenized and suspended in 100 μl of RIPA buffer (50 mM Tris, 150 mM NaCl, 1% NP-40, 0.25% Na-deoxycholate, 1 mM EDTA, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepsatin, 1 mM PMSF,1 mM Na_3VO_4,1 mM NaF). Samples were incubated on ice for 15 minutes and centrifuged at 4ºC for 5 min at 150x g to pellet insoluble material. Protein concentrations of the supernate were determined in triplicate using BSA as a standard and the Bradford method (Pierce, Rockford, IL). Samples were

diluted to a concentration of 3mg/ml in SDS-loading buffer and after boiling for 5 minutes, 30 μg of total protein for each sample was separated on a 10% SDS-PAGE gel. Western blot transfer of protein onto nitrocellulose membranes was performed using standard conditions. (117, 126-128) To verify transfer of proteins and equal loading of lanes the membranes were stained with Ponceau S. For immunodetection, membranes were blocked in 5% Milk TBST for 1 hour at room temperature and then incubated with the appropriate primary antibody overnight. After washing in TBST, 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 nonsaturated range, and band signal intensity was quantified by densitometry using a flatbed scanner (Epson Pefection 3200 PHOTO) and Imaging software (AlphaEaseFC). Molecular weight markers (Cell Signaling) were used as molecular mass standards and NIH 3T3 cell lysates were included as positive controls. A total of three SDS-PAGE gels were run for each experimental set to evaluate; MAPK tissue content, basal phosphorylation, and stretch-induced phosphorylation. In order to obtain direct comparisons between expression and phosphorylation levels of different signaling molecules, immunoblots were stripped with Restore western blot stripping buffer as detailed by the manufacturer. After verifying the absence of residual HRP activity by reacting the membrane with the ECL reagent, membranes were washed and reprobed. To minimize potential experimental error associated with membrane stripping, the order of immunostaining was randomized between experiments.

Histology and Morphometry

Aortic specimens were serially sectioned (8 μm) using an IEC Minotome cryostat and collected on poly-lysine coated slides. After fixing in acetone, (-20°C for 2 min) sections were stained with hematoxylin and eosin, mounted and cover slipped. Morphometric evaluation was performed with the use of a computerized imaging analysis system (Olympus MicroSuite™ Basic). Medial thickness in μm was calculated from the average of eight different points of cross section.

Data Analysis

Results are presented as mean ± SEM. Multiple group comparisons were performed by one-way ANOVA followed by Student's t-test where appropriate. Regression analysis of the relationship between MAPK expression or basal phosphorylation levels and tunica media thickness was performed across age groups using values from four individual aortae from each group. Significance of correlation was analyzed by one-way ANOVA. For all comparisons, the alpha level was set at $P \leq 0.05$ outside of age groups, and $P \leq 0.01$ for within-group analysis.

Results

Verification of loading stimulus

During the isolated vessel experiments, the isolated vessels responded to incremental increases in pressure in a passive manner. To examine if the loading stimulus was constant throughout the loading procedure, we constantly recorded system pressure before entry into and exit from the mounted aorta (Fig 3-1B). If fluctuations in

loading pressure occurred, the vessel was immediately discarded. At 200 mm Hg intraluminal pressure, the aortic diameter of the 6- , 30- and 36-month old animals increased 44.6 %, 67.0 %, and 61.1 %, respectively.

Aging effects on aortic pressure and wall morphology

No evidence of age-associated pathology was observed in any of the cross sections (Fig 3-2). Compared to the 6 month old animals, aging increased the tunica media thickness of the aorta ~11.2% and 21.1% at 30- and 36-months, respectively *(P<0.01)* (Fig 3-2B). Systolic blood pressure was not significantly different among the three age groups (Table 3-1). Body weights of aged and very aged rats were not significantly different but were increased compared to 6 month values.

Aging effects on MAPK expression and phosphorylation

To explore whether aging influenced the total amount of ERK 1/2, p38 and JNK MAPK present in the aorta, gel electrophoresis and Western blot analysis were performed using antibodies which recognize both the unphosphorylated and phosphorylated forms of the proteins. Western blot analysis failed to demonstrate any changes in the expression of the ERK1/2 MAPK with aging (Fig. 3-3, Table 3-2). However, in the 30-month aortas, total p38 MAPK and JNK MAPK expression increased 83.4% and 81.4%, respectively ($P<0.01$). Compared to 6-month aortas, total JNK MAPK expression in 36 month aortas increased 72.3% ($P<0.01$).

Because the MAPK proteins are activated by phosphorylation it was important to determine if aging in the aorta was characterized by changes in the basal level of MAPK

protein phosphorylation. Similar to our analysis of the total MAPK expression, it appeared that the phosphorylation status of the MAPK proteins was also regulated differently with aging. Compared to 6-month aortae, basal phosphorylation of the ERK 1/2 MAPK decreased 31.3% and 26.2% at 30- and 36-months, respectively ($P<0.01$) (Fig. 3-4, Table 3-2). In contrast, the phosphorylation of the p38 MAPK increased 128.7% at 30-months and 97.0% at 36-months ($P<0.01$) (Fig. 3-5, Table 3-2). In a similar fashion, the phosphorylation of the JNK MAPK increased with aging 23.9% and 14.5 % at 30- and 36-months, respectively (Fig. 3-6, Table 3-2). Regression analysis indicated significant correlation between the tissue content of JNK, the phosphorylation levels of ERK 1/2 and p38 and the thickness of the tunica media (Table 3-4).

Aging effects on MAPK phosphorylation in response to intraluminal pressure

In parallel studies, the effect of pressurization of aortae on ERK 1/2, p38 and JNK MAPK phosphorylation was determined. In aortae subjected to 15 minutes of pressure loading (200 mm Hg), phosphorylation of the ERK1/2 MAPK increased 27%, 73%, and 42 % for the 6-, 30-, and 36-month age groups, respectively ($P<0.05$) (Fig. 3-4, Table 3-3). In a similar fashion, pressure loading increased p38 MAPK phosphorylation in the 6-, 30-, and 36-month aortas by 320.2%, 98.55, and 45.7%, respectively ($P<0.05$) (Fig. 3-5, Table 3-3). Increased JNK MAPK phosphorylation of 51.2% and 21.4% with pressure loading was found in the 6- and 30- month aortas ($P<0.05$). However, pressure loading failed to increase JNK MAPK phosphorylation in the 36-month old aortas (Fig. 3-6, Table 3-3).

Discussion

Arteries are capable of structural and functional changes in response to alterations within their milieu or to changes in hemodynamic variables. Vascular remodeling may be considered as an adaptive process in response to long-lasting changes in arterial blood flow and/or pressure, in which the ultimate effect tends to be maintenance of the constancy of tensile and/or shear stresses. Similar to previous reports (27, 33, 37), tunica media thickness was found to significantly increase with age. This age-associated increase in aorta media thickness is thought to occur via smooth muscle cell hypertrophy. (120, 121) Both human and animal studies have demonstrated that hypertension, if not controlled, can cause wall thickening (15, 30)and it is thought that this wall hypertrophy in turn acts to normalize wall tension. The exact mechanism(s) underlying hypertension-associated remodeling are not known; however, an elevation in blood pressure will cause an increase in the amount of tension experienced by vascular smooth muscle cells (VSMC) residing in the vessel wall. It has been demonstrated that stretch is an important hypertrophic stimulus for both smooth and striated (cardiac and skeletal) muscle. Indeed, a number of *in vitro* studies using VSMC cultured on deformable substrates have demonstrated that direct mechanical loading of the cell is capable of inducing cell growth.(60, 76) A previous report has indicated systolic blood pressure is increased in 30- vs. 6-month old F1 hybrid rats.(80) However, our results show no age-associated elevation of blood pressure in these animals, suggesting the described changes in vessel morphology is not a result of hypertensive stimuli.

A major finding of the present study is that when normalized based on total protein, MAPK concentrations and basal levels of phosphorylation appear to be regulated differently with aging in the rat aorta. For example, total ERK 1/2 MAPK content remains constant with age, while p38 MAPK levels are increased at 30 months, and JNK MAPK is elevated at both 30- and 36-months. To our knowledge, age-associated change in the concentration of these molecules has not been reported before in vascular smooth muscle. However, similar to our findings in rat smooth muscle, Williamson *et al*. (139) found ERK 1/2 MAPK expression levels were unchanged with aging in the skeletal muscle of humans. The physiological impact of increased p38 and JNK MAPK expression in vascular smooth muscle with aging is unknown. However, these kinases are both characterized as stress-activated proteins.(9) Given our finding of normotensive blood pressure in aged F1 hybrid rats, the results suggest the vessel hypertrophy seen in these animals is due to regulatory dysfunction not directly related to changes in pressure and hemodynamic conditions. Our finding of an increased basal activation of p38 and JNK MAPK family members with aging (Table 3-2) is consistent with significant alteration in regulatory function. One possibility is that increased basal MAPK activation in aged rats could result from non-mechanical stimuli (7) including; H_2O_2, reactive oxygen species, cytokines, inflammation, angiotensin II, endothelin-1, and receptor tyrosine kinases (insulin-like growth factor, transforming growth factor-β, and fibroblast growth factor). (8, 44, 45, 64, 75) Previous investigations have demonstrated an increased H_2O_2 production in the aorta from older animals which is not surprising in light of the general increase in reactive oxygen species in other tissues with aging. (124) Finally, increased basal p38 and JNK MAPK expression may be causally linked to

increases in tunica media thickening. Indeed, a number of studies have found that these two MAPK family members are required for cardiac hypertrophy. (102) However, only JNK expression showed significant correlation with medical thickness in the present study (Table 3-4). Conversely, the lack of an increase in ERK1/2 expression with aging and poor correlation of the expression of this protein with medial thickness may suggest that this MAPK is not directly involved in smooth muscle cell hypertrophy. In any case, our results clearly indicated changes in MAPK protein expression and signaling with aging suggesting exaggerated basal activity in at least two pathways. Further information on the individual or combined effects of these changes in tissue content and activity of the MAPKs on downstream signaling may reveal their overall impact on VSMC status.

A novel finding is that activation (phosphorylation) of p38 and JNK MAPK proteins in response to acute mechanical stress is blunted while ERK1/2 is enhanced in aortae from aged animals. The smooth muscle cells of the vasculature are constantly exposed to mechanical strain from superimposed pulsatile and mean pressure loads by the cardiac contractile cycle *in vivo*. It is thought that this mechanical strain modulates cellular orientation, synthesis of extracellular matrix, myosin isoform expression, and cellular proliferation. (60, 76, 84, 87) Although the cellular mechanism by which mechanical strain stimulates VSMC growth remains obscure, recent *in vitro* studies have focused on the potential involvement of MAPK family members in the long-term responses including cell proliferation, apoptosis and differentiation. (102) In this study, we measured pressure-induced phosphorylation of the ERK 1/2, p38, and JNK MAPKs in freshly isolated aortae from animals of different ages. Similar to results from previous *in vivo* studies using acute hypertension (75, 146)and balloon-overstretched injury (61)in

young adult animals, we demonstrate activation (phosphorylation) of the MAPK cascade in adult (6-month) vessels. We further demonstrate an increased ERK 1/2 MAPK activation and reduced p38 and JNK MAPK activation with increasing age which, to our knowledge, has not been previously reported in vascular tissue. Our findings of decreased p38 and JNK MAPK phosphorylation in the aorta in response to mechanical stimuli are similar to results others have reported using mitogenic activation in other cell and tissue types. (61, 77, 78, 100, 137) Gennanro and colleagues (30) recently noted one exception to this trend. These researchers used VSMC isolated from the aorta of young and old rabbits and, similar to the present study, demonstrated an increased degree of ERK 1/2 MAPK phosphorylation following serum stimulation of aged cells. Their findings, like ours, suggest that VSMC cells are different from a variety of cell types which have all been shown to be characterized by reductions in ERK 1/2 MAPK activation with aging. Why aging in VSMC may be associated with an increased ability to activate the ERK 1/2 MAPK is not known. However, it is possible that augmented ERK 1/2 MAPK activity in response to cellular insult may be advantageous in protecting aged cells from apoptosis, or perhaps play a role in the enhanced cellular proliferation and neointimal formation seen in aged vascular tissues subsequent to wall injury. (121) Because most of our knowledge concerning the regulation and function of MAPK has resulted from studies on cultured cells, little is known about their activation *in vivo*. However, in adult tissues, the mechanisms regulating ERK1/2, p38, and JNK MAPK activation by mechanical stress have been linked to stretch-induced release of angiotensin II, phenylephrine, and endothelin-1 in cardiac myocytes, and PDGF, fibroblast growth factor, and ATP in VSMC. (8, 20, 44, 45, 64, 75) It is thought that many of these factors

may activate MAPK family members in an autocrine and/or paracrine manner. The presence of such events cannot be excluded in the present study. However, previous investigations showed that cultured VSMC exposed to the conditioned media collected from VSMC after stretching failed to produce increases in ERK1/2 MAPK phosphorylation suggesting that the release of these factors does not play a significant role in vascular smooth muscle.(120) Other studies investigating MAPK activation with mechanical load have proposed that signaling pathways converging at nonreceptor tyrosine kinases may play a role. (64) Whether two prototypical members of this family, proline-rich tyrosine kinase 2 or focal adhesion kinase participate in pressure-induced ERK 1/2 activation is at present being intensely investigated in our laboratory.

We feel that one strength of the present investigation was our use of intact vessels for loading experiments. As such, our preparations included both a smooth muscle and endothelial layer thus mimicking in vivo conditions as closely as possible. Hence, the possibility exists that non-vascular smooth muscle tissue contributed to results. Previous investigations utilizing differentiated vascular tissues have demonstrated that activated MAPK family members are found in the vascular smooth muscle cells (141, 146) and we consider it unlikely that endothelial cells, which comprise less than 5% of the artery cell population, accounted for a significant proportion of the response we observe. Nonetheless, such an issue in our preparations and in animals of different ages requires further investigation.

In summary, we have demonstrated the *in vitro* activation of ERK1/2, p38 and JNK in the arteries of rats following an acute elevation in loading pressure. Protracted loading, such as that induced by hypertension or the cellular response subsequent to

injury, is characterized by the growth and proliferation of smooth muscle cells. That MAPK activation occurs with increases in pressure suggests an important role for these kinases in the arterial adaptation to fluctuations in blood pressure. Our findings provide evidence for the possibility that MAPK activation may also contribute directly to age-associated alterations in aortic vessel remodeling.

Figures and Legends

Table 3-1

Body weight and systolic blood pressure of young adult (6 month), aged (30 month), and very aged (36 month) Fischer 344/Brown Norway F1 hybrid rats.

Group	N	Body Weight, g	Systolic BP, mmHg
6 month	6	421 ± 17	143 ± 3
30 month	6	549 ± 22	131 ± 8
36 month	6	482 ± 28	135 ± 4

Table 3-2

Tissue concentration and basal levels of phosphorylated MAPKs in aortae excised from young adult (6 month), aged (30 month) and very aged (36 month) Fischer 344X Brown Norway F1 rats. Results were obtained by Western blot analysis using antibodies which recognized the unphosphorylated and phosphorylated forms of the proteins. Data are presented as percent of the young adult value. An (n.s.) indicates the value was not significantly different from the young adult.

	6 mo. basal level	**30 mo. basal level**	**36 mo. basal level**
ERK1/2	100.0 ± 6.3	n.s.	n.s.
p-ERK1/2	100.0 ± 1.1	68.7 ± 2.4	73.8 ± 6.3
p38	100.0 ± 7.9	183.4 ± 17.6	n.s.
p-p38	100.0 ± 3.2	228.7 ± 2.6	197.0 ± 13.6
JNK	100.0 ± 23.2	181.3 ± 9.5	172.3 ± 3.8
p-JNK	100.0 ± 2.9	123.9 ± 4.1	114.5 ± 1.6

Table 3-3

Pressure-induced, percent change in phosphorylation of MAPKs in aortae obtained from young adult (6 month), aged (30 month), and very aged (36 month) Fischer 344X Brown Norway F1 rats. Values were obtained by Western analysis utilizing antibodies that recognized the phosphorylated forms of the proteins. An asterisk (*) indicates a significant difference from the 6 month value, $p < 0.05$.

	6 mo.	**30 mo**	**36 mo**
p-ERK1/2	+27.0 ± 1.8	+50.3 ± 11.6*	+30.6 ± 17.3
p-p38	+320.0 ± 12.2	+225.1 ± 17.6*	+89.9 ± 22.7*
p-JNK	+51.2 ± 4.3	+26.5 ± 5.0*	-17.0 ± 2.5*

Table 3-4

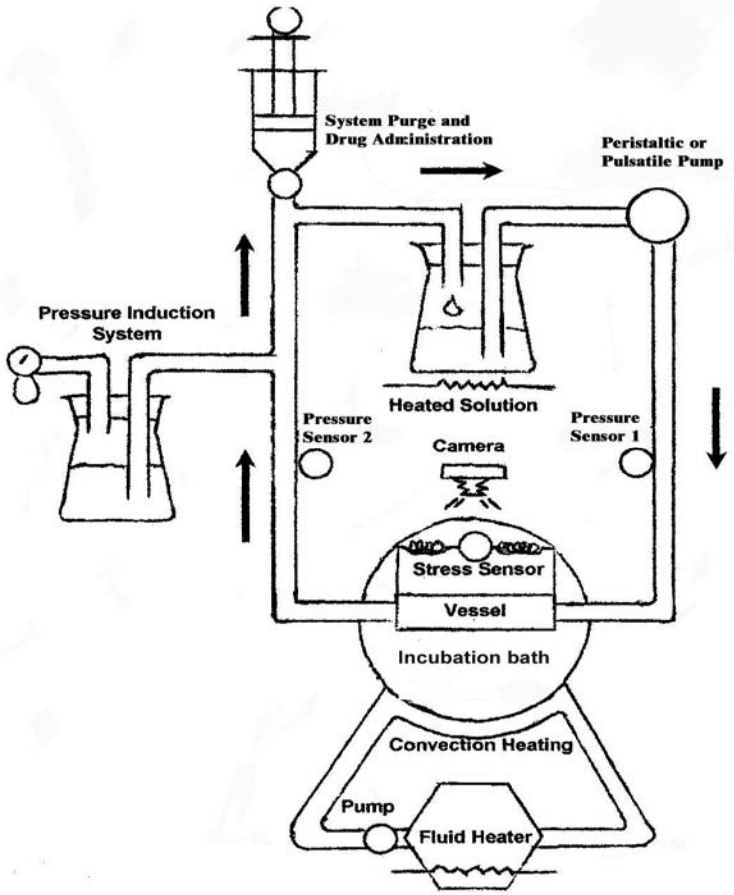
Regression analysis of the relationship between basal levels of expression and phosphorylation of ERK 1/2, p38, and JNK MAPKs and the thickness of the tunica media of aortae obtained young adult (6 month), aged (30 month), and very aged (36 month) Fischer 344/Brown Norway F1 hybrid rats. The results reflect the analysis of combined MAPK and thickness comparisons of four individual aortas from each age group. An asterisk (*) indicates significant correlation between parameters, $p < 0.01$ or greater.

Total Tissue Content	**R**
ERK 1/2	0.005
P38	0.011
JNK	0.522*

Percent Phosphorylation	
ERK 1/2	0.628*
P38	0.572*
JNK	0.383

Figure 3-3

A.


System Purge and
Drug Administration
Peristaltic or
Pulsatile Pump
Pressure Induction
System
Heated Solution
Pressure
Sensor 2
Camera
Pressure
Sensor 1
Stress Sensor
Vessel
Incubation bath
Convection Heating
Pump
Fluid Heater

B.

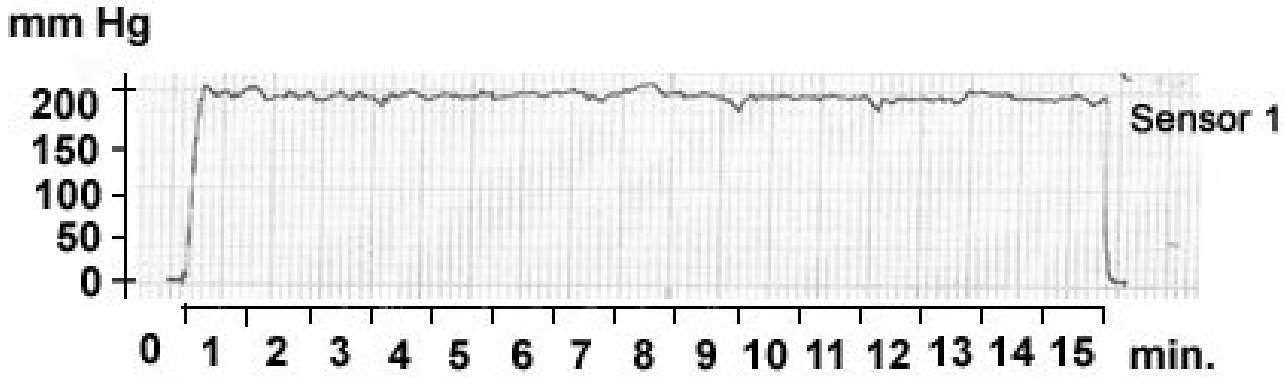
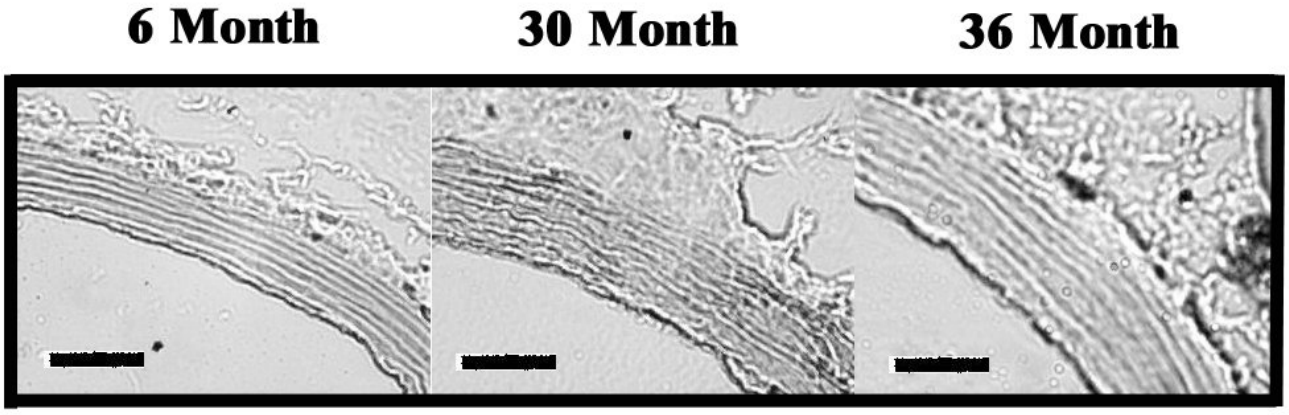

mm Hg
200
150
100
50
0
Sensor 1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 min.

Figure3-1. A: Schematic of experimental setup. A reservoir positioned upstream of the peristaltic pump maintained perfusate and was temperature controlled. A second fluid reservoir served as a hydraulic mechanism for increasing pressure in the system. A sphygmometer bulb (Welch Allyn) was used to pressurize the system. Two inline transducers measured pre- and post-aortic pressure levels. A force transducer (PCB, Piezoelectronics) located on an adjustable micrometer measured the force experienced by the vessel perpendicular to the flow of the system. Vessel intraluminal pressure was controlled by adjusting the air pressure introduced into the fluid reservoir. System calibration was performed before all experiments. **B**: **Representative pressure-time tracing of aortic loading.** Tracing obtained from force transducer positioned proximal to aortic vessel. Note the consistency of pressure maintenance over the interval of experimentation.

Figure 3-4

A.


6 Month
30 Month
36 Month

B.

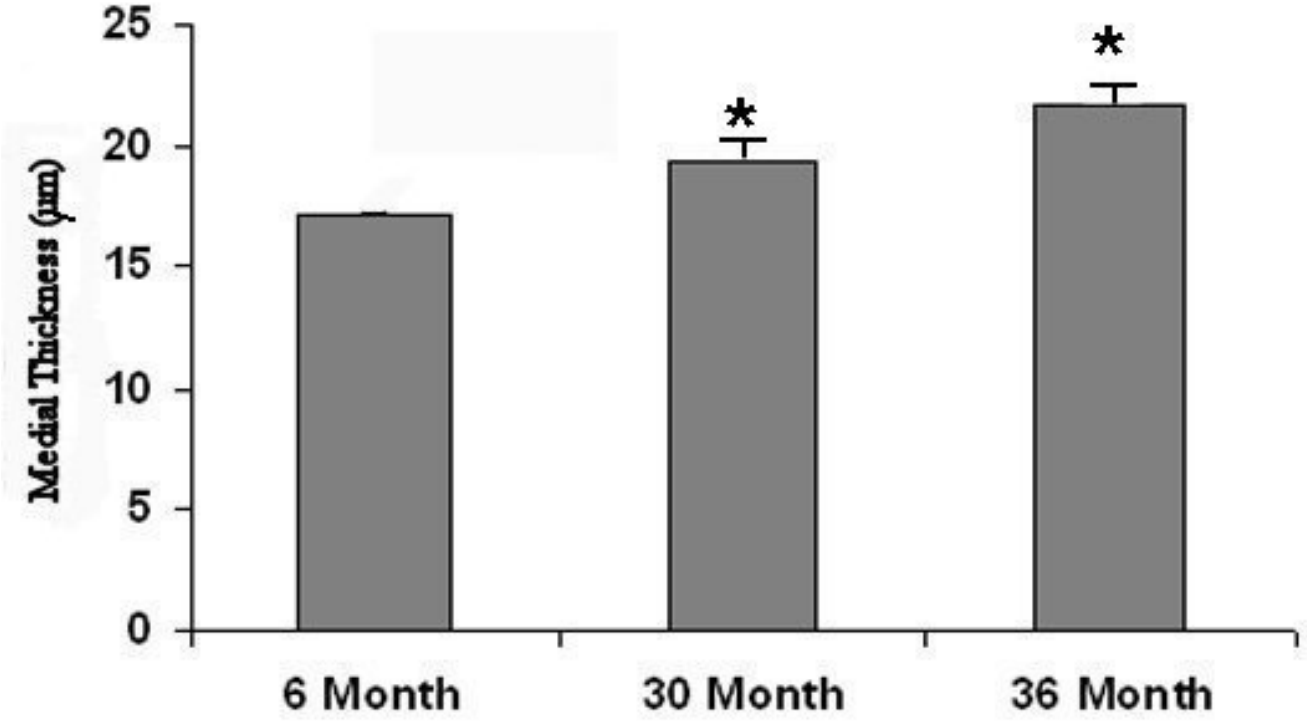

Medial Thickness (μm)
25
20
15
10
5
0
*
*
6 Month
30 Month
36 Month

Figure 3-2. Morphometric changes in the aortic wall of young adult (6 month), aged (30 month) and very aged (36 month) rats. A) Sections stained with hematoxylin and eosin demonstrate the progressive thickening of the medial layer of the aortic wall with aging. Bar indicates 100 :m. B) Bar graph indicating significant increases (*) in the thickness of the tunica media of aortae from aged and very aged rats compared to young adult animals. Thickness was measured from serial cross-sections of each vessel with data averaged from eight different points of cross-section.

Figure 3-5

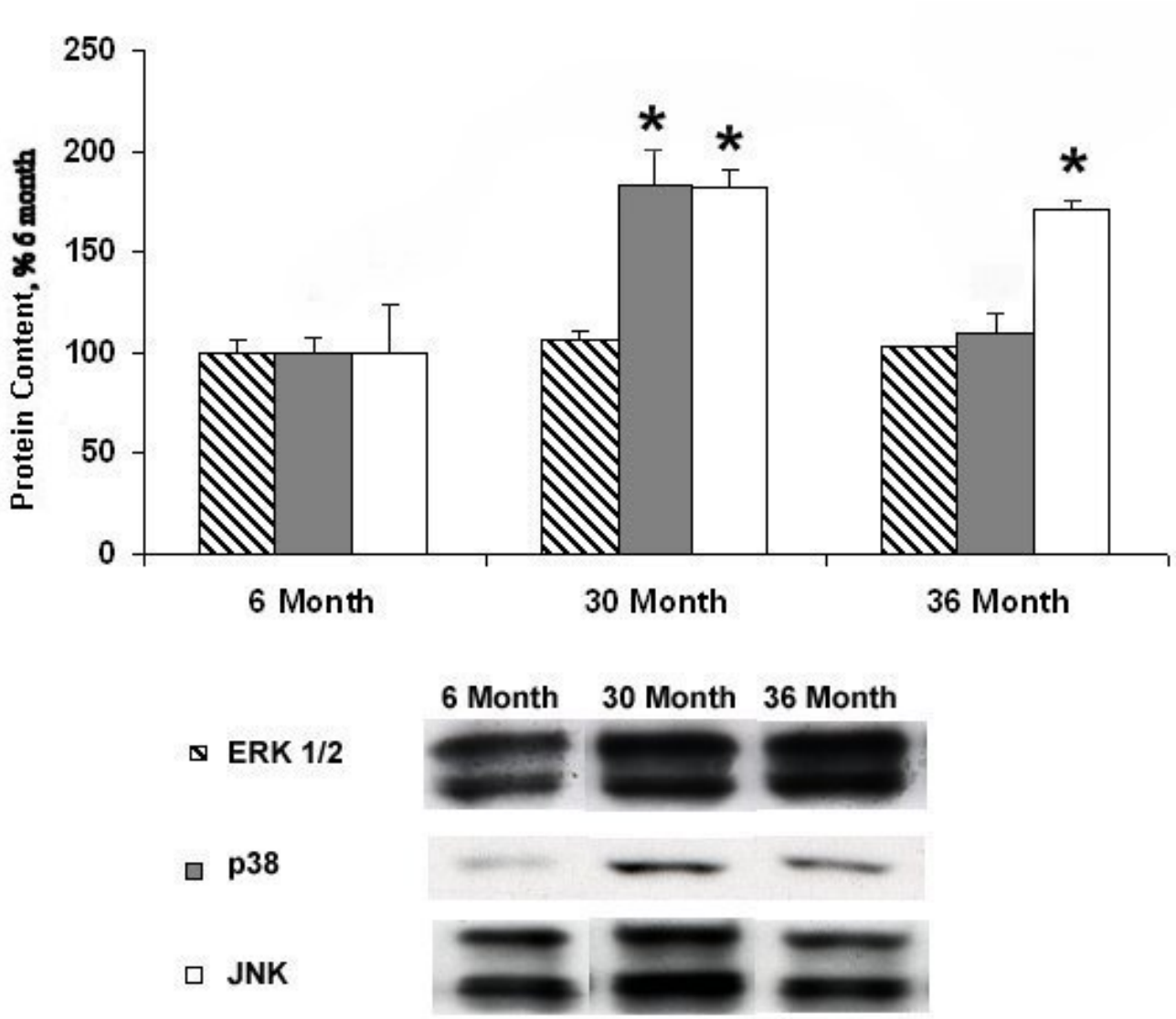

250
200
150
100
50
0
Protein Content, % 6 month
*
*
*
6 Month
30 Month
36 Month
6 Month
30 Month
36 Month
ERK 1/2
p38
JNK

Figure 3-3. Aging differentially affects MAPK protein expression. Aortic segments from young adult (6 month), aged (30 month), and very aged (36 month) rats were analyzed by Western analysis for age-related changes in total ERK 1/2, p38, and JNK MAPK protein expression. Results are expressed as percent of the 6 month value. An asterisk (*) indicates significant difference from the young adult (6 month) value, $p < 0.05$ or greater.

Figure 3-6

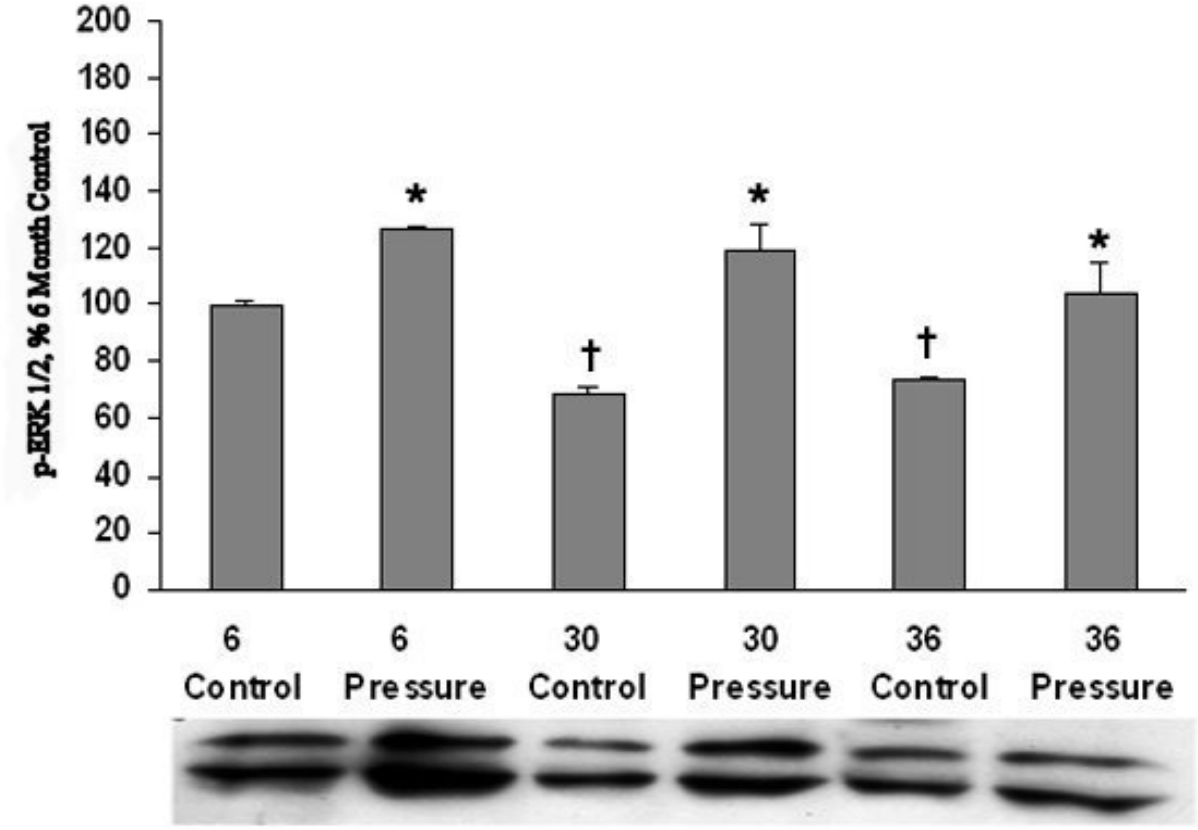


Figure 3-4. Effects of aortic pressurization on phosphorylation of ERK 1/2 MAPK. Aortae were removed from 6, 30 and 36-month old animals, cannulated and pressurized to 200 mm Hg for 15 minutes. ERK 1/2 MAPK phosphorylation was determined by Western analysis and immunodetection for ERK 1/2 MAPK phosphorylated on Thr ^{202} and Tyr ^{204} (phospho-ERK 1/2 MAPK). Phosphorylation status was calculated as phospho-specific optical density divided by the 6 month value. An asterisk (*) or (+) indicates significant difference from the non-pressurized control of that group or the corresponding 6 month value, respectively, $p < 0.01$, $n = 4$ observations for each group.

Figure 3-7

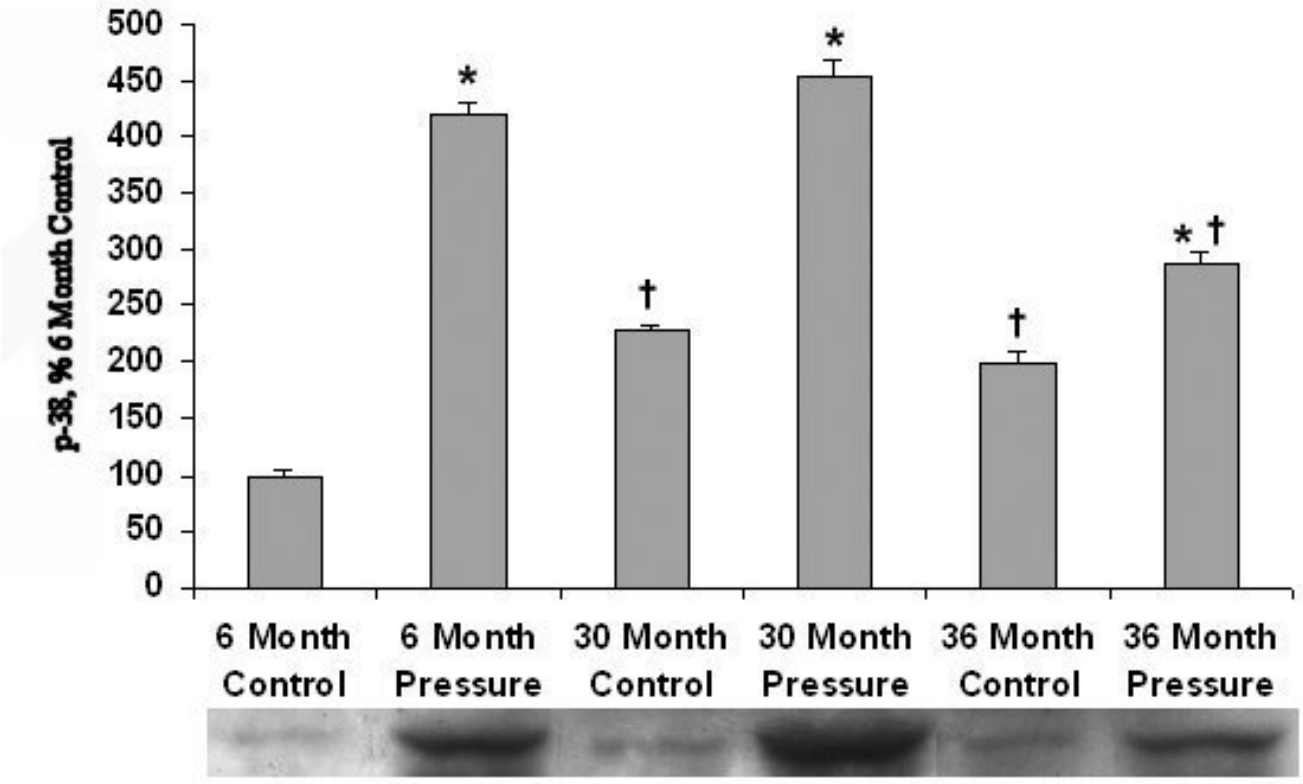


Figure 3-5. Effects of aortic pressurization on phosphorylation of p38 MAPK. Aortae obtained from 6, 30, and 36 month old rats were cannulated and pressurized to 200 mm Hg. p38 MAPK phosphorylation was determined by Western analysis and immunodetection for p38 MAPK phosphorylated on Thr ^{180} and Tyr ^{182} (phospho- p38α MAPK). Phosphorylation status was calculated as phospho-specific optical density divided by the 6 month value. An asterisk (*) or (+) indicates a significant difference from the non-pressurized control of that age group or the corresponding 6 month value, respectively, $p < 0.01$, $n = 4$ observations per group.

Figure 3-8

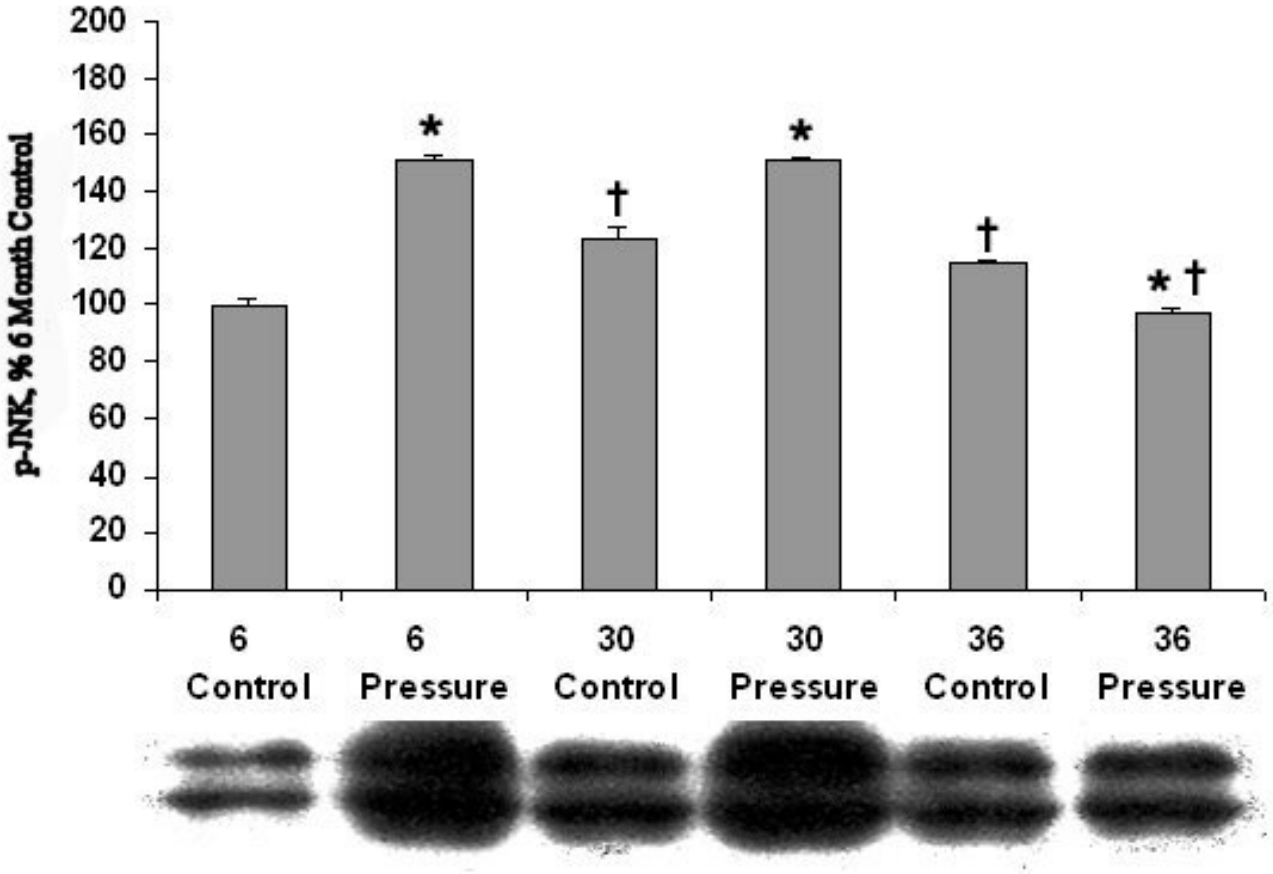


Figure 3-6. Effects of aortic pressurization on JNK MAPK phosphorylation. Aortae obtained from 6, 30, and 36-month old rats were cannulated and pressurized to 200 mm Hg. JNK MAPK phosphorylation was determined by Western analysis and immunodetection for JNK MAPK phosphorylated on Thr^{183} and Tyr^{185} (phospho-JNK MAPK). Phosphorylation status was calculated as phospho-specific optical density divided by the 6 month value. An asterisk (*) or (+) indicates a significant difference from the non-pressurized control of that age group or the corresponding 6 month value, respectively, $p < 0.01$, $n = 4$ observations for each group.

Acknowledgments:

This study was supported by National Institute on Aging Grant AG-20370 to E.B., NIH Grant RR16477 to R.H., and by NSF EPSCOR to Marshall University.

Chapter 4

CONCLUSIONS

1. Aging was found to significantly alter basal expression of p38 and JNK in the 30 month animals, how ever on JNK remained scientifically altered in the 36 month aortas.

2. Aging was found to significantly alter the basal phosphorylation of ERK 1/2, p38, and JNK.

3. Aging was found to alter pressure induced signaling in the aortas. ERK 1/2, and p38 pressure induced phosphorylation was slightly diminished with age. However pressure induced JNK phosphorylation was lost in the very aged animals.

4. We observed age associated increase in tunica medial thickness similar to age-associated vascular structure seen in humans, these data support the utility of the F1 strain as a tool to investigate the age associated alterations cardiovascular seen in aging humans.

Further directions

Future directions for research based on this study should focus on the mechanisms associated with the differences in load induced MAPK activation with aging in the F344BN aorta.

MAPK activation is a complex process necessitating the integration of many different participants. The mechanisms associated with differences in MAPK activation with aging may lie in differences in aortic wall structure. A study designed to determine how mechanical properties are altered with age may be invaluable to understanding age-associated changes in aortic mechanotransduction. This study could be done by subjecting aortas from different aged animals to increased pressure and examining the physical response. Alterations in compliance, contractile response, K+ sensitivity, and numerous other factors could be examined to address age related alterations. Conversely endothelia damage has been associated with many pathological conditions with aging, in addressing the above mentioned mechanical properties of the aortic tissue the removal of the endothelium may shine light on the age related loss or damage of this tissue layer. Overall many age-associated changes involving mechano-sensing, mechano-stimulation, mechano-transduction, and force perception have not been addressed. If age alters the tissues ability to perceive and respond to mechanical stimuli, knowing if these alteration are due to changes in tissue structure and architecture or in proteins associated with specific signaling pathways may provide an avenue to inhibit age-associated changes in mechanotrasduction.

Although the effects of mechanical stress could be mediated in part, by activation of mechanosensitive ion channels or by locally and systemically released growth factors,

the seminal studies of Ingber and others (50-57) have demonstrated that mechanical input itself is able to trigger cellular signaling mechanisms. Increasing evidence supports the notion that living cells transduce and transmit forces into biochemical signals through specialized focal sites of the membrane known as focal adhesion complexes. These regions are rich in a variety of signaling molecules including focal adhesion kinase (FAK), RhoA, c-Src family kinases, paxillin, cdc42, Rac1, Ras family proteins, phosphatidylinositol 3-kinase (PI3-K), PKC, and MAPK. It is thought that these signaling molecules may act as the "transducers" of mechanical stimuli that integrin dependent signals use to regulate alterations in gene transcription and translation (1, 86, 109). To investigate the possibility that age-associated alterations in FAK signaling may be involved in the different MAPK phosphorylation we see with aging, we could compare resting levels and the phosphorylation (activation) of the FAK signaling pathway after increases in intraluminal pressure in isolated aortae from different age groups of F344XBN rats.

Alternatively, another potent activator of MAPK phosphorylation may be reactive oxygen species (ROS). Defined as an imbalance between the generation of ROS and the existing antioxidative defense mechanisms, oxidative stress has been implicated to play important roles in tissue injury, vascular remodeling, apoptosis, and aging. Recent investigations have identified that oxidative stress is capable of activating MAPK proteins (5, 6, 108). To investigate whether alterations in ROS contribute to age-associated differences in MAPK signaling we could repeat the experiments performed in this study in the absence and presence of ROS scavengers. A similar MAPK response to increased intraluminal pressure in the presence of ROS scavengers with aging would

suggest that ROS are not factor involved in age associated changes of pressure induced MAPK activation. Conversely, if introduction of ROS in the 6-month animals altered MAPK pathway activation to resemble that of the 36-month age group, we could postulate that age related increase in ROS alter the pressure induced activation of MAPKs pathway related proteins.

Reference

1. **Alenghat FJ and Ingber DE.** Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. *Sci STKE* 2002: PE6, 2002.
2. **Berk BC, Corson MA, Peterson TE, and Tseng H.** Protein kinases as mediators of fluid shear stress stimulated signal transduction in endothelial cells: a hypothesis for calcium-dependent and calcium-independent events activated by flow. *J Biomech* 28: 1439-1450, 1995.
3. **Bey L, Areiqat E, Sano A, and Hamilton MT.** Reduced lipoprotein lipase activity in postural skeletal muscle during aging. *J Appl Physiol* 91: 687-692, 2001.
4. **Birukov KG, Lehoux S, Birukova AA, Merval R, Tkachuk VA, and Tedgui A.** Increased pressure induces sustained protein kinase C-independent herbimycin A-sensitive activation of extracellular signal-related kinase 1/2 in the rabbit aorta in organ culture. *Circ Res* 81: 895-903, 1997.
5. **Blanc A, Pandey NR, and Srivastava AK.** Distinct roles of Ca2+, calmodulin, and protein kinase C in H2O2-induced activation of ERK1/2, p38 MAPK, and protein kinase B signaling in vascular smooth muscle cells. *Antioxid Redox Signal* 6: 353-366, 2004.
6. **Blanc A, Pandey NR, and Srivastava AK.** Synchronous activation of ERK 1/2, p38mapk and PKB/Akt signaling by H2O2 in vascular smooth muscle cells: potential involvement in vascular disease (review). *Int J Mol Med* 11: 229-234, 2003.

7. **Blough ER and Linderman JK.** Lack of skeletal muscle hypertrophy in very aged male Fischer 344 x Brown Norway rats. *J Appl Physiol* 88: 1265-1270, 2000.

8. **Bogoyevitch MA, Ketterman AJ, and Sugden PH.** Cellular stresses differentially activate c-Jun N-terminal protein kinases and extracellular signal-regulated protein kinases in cultured ventricular myocytes. *J Biol Chem* 270: 29710-29717, 1995.

9. **Bokemeyer D, Sorokin A, and Dunn MJ.** Multiple intracellular MAP kinase signaling cascades. *Kidney Int* 49: 1187-1198, 1996.

10. **Carson JA, Lee WJ, McClung J, and Hand GA.** Steroid receptor concentration in aged rat hindlimb muscle: effect of anabolic steroid administration. *J Appl Physiol* 93: 242-250, 2002.

11. **Carson JA, Nettleton D, and Reecy JM.** Differential gene expression in the rat soleus muscle during early work overload-induced hypertrophy. *Faseb J* 16: 207-209, 2002.

12. **Cartee GD.** Myocardial GLUT-4 glucose transporter protein levels of rats decline with advancing age. *J Gerontol* 48: B168-170, 1993.

13. **Cartee GD, Bohn EE, Gibson BT, and Farrar RP.** Growth hormone supplementation increases skeletal muscle mass of old male Fischer 344/brown Norway rats. *J Gerontol A Biol Sci Med Sci* 51: B214-219, 1996.

14. **Cartee GD, Briggs-Tung C, and Kietzke EW.** Persistent effects of exercise on skeletal muscle glucose transport across the life-span of rats. *J Appl Physiol* 75: 972-978, 1993.

15. **Chang KC, Hsu KL, Peng YI, Lee FC, and Tseng YZ.** Aminoguanidine prevents age-related aortic stiffening in Fisher 344 rats: aortic impedance analysis. *Br J Pharmacol* 140: 107-114, 2003.

16. **Chang L and Karin M.** Mammalian MAP kinase signalling cascades. *Nature* 1;410(6824): 37-40, 2001.

17. **Chen KD and Alway SE.** A physiological level of clenbuterol does not prevent atrophy or loss of force in skeletal muscle of old rats. *J Appl Physiol* 89: 606-612, 2000.

18. **Chicurel ME, Chen CS, and Ingber DE.** Cellular control lies in the balance of forces. *Curr Opin Cell Biol* 10: 232-239, 1998.

19. **Chockalingam A, Balaguer-Vintro I, Achutti A, de Luna AB, Chalmers J, Farinaro E, Lauzon R, Martin I, Papp JG, Postiglione A, Reddy KS, and Tse TF.** The World Heart Federation's white book: impending global pandemic of cardiovascular diseases: challenges and opportunities for the prevention and control of cardiovascular diseases in developing countries and economies in transition. *Can J Cardiol* 16: 227-229, 2000.

20. **Crowley ST, Ray CJ, Nawaz D, Majack RA, and Horwitz LD.** Multiple growth factors are released from mechanically injured vascular smooth muscle cells. *Am J Physiol* 269: H1641-1647, 1995.

21. **Davis MJ, Wu X, Nurkiewicz TR, Kawasaki J, Davis GE, Hill MA, and Meininger GA.** Integrins and mechanotransduction of the vascular myogenic response. *Am J Physiol Heart Circ Physiol* 280: H1427-1433, 2001.

22. **Degens H and Alway SE.** Skeletal muscle function and hypertrophy are diminished in old age. *Muscle Nerve* 27: 339-347, 2003.

23. **Eddinger TJ, Moss RL, and Cassens RG.** Fiber number and type composition in extensor digitorum longus, soleus, and diaphragm muscles with aging in Fisher 344 rats. *J Histochem Cytochem* 33: 1033-1041, 1985.

24. **English J, Pearson G, Wilsbacher J, Swantek J, Karandikar M, Xu S, and Cobb MH.** New insights into the control of MAP kinase pathways. *Exp Cell Res* 253: 255-270, 1999.

25. **Fox J, Garber P, Hoffman M, Johnson D, Schaefer P, Vien J, Zeaton C, and Thompson LV.** Morphological characteristics of skeletal muscles in relation to gender. *Aging Clin Exp Res* 15: 264-269, 2003.

26. **Frisch SM, Vuori K, Ruoslahti E, and Chan-Hui PY.** Control of adhesion-dependent cell survival by focal adhesion kinase. *J Cell Biol* 134: 793-799, 1996.

27. **Furnari FB, Huang HJ, and Cavenee WK.** The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. *Cancer Res* 58: 5002-5008, 1998.

28. **Gaballa MA, Jacob CT, Raya TE, Liu J, Simon B, and Goldman S.** Large artery remodeling during aging: biaxial passive and active stiffness. *Hypertension* 32: 437-443, 1998.

29. **Garrington TP and Johnson GL.** Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol* 11: 211-218, 1999.

30. **Gennaro G, Menard C, Giasson E, Michaud SE, Palasis M, Meloche S, and Rivard A.** Role of p44/p42 MAP kinase in the age-dependent increase in vascular smooth muscle cell proliferation and neointimal formation. *Arterioscler Thromb Vasc Biol* 23: 204-210, 2003.

31. **Goldspink G, Scutt A, Loughna PT, Wells DJ, Jaenicke T, and Gerlach GF.** Gene expression in skeletal muscle in response to stretch and force generation. *Am J Physiol* 262: R356-363, 1992.

32. **Gomes RR, Jr. and Booth FW.** Expression of acetylcholine receptor mRNAs in atrophying and nonatrophying skeletal muscles of old rats. *J Appl Physiol* 85: 1903-1908, 1998.

33. **Grimes CA and Jope RS.** The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol* 65: 391-426, 2001.

34. **Groskreutz JJ and Thompson LV.** Enzymatic alterations in single type IIB skeletal muscle fibers with inactivity and exercise in 12- and 30-month-old rats. *Aging Clin Exp Res* 14: 347-353, 2002.

35. **Hagen JL, Krause DJ, Baker DJ, Fu MH, Tarnopolsky MA, and Hepple RT.** Skeletal Muscle Aging in F344BN F1-Hybrid Rats: I. Mitochondrial Dysfunction Contributes to the Age-Associated Reduction in VO2max. *J Gerontol A Biol Sci Med Sci* 59: 1099-1110, 2004.

36. **Hamada K, Takuwa N, Yokoyama K, and Takuwa Y.** Stretch activates Jun N-terminal kinase/stress-activated protein kinase in vascular smooth muscle cells through mechanisms involving autocrine ATP stimulation of purinoceptors. *J Biol Chem* 273: 6334-6340, 1998.

37. **Haq S, Choukroun G, Kang ZB, Ranu H, Matsui T, Rosenzweig A, Molkentin JD, Alessandrini A, Woodgett J, Hajjar R, Michael A, and Force T.** Glycogen synthase kinase-3beta is a negative regulator of cardiomyocyte hypertrophy. *J Cell Biol* 151: 117-130, 2000.

38. **Hariri RJ, Alonso DR, Hajjar DP, Coletti D, and Weksler ME.** Aging and arteriosclerosis. I. Development of myointimal hyperplasia after endothelial injury. *J Exp Med* 164: 1171-1178, 1986.

39. **Hazzalin C and Mahadevan L.** MAPK-regulated transcription: a continuously variable gene switch? *Nat Rev Mol Cell Biol* 3(1): 30-40, 2002.

40. **Hepple RT, Hagen JL, Krause DJ, and Jackson CC.** Aerobic power declines with aging in rat skeletal muscles perfused at matched convective O2 delivery. *J Appl Physiol* 94: 744-751, 2003.

41. **Hepple RT, Ross KD, and Rempfer AB.** Fiber atrophy and hypertrophy in skeletal muscles of late middle-aged Fischer 344 x Brown Norway F1-hybrid rats. *J Gerontol A Biol Sci Med Sci* 59: 108-117, 2004.

42. **Hepple RT and Vogell JE.** Anatomic capillarization is maintained in relative excess of fiber oxidative capacity in some skeletal muscles of late middle-aged rats. *J Appl Physiol* 96: 2257-2264, 2004.

43. **Holloszy J, Chen M, Cartee G, and Young J.** Skeletal muscle atrophy in old rats: differential changes in the three fiber types. *Mech Aging Dev* 60: 199-213, 1991.

44. **Hong H, Chan P, Liu J, Juan S, Huang M, Lin J, and Cheng T.** Angiotensin II induces endothelin-1 gene expression via extracellular signal-regulated kinase

pathway in rat aortic smooth muscle cells. *Cardiovasc Res* 1;61(1): 159-168, 2004.

45. **Hosokawa H, Aiuchi S, Kambe T, Hagiwara Y, and Kubo T.** Mechanical stretch-induced mitogen-activated protein kinase activation is mediated via angiotensin and endothelin systems in vascular smooth muscle cells. *Biol Pharm Bull* 25(12): 1588-1592, 2002.

46. **Hu Y, Cheng L, Hochleitner B, and Xu Q.** Activation of mitogen-activated protein kinases (ERK/JNK) and AP-1 transcription factor in rat carotid arteries after balloon injury. *Arterioscler Thromb Vasc Biol* 17(11): 2808-2816, 1997.

47. **Hung CT, Henshaw DR, Wang CC, Mauck RL, Raia F, Palmer G, Chao PH, Mow VC, Ratcliffe A, and Valhmu WB.** Mitogen-activated protein kinase signaling in bovine articular chondrocytes in response to fluid flow does not require calcium mobilization. *J Biomech* 33: 73-80, 2000.

48. **Ihlemann J, Ploug T, Hellsten Y, and Galbo H.** Effect of tension on contraction-induced glucose transport in rat skeletal muscle. *Am J Physiol* 277: E208-214, 1999.

49. **Ikeda M, Takei T, Mills I, and Sumpio BE.** Calcium-independent activation of extracellular signal-regulated kinases 1 and 2 by cyclic strain. *Biochem Biophys Res Commun* 247: 462-465, 1998.

50. **Ingber D.** Mechanical signaling. *Ann N Y Acad Sci* 961: 162-163, 2002.

51. **Ingber DE.** Integrins, tensegrity, and mechanotransduction. *Gravit Space Biol Bull* 10: 49-55, 1997.

52. **Ingber DE.** Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ Res* 91: 877-887, 2002.

53. **Ingber DE.** Mechanobiology and diseases of mechanotransduction. *Ann Med* 35: 564-577, 2003.

54. **Ingber DE.** Mechanosensation through integrins: cells act locally but think globally. *Proc Natl Acad Sci U S A* 100: 1472-1474, 2003.

55. **Ingber DE.** Tensegrity I. Cell structure and hierarchical systems biology. *J Cell Sci* 116: 1157-1173, 2003.

56. **Ingber DE.** Tensegrity II. How structural networks influence cellular information processing networks. *J Cell Sci* 116: 1397-1408, 2003.

57. **Ingber DE.** Tensegrity: the architectural basis of cellular mechanotransduction. *Annu Rev Physiol* 59: 575-599, 1997.

58. **Iwasaki H, Yoshimoto T, Sugiyama T, and Hirata Y.** Activation of cell adhesion kinase beta by mechanical stretch in vascular smooth muscle cells. *Endocrinology* 144(6): 2304-2310, 2003.

59. **Johnson TE.** Aging can be genetically dissected into component processes using long-lived lines of Caenorhabditis elegans. *Proc Natl Acad Sci U S A* 84: 3777-3781, 1987.

60. **Joki N, Kaname S, Hirakata M, Hori Y, Yamaguchi T, Fujita T, Katoh T, and Kurokawa K.** Tyrosine-kinase dependent TGF-beta and extracellular matrix expression by mechanical stretch in vascular smooth muscle cells. *Hypertens Res* Mar;23(2): 91-99, 2000.

61. **Kirk C, Freilich A, and Miller R.** Age-related decline in activation of JNK by TCK- CD28- mediated signals in murine T-lymphocytes. *Cell Immunol* 197(2): 75-82, 1999.

62. **Komuro I, Kudo S, Yamazaki T, Zou Y, Shiojima I, and Yazaki Y.** Mechanical stretch activates the stress-activated protein kinases in cardiac myocytes. *Faseb J* 10: 631-636, 1996.

63. **Kumar A, Chaudhry I, Reid M, and Boriek A.** Distinct signaling pathways are activated in responce to mechanical stress applied axially and transversely to skeletal muscle fibers. *J Biological Chem* 277(48): 46493-46503, 2002.

64. **Kyosseva S.** Mitogen-activated protein kinase signaling. *Int Rev Neurobiol* 59: 201-220, 2004.

65. **Lagaud GJ, Lam E, Lui A, van Breemen C, and Laher I.** Nonspecific inhibition of myogenic tone by PD98059, a MEK1 inhibitor, in rat middle cerebral arteries. *Biochem Biophys Res Commun* 257: 523-527, 1999.

66. **Lakatta E.** Aging Effects on the Vasculature in Health: Risk Factors for Cardiovascular Disease. *Am J Geriatr Cardiol* 3: 11-17, 1994.

67. **Lakatta EG.** Cardiovascular aging research: the next horizons. *J Am Geriatr Soc* 47: 613-625, 1999.

68. **Lakatta EG.** Cardiovascular Aging: Perspectives From Humans to Rodents. *Am J Geriatr Cardiol* 7: 32-45, 1998.

69. **Lakatta EG, MD and Najjar SS, MD.** Vascular Aging: An emerging New global cardiovascular risk: Gerontology Research Center, Intramural Research Program, National Institute on Aging, National Institutes of Health.

70. **Lakatta EG and Sollott SJ.** Perspectives on mammalian cardiovascular aging: humans to molecules. *Comp Biochem Physiol A Mol Integr Physiol* 132: 699-721, 2002.

71. **Lakatta EG, Sollott SJ, and Pepe S.** The old heart: operating on the edge. *Novartis Found Symp* 235: 172-196; discussion 196-201, 217-120, 2001.

72. **Larkin LM, Halter JB, and Supiano MA.** Effect of aging on rat skeletal muscle beta-AR function in male Fischer 344 x brown Norway rats. *Am J Physiol* 270: R462-468, 1996.

73. **Larkin LM, Reynolds TH, Supiano MA, Kahn BB, and Halter JB.** Effect of aging and obesity on insulin responsiveness and glut-4 glucose transporter content in skeletal muscle of Fischer 344 x Brown Norway rats. *J Gerontol A Biol Sci Med Sci* 56: B486-492, 2001.

74. **Leeuwenburgh C, Gurley CM, Strotman BA, and Dupont-Versteegden EE.** Age-related differences in apoptosis with disuse atrophy in soleus muscle. *Am J Physiol Regul Integr Comp Physiol*, 2005.

75. **Lehoux S, Esposito B, Merval R, Loufrani L, and Tedgui A.** Pulsatile stretch-induced extracellular signal-regulated kinase 1/2 activation in organ culture of rabbit aorta involves reactive oxygen species. *Arterioscler Thromb Vasc Biol* 20(11): 2366-2372, 2000.

76. **Li C and Xu Q.** Mechanical stress-initiated signal transductions in vascular smooth muscle cells. *Cell Signal* Jul;12(7): 435-445, 2000.

77. **Li M, Torres C, Acuna-Castillo C, Walter R, Gardner E, Murasko D, and Sierra F.** Defect in ERK2 and p54(JNK) activation in aging mouse splenocytes. *Biol Sci Med Sci* 57(2):B4: 1-7, 2002.

78. **Li M, Walter R, Torres C, and Sierra F.** Impaired signal transduction in mitogen activated rat splenic lymphocytes during aging. *Mech Ageing Dev* 113(2): 85-99, 2000.

79. **Li S, Kim M, Hu YL, Jalali S, Schlaepfer DD, Hunter T, Chien S, and Shyy JY.** Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. *J Biol Chem* 272: 30455-30462, 1997.

80. **Li Z, Froehlich J, Galis ZS, and Lakatta EG.** Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats. *Hypertension* 33: 116-123, 1999.

81. **Lipman RD, Chrisp CE, Hazzard DG, and Bronson RT.** Pathologic characterization of brown Norway, brown Norway x Fischer 344, and Fischer 344 x brown Norway rats with relation to age. *J Gerontol A Biol Sci Med Sci* 51: B54-59, 1996.

82. **Loufrani L, Lehoux S, Tedgui A, Levy BI, and Henrion D.** Stretch induces mitogen-activated protein kinase activation and myogenic tone through 2 distinct pathways. *Arterioscler Thromb Vasc Biol* 19: 2878-2883, 1999.

83. **Lowe DA, Degens H, Chen KD, and Alway SE.** Glyceraldehyde-3-phosphate dehydrogenase varies with age in glycolytic muscles of rats. *J Gerontol A Biol Sci Med Sci* 55: B160-164, 2000.

84. **MacKenna D, Dolfi F, Vuori K, and Ruoslahti E.** Extracellular signal-regulated kinase and c-Jun NH2-terminal kinase activation by mechanical stretch is integrin-dependent and matrix-specific in rat cardiac fibroblasts. *J Clin Invest* 101(2): 301-310, 1998.

85. **Malek AM and Izumo S.** Control of endothelial cell gene expression by flow. *J Biomech* 28: 1515-1528, 1995.

86. **Malik R and Parsons J.** Integrin-dependent activation of the p70 ribosomal S6 kinase signaling pathway. *J Biol Chem* 271: 29785-29791, 1996.

87. **Mangiarua E, Galagedera N, and Patterson J.** Increased intraluminal pressure induces DNA synthesis and c-fos expression in perfused rat aorta. *Archives of Physiology and Biochem* 104(7): 838-844, 1996.

88. **Marin J.** Age-related changes in vascular responses: a review. *Mech Ageing Dev* 14;79(2-3): 71-114, 1995.

89. **Marsh DR, Criswell DS, Carson JA, and Booth FW.** Myogenic regulatory factors during regeneration of skeletal muscle in young, adult, and old rats. *J Appl Physiol* 83: 1270-1275, 1997.

90. **Marsh DR, Hinds LR, Lester WS, Reinking BE, and Booth FW.** The force-frequency relationship is altered in regenerating and senescent rat skeletal muscle. *Muscle Nerve* 21: 1265-1274, 1998.

91. **Matrougui K, Eskildsen-Helmond YE, Fiebeler A, Henrion D, Levy BI, Tedgui A, and Mulvany MJ.** Angiotensin II stimulates extracellular signal-regulated kinase activity in intact pressurized rat mesenteric resistance arteries. *Hypertension* 36: 617-621, 2000.

92. **Mayhew M, Renganathan M, and Delbono O.** Effectiveness of caloric restriction in preventing age-related changes in rat skeletal muscle. *Biochem Biophys Res Commun* 251: 95-99, 1998.

93. **McCaffrey T, Nicholson A, Szabo P, Weksler M, and Weksler B.** Aging and arteriosclerosis. The increased proliferation of arterial smooth muscle cells isolated from old rats is associated with increased platelet-derived growth factor-like activity. *Exp Med* 1;167(1): 163-174, 1988.

94. **Meloche S, Landry J, Huot J, Houle F, Marceau F, and Giasson E.** p38 MAP kinase pathway regulates angiotensin II-induced contraction of rat vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 279: H741-751, 2000.

95. **Mikuni-Takagaki Y.** Mechanical responses and signal transduction pathways in stretched osteocytes. *J Bone Miner Metab* 17: 57-60, 1999.

96. **Morris RT, Spangenburg EE, and Booth FW.** Responsiveness of cell signaling pathways during the failed 15-day regrowth of aged skeletal muscle. *J Appl Physiol* 96: 398-404, 2004.

97. **Ng YC, Nagarajan M, Jew KN, Mace LC, and Moore RL.** Exercise training differentially modifies age-associated alteration in expression of Na+-K+-ATPase subunit isoforms in rat skeletal muscles. *Am J Physiol Regul Integr Comp Physiol* 285: R733-740, 2003.

98. **Numaguchi K, Eguchi S, Yamakawa T, Motley ED, and Inagami T.** Mechanotransduction of rat aortic vascular smooth muscle cells requires RhoA and intact actin filaments. *Circ Res* 85: 5-11, 1999.

99. **Osol G.** Mechanotransduction by vascular smooth muscle. *J Vasc Res* 32: 275-292, 1995.

100. **Palmer H, Tuzon C, and Paulson K.** Age-dependent decline in mitogenic stimulation of hepatocytes. Reduced association between Shc and the epidermal growth factor receptor is coupled to decreased activation of Raf and extracellular signal-regulated kinases. *J Biol Chem* 16;274(16): 11424-11430, 1999.

101. **Parkington JD, LeBrasseur NK, Siebert AP, and Fielding RA.** Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle. *J Appl Physiol* 97: 243-248, 2004.

102. **Petrich B and Wang Y.** Stress-activated MAP kinases in cardiac remodeling and heart failure; new insights from transgenic studies. *Trends Cardiovasc Med* Feb;14(2): 50-55, 2004.

103. **Peyssonnaux C and Eychene A.** The Raf/MEK/ERK pathway: new concepts of activation. *Biol Cell* 93(1-2): 53-62, 2001.

104. **Roberts D, Gelperin D, and Wiley JW.** Evidence for age-associated reduction in acetylcholine release and smooth muscle response in the rat colon. *Am J Physiol* 267: G515-522, 1994.

105. **Ruoslahti E.** Stretching is good for a cell. *Science* 276: 1345-1346, 1997.

106. **Russell JA, Kindig CA, Behnke BJ, Poole DC, and Musch TI.** Effects of aging on capillary geometry and hemodynamics in rat spinotrapezius muscle. *Am J Physiol Heart Circ Physiol* 285: H251-258, 2003.

107. **Sadoshima J and Izumo S.** Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: potential involvement of an autocrine/paracrine mechanism. *Embo J* 12: 1681-1692, 1993.

108. **Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, Piao JH, Yagita H, Okumura K, Doi T, and Nakano H.** NF-kappaB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. *Embo J* 22: 3898-3909, 2003.

109. **Schmidt C, Pommerenke H, Durr F, Nebe B, and Rychly J.** Mechanical stressing of integrin receptors induces enhanced tyrosine phosphorylation of cytoskeletally anchored proteins. *J Biol Chem* 273: 5081-5085, 1998.

110. **Seko Y, Takahashi N, Sabe H, Tobe K, Kadowaki T, and Nagai R.** Hypoxia induces activation and subcellular translocation of focal adhesion kinase (p125(FAK)) in cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 262: 290-296, 1999.

111. **Seko Y, Takahashi N, Tobe K, Kadowaki T, and Yazaki Y.** Pulsatile stretch activates mitogen-activated protein kinase (MAPK) family members and focal adhesion kinase (p125(FAK)) in cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 259: 8-14, 1999.

112. **Sharp WW, Simpson DG, Borg TK, Samarel AM, and Terracio L.** Mechanical forces regulate focal adhesion and costamere assembly in cardiac myocytes. *Am J Physiol* 273: H546-556, 1997.

113. **Smith AR and Hagen TM.** Vascular endothelial dysfunction in aging: loss of Akt-dependent endothelial nitric oxide synthase phosphorylation and partial restoration by (R)-alpha-lipoic acid. *Biochem Soc Trans* 31: 1447-1449, 2003.

114. **Smith PG, Garcia R, and Kogerman L.** Strain reorganizes focal adhesions and cytoskeleton in cultured airway smooth muscle cells. *Exp Cell Res* 232: 127-136, 1997.

115. **Smith PG, Moreno R, and Ikebe M.** Strain increases airway smooth muscle contractile and cytoskeletal proteins in vitro. *Am J Physiol* 272: L20-27, 1997.

116. **Spangenburg EE, Abraha T, Childs TE, Pattison JS, and Booth FW.** Skeletal muscle IGF-binding protein-3 and -5 expressions are age, muscle, and load dependent. *Am J Physiol Endocrinol Metab* 284: E340-350, 2003.

117. **Spier A, Meurs K, Coovert D, Lehmkuhl L, O'Grady M, Freeman L, Burghes A, and Towbin J.** Use of western immunoblot for evaluation of myocardial dystrophin, alpha-sarcoglycan, and beta-dystroglycan in dogs with idiopathic dilated cardiomyopathy. *Am J Vet Res* Jan;62(1): 67-71, 2001.

118. **Spinetti G, Wang M, Monticone R, Zhang J, Zhao D, and Lakatta EG.** Rat aortic MCP-1 and its receptor CCR2 increase with age and alter vascular smooth muscle cell function. *Arterioscler Thromb Vasc Biol* 24: 1397-1402, 2004.

119. **Sprott R.** Development of animal models of aging at the National Institute on Aging. *Neurobiol Aging* 12: 635-638, 1991.

120. **Spurrell BE, Murphy TV, and Hill MA.** Intraluminal pressure stimulates MAPK phosphorylation in arterioles: temporal dissociation from myogenic contractile response. *Am J Physiol Heart Circ Physiol* 285: H1764-1773, 2003.

121. **Sumitani M, Cabral AM, Michelini LC, and Krieger EM.** In vivo adaptive responses of the aorta to hypertension and aging. *Am J Physiol* 273: H96-103, 1997.

122. **Sun X, Nagarajan M, Beesley PW, and Ng YC.** Age-associated differential expression of Na(+)-K(+)-ATPase subunit isoforms in skeletal muscles of F-344/BN rats. *J Appl Physiol* 87: 1132-1140, 1999.

123. **Suzuma I, Suzuma K, Ueki K, Hata Y, Feener E, King G, and Aiello L.** Stretch-induced Retinal Vascular Endothelial Growth Factor Expression Is Mediated by Phosphatidylinositol 3-Kinase and Protein Kinase C (PKC)- but Not by Stretch-induced ERK1/2, Akt, Ras, or Classical/Novel PKC Pathways. *J Biol Chem* 277(2): 1047-1057, 2002.

124. **Tanguy S, Boucher F, Toufektsian M, Besse S, and de Leiris J.** Aging exacerbates hydrogen peroxide-induced alteration of vascular reactivity in rats. *Antioxid Redox Signal* Summer;2(2): 363-368, 2000.

125. **Thompson LV, Johnson SA, and Shoeman JA.** Single soleus muscle fiber function after hindlimb unweighting in adult and aged rats. *J Appl Physiol* 84: 1937-1942, 1998.

126. **Towbin H, Staehelin T, and Gordon J.** Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76: 4350-4354, 1979.

127. **Towbin H, Staehelin T, and Gordon J.** Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. 1979. *Biotechnology* 24: 145-149, 1992.

128. **Towbin H, Staehelin T, and Gordon J.** Immunoblotting in the clinical laboratory. *J Clin Chem Clin Biochem* Aug;27(8): 495-501, 1989.

129. **Tucker MZ and Turcotte LP.** Aging is associated with elevated muscle triglyceride content and increased insulin-stimulated fatty acid uptake. *Am J Physiol Endocrinol Metab* 285: E827-835, 2003.

130. **Tucker MZ and Turcotte LP.** Impaired fatty acid oxidation in muscle of aging rats perfused under basal conditions. *Am J Physiol Endocrinol Metab* 282: E1102-1109, 2002.

131. **van der Loo B, Labugger R, Aebischer CP, Bachschmid M, Spitzer V, Kilo J, Altwegg L, Ullrich V, and Luscher TF.** Age-related changes of vitamin A status. *J Cardiovasc Pharmacol* 43: 26-30, 2004.

132. **Vandenburgh HH, Solerssi R, Shansky J, Adams JW, and Henderson SA.** Mechanical stimulation of organogenic cardiomyocyte growth in vitro. *Am J Physiol* 270: C1284-1292, 1996.

133. **Wang S, Desai D, Wright G, Niles RM, and Wright GL.** Effects of protein kinase C alpha overexpression on A7r5 smooth muscle cell proliferation and differentiation. *Exp Cell Res* 236: 117-126, 1997.

134. **Watson PA.** Function follows form: generation of intracellular signals by cell deformation. *Faseb J* 5: 2013-2019, 1991.

135. **Watts SW.** Activation of the mitogen-activated protein kinase pathway via the 5-HT2A receptor. *Ann N Y Acad Sci* 861: 162-168, 1998.

136. **Weindruch R and Masoro EJ.** Concerns about rodent models for aging research. *J Gerontol* 46: B87-88, 1991.

137. **Whisler R, Newhouse Y, and SE B.** Age-related reduction in the activation of mitogen-activated protein kinases p44mapk/ERK1 and p42mapk/ERK2 in human T cells stimulated via ligation of the T cell receptor complex. *Cell Immunol* 168: 201-210, 1996.

138. **Widmann C, Gibson S, Jarpe MB, and Johnson GL.** Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* 79: 143-180, 1999.

139. **Williamson D, Gallagher P, Harber M, Hollon C, and Trappe S.** Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. *J Physiol* 15;547(Pt 3): 977-987, 2003.

140. **Wright MO, Nishida K, Bavington C, Godolphin JL, Dunne E, Walmsley S, Jobanputra P, Nuki G, and Salter DM.** Hyperpolarisation of cultured human chondrocytes following cyclical pressure-induced strain: evidence of a role for alpha 5 beta 1 integrin as a chondrocyte mechanoreceptor. *J Orthop Res* 15: 742-747, 1997.

141. **Xu Q, Liu Y, Gorospe M, Udelsman R, and Holbrook N.** Acute hypertension activates mitogen-activated protein kinases in arterial wall. *J Clin Invest* 15;97(2): 508-514, 1996.

142. **Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Hiroi Y, Mizuno T, Maemura K, Kurihara H, Aikawa R, Takano H, and Yazaki Y.** Endothelin-1 is involved in mechanical stress-induced cardiomyocyte hypertrophy. *J Biol Chem* 271: 3221-3228, 1996.

143. **Yamazaki T, Komuro I, and Yazaki Y.** Molecular aspects of mechanical stress-induced cardiac hypertrophy. *Mol Cell Biochem* 163-164: 197-201, 1996.

144. **Yamboliev IA, Hedges JC, Mutnick JL, Adam LP, and Gerthoffer WT.** Evidence for modulation of smooth muscle force by the p38 MAP kinase/HSP27 pathway. *Am J Physiol Heart Circ Physiol* 278: H1899-1907, 2000.

145. **Yano Y, Geibel J, and Sumpio BE.** Cyclic strain induces reorganization of integrin alpha 5 beta 1 and alpha 2 beta 1 in human umbilical vein endothelial cells. *J Cell Biochem* 64: 505-513, 1997.

146. **Yau L and Zahradka P.** Immunodetection of activated mitogen-activated protein kinase in vascular tissues. *Mol Cell Biochem* 172(1-2): 59-66, 1997.

147. **Zou Y, Hu Y, Metzler B, and Xu Q.** Signal transduction in arteriosclerosis: mechanical stress-activated MAP kinases in vascular smooth muscle cells (review). *Int J Mol Med* 1: 827-834, 1998.

Appendix A

Subsection A (Pressure traces)

Preliminary Pressurization Experiments

The following is the preliminary pressurization experiments performed to test the validity of our project design.

Figure A -9

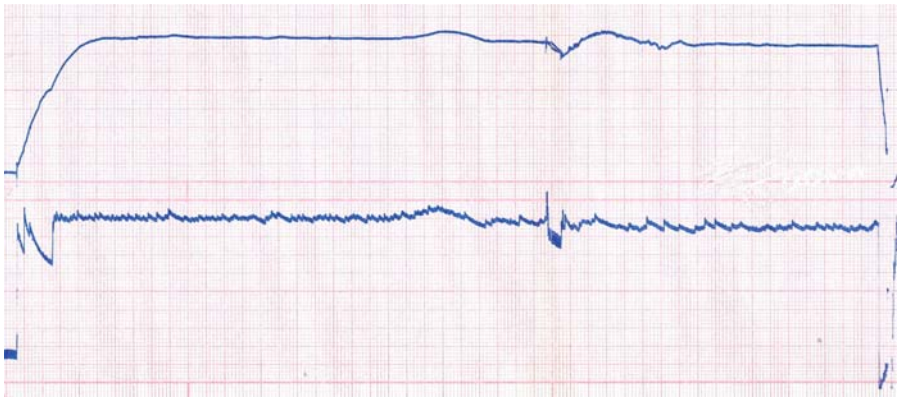


Figure
Preliminary experiment on 3-11-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 10

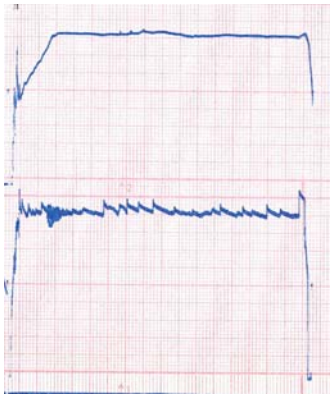


Figure
Preliminary experiment on 3-11-2004, this represents a 6-month-old F1 male rat aorta pressurized for 5 min at 200mm hg.

Figure A - 11

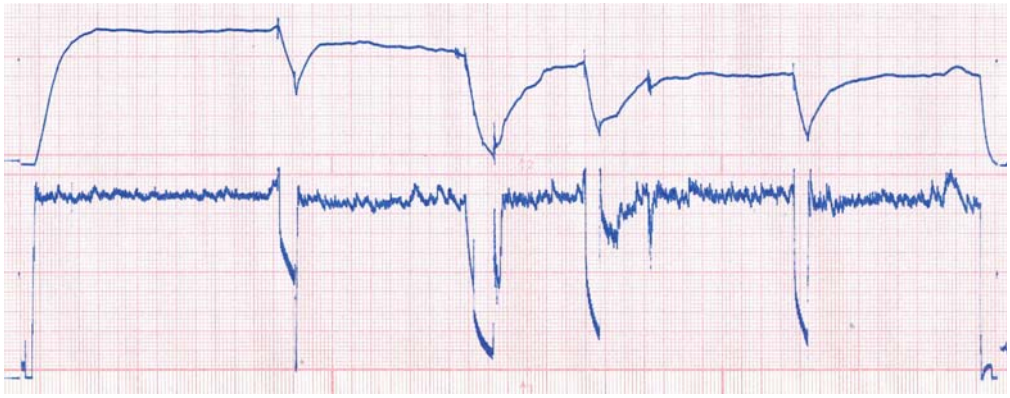


Figure
Preliminary experiment on 3-16-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 12

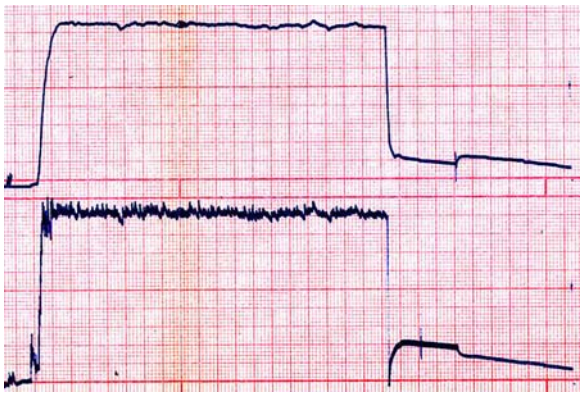


Figure
Preliminary experiment on 3-22-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

6 Month Pressure Experiments

The following represent the graphical output of the force-transducers for the designated experiment.

15 Minute Pressure Experiment

Figure A - 13

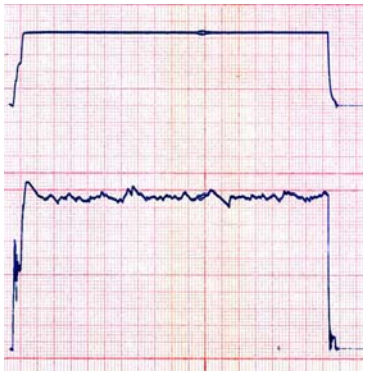


Figure
Experiment on 5-11-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 14

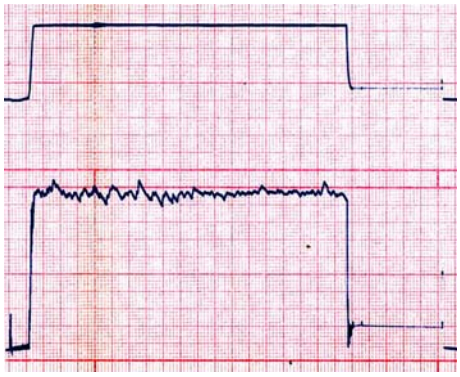


Figure
Experiment on 5-11-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 15

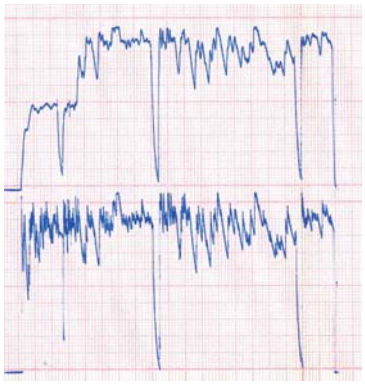


Figure
Experiment on 4-5-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 16

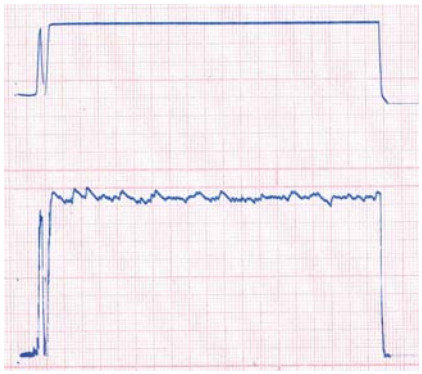


Figure
Experiment on 5-25-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 17

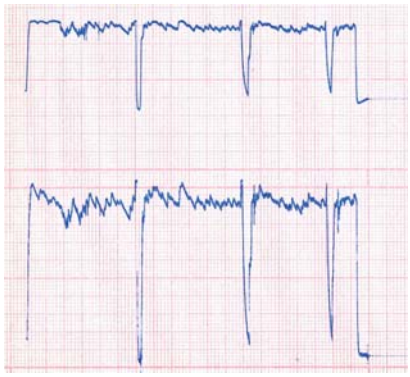


Figure
Experiment on 5-26-2004, this represents a 6-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

30 Month Pressure Experiments

15 Minute Pressure Experiment

Figure A - 18

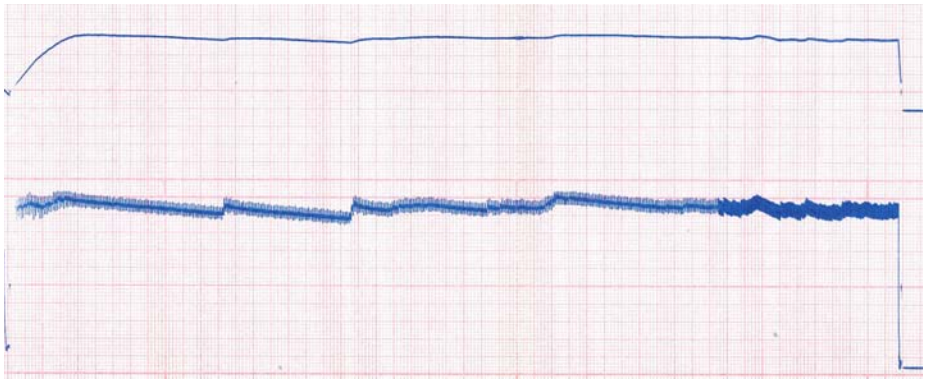


Figure
Experiment on 5-7-04, this represents a 30-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 19

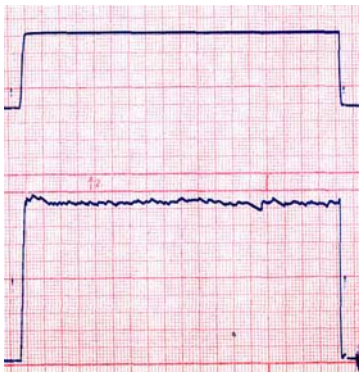


Figure
Experiment on 5-10-04, this represents a 30-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 20

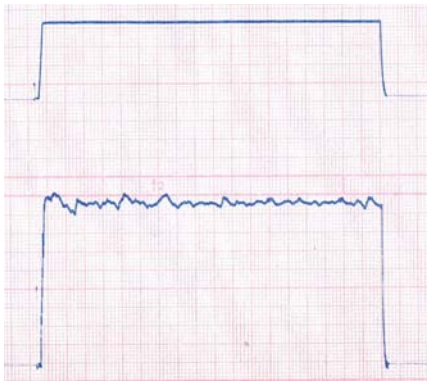


Figure
Experiment on 5-27-04, this represents a 30-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 21

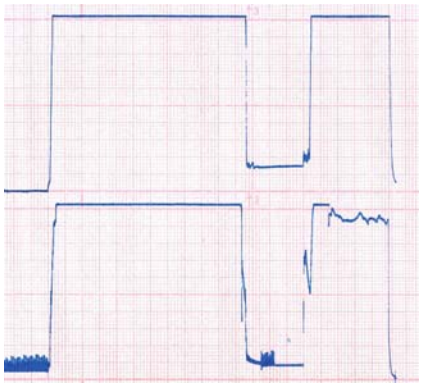


Figure
Experiment on 5-28-04, this represents a 30-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 22

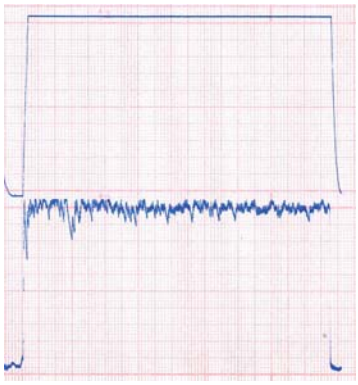
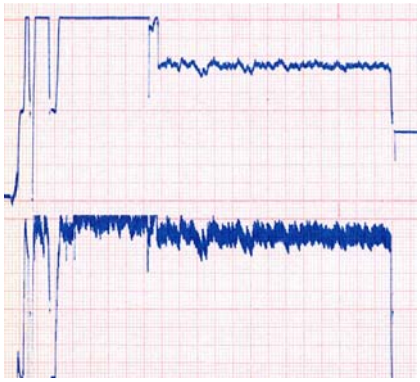


Figure
Experiment on 6-3-04, this represents a 30-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

36 Month Pressure Experiments

15 Minute Pressure Experiment

Figure A - 23



Figure

Experiment on 4-14-2004, this represents a 36-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 24

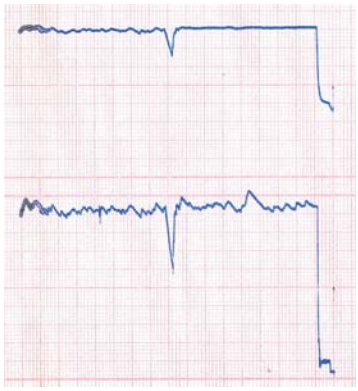


Figure
Experiment on 5-25-2004, this represents a 36-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 25

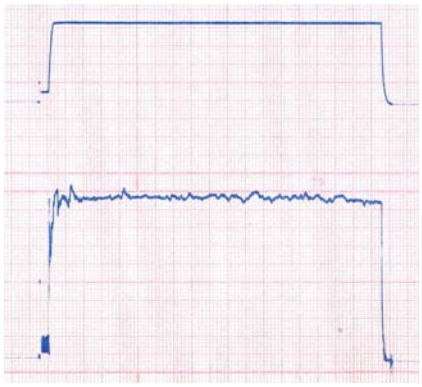


Figure
Experiment on 5-27-2004, this represents a 36-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Figure A - 26

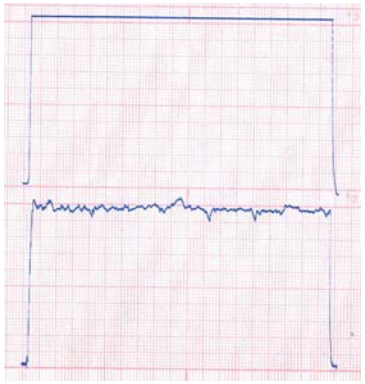


Figure
Experiment on 5-28-2004, this represents a 36-month-old F1 male rat aorta pressurized for 15 min at 200mm hg.

Appendix B

Film interpretation

Working guidelines:

1. Shoot for a film exposure that gives "scan worthy" bands when the exposure is > 1 min. The longer the exposure, the better, because this suggests that the slope of the enzyme reaction rate line is < 1.0.

2. Examine the bands- is any one of them "saturated"? If so, this film cannot be used without justification.

3. Examine the background- is it "excessive"? Does it compromise the interpretation? If so, this film cannot be used without justification.

4. Examine the band shape present in each and all lanes. "A band should be a band" and it should "look like a band" e.g. a line. Not a "blob", "smudge", "or something else.

5. Refer to "Figure worthy" data point criteria before proceeding further.

“Figure worthy” data point

Definition:

1. If using a pooled sample (minimum n=3 separate samples), each sample must be run on a minimum of 3 lanes (preferably ≥ 4). Take this mean value (IDV or area or some other assessment) and find the mean value.

2. If this mean value, when subjected to statistical analysis, does not “work” then data point must be re-done.

3. * A “lane” or “gel” cannot be displaced without adherence to sampling assumptions.

In an ideal situation, each and every antibody should have standard conditions for dilution, blocking, washes, exposures etc. It is recommended to start with the manufactures’ protocol as an initial starting point. Everyone’s input is encouraged and expected.

Subsection A (Film Reports)

The following are the film reports will immunoblot films, for the appropriate protein molecule.

JNK

Film Properties Report JNK (1)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: JNK (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 2°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 46, 54 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

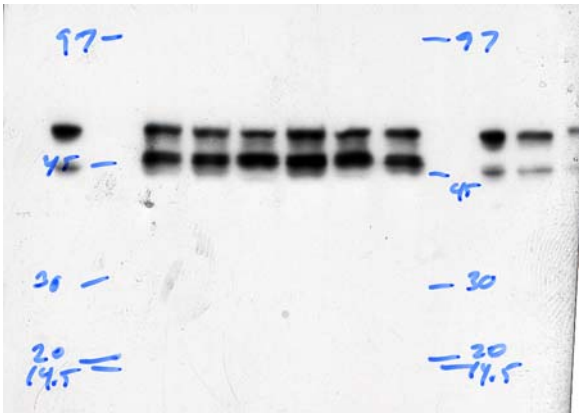
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report JNK (2)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: JNK (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 2°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 46, 54 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

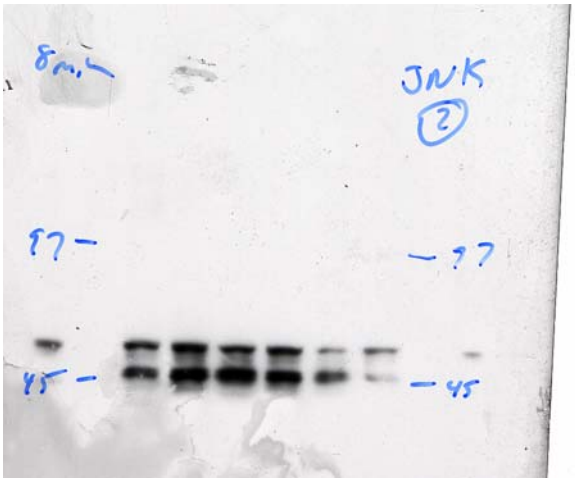
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report p-JNK (1)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-JNK (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 2°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 46, 54 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

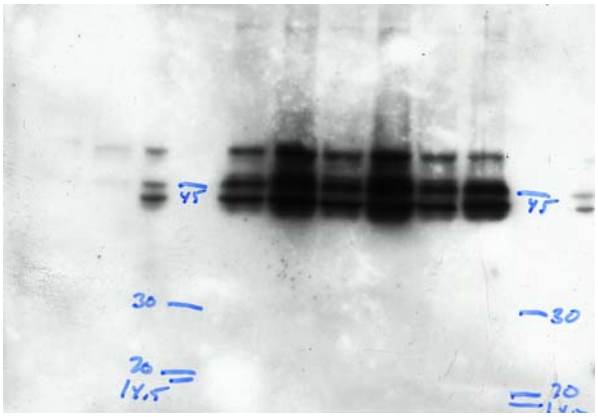
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report p-JNK (2)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-JNK (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 2°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 46, 54 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

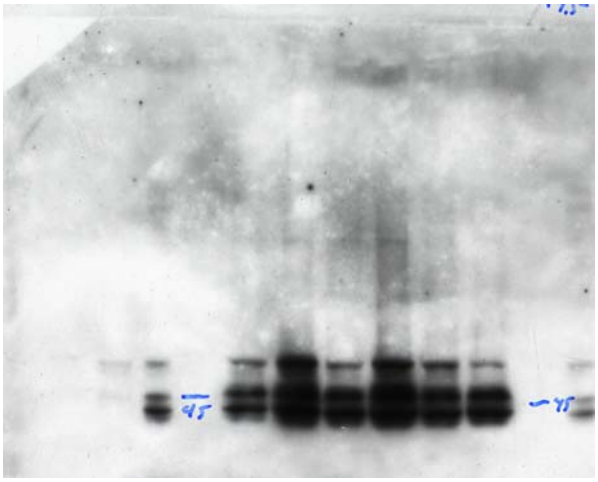
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



P38

Film Properties Report p38 (1)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p38 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 2°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 38 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

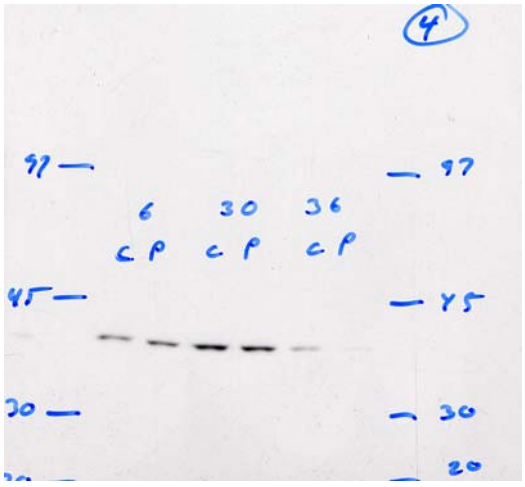
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report p38 (2)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p38 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 4°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 38 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

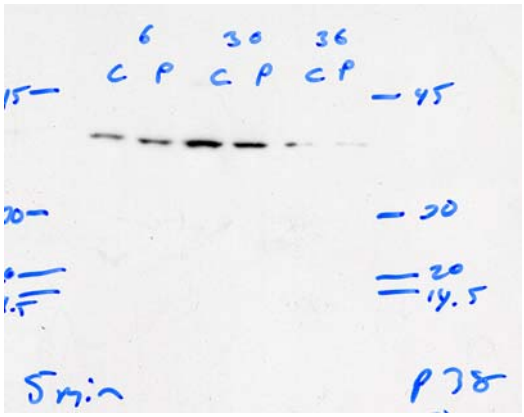
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report p-p38 (1)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-p38 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 2°C Medium: 10% BSA

Secondary Antibody: Anti Mouse Secondary Antibody Dilution: 1/1000

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

Exposure Time 1 min Molecular weight: 38 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

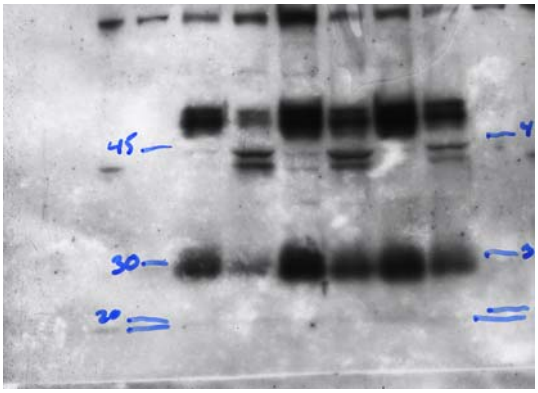
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report p-p38 (2)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-p38 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 4°C Medium: 10% BSA

Secondary Antibody: Anti Mouse Secondary Antibody Dilution: 1/1000

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

Exposure Time 1 min Molecular weight: 38 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

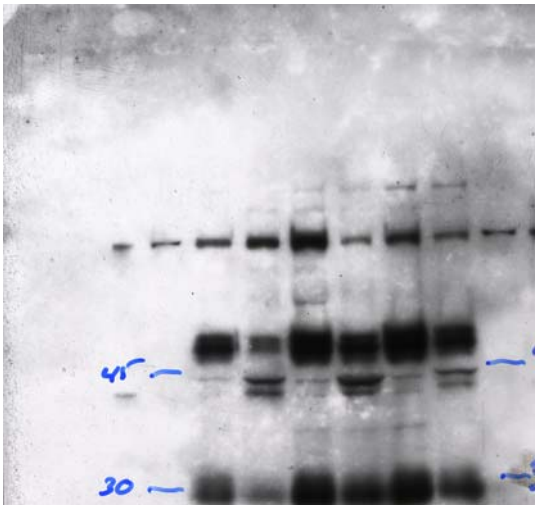
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



P44/42 MAPK (ERK 1/2)

Film Properties Report ERK1/2 (1)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 4°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 42, 44 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

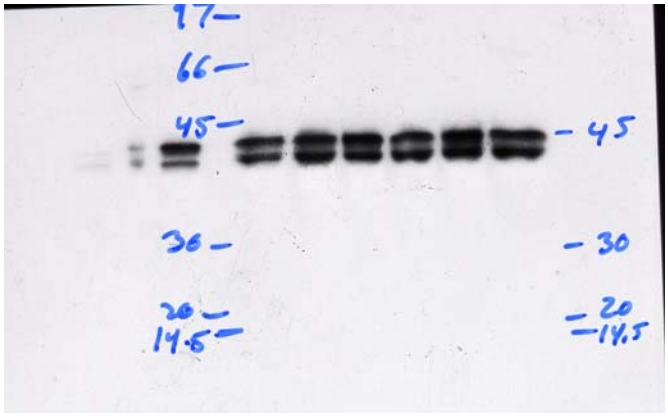
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report ERK1/2 (2)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 4°C Medium: 10% BSA

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

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

Exposure Time 1 min Molecular weight: 42, 44 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

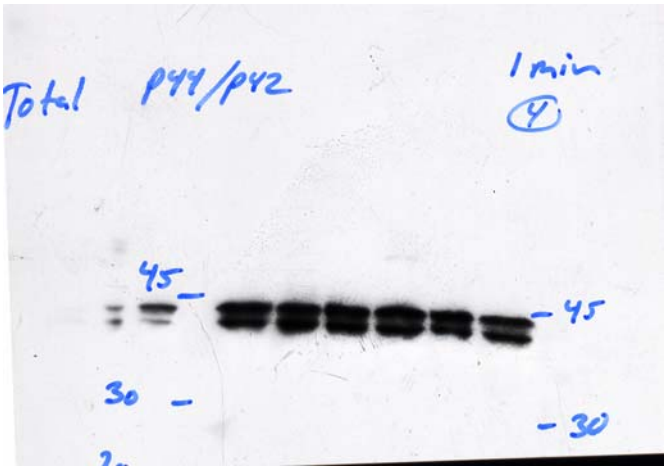
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report p-ERK1/2 (1)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 4°C Medium: 10% BSA

Secondary Antibody: Anti Mouse Secondary Antibody Dilution: 1/1000

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

Exposure Time 1 min Molecular weight: 42, 44 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

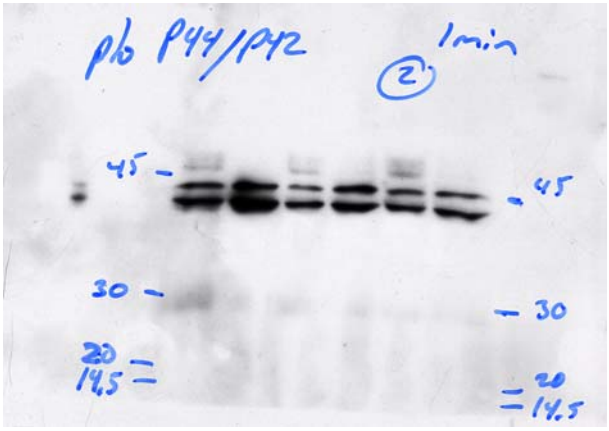
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Film Properties Report p-ERK1/2 (2)

Experimenter: Kevin M. Rice

Muscle / Tissue: Aorta Species: F1 rat F344 X BN

Protein concentration: 30 µg/ml Gel type: 10% Tris-HCL SDS-PAGE

Electrophoresis Voltage: 120V Transfer Voltage: 24V Duration: 45 min

Primary Antibody: p-ERK 1/2 (Cell Signaling) Primary Antibody Dilution: 1/500

Incubation Time: overnight @ 4°C Medium: 10% BSA

Secondary Antibody: Anti Mouse Secondary Antibody Dilution: 1/1000

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

Exposure Time 1 min Molecular weight: 42, 44 kDa

Lane 1: Hela Cell Extract 3 µl

Lane 2: NIH 3T3 Activated Cell Extract 3 µl

Lane 3: L6 IGF Cell Extract 3 µl

Lane 4: Rainbow Marker RPN756 3 µl

Lane 5: 6 month control 20 µl

Lane 6: 6 month 15 min pressure 20µl

Lane 7: 30 month control 20 µl

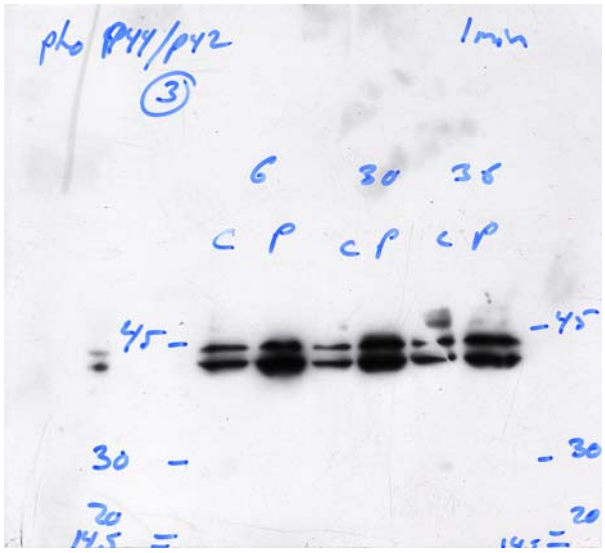
Lane 8: 30 month 15 min pressure 20 µl

Lane 9: 36 month control 20 µl

Lane 10: 36 month 15 min pressure 20 µl

Lane 11: Rainbow Marker RPN756 3 µl

Lane 12: Biotinylated Ladder 3 µl



Subsection B (Raw data)

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

JNK Data Set

Total JNK IDV values

	6 Control	6 Press	30 Control	30 Press	36 Control	36 Press
IDV	689472	756000	883008	731808	719712	743904
IDV	187488	338688	683424	725760	798336	562464
IDV	696348	756378	906453	726363	714357	750375
IDV	186093	342171	696348	738369	792396	552276
IDV	692208	752928	916872	728640	722568	765072
IDV	188232	346104	704352	734712	807576	552552
IDV	702032	750448	907800	726240	720188	756500
IDV	188232	346104	704352	734712	807576	552552
N	8	8	8	8	8	8
Mean	441263.1	548602.6	800326.13	730825.5	760338.63	654461.9
Standard Deviation	271296.6	219533.4	110926.23	4772.77817	44300.5	106584.9
Standard Error of the mean	102540.5	82975.81	41926.175	1803.94058	16744.015	40285.31

	6 Control	6 Pressure	30 Control	30 Pressure	36 Control	36 Pressure
Relative Expression Level	1	1.243255	1.8137163	1.65621249	1.7230958	1.483156
Standard error of the mean	0.232379	0.188042	0.095014	0.00408813	0.0379456	0.091295

Phosphorylated JNK IDV Values

	6 Control	6 Press	30 Control	30 Press	36 Control	36 Press
IDV	981558	1399629	1260272	1375393	999735	896732
IDV	805847	1314803	945204	1308744	1054266	830083
IDV	990000	1392000	1230000	1356000	990000	882000
IDV	798000	1302000	960000	1314000	1080000	882000
IDV	960640	1392928	1242828	1380920	936624	906604
IDV	786524	1308872	990660	1320880	1098732	834556
IDV	1003002	1387386	1207206	1369368	984984	888888
IDV	810810	1291290	1003002	1309308	1027026	834834
N	16	16	16	16	16	16
Mean	892047.6	1348614	1104897	1341827	1021421	869462.1
Strandard Deviation	99018.65	48008.02	141023.8	31576.23	54108.95	31129.19
Standard Error of the mean	25566.51	12395.62	36412.18	8152.948	13970.87	8037.523

	6 Control	6 Pressure	30 Control	30 Pressure	36 Control	36 Pressure
Relative Expression Level	1	1.511818	1.238607	1.50421	1.14503	0.974681
Standard error of the mean	0.02866	0.013896	0.040819	0.00914	0.015662	0.00901

P38 Data Set

Total p38 IDV values

	6 Control	6 Press	30 Control	30 Press	36 Control	36 Press
IDV	32256	30240	64512	52416	36288	36288
IDV	30375	30375	62775	46575	38475	36450
IDV	34510	32480	60900	50750	36540	34510
IDV	34680	32640	63240	48960	36720	36720
IDV	22176	32256	38304	58464	24192	28224
IDV	22528	32768	38912	57344	24576	26624
IDV	22880	33280	39520	58240	22880	27040
IDV	22165	32240	38285	56420	24180	26195
N	8	8	8	8	8	8
Mean	27696.25	32034.88	50806	53646.13	30481.38	31506.38
Standard Deviation	5783.498	1116.106	12925.73	4591.072	7022.009	4873.805
Standard Error of the mean	2185.957	421.8486	4885.466	1735.262	2654.07	1842.125

	6 Control	6 Pressure	30 Control	30 Pressure	36 Control	36 Pressure
Relative Expression Level	1	1.15665	1.8344	1.936945	1.10056	1.137568
Standard error of the mean	0.078926	0.015231	0.176394	0.062653	0.095828	0.066512

Phosphorylated p38 IDV Values

	6 Control	6 Press	30 Control	30 Press	36 Control	36 Press
IDV	652080	797544	403200	614880	463680	491568
IDV	660240	801360	396975	608025	462300	498960
IDV	650160	796320	399424	616832	475264	493920
IDV	655200	801360	401280	616968	461472	488880
IDV	624102	816914	472350	909525	477375	842284
IDV	614880	826560	483840	897120	473760	851760
IDV	619028	821988	481650	907530	476580	842284
IDV	624960	816914	466488	897864	471504	816480
N	8	8	8	8	8	8
Mean	637581.3	809870	438150.9	758593	470241.9	665767
Standard Deviation	18488.74	11982.19	40932.86	154470	6689.944	184628.8075
Standard Error of the mean	6988.086	4528.844	15471.17	58384.16	2528.561	69783.12995

	6 Control	6 Pressure	30 Control	30 Pressure	36 Control	36 Pressure
Relative Expression Level	1	1.270222	0.687208	1.189798	0.73754	1.044207307
Standard error of the mean	0.01096	0.007103	0.024265	0.091571	0.003966	0.109449784

P44/42 MAPK (ERK 1/2) Data Set

Total ERK 1/2 IDV values

	6 Control	6 Press	30 Control	30 Press	36 Control	36 Press
IDV	582552	690480	650160	645120	720720	778410
IDV	808542	821520	821520	887040	700560	778410
IDV	573249	632875	643001	627812	703757	750804
IDV	791388	774639	830332	891088	713883	776169
IDV	593541	683505	648064	653127	708820	776169
IDV	796461	830332	825269	896151	718946	776169
IDV	583395	709800	664170	648960	725010	781242
IDV	796461	821340	811200	882180	709800	776169
N	8	8	8	8	8	8
Mean	690698.6	745561.4	736714.5	766434.8	712687	774192.8
Standard Deviation	115164.8	75975.3	91606.08	131409.8	8511.24	9620.797
Standard Error of the mean	43528.21	28715.97	34623.84	49668.25	3216.946	3636.32

	6 Control	6 Pressure	30 Control	30 Pressure	36 Control	36 Pressure
Relative Expression Level	1	1.079431	1.066622	1.109651	1.031835	1.120884
Standard error of the mean	0.063021	0.041575	0.050129	0.07191	0.004658	0.005265

Phosphorylated ERK 1/2 IDV Values

	6 Control	6 Press	30 Control	30 Press	36 Control	36 Press
IDV	652080	797544	403200	614880	463680	491568
IDV	660240	801360	396975	608025	462300	498960
IDV	650160	796320	399424	616832	475264	493920
IDV	655200	801360	401280	616968	461472	488880
IDV	624102	816914	472350	909525	477375	842284
IDV	614880	826560	483840	897120	473760	851760
IDV	619028	821988	481650	907530	476580	842284
IDV	624960	816914	466488	897864	471504	816480
N	8	8	8	8	8	8
Mean	637581.3	809870	438150.9	758593	470241.9	665767
Standard Deviation	18488.74	11982.19	40932.86	154470	6689.944	184628.8075
Standard Error of the mean	6988.086	4528.844	15471.17	58384.16	2528.561	69783.12995

	6 Control	6 Pressure	30 Control	30 Pressure	36 Control	36 Pressure
Relative Expression Level	1	1.270222	0.687208	1.189798	0.73754	1.044207307
Standard error of the mean	0.01096	0.007103	0.024265	0.091571	0.003966	0.109449784

Subsection C (Statistics)

JNK

One Way Analysis of Variance

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
6 Control	8	0	438852.000	187860.000	694278.000
6 Press	8	0	548276.000	344137.500	754464.000
30 Control	8	0	793680.000	700350.000	907126.500
30 Press	8	0	730224.000	726301.500	734712.000
36 Control	8	0	757482.000	719950.000	802956.000
36 Press	8	0	653184.000	552552.000	753437.500

H = 15.432 with 5 degrees of freedom. (P = 0.009)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.009)

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

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

Comparison	**Diff of Ranks**	**q**	**P<0.05**
36 Control vs 6 Control	186.500	4.710	Yes
36 Control vs 6 Press	88.000	2.661	No
36 Control vs 36 Press	72.000	2.714	Do Not Test
36 Control vs 30 Press	32.000	1.600	Do Not Test
36 Control vs 30 Control	5.500	0.408	Do Not Test
30 Control vs 6 Control	181.000	5.474	Yes
30 Control vs 6 Press	82.500	3.109	Do Not Test
30 Control vs 36 Press	66.500	3.325	Do Not Test
30 Control vs 30 Press	26.500	1.968	Do Not Test
30 Press vs 6 Control	154.500	5.823	Yes
30 Press vs 6 Press	56.000	2.800	Do Not Test
30 Press vs 36 Press	40.000	2.970	Do Not Test
36 Press vs 6 Control	114.500	5.725	Yes
36 Press vs 6 Press	16.000	1.188	Do Not Test
6 Press vs 6 Control	98.500	7.315	Yes

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	438852.000	187860.000	694278.000
30 Control	8	0	793680.000	700350.000	907126.500

T = 41.500 n(small)= 8 n(big)= 8 P(est.)= 0.006 P(exact)= 0.003

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.003)

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	438852.000	187860.000	694278.000
36 Control	8	0	757482.000	719950.000	802956.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	793680.000	700350.000	907126.500
36 Control	8	0	757482.000	719950.000	802956.000

T = 68.000 n(small)= 8 n(big)= 8 P(est.)= 0.958 P(exact)= 1.000

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 1.000)

P-JNK

One Way Analysis of Variance p-JNK

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
6 Control	8	0	885725.000	801923.500	985779.000
6 Press	8	0	1351094.500	1305436.000	1392464.000
30 Control	8	0	1105104.000	975330.000	1236414.000
30 Press	8	0	1338440.000	1311654.000	1372380.500
36 Control	8	0	1013380.500	987492.000	1067133.000
36 Press	8	0	882000.000	834695.000	892810.000

H = 39.286 with 5 degrees of freedom. (P = <0.001)

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

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

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

Comparison	**Diff of Ranks**	**q**	**P<0.05**
6 Press vs 36 Press	260.000	6.566	Yes
6 Press vs 6 Control	242.000	7.319	Yes
6 Press vs 36 Control	149.500	5.634	Yes
6 Press vs 30 Control	132.500	6.625	Yes
6 Press vs 30 Press	8.000	0.594	No
30 Press vs 36 Press	252.000	7.621	Yes
30 Press vs 6 Control	234.000	8.819	Yes
30 Press vs 36 Control	141.500	7.075	Yes
30 Press vs 30 Control	124.500	9.246	Yes
30 Control vs 36 Press	127.500	4.805	Yes
30 Control vs 6 Control	109.500	5.475	Yes
30 Control vs 36 Control	17.000	1.262	No
36 Control vs 36 Press	110.500	5.525	Yes
36 Control vs 6 Control	92.500	6.869	Yes
6 Control vs 36 Press	18.000	1.337	No

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	885725.000	801923.500	985779.000
6 Press	8	0	1351094.500	1305436.000	1392464.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	1105104.000	975330.000	1236414.000
30 Press	8	0	1338440.000	1311654.000	1372380.500

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.151)

Group Name	N	Missing	Mean	Std Dev	SEM
36 Control	8	0	1021420.875	54108.948	19130.402
36 Press	8	0	869462.125	31129.192	11005.831

Difference 151958.750

t = 6.885 with 14 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 104622.544 to 199294.956

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

Power of performed test with alpha = 0.050: 1.000

t-test

Normality Test: Failed (P = 0.010)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	885725.000	801923.500	985779.000
30 Control	8	0	1105104.000	975330.000	1236414.000

T = 45.500 n(small)= 8 n(big)= 8 P(est.)= 0.021 P(exact)= 0.015

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.015)

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	885725.000	801923.500	985779.000
36 Control	8	0	1013380.500	987492.000	1067133.000

T = 44.500 n(small)= 8 n(big)= 8 P(est.)= 0.016 P(exact)= 0.010

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.010)

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	1105104.000	975330.000	1236414.000
36 Control	8	0	1013380.500	987492.000	1067133.000

T = 77.000 n(small)= 8 n(big)= 8 P(est.)= 0.372 P(exact)= 0.382

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.382)

P38

One Way Analysis of Variance

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
6 Control	8	0	26627.500	22352.000	33383.000
6 Press	8	0	32368.000	31307.500	32704.000
30 Control	8	0	50210.000	38608.000	63007.500
30 Press	8	0	54418.000	49855.000	57792.000
36 Control	8	0	30432.000	24186.000	36630.000
36 Press	8	0	31367.000	26832.000	36369.000

H = 32.354 with 5 degrees of freedom. (P = <0.001)

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

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

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

Comparison	**Diff of Ranks**	**q**	**P<0.05**
30 Press vs 6 Control	233.000	5.884	Yes
30 Press vs 6 Press	181.000	5.474	Yes
30 Press vs 36 Control	178.500	6.727	Yes
30 Press vs 36 Press	173.500	8.675	Yes
30 Press vs 30 Control	2.000	0.149	No
30 Control vs 6 Control	231.000	6.986	Yes
30 Control vs 6 Press	179.000	6.746	Yes
30 Control vs 36 Control	176.500	8.825	Yes
30 Control vs 36 Press	171.500	12.736	Yes
36 Press vs 6 Control	59.500	2.242	No
36 Press vs 6 Press	7.500	0.375	Do Not Test
36 Press vs 36 Control	5.000	0.371	Do Not Test
36 Control vs 6 Control	54.500	2.725	Do Not Test
36 Control vs 6 Press	2.500	0.186	Do Not Test
6 Press vs 6 Control	52.000	3.862	Do Not Test

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	26627.500	22352.000	33383.000
30 Control	8	0	50210.000	38608.000	63007.500

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Failed (P = 0.002)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	26627.500	22352.000	33383.000
36 Control	8	0	30432.000	24186.000	36630.000

T = 52.500 n(small)= 8 n(big)= 8 P(est.)= 0.115 P(exact)= 0.105

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.105)

t-test

Normality Test: Failed (P = 0.047)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	50210.000	38608.000	63007.500
36 Control	8	0	30432.000	24186.000	36630.000

T = 98.000 n(small)= 8 n(big)= 8 P(est.)= 0.002 P(exact)= <0.001

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

p-p38

One Way Analysis of Variance

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
6 Control	8	0	51650.000	47540.000	54060.000
6 Press	8	0	216452.000	207268.000	218196.000
30 Control	8	0	115674.000	114240.000	119806.000
30 Press	8	0	232044.000	212160.000	251030.000
36 Control	8	0	103020.000	82560.000	117416.000
36 Press	8	0	143960.000	138216.000	157884.000

H = 43.560 with 5 degrees of freedom. (P = <0.001)

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

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

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

Comparison	**Diff of Ranks**	**q**	**P<0.05**
30 Press vs 6 Control	301.000	7.601	Yes
30 Press vs 36 Control	218.500	6.608	Yes
30 Press vs 30 Control	191.500	7.217	Yes
30 Press vs 36 Press	109.000	5.450	Yes
30 Press vs 6 Press	26.000	1.931	No
6 Press vs 6 Control	275.000	8.317	Yes
6 Press vs 36 Control	192.500	7.255	Yes
6 Press vs 30 Control	165.500	8.275	Yes
6 Press vs 36 Press	83.000	6.164	Yes
36 Press vs 6 Control	192.000	7.236	Yes
36 Press vs 36 Control	109.500	5.475	Yes
36 Press vs 30 Control	82.500	6.127	Yes
30 Control vs 6 Control	109.500	5.475	Yes
30 Control vs 36 Control	27.000	2.005	No
36 Control vs 6 Control	82.500	6.127	Yes

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.189)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
6 Control	8	0	51062.500	4380.299	1548.669
6 Press	8	0	214539.000	12168.622	4302.258

Difference -163476.500

t = -35.752 with 14 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -173283.547 to -153669.453

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

Power of performed test with alpha = 0.050: 1.000

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	115674.000	114240.000	119806.000
30 Press	8	0	232044.000	212160.000	251030.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = 0.027)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
36 Control	8	0	103020.000	82560.000	117416.000
36 Press	8	0	143960.000	138216.000	157884.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.732)

Group Name	N	Missing	Mean	Std Dev	SEM
6 Control	8	0	51062.500	4380.299	1548.669
30 Control	8	0	116755.000	3562.867	1259.664

Difference -65692.500

t = -32.907 with 14 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -69974.092 to -61410.908

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

Power of performed test with alpha = 0.050: 1.000

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	51650.000	47540.000	54060.000
36 Control	8	0	103020.000	82560.000	117416.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	115674.000	114240.000	119806.000
36 Control	8	0	103020.000	82560.000	117416.000

T = 81.500 n(small)= 8 n(big)= 8 P(est.)= 0.172 P(exact)= 0.161

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.161)

P44/42 MAPK (ERK 1/2)

One Way Analysis of Variance

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
6 Control	8	0	692464.500	582973.500	796461.000
6 Press	8	0	742219.500	686992.500	821430.000
30 Control	8	0	737685.000	649112.000	823394.500
30 Press	8	0	767653.500	647040.000	889064.000
36 Control	8	0	711841.500	706288.500	719833.000
36 Press	8	0	776169.000	776169.000	778410.000

H = 3.487 with 5 degrees of freedom. (P = 0.625)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.625)

t-test

Normality Test: Failed (P = 0.004)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	692464.500	582973.500	796461.000
30 Control	8	0	737685.000	649112.000	823394.500

T = 52.000 n(small)= 8 n(big)= 8 P(est.)= 0.104 P(exact)= 0.105

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.105)

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	692464.500	582973.500	796461.000
36 Control	8	0	711841.500	706288.500	719833.000

T = 68.000 n(small)= 8 n(big)= 8 P(est.)= 0.958 P(exact)= 1.000

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 1.000)

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	737685.000	649112.000	823394.500
36 Control	8	0	711841.500	706288.500	719833.000

T = 68.000 n(small)= 8 n(big)= 8 P(est.)= 0.958 P(exact)= 1.000

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 1.000)

p-P44/42 MAPK (ERK 1/2)

One Way Analysis of Variance

Normality Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
6 Control	8	0	637560.000	621565.000	653640.000
6 Press	8	0	809137.000	799452.000	819451.000
30 Control	8	0	434844.000	400352.000	477000.000
30 Press	8	0	757044.000	615856.000	902697.000
36 Control	8	0	472632.000	462990.000	475922.000
36 Press	8	0	657720.000	492744.000	842284.000

H = 33.517 with 5 degrees of freedom. (P = <0.001)

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

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

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

Comparison	**Diff of Ranks**	**q**	**P<0.05**
6 Press vs 30 Control	237.000	5.985	Yes
6 Press vs 36 Control	219.000	6.623	Yes
6 Press vs 6 Control	70.500	2.657	No
6 Press vs 36 Press	56.000	2.800	Do Not Test
6 Press vs 30 Press	17.500	1.300	Do Not Test
30 Press vs 30 Control	219.500	6.638	Yes
30 Press vs 36 Control	201.500	7.594	Yes
30 Press vs 6 Control	53.000	2.650	Do Not Test
30 Press vs 36 Press	38.500	2.859	Do Not Test
36 Press vs 30 Control	181.000	6.822	Yes
36 Press vs 36 Control	163.000	8.150	Yes
36 Press vs 6 Control	14.500	1.077	Do Not Test
6 Control vs 30 Control	166.500	8.325	Yes
6 Control vs 36 Control	148.500	11.028	Yes
36 Control vs 30 Control	18.000	1.337	No

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = 0.007)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	637560.000	621565.000	653640.000
6 Press	8	0	809137.000	799452.000	819451.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	434844.000	400352.000	477000.000
30 Press	8	0	757044.000	615856.000	902697.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Failed (P = 0.025)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
36 Control	8	0	472632.000	462990.000	475922.000
36 Press	8	0	657720.000	492744.000	842284.000

T = 36.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	637560.000	621565.000	653640.000
30 Control	8	0	434844.000	400352.000	477000.000

T = 100.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
6 Control	8	0	637560.000	621565.000	653640.000
36 Control	8	0	472632.000	462990.000	475922.000

T = 100.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

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

t-test

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
30 Control	8	0	434844.000	400352.000	477000.000
36 Control	8	0	472632.000	462990.000	475922.000

T = 59.000 n(small)= 8 n(big)= 8 P(est.)= 0.372 P(exact)= 0.382

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.382)

Table of Measures

Systeme Internationale (SI) Unit Chart - International System of weights and measures.

Yotto	[Y]	1,000,000,000,000,000,000,000,000	= $1x10^{24}$
Zeta	[Z]	1,000,000,000,000,000,000,000	= $1x10^{21}$
Exa	[E]	1,000,000,000,000,000,000	= $1x10^{18}$
Peta	[P]	1,000,000,000,000,000	= $1x10^{15}$
Tera	[T]	1,000,000,000,000	= $1x10^{12}$
Giga	[G]	1,000,000,000	= $1x10^{9}$
Mega	[G]	1,000,000	= $1x10^{6}$
Kilo	[k]	1,000	= $1x10^{3}$
Hecto	[h]	100	= $1x10^{2}$
Deca	[da]	10	= $1x10^{1}$
basic unit		1	= 1
Deci	[d]	0.1	= $1x10^{-1}$
Centi	[c]	0.01	= $1x10^{-2}$
Milli	[m]	0.001	= $1x10^{-3}$
Micro	[µ]	0.000 001	= $1x10^{-6}$
Nano	[n]	0.000 000 001	= $1x10^{-9}$
Pico	[p]	0.000 000 000 001	= $1x10^{-12}$
Femto	[f]	0.000 000 000 000 001	= $1x10^{-15}$
Atto	[a]	0.000 000 000 000 000 001	= $1x10^{-18}$
Zepto	[z]	0.000 000 000 000 000 001	= $1x10^{-21}$
Yocto	[y]	0.000 000 000 000 000 001	= $1x10^{-24}$

Basic SI units

Category	*Name*	*Abbreviation*
Length	meter	m
Mass	gram	g
Time	second	s
Electric current	ampere	A
Temperature	kelvin	K
Temperature	Celsius	C
Amount of substance	mole	mol
Luminous intensity	candela	cd
Electrical capacitance	farad	F
Frequency	hertz	Hz
Energy (work)	joule	J
Force	newton	N
Electrical conductor resistance	ohm	Ω
Pressure generated by force	pascal	Pa
Electric potential	volt	V
Power or rate of work	watt	W
Speed		
Volume	liter	L

Metric System of Measurements

Length

10 millimeters (mm) = 1 centimeter (cm)

10 centimeters (cm) = 1 decimeter (dm)

10 decimeters (dm) = 1 meter (m)

10 meters (m) = 1 decameter (dam)

10 decameters(dam)= 1 hectometer (hm)

10 hectometers(hm) = 1 kilometer (km)

1,000 meters (m) = 1 kilometer (km)

Volume

1,000 cubic mm = 1 cubic cm

1,000 cubic cm = 1 cubic dm

1,000 cubic dm = 1 cubic meter

1 million cm^3 = 1 cubic meter

Capacity

10 milliliters (mL) = 1 centiliter (cL)

10 centiliter (cL) = 1 deciliter (dL)

10 deciliters (dL) = 1 liter (L)

1,000 liters (L) = 1 m^3

Area

100 square mm = 1 square cm

10,000 cm^2 = 1 m^2

100 m^2 = 1 are

100 ares = 1 hectare

10,000 m^2 = 1 hectare

100 hectares = 1 km^2

1,000,000 m^2 = 1 sq.

Mass

1,000 grams (g) = I kilogram (kg)

1,000 kilograms (kg) = 1 tonne

Time

1 minute (min) = 60 seconds (s)

1 hour (h) = 60 minutes (min)

1 day (d) = 24 hours (h)

1 week (wk) = 7 days (d)

1 year (a) = 12 months (mo)

The UK (imperial) System of Measurements

Length

12 inches = 1 foot

3 feet = 1 yard

22 yards = 1 chain

10 chains = 1 furlong

8 furlongs = 1 mile

5,280 feet = 1 mile

1,760 yards = 1 mile

Volume

1,728 cubic inches = 1 cubic foot

27 cubic feet = 1 cubic yard

Mass

437.5 grains = 1 ounce

16 ounces = 1 pound (7,000 grains)

14 pounds = 1 stone

8 stones = 1 hundredweight [cwt]

20 cwt = 1 ton (2,240 pounds)

Area

144 square inches = 1 square foot

9 square feet = 1 square yard

4,840 square yards = 1 acre

640 acres = 1 square mile

Capacity

20 fluid ounces = 1 pint

4 gills = 1 pint

2 pints = 1 quart

4 quarts = 1 gallon (8 pints)

Troy Weights

24 grains = 1 pennyweight

20 pennyweights = 1 ounce (480 grains)

12 ounces = 1 pound (5,760 grains)

Apothecaries' Measures

20 minims = 1 fluid scruple

3 fluid scruples = 1 fluid drachm

8 fluid drachms = 1 fluid ounce

20 fluid ounces = 1 pint

Apothecaries' Weights

20 grains = 1 scruple

3 scruples = 1 drachm

8 drachms = 1 ounce (480 grains)

12 ounces = 1 pound

Conversions

1 yard = 0.9144 meters –same as U.S.

1 pound = 0.453 593 37 kg-same as U.S.

1 gallon = 4.536 09 liters-different U.S

The US System of Measurements

Length

12 inches	= 1 foot
3 feet	= 1 yard
220 yards	= 1 furlong
8 furlongs	= 1 mile
5,280 feet	= 1 mile
1,760 yards	= 1 mile

Area

144 square inches	= 1 square foot
9 square feet	= 1 square yard
4,840 square yards	= 1 acre
640 acres	= 1 square mile
1 square mile	= 1 section
36 section	= township

Volume

1,728 cubic inches	= 1 cubic foot
27 cubic feet	= 1 cubic yard

Mass

437.5 grains	= 1 ounce
16 ounces	= 1 pound (7,000 grains)
14 pounds	= 1 stone
100 pounds	= 1 hundredweight [cwt]
20 cwt	= 1 ton (2,000 pounds)

Capacity (Liquid)

16 fluid ounces	= 1 pint
4 gills	= 1 pint
2 pints	= 1 quart
4 quarts	= 1 gallon (8 pints)

Troy Weights

24 grains	= 1 pennyweight
20 pennyweights	= 1 ounce (480 grains)
12 ounces	= 1 pound (5,760 grains)

Apothecaries' Measures

60 minims	= 1 fluid dram
8 fluid drams	= 1 fluid ounce
16 fluid ounces	= 1 pint

Apothecaries' Weights

20 grains	= 1 scruple
3 scruples	= 1 dram
8 drams	= 1 ounce (480 grains)
12 ounces	= 1 pound

Capacity (Dry)

2 pints	= 1 quart
8 quarts	= 1 peck
4 pecks	= 1 bushel

Conversions

1 yard = 0.9144 meters –same as UK

1 pound = 0.453 593 37 kg-same as UK

1gallon=3.785411784litersdifferentUK

1 bushel = 35.239 070 166 88 liters

Conversions

Length

1 m	= 100 cm
1 m	= 1.0936 yards (yd)
1 cm	= 0.3937 inches (in)
1 inch (in)	= 2.54 cm exactly
1 inch (in)	= 0.0254 km
1 angstrom (Å)	= 10^{-8} cm
1 mile	= 1.6093 km

Time

1 day (d)	= 86,400 s
1 hour (hr)	= 3,600 s
1 minute (min)	= 60 s
1 day (d)	= 1,440 min
1 year	= 525,600 min
1 year	= 31,536,000 s

Mass

1 kg	= 1,000 g
1 kg	= 2.205 pounds (lb)
1 lb	= 453.6 g

1atomic mass unit(amu)=1.66054×10^{-24}g

Temperature

0 K	= -273.15 ° Celsius (C)
0 K	= -459.67° Fahrenheit (F)
° F	= (9/5) ° C + 23 °
° C	= (5/9) (° F - 23 °)
K	= ° C + 273.15
° C	= K – 273.15
° F	= K – 459.67

Volume (derived)

1 L = 10-3 m^3

1 L = 1.057 quarts (qt)

1 $in.^3$ = 16.4 cm^3

1 cm^3 = 1 mL

Force (derived)

Newton (N = m-kg/s^2)

1 dyne (dyn) = 10^{-5} N

Pressure (derived)

Pascal (Pa =N/m^2)

1 atmosphere (atm) = 101,325 Pa

1 atm = 760 mm Hg

1 atm = 14.70 b/$in.^2$

1 atm = 1.013x10 6 dyn/cm^2

Energy (derived)

Joule (J=N-m)

1 calorie (cal) = 4.184 J

1 electron volt (eV) = 96.485 kJ/mol

1 liter-atmosphere (L-atm) = 101.325 J

1 J = 10^7 ergs

Index

Shift alt x to mark word.

Curriculum Vitae

Kevin Matthew Rice

Office: Science Building 311, College of Science
Marshall University - COS, 1 John Marshall Drive, Huntington, WV 25702
304.696.3267 rice9@marshall.edu
Home: 3326 County Rd. 73, Crown City, Oh 45623
(740)256-1874
Cell (304)638-2982

ACADEMIC PREPARATION:

M.S. in Biology,

College of Science, Marshall University of West Virginia, Huntington, 2005

Concentrations: Molecular Biology, Physiology

Dissertation: ***EFFECTS OF AGING ON PRESSURE-INDUCED MAPK ACTIVATION IN THE RAT AORTA***

Advisor: Dr. Eric R. Blough

Committee members: Dr Eric Blough, Dr. David Mallory, Dr. Nicoli Locassio

In –Service Training Certificate,

Common Pleas Court – Constable Division, Ohio,

FBI Domestic and International Terrorism, and Weapons of Mass Destruction Seminar at Ohio University Southern Campus, Ironton, 2002

Basic Spotter Training Certification,

The National Weather Service (SKYWARN) 2002, 2 hours training

B.A. in Psychology, Minor Biological Science,

College of Arts and Sciences, Ohio University Southern Campus, Ironton, 1998

Concentrations: Educational Psychology, Developmental Psychology, Clinical and Experimental Psychology

Confined Space Rescuer Training and Certification OSHA 29 CFR 1910.146,

Hazardous Tek-knowledgies In. Heiner's Bakery 1995 16 hours training

Certified Bakery Maintenance Engineering,

American Institute of Baking, Manhattan, Kansas, 1996 21.5 Continuing Education Units

Received training in:

- Overall Baking industry
- Mathematics
  - Basic Computations
  - Weights, measures, and formulas
- Applied mechanics
  - Forces and energy
  - Solids, liquids, and gases
  - Heat and transfer
- Basic electricity
- Electrical energy and power
- R C L Circuits
- Electric motors and generators
- Motor control
- Basic electronics
- Refrigeration
- Refrigerants, accessories, and piping
- Charging and troubleshooting a refrigeration system
- Principles of hydraulics
- Hydraulic components
- Hydraulic components, operation and maintenance
- Bearings and lubrication
- Oxyacetylene welding and brazing
- Arc welding, mig and tig gelding
- Boilers
- Bread processing equipment
- Material handling equipment
- Bakery ovens
- Baking facility – machines and systems
- Preventive maintenance

Certified Heating and Air-conditioning Mechanic,

Lawrence County Vocational School, Chesapeake, OH, 1991

Proficient in the following areas:

- Design and layout of electrical circuits
- Installing electrical components of the electrical trade
- Repair and maintain electrical equipment
- Apply all troubleshooting procedures
- Use amprobe to check proper amperage
- Use voltmeter to take proper colt readings
- Use ohm meter to take proper resistance reagins
- Identify an open electrical circuit
- Identify a shorted electrical circuit
- Read and understand basic wiring schematics
- Correctly match wire sizes to their related amperage
- Use good judgment in the repair of electrical equipment
- Solder joints used in the residential plumbing
- Use stick of sil-flos for commercial work
- Solder joint using 50/50, 95/5 staybrite or stick
- Make flares or swedges used in working copper tubing
- Cut and tread different types of pipe used in the field

Layout and wire the following equipment:

- Up-flow, down-flow and horizontal gas furnaces
- Oil furnace
- Electric furnaces
- Split heat pump units
- Electric water heaters
- Window air conditioning
- Freezers
- Refrigerators
- Package heat pumps
- Central air conditioning
- Sheet metal and duct work designs
- Commercial refrigeration systems
    - Walk-in coolers
    - Walk-in Freezers
    - Etc.
- Layout design air, gas, water, septic piping

Course work in progress

Institute of Children's Literature, West Redding, CT, at present

Course work in progress

International Bible College & Seminary**,** Independence, Missouri, at present

Transferred course work to IBC&S,

Kent Christian College, Dover, Delaware Attended in 1992.

Ohio University Southern Campus

Ironton, Ohio Study Skills Workshop 1998

RESEARCH SKILLS:

Proficient in:

- Computer based statistics programs
- Computer based data analysis software
    - Microsoft Office package software
    - Etc.
- Figure generation and data presentation
- Spot densitometry
- Immunoblotting
- Immunohistochemistry
- Tissue sectioning
- Cryostat operation and maintenance
- Wet and dry mount slide preparation
- Multiple tissue staining procedures
- Gel electrophoresis
- Cell culture
    - Passage cell
    - Cell counting
    - Sterile conditions
    - Cell collection and storage
    - Preparing media and cell culture materials
- Tissue preparation
- Protein analysis
- Cell and tissue lysis procedures
- Designed and constructed vessel perfusion system
- Small animal thoracic surgery
- Skeletal muscle isolation
- Development and evaluation research models and techniques
- Microbiology media preparation

LANGUAGES:

- Can read and speak some Spanish
- Basic computer language
- Cobol computer language
- Pascal computer language
- Visual Basic
- Assembly computer language

PROFESSIONAL EXPERIENCE:

Research Assistant, spring 2005
Dr. Eric Blough, Marshall University

Teaching Assistant, fall 2004
Faculty in Biology, Marshall University
Courses:
Microbiology

Volunteer Research Assistant, fall 2004
Faculty in Biology, Marshall University
- Assisted Dr. Eric R. Blough on Cardiac Research Grant
- Created mechanical device for mounting and pressurizing rat aortic section.

Summer Research Assistant, Summer 2004
Faculty in Biology, Marshall University
- Assisted Dr. Eric R. Blough on Cardiac Research Grant
- Collected and analyzed data, dealing with MAPK and p70S6k activation in rat aortic vascular smooth muscle.

Volunteer Research Assistant, spring 2004
Faculty in Biology, Marshall University
- Assisted Dr. Eric R. Blough on Cardiac Research Grant
- Maintained cell culture line of A7R5 vascular smooth muscle cells.
- Collected and analyzed data, dealing with Fluprostenol activation of MAPK and p70S6k activation in A7R5 aortic vascular smooth muscle.

Instructor, winter 2004
Division of Continuing Education, Marshall Community & Technical College
Courses:
The Art of Crochet, and Intermediate Crocheting

Skilled Construction Worker 1998 - 2002

Riedel Wilks Building Structures, Huntington, WV

- Commercial construction
- General plumbing
- Electrical
- Steel building erection
- Metal framework,
- Conveyor systems design and installation for industry,
- Mechanical equipment manufacturing
- Welding
- Conventional construction
- Concrete work
- Sight excavation
- Building layout and design
- Heavy equipment operation Backhoe, bulldozer, bobcat, front-end loader, fork truck,

Merchandising Assistant 1997 - 1999

J. C. Penny's, Barbersville, WV

- Department organization
- Floor set up and display
- Stock organization
- Customer service

Peer Adviser, 1997

Director of Admissions, Admission Department
Ohio University, Ironton, OH

- Assisted Dr. Charles Jerret, Director of admission
- Advised incoming students in course offerings and student financial aid
- Developed programs to increase enrollment

Tutor, 1997

Faculty in all Departments
Ohio University, Ironton, OH, Marshall University
Courses:

- Chemistry
- Physics
- Economics
- Algebra
- Psychology
- Biology
- General Math
- Statistics

PUBLICATIONS:

- *Effects of Aging on Pressure-Induced MAPK Activation in the Rat Aorta***;** K. M. Rice[1], R. S. Kinnard[1], R. Harris[2, 3], G. L. Wright[3] and E. R. Blough[1, 3] ; [1] Department of Biological Sciences, Marshall University, [2] Department of Biological Sciences, West Virginia State University, [3] Department of Physiology, Joan C. Edwards School of Medicine, Marshall University (Accepted by Pflüger's Arch.)

RESEARCH SUBMITTED AND IN PREPARATION:

- *Effects of Aging on Tissue Content and Pressure-Induced Regulation of p70S6k in the Rat Aorta;* K. M. Rice[1], R. S. Kinnard[1], G. L. Wright[2], and E. R. Blough[1,2] ; [1] Department of Biological Sciences, Marshall University, [2] Department of Physiology, Marshall University, Joan C. Edwards School of Medicine (Currently in review at Mechanisms of Aging)

-*The Effects of Age on Muscle Physiology in Fischer 344 and Fischer 344 X Brown Norway Rats;* Kevin M. Rice[1], Jon K. Linderman[2], Eric R. Blough[1, 3] ; [1] Department of Biological Sciences, Marshall University, [2]Department of Health and Sport Science, University of Dayton, [3] Department of Physiology, Joan C. Edwards School of Medicine, Marshall University (Currently in review with Canadian Journal of Physiology)

- *Prostaglandin $F_{2\alpha}$ Signaling in A7R5 Hypertrophy.* K. M. Rice[1], S. Uddemarri [1], F. Chan[1], R. S. Kinnard[1], (R. Harris[2,3]), G. L. Wright[3] and E. R. Blough[1,3] ; [1] Department of Biological Sciences, Marshall University, [2] Department of Biological Sciences, West Virginia State University, [3] Department of Physiology, Joan C. Edwards School of Medicine, Marshall University (In preparation)

- *Effects of FP Agonist on MAPK Activation in the A7R5 Smooth Muscle Cell Line;* Kevin M. Rice[1], S. Uddemarri[1], Randy Kinnard[1], Robert Harris[2], Gary Wright[3] and Eric R. Blough[1,3] ; [1] Department of Biological Sciences, Marshall University, [2] Department of Biological Sciences, West Virginia State University, [3] Department of Physiology, Joan C. Edwards School of Medicine, Marshall University (In preparation)

- *Effects of Aging on Pressure-Induced FAK Activation in the Rat Aorta.* K. M. Rice[1], R. S. Kinnard[1], R. Harris[2,3], G. L. Wright[3] and E. R. Blough[1,3] : [1] Department of Biological Sciences, Marshall University, [2] Department of Biological Sciences, West Virginia State University, [3] Department of Physiology, Joan C. Edwards School of Medicine, Marshall University (In preparation)

- *Effects of Aging on Contractile properties in the Aorta of Fischer 344 X Brown Norway Rats;* K. M. Rice[1], G. L. Wright[2], and E. R. Blough[1,2] ; [1] Department of Biological Sciences, Marshall University, [2] Department of Physiology, Marshall University, Joan C. Edwards School of Medicine (In preparation)

- *Effects of Aging on ROS Indices in the Aorta of Fischer 344 X Brown Norway Rats;* K. M. Rice[1], G. L. Wright[2], and E. R. Blough[1,2] ; [1] Department of Biological Sciences, Marshall University, [2] Department of Physiology, Marshall University, Joan C. Edwards School of Medicine (In preparation)

PAPERS PRESENTED AT CONFERENCES:

ERK-1/2 and p38 MAPK signaling in the A7R5 cell line following Fluprostenol stimulation. Kevin M. Rice[1], Sreevani Uddemaari[1], Devashish Desai[3], Randy S. Kinnard[1], Robert Harris[2], G. L. Wright[3], and E. R. Blough[1, 3]. [1]Department of Biological Sciences, Marshall University, [2] Department of Biological Sciences, West Virginia State University, [3] Department of Physiology, Joan C. Edwards School of Medicine, Marshall University. To be Presented at the 52nd Annual Meeting of the American College of Sport Medicine(ACSM), Nashville, TN, 2005

The PGF2α analog Fluprostenol activates mTOR and GSK-3β in the A7R5 smooth muscle cell line. Sreevani Uddemaari[1], Kevin M. Rice[1], Randy S. Kinnard[1], Robert Harris[2], G. L. Wright[3], and E. R. Blough[1, 3]. [1]Department of Biological Sciences, Marshall University, [2] Department of Biological Sciences, West Virginia State University, [3]Department of Physiology, Joan C. Edwards School of Medicine, Marshall University. To be Presented at the 52nd Annual Meeting of the American College of Sport Medicine, Nashville, TN, 2005

Comparison between stretch-induced p42/44 MAPK signaling in rat aortic smooth muscle and A7R5 vascular cells. Devashish H. Desai, M.S.[1], Robert T. Harris, Ph.D.[2], Gary L. Wright, Ph.D.[3], Kevin M. Rice, B.S.[3], Deepak B. Mylabathula, M.B.B.S.[3], Eric R. Blough, Ph.D.[1,3]. [1]Department of Physiology, Marshall University School of Medicine, Huntington, WV; [2]West Virginia State University, Charleston, WV; [3]Department of Biology, Marshall University, Huntington, WV. To be Presented at the 52nd Annual Meeting of the American College of Sport Medicine, Nashville, TN, 2005

Comparison between stretch-induced p42/p44 MAPK signaling in rat aortic smooth muscle and A7R5 vascular cells. Devashish H. Desai[1], Robert T. Harris[2], Gary L. Wright[1], Kevin M. Rice[3], Eric R Blotigh[1,3], [1]Department of Physiology, Marshall University School of Medicine, Huntington, WV; [2]West Virginia State University, Charleston, WV; [3]Department of Biology, Marshall University, Huntington, WV. Presented at the Marshall University School of Medicine Research Day, Huntington, WV, 2005

Differential effects of aging on basal expression level and stretch-induced activation of ERK1/2 in rat soleus and EDL muscles. Kevin M. Rice[1], Zihao Wang[1], Deepak benjamin Mylabathula[1], Eric R Blotigh[1,2], [1]Department of Biology, Marshall University, 311 Science Building, One John Marshall Drive, Huntington, WV, 25702, Marshall University; [2]Department of Physiology, Marshall University School of Medicine, Huntington, WV; Presented at the Integrative Biology of Exercise International meeting of the American Physiological Society, the Canadian Society for Exercise Physiology, and the American College of Sports Medicine, Austin, TX, 2004

CURRENT RESEARCH INTERESTS:

I am currently interested in researching the isolation of skeletal myocytes and development aortic replacement vessel from the isolated cells.

I am currently investigating the biochemical responses of A7R5 vascular smooth muscle to prostaglandin F2α stimulation.

Presently, I am looking at developing an aortic transplant developed from explants of skeletal muscle myocytes.

I am currently performing a studying an alterations in molecular signaling in diabetic and non-diabetic aortas of Zuker rats exposed to a hypertensive pressure of 200 mm hg for 30 min.

I am currently performing a studying an alterations in molecular signaling in diabetic and non-diabetic vena cava of Zuker rats exposed to a systolic pressure of 120 mm hg for 30 min.

GRANTS RECEIVED:

Travel award from Marshall University – COS to attend the Integrative Biology of Exercise International meeting of the American Physiological Society, the Canadian Society for Exercise Physiology, and the American College of Sports Medicine, Austin, TX, 2004

PROFESSIONAL MEMBERSHIPS:

American College of Sport Medicine (ACSM)

PROFESSIONAL SERVICE:

- Founder and President, Campus Ministries International, Marshall University, 2005
- President, Graduate Student Council, Marshall University, 2004-2005
  Reestablished this group after numerous years of non-existence
  - Established cancer scarf drive 2005
- Member of the Lawrence County ARES (Amateur Radio Emergency Services) 1999 - Present
- Member of the Lawrence County RACES (Radio Amateur Civil Emergency Services) 1999 - Present

- President, Student Government, Ohio University Southern Campus, 1997-98
  - Developed numerous events and student activites which still occur to date
- Vice President, Student Government, Ohio University Southern Campus, 1996-1997
- Tobacco Chair, American Cancer Society, Lawrence County Ohio Chapter, 1996-1998
- Member, Los Amigos Internacionales Club May 1997 – May 1998
- President, Vocational Industrial Clubs of America (V.I.C.A.) Lawrence County Joint Vocational School (Now Collins Career Center) 1990-91
- Chaplin, Vocational Industrial Clubs of America (V.I.C.A.) Lawrence County Joint Vocational School (Now Collins Career Center) 1989-90
- Third place, VICA Regional HVAC competition 1990
- Second place, VICA Regional HVAC competition 1991

COMMUNITY SERVICE:

- Taught crochet at ladies groups at New Beginning Apostolic Church 2005
- Church Librarian for 4 years
- Church musician (Bass, guitar, keyboard, and singer) for 15+ years
- Licensed Minister New Beginning Apostolic Church 2000-pressent
- Evangelized intermittently for 14 years
- Licensed Minister UPCI 1999-2000
- Authority to Solemnize Marriages in the State of Ohio 1997- present
- Licensed Minister Universal Life Church 1997- present
- Leader BOYZ Club New Beginning Apostolic Church 1998-1999
- Director of High Rise Ministry (Nursing Home) New Beginnings Apostolic Church 1998 - 1999
- Assistant Director of High Rise Ministry (Nursing Home) New Beginnings Apostolic Church 1997 - October 1998
- Pastor, New Life Apostolic Church, Scottown Ohio, 1997 –1998
- Assistant to Pastor (during illness and recovery), Decator Apostolic Church, Decator, OH, 1996 –1997
- Church Camp Staff Member, UPCI WV District youth camp, Point Pleasant, WV, 1995 –1996
- Assistant to Teen Department Sunday school director, Apostolic Life Cathedral, Huntington, WV, 1995 –1996
- Sunday school teacher, Apostolic Life Cathedral, Huntington, WV, 1993 –1995

HONORS AND AWARDS:

Recognition Award for work in the 134th Lawrence County Memorial Day Parade
Amateur Radio Operator and division leader
Certificate of Effective Alter Working New Beginning Apostolic Church 2002
Certificate of Completion Ohio Project Wild 1998
Certificate of Achievement Los Amigos Internacionales 1998
Certificate of Appreciation Apostolic Life Cathedral Sunday School Department 1997
Who's Who American Colleges and University's 1997-98
Who's Who American Colleges and University's 1996-97
Certificate of Appreciation Sunday School Music Ministry, Sunday School Department Apostolic Life Cathedral 1995
Certificate of Appreciation Teacher's Assistant Sunday School Department Apostolic Life Cathedral 1995
Certificate of Recognition in Voice, Kent Christian College Music Department, Dover, DE 1992
Presidential Scholarship Kent Christian College Dover, DE 1992
Honor Award Fairland High School 1991
Achievement Award Fairland/ Lawrence County Joint Vocational School, Certificate of Achievement for heating-ventilation/air conditioning 1991
Award of Distinction, State Board of Education 1991
Who's Who in American High Schools 1990-91
Who's Who in American High Schools 1989-90

REFERENCES:

The following persons have written letters of recommendation on my behalf:

Dr. Eric R. Blough, Assistant Professor
Department of Biology
College of Science
Marshall University, Huntington, WV
(304)696-3267

Dr. David Mallory, Professor
Department of Biology
College of Science
Marshall University, Huntington, WV
(304)696-3253

Dr. Nicki, Locascio, Professor
Department of Biology
College of Science
Marshall University, Huntington, WV
(304)696-3975

Dr. Robert Culp, Professor

Ohio University Southern Campus,
Chemistry Department Chair
Ironton, OH
(740) 533-4563