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ROLE OF RAC GUANOSINE TRIPHOSPHATASE IN SIMVASTATIN-MEDIATED ENDOTHELIAL CELL SIGNALING. J.R. Jacobson, W. Chen, J.G.N. Garcia, Section of Pulmonary and Critical Care Medicine, The University of Chicago, Chicago, IL.

The statins are a class of HMG CoA-reductase inhibitors used clinically for their ability to reduce serum cholesterol levels via inhibition of the prenylation pathway. However, not all of their clinical benefits, including both vascular barrier protection and reduced superoxide generation, can be attributed to their lipid-lowering properties. One potential mechanism of these effects is via inhibition of geranylgeranylation, a covalent modification that allows translocation to the cell membrane and activation of the small GTPases Rho and Rac, mediators of cytoskeletal rearrangement. While statins inhibit Rho, we previously reported the paradoxical activation of Rac (Rac-GTP) in endothelial cell (EC) after prolonged treatment with simvastatin (5 μM, 16 hours). In this study, upon membrane fractionation and subsequent Western blotting, we report a 37% reduction in Rac translocation to the EC membrane by simvastatin (5 μM, 16 hours) relative to untreated control cells, consistent with the inhibition of geranylgeranylation and evidence of a separate mechanism of simvastatin-mediated Rac activation. In addition, as Rac is required for activation of the NADPH oxidase complex and subsequent superoxide anion generation, we examined the effect of simvastatin on the peripheral translocation of $P47^{phox}$, an NADPH oxidase component. Simvastatin affected a 47% reduction in $P47^{phox}$ at the EC membrane relative to control cells. Finally, we measured transendothelial electrical resistance and quantified the effects of simvastatin pretreatment on sphingosine 1-phosphate–induced barrier enhancement, an event dependent on Rac. S1P-induced (1 μM) EC barrier function was increased by simvastatin (5 μM, 16 hours) 70% at peak effect relative to S1P-treated control cells. These data are consistent with a functional role for Rac in simvastatin-mediated EC barrier protection and further define the mechanism by which simvastatin is able to directly modulate EC.

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HYPOXIC VENTILATORY RESPONSE IN EARLY LUNG INJURY IS AUGMENTED BY OXYGEN-INDEPENDENT SENSITIZATION OF THE CAROTID BODY. F.J. Jacono,[1] Y. Peng,[2] D. Nethery,[1] J.A. Faress,[1] J.A. Kern,[1,2] N.R. Prabhakar,[2] [1]Division of Pulmonary and Critical Care Medicine and [2]Department of Physiology, Case Western Reserve University, Cleveland, OH.

Rationale: Acute lung injury alters ventilatory control by impairing gas exchange. However, even before hypoxemia develops, lung inflammation itself may alter ventilatory control. The objective of the present study was to examine the impact of acute lung injury on ventilatory control by hypoxia and hypercapnia. **Methods:** Experiments were performed on adult male Sprague-Dawley rats challenged with intratracheal injections of either bleomycin (BM; 1 unit) or PBS. Five days after the injections, the extent of lung injury was evaluated, and ventilatory responses to hypoxia (12% O_2) or hypercapnia (7% CO_2) were measured by plethysmography in unanesthetized animals and by diaphragmatic EMG in anesthetized animals. Contribution of carotid body sensory afferents to ventilatory patterns was evaluated by comparing responses before and after glomectomy in anesthetized animals. **Results:** BM-treated animals had increased total cell count, percent neutrophils, and protein levels in lavage fluid with no alterations in lung collagen content suggesting acute lung injury but not fibrosis. Core body temperature, PaO_2, and $PaCO_2$ were comparable between both groups of animals. In unanesthetized animals (n = 16), baseline ventilation and the hypoxic ventilatory responses were significantly higher in BM-injected animals compared to control animals (average increases in minute ventilation [V_E]: BM +214 ± 59 mL/kg/min vs Control +60 ± 8 mL/kg/min; p = .003), whereas respiratory stimulation by hypercapnia was not altered to the same degree (p = .672). The selective enhancement of hypoxic ventilatory drive was also present in anesthetized, spontaneously breathing animals (n = 12) where average increases in respiratory rate [RR] were greater in animals with lung injury (p = .036). In contrast, this difference between control and BM-exposed animals was abolished following bilateral glomectomy (p = .786). In these same animals, average decreases in RR in response to sudden administration of hyperoxia (FiO_2 change from 0.12→1.0) was significantly greater in the BM-exposed group compared to control animals (BM −13.0 ± 1.0 % vs control −9.7 ± 1.0 %; p = .041), and these differences were abolished following glomectomy (p = .128). **Conclusions:** These data demonstrate that afferent sensory input from the carotid body contributes to a selective enhancement of hypoxic ventilatory drive in the absence of pulmonary fibrosis and arterial hypoxemia in early BM-induced lung injury.

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THE SOY ISOFLAVONE GENISTEIN BLOCKS TRANSFORMING GROWTH FACTOR β_1–STIMULATED LUNG FIBROBLAST TO MYOFIBROBLAST TRANSFORMATION. R. Kalhan, K. Thavarajah, M.C. Nlend, A. Nair, L.J. Smith, P.H.S. Sporn, Northwestern University Feinberg School of Medicine, Chicago, IL.

Rationale: Genistein is a dietary isoflavone and a broad-spectrum tyrosine kinase inhibitor contained in soy products. An epidemiologic study of asthma revealed that subjects with high consumption of dietary soy isoflavones had better lung function than those with lower intake. In a guinea pig model of allergic asthma, genistein reduced methacholine-induced bronchoconstriction. To explore the mechanisms underlying these observations, we tested genistein's ability to block lung myofibroblast differentiation, a key phenotypic change in asthmatic airway remodeling. **Methods:** Human fetal lung fibroblasts (IMR-90) were grown to subconfluence in DMEM containing 10% FBS, serum-deprived for 24 hours, and treated with genistein (10 μM) for an additional 24 hurs. Cells were then stimulated with TGF-β_1 (2 ng/mL) for 24 hours, and α-smooth muscle actin (αSMA) expression, a marker of the myofibroblast phenotype, was assessed by both immunoblot and immunofluorescence microscopy. To determine the intracellular mechanism of inhibitory actions by genistein, we also assessed phosphorylation of Smad2 by immunoblot in the presence and absence of genistein after 30-minute stimulation with TGF-β_1. **Results:** Treatment with genistein resulted in 54.9 ± 17.1% reduction in TGF-β_1 induced expression of αSMA. Immunofluorescence microscopy revealed a decrease in αSMA staining intensity and stress fiber formation in genistein-treated cells. In addition, Smad2 phosphorylation was inhibited by 71.1 ± 16.7% in genistein-treated cells. **Conclusions:** These results demonstrate that genistein interferes with TGF-β_1-stimulated myofibroblast differentiation and suggest that tyrosine kinase inhibition may have a role in modulation of asthmatic airway remodeling.

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A DIRECT HAPLOTYPING METHOD OF PUTATIVE FUNCTIONAL VARIANTS IN PROMOTER REGIONS OF THREE CANDIDATE GENES IN ACUTE LUNG INJURY. M.M. Kalscheur,[1] C. Flores,[2] S.F. Ma,[2] M. Burke,[3] W.J.Buikema,[3] J.G.N. Garcia,[2] [1]Pritzker School of Medicine,[2]Pulmonary and Critical Care, [3]Cancer Research Center DNA Sequencing Facility, The University of Chicago, Chicago, IL.

Rationale: Due to their functional relevance, single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) have been used in association studies of complex diseases. The combination of both types of polymorphisms in short genomic regions (< 500 bp), termed SNPSTRs, constitutes a powerful tool as it allows for the empiric determination of gametic phase, or haplotype, thus adding a new level of complexity over single variants. Our aim is to select a set of SNPSTR pairs in candidate genes in acute lung injury (ALI) and to develop a multiplex method to directly determine the haplotypes of each of these SNPSTR pairs. **Methods:** Candidate genes identified by microarray analysis of animal models of ALI were selected. Functional relevance of SNPs and STRs in the promoter region was established for the selected candidate genes using *PubMed*. *Primer3* software (<http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi>) was used to design SNPSTR primers as well as the complimentary fluorescently labeled primers for SNP alleles. A three-step direct haplotyping method was performed as follows: (1) coamplification of the genomic regions of interest by multiplex PCR, (2) linear amplification of the amplicons with FAM- and HEX-labeled fluorescent primers, and (3) identification of haplotypes by *GeneMapper* software v3.7. **Results:** Three candidate genes (*IL-10*, *HMOX-1*, and *CXCL2*) were identified with reported functionally relevant SNPSTRs and were arranged into a multiplex protocol. Genotyped variants were −1087 G/A SNP and the −1121 CA_n STR for *IL-10*, −413 A/T SNP and the −199 GT_n for *HMOX-1*, and −437 A/G SNP and the −665 CA_n STR for *CXCL2*. A Coriell CEPH DNA panel, consisting of individuals of European and African American decent (23 and 24 individuals, respectively), was then used to develop our haplotyping method. SNP alleles and STR repeats were confirmed with direct DNA sequencing. **Conclusion:** This SNPSTR method represents an efficient and robust approach to obtain direct haplotypes in three candidate genes, which will advantage association studies of genetic variants in ALI.

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ABNORMAL CYCLIC ADENOSINE MONOPHOSPHATE PRODUCTION IN HUMAN CORTICAL FRAGILE X NEURAL TISSUE: A PROOF OF PRINCIPLE STUDY. D.J. Kelley, A. Bhattacharyya, J.C. Yin, T.R. Oakes, M.L. Chung, K.M. Dalton, B.T. Christian, R.J. Davidson, Waisman Center, University of Wisconsin-Madison, Madison, WI.

Fragile X syndrome, the most common inheritable cause of mental retardation, is due to a CGG trinucleotide amplification on the X chromosome (Xq27.3) in the 5′ untranslated region of the fragile X mental retardation 1 gene (FMR1) that suppresses production of the fragile X mental retardation protein (FMRP). Based on a decade of studies by Berry-Kravis who established the cyclic AMP (cAMP) cascade as dysfunctional in fragile X using a variety of non-neuronal cell types and who identified a direct relationship between levels of FMRP expression and cAMP levels in a mouse neural cell line overexpressing FMRP (Berry-Kravis and Ciurlionis, 1998), we hypothesized that human fragile X neural tissue would produce less cyclic AMP upon stimulation. Using an assay in which reductions in fluorescence intensity are associated with increasing cAMP levels (Mediomics, LLC), we quantified cAMP levels in neurospheres (NS) and differentiated cells (DC) from the human cortical fragile X (M049) and control (M037, M045, M046) fetal stem cell lines incubated with the phosphodiesterase inhibitor IBMX (3-isobutyl-1-methyl≠xanthine) in the presence or absence of forskolin, an adenylate cyclase agonist. As hypothesized, the fragile X differentiated cell line (fractional decrease in raw fluorescence [FDRF] = 0.32) shows a stimulated cAMP production that is reduced relative to the three differentiated control stem cell lines (mean FDRF ± SD = 0.70 ± 0.09). When differentiated cells are compared to neurospheres, all three control cell lines showed a marked increase in the levels of cAMP production in differentiated cells compared to undifferentiated neurospheres (mean FDRF difference [DC-NS] = 0.52); however, stimulated cAMP levels in differentiated cells and neurospheres are comparable in the fragile X line (FDRF difference [DC-NS] = 0.05). To our knowledge, these results are the first demonstration of an altered cAMP cascade in human fragile X neural tissue and suggest a developmental role for FMRP in the cAMP cascade. With only one human fragile X fetal stem cell line available, these results require replication with more fragile X neural tissue samples as they become available. Nevertheless, this proof of principle study identifies the human fragile X cAMP cascade as a potentially useful pharmacotherapeutic target that deserves further investigation.

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THE EFFECT OF ACIDOSIS AND HYPERKALEMIA ON THE IKr BLOCKING ACTION OF ANTIARRHYTHMIC DRUGS. C. Lin, X. Ke, I. Cvetanovic, V. Ranade, J.C. Somberg, Department of Pharmacology, Rush University Medical Center, Chicago, IL.

Regional myocardial acidosis resulting from the impaired coronary blood flow has been observed in both animal models and in man. The ischemic myocardium also releases potassium into the extracellular space, which can cause regional hyperkalemia. Antiarrhythmic agents are frequently prescribed for patients with ischemic heart disease and regional changes in pH and potassium may alter the effect of these agents. In this study, we evaluated the effect of extracellular acidosis and hyperkalemia on the action of IKr blocking antiarrhythmic drug-quinidine (Q). The IKr channel was studied at room temperature by employing human-ether-a-go-go-related gene (HERG) expressed in *Xenopus oocytes* and two-electrode voltage clamp technique was employed for recording. The pH of the recording bath solution was adjusted with NaOH to 6.8 or 7.4 and the recording bath solutions contained either 5 or 7.5 mmol/L KCl (5 or 7.5 K). The recording solution with 5 K, pH 7.4 represented the normal condition and 7.5 K, pH 6.8 represented acidic and hyperkalemic conditions. Q 3, 10, and 30 μM when applied at 5 K, pH 7.4 inhibited current by 17 ± 3, 39 ± 3, and 63 ± 4%. The percentage current block by Q at 7.5 K, pH 7.4 was similar to current block at 5 K, pH 7.4. Q at 7.5 K, pH 7.4 decreased HERG current by 18 ± 1, 42 ± 3, and 65 ±3%. But if Q was applied at 5 K, pH 6.8, the HERG inhibitory effect of Q was decreased, and 3, 10, and 30 μM Q produced 8 ± 2, 24 ± 3, and 50 ± 4% current block. Q 3, 10, 30 μM administered in acidic and hyperkalemic condition (7.5 K, pH 6.8) caused 13 ± 2, 25 ± 2, and 47 ± 2% current inhibition, which was similar to the inhibitory effect of Q at 5 K, pH 6.8. There was a significant difference in current block by Q between 5 K, pH 7.4 and 5 K, pH 6.8, and there was also a significant difference in current inhibition by Q between 5 K, pH 7.4 and 7.5 K, pH 6.8 (p < .05). Our data suggest that extracellular acidosis (pH 6.8)

attenuates the HERG inhibitory effect of Q, and when extracellular acidosis is combined with hyperkalemia (7.5 K), the effect on IKr inhibition is similar to acidosis alone. The attenuated effect of Q at low pH may cause heterogeneity of repolarization between ischemic and normal regions, and this may set the stage for reentrant arrhythmias, contributing to Q's proarrhythmic toxicity.

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THE EFFECT OF NARINGENIN (GRAPEFRUIT JUICE) AND ANTIARRHYTHMIC DRUGS ON IKr INHIBITION. C. Lin, X. Ke, I. Cvetanovic, V. Ranade, J.C. Somberg, Department of Pharmacology, Rush University Medical Center, Chicago, IL.

Grapefruit juice has been reported to cause significant QT prolongation in healthy volunteers. Naringenin (N), the principal flavonoid in grapefruit juice, has been identified as the most potent HERG channel blocker among several dietary flavonoids. In light of these reports, we thought that combining naringenin with Ikr-inhibiting antiarrhythmic drugs would increase IKr inhibition and possibly pose an increased health risk by increasing repolarization delay and ensuing arrhythmias. In this study, we investigated the effect of N combined with quinidine (Q) on IKr inhibition. The study was performed in an oocyte system with heterogeneously expressed human-ether-a-go-go-related gene (HERG) employing two electrodes voltage clamp technique for recording. The experiments were performed at room temperature. Doses of 10 μM and 100 μM N were found to inhibit HERG channel by 15 ± 4 and 40 ± 7%. Q at 1 and 10 μM caused 9 ± 1 and 39 ± 3% inhibition of HERG current. When 10 μM N was combined with 1 μM Q, 9 ± 3% current was blocked. HERG current was blocked by 29 ± 2% when 10 μM N was combined with 10 μM Q. N 100 μM combined with 1 μM Q and N 100 μM combined with 10 μM Q caused 21 ± 2 and 36 ± 2% inhibition in HERG current, respectively. Combining naringenin and quinidine does not show an additive effect but rather a diminution in IKr inhibition. Further studies on the interaction of N with other known IKr channel blockers are indicated.

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GEFITINIB RESPONSE OF ERLOTINIB-REFRACTORY LUNG CANCER WITH LEPTOMENINGEAL METASTASIS. N.W. Choong,[1] S. Dietrich,[1] T.Y. Seiwert,[1] M.S. Tretiakova,[2] V. Nallasura,[1] G.C. Davies,[3] S. Lipkowitz,[3] A.N. Husain,[2] R. Salgia,[1] P.C. Ma,[4] [1]Section of Hematology/Oncology, Departments of Medicine and [2]Pathology, The University of Chicago, Chicago, IL; [3]National Cancer Institute, NIH, Bethesda, MD; [4]Division of Hematology/Oncology, Case Western Reserve University, Case Comprehensive Cancer Center, Cleveland, OH.

Although various mutations of the epidermal growth factor receptor (*EGFR*) gene, most commonly L858R (exon 21) and short exon 19 deletions, have been identified to confer sensitivity toward EGFR tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, it is not known if there are mutations that may result in differential activities of the two inhibitors. We describe a 70-year-old Japanese American woman diagnosed with stage IV non–small-cell lung cancer (NSCLC) with rib metastasis. While receiving treatment with the EGFR small molecule TKI erlotinib, she progressed and developed new brain metastases. She failed further chemotherapy treatments and subsequently developed symptomatic extensive leptomeningeal carcinomatosis associated with diplopia, hemiparesis, weight loss, and incontinence. Monotherapy gefitinib 250 mg daily was initiated, and she showed striking response both clinically and radiographically within the first few weeks. Using laser microdissection (LMD), we performed genomic DNA extraction and *EGFR* gene sequencing from the enriched tumor cells in her pretreatment tumor biopsy specimen and tumor cells found in her cerebrospinal fluid. Two heterozygous somatic *EGFR* mutations, L858R (exon 21) and E884K (exon 22), were identified in both specimens. In vitro transfection and biochemical studies revealed that the novel E884K mutation confers opposite effects in sensitivity to the two EGFR inhibitors. $EGFR^{E884K}$ and $EGFR^{L858R+E884K}$ enhanced the sensitivity of the mutated receptor to gefitinib inhibition. Conversely, the E884K mutation resulted in decreased responsiveness of the receptor to erlotinib, and it significantly abrogated the drug sensitivity conferred by L858R ($EGFR^{L858R+E884K}$). This study demonstrates that it is possible to have differential response to alternative EGFR TKIs. This also represents the first report of a response of leptomeningeal metastases to EGFR inhibition by small molecule inhibitor gefitinib alone in NSCLC. Further structural studies of the mutant EGFR are warranted to improve individualized targeted therapy and small molecule inhibitors' design in lung cancer in the future.

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SIMVASTATIN ATTENUATES ACUTE LUNG INJURY INDUCED BY ISCHEMIA-REPERFUSION IN RODENTS. L. Moreno,[1] J. Jacobson Jr,[1] P. Bonde,[2] J.G.N. Garcia,[1] [1]Department of Medicine, The University of Chicago, IL; [2]Department of Cardio-Thoracic Surgery, Royal Victoria Hospital, Belfast, United Kingdom.

Ischemia-reperfusion (IR) lung injury, a common cause of lung transplant failure, is characterized by hypoxemia, alveolar damage, inflammation, and edema. As novel lung preservation techniques could have a significant clinical impact in this setting, we employed an animal model of IR injury to investigate the potential therapeutic role of simvastatin, an agent we have previously characterized as a potent vascular barrier protectant (Jacobson JR et al, 2004 and 2005). Ischemia was induced in anesthetized Sprague-Dawley rats by ligation of the left pulmonary artery for a period of 1 hour followed by 4 hours of reperfusion. Indices of inflammation and vascular leak, including bronchoalveolar lavage (BAL) cell counts and protein content and lung tissue myeloperoxidase activity, were then assessed. Similar to the protective effects we previously observed with sphingosine 1-phosphate in this model (Moreno L, 2004), BAL from animals pretreated with simvastatin (20 mg/kg, intraperitoneal injection, 16 hours prior to ischemia) demonstrated a reduced number of total cells (48.3% decrease), neutrophils (33% decrease), and albumin concentration (20.7% decrease) compared to controls. A single dose of simvastatin also resulted in reduced (49% decrease) lung tissue MPO. These data indicate that simvastastin significantly attenuates the protein leakage and inflammation associated with IR lung injury in our animal model. Ultimately, our results could have profound clinical implications as simvastatin treatment may represent a potential agent to reduce the incidence and severity of IR injury associated with lung transplantation.

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MAST CELLS AND EOSINOPHILS STIMULATE MYOFIBROBLAST DIFFERENTIATION BY SIGNALING THROUGH THE TRANSFORMING GROWTH FACTOR β TYPE I (ALK5) RECEPTOR. M.C. Nlend,[1] A. Nair,[1] A. Talati,[1] C.H. Sheen,[1] R. Kalhan,[1] K. Thavarajah,[1] M. Kulka,[1] P.H.S. Sporn,[1] [1]Feinberg School of Medicine, Northwestern University, Chicago, IL.

Rationale: Mast cells and eosinophils synthesize various mediators, including transforming growth factor β (TGF-β) and cysteinyl leukotrienes (cysLTs), that may promote subepithelial fibrosis in asthma. We sought to determine the roles of TGF-β and cysLTs in myofibroblast differentiation stimulated by coculture of fibroblasts with mast cells or eosinophils. **Methods:** IMR-90 human lung fibroblasts were serum starved and cocultured for 48 hours with either LAD-2 human mast cells or freshly isolated human blood eosinophils in the absence or presence of SB431542 (10 M) to block signaling through the TGF-β type I (ALK5) receptor; MK886 (1 M) to block leukotriene synthesis; MK571 (100 nM) to block the cysLT1 receptor; or Bay u9773 (3 M) to block the cysLT1 and cysLT2 receptors. α-Smooth muscle actin (α-SMA) expression was assessed as an index of myofibroblast differentiation. **Results:** Mast cells and eosinophils stimulated 2.50.2 (n = 5, p < .005)- and 1.80.2 (n = 5, p < .0001)-fold increases, respectively, in fibroblast α-SMA expression, determined by immunoblot analysis. In comparison, TGF-β (2.5 ng/mL) increased α-SMA expression by 5.50.5-fold (n = 6, p < .0001). SB431542 inhibited the increase in α-SMA expression stimulated by mast cells, eosinophils and TGF-β by 6,019%, 8,221% and 9,210%, respectively (all p < .0001). By contrast, none of the leukotriene pathway inhibitors significantly affected α-SMA expression. Immunofluorescence microscopy for α-SMA confirmed the immunoblot results. **Conclusion:** Our data indicate that mast cells and eosinophils stimulate differentiation of myofibroblasts by TGF-β signaling through the ALK5 receptor and that cysLTs are less important in this process. Inhibition of ALK5 receptor signaling may be a useful strategy to interrupt airway remodeling in asthma.

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HYPERTENSION-INDUCED CONTRACTILE DYSFUNCTION, LEFT VENTRICULAR HYPERTROPHY, AND DOWN-REGULATION OF MITOCHONDRIAL METABOLIC ENZYME ACTIVITY ARE ATTENUATED BY HIGH-FAT/LOW-CARBOHYDRATE FEEDING. I.C. Okere,* D.J. Chess,* T.A. McElfresh,* M.E. Young,** P. Ernsberger,* B.D. Hoit,* M.P. Chandler,* W.C. Stanley,* *Case Western Reserve University, Cleveland, OH; **Baylor University, Houston, TX.

Cardiac hypertrophy and heart failure are characterized by increased expression of atria natriuretic peptide (ANP), a decrease in the activity of fatty acid oxidation (FAO) enzymes, impaired mitochondrial respiration, and LV remodeling and systolic dysfunction. Fatty acids up-regulates the expression of FAO enzymes via activation of peroxisomal proliferator activator receptors. We hypothesized that a high-fat diet would prevent down-regulation of FAO enzymes activity and slow development of cardiac dysfunction in a model of hypertension-induced hypertrophic cardiomyopathy. Three groups (n = 10) of 11-week-old male Dahl salt-sensitive rats were fed (1) low-fat/low-salt rodent chow (10% calories from fat) (LF-LS), (2) low-fat/high-salt chow (6% NaCl) (LF-HS), or a high-fat/high-salt diet (60% calories from fat, 6% NaCl) (HF-HS) for 12 weeks. Tail cuff blood pressure and echocardiographic assessment of LV dimensions and function were performed before initiation of diets and after 11 weeks on diets. Myocardial activities of citrate synthase (CS) and medium chain acyl dehydrogenase (MCAD) activities were measured, and the mRNA expressions for ANP was measured. **Results:** There were no differences in body weight among groups. Salt feeding caused similar elevations in systolic blood pressure in LF-HS and HF-HS groups (≈190 mm Hg compared to 120 mm Hg in LF-LS). LV mass was increased in LF-HS (1.30 ± 0.06 g) compared to LF-LS (0.90 ± 0.02 g). Hypertension down-regulated CS and MCAD activities in the LF-HS group but not in the HF-HS group. LV end-diastolic volume and ANP expression were elevated and fractional shortening reduced in HS-LF compared to LS-LF. The HF-HS group had reduced LV hypertrophy (1.16 ± 0.05 g) and ANF expression and showed no LV remodeling or systolic dysfunction compared to LF/HS fed rats. **Conclusion:** High-fat diet prevented the down-regulation of the activity of metabolic enzymes and preserved contractile function and LV chamber dimensions in this model of hypertrophic cardiomyopathy.

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A POTENTIAL ROLE FOR 5-LIPOXYGENASE IN THE PATHOGENESIS OF CEREBELLAR DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS. O. Ostmann, T.J. Santoro, M. Tomita, Department of Medicine, University of North Dakota School of Medicine and Health Sciences, Fargo, ND.

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology associated with inflammation involving the lungs, kidneys, joints, skin, and brain. Central nervous system (CNS) involvement in human and experimental SLE is characterized, in part, by disturbances in posture, balance, and tremulousness, which map to the cerebellum (J Neurosci Res 2001;64:26). Five-lipoxygenase (5-LO) activity is increased in the setting of neurodegeneration associated with aging, ischemia, and other CNS insults, which may be accompanied by cerebellar dysfunction. To determine whether 5-LO contributes to CNS inflammation/neurodegeneration in neuropsychiatric lupus, we studied 5-LO and 5-LO activating protein (FLAP) in the cerebellum of MRL-*lpr*/*lpr* mice, which spontaneously develop a lupus-like illness associated with disturbances in cerebellar function. Relative to age- and sex-matched, immunologically and neurologically normal, H-2 identical CBA/CaJ mice, cerebellar homogenates from 20- to 30-week-old clinically affected MRL-*lpr*/*lpr* mice exhibited enhanced expression of genes encoding both 5-LO and FLAP as assessed by reverse transcriptase polymerase chain amplification. In contrast, no differences were found in the expression of genes encoding cytosolic phospholipase A2 and neuronal nitric oxide synthase in the cerebellum of autoimmune and control mice. Western blotting of homogenates indicated that MRL-*lpr*/*lpr* mice had a greater content of 5-LO but not of FLAP protein compared with control CBA/CaJ mice. These data suggest a potential role for 5-LO in the pathophysiology of cerebellar dysfunction in lupus.