necrosis factor α and interleukin-1β generation of ROS and subsequent activation of the transcription factor NF-κB. We propose that ClC-3 functions as a chloride-proton exchanger and thereby influences ROS production via charge neutralization of the electron flow generated by Nox1 in the endosome. These findings identify ClC-3 as a critical component of the signaling endosome and a novel intermediate in redox-dependent control of gene expression.

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A NOVEL MYOSIN LIGHT CHAIN KINASE INHIBITOR, PIK, PROTECTS FROM LIPOPOLYSACCHARIDE-INDUCED ACUTE LUNG INJURY. T. Mirzapoiazova, S. Sammani, L. Moreno, S.M. Dudek, J.R. Turner, J.G. Garcia, University of Chicago, Chicago, IL.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are major causes of acute respiratory failure that are associated with high morbidity and mortality. These disorders are characterized by a significant pulmonary inflammatory response resulting in injury to alveolar epithelial and endothelial barriers followed by pulmonary edema. Our prior studies demonstrated that myosin light chain kinase phosphorylation of myosin light chains (MLC) is associated with increases in both epithelial and endothelial barrier permeability. We previously have identified a novel oligopeptide, PIK, that inhibits MLC kinase in vitro, is membrane permeant, decreases intracellular MLC phosphorylation, and causes increased intestinal epithelial cell barrier function (Turner et al. Gastroenterology 2002). We hypothesized that PIK-mediated inhibition of MLC kinase activity would attenuate inflammation and vascular leak associated with acute lung injury. To test this hypothesis, we used a murine acute lung injury model with intratracheal administration of endotoxin/lipopolysaccharide (LPS, 2.5 mg/kg). Optimal PIK responses were observed at 125 μM PIK when tested over a range of PIK concentrations. PIK administered intravenously simultaneously with LPS resulted in significantly attenuated lung inflammation reflected by decreasing accumulation of bronchoalveolar lavage (BAL) proteins (25% reduction, $p < .02$) and BAL cells (25% reduction, $p < .05$). IV administration of PIK decreased LPS-induced tissue MPO activity (a reflection of leukocytes in lung tissue) (15% reduction) and LPS-mediated MLC phosphorylation in lung homogenates (25% reduction). Our data suggest that PIK stabilizes epithelial-endothelial permeability and has significant therapeutic potential in ALI.

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CARDIOVASCULAR EVENTS ≤ AGE 45: ATHEROTHROMBOSIS. J. Munjal, G.J. Charles, D.M. Aregawi, P. Wang, The Jewish Hospital, Cincinnati, OH.

The population that develops occlusive arterial circulatory events at a young age (≤ 45 years), particularly if they are normolipidemic, represents a rare population in which a heightened hemostatic system may play an important mechanistic role in thrombus formation and in arterial events. We assessed whether thrombophilia-hypofibrinolysis contributed to premature atherothrombotic cardiovascular disease (ATCVD) in 78 men and 40 women with 230 ATCVD events (≤ age 45 years). ATCVD events included ≥ 1 myocardial infarction ($n = 60$), CABG ($n = 33$), angioplasty ($n = 52$), chronic angina ($n = 41$), ischemic stroke ($n = 11$), TIA ($n = 24$), and claudication ($n = 9$). Hereditary thrombophilia was assessed: G1691A factor V Leiden, G20210A prothrombin, MTHFR C677T-A1298C, and platelet glycoprotein PL A1/A2 mutations, with serologic measures of ACLA IgG and IgM, lupus anticoagulant, proteins C and S, antithrombin III, homocysteine, and factors VIII and XI. Hypofibrinolysis was assessed: 4G4G plasminogen activator inhibitor 1 mutation (PAI), PAI activity (PAI-Fx), and Lp(a). Cases were compared with healthy normal controls (149 men for PCR, 40 men for serologic tests, 109 women for PCR and serologic tests). Cases did not differ from controls by race or age ($p > .05$) Hypertension was present in 44% of men and 25% of women, diabetes in 17% and 8%, and cigarette smoking in 25% and 20%. In the 78 male cases, mean ± SD age was 48 ± 11 years, BMI 29.5 ± 4.5, LDLC 103 ± 44, HDLC 41 ± 14, and TG 195 ± 219 mg/dL. In 40 female cases, mean ± SD age was 38 ± 10 years, BMI 28.3 ± 7.7, LDLC 105 ± 31, HDLC 50 ± 13, and TG 147 ± 121 mg/dL. High factor VIII (> 150%) was present in 15 of 59 (25%) male cases versus 1 of 38 (3%) male controls, $p = .003$. High factor XI (> 150%) was present in 9 of 56 (16%) male cases versus 0 of 38 (0%) male controls, $p = .01$. High PAI-Fx (> 21.1 U/mL) was present in 24% of male cases (15 of 63) versus 8% (3 of 39) of male controls, $p = .038$. Low protein C (< 73%) was present in 4 of 25 (16%) female cases versus 2 of 107 (2%) female controls, $p = .012$. Low free protein S (< 66%) was present in 5 of 27 (19%) female cases versus 3 of 107 (3%) female controls, $p = .009$. High factor XI (> 150%) was present in 3 of 26 (12%) female cases versus 2 of 107 (2%) female controls, $p = .051$. The lupus anticoagulant was present in 9 of 37 (24%) female cases versus 6 of 84 (7%) female controls, $p = .014$. In patients with ATCVD ≤ age 45, thrombophilias (factor VIII, factor XI, protein C and S deficiency, and the lupus anticoagulant) and hypofibrinolysis (PAI-Fx) may promote arterial thrombosis, synergistic with atherosclerotic endothelial injury. If thrombophilia-hypofibrinolysis accompanies ATCVD at ≤ age 45, thromboprophylaxis may have value in secondary prevention of subsequent ATCVD.

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MYOCARDIAL INFARCTION-ANGIOPLASTY-CORONARY ARTERY BYPASS GRAFTS ≤ AGE 45: ATHEROTHROMBOSIS. J. Munjal, C.J. Glueck, D.M. Aregawi, P. Wang, The Jewish Hospital, Cincinnati, OH.

Although heritable thrombophilias and hypofibrinolysis predominantly cause venous thrombosis, they may also promote arterial thrombosis, synergistic with atherosclerosis, producing atherothrombotic cardiovascular (ATCVD) events. In 70 men and 16 women with ≥ 1 premature myocardial infarction ($n = 60$)-angioplasty ($n = 52$)-coronary artery bypass grafts ($n = 33$) (≤ age 45 years), we assessed whether and to what degree heritable thrombophilia-hypofibrinolysis contributed to 145 ATCVD events ≤ age 45. Hereditary thrombophilias studied by PCR included the G1691A factor V Leiden, G20210A prothrombin, MTHFR C677T-A1298C, and platelet glycoprotein PL A1/A2 mutations, with serologic studies of ACLA IgG and IgM, the lupus anticoagulant, proteins C and S, antithrombin III, homocysteine, and factors VIII and XI. Hypofibrinolysis studies included the 4G4G plasminogen activator inhibitor 1 mutation (PAI), PAI activity (PAI-Fx), and Lp(a). Cases were compared with healthy normal controls (149 men for PCR, 40 for serologic tests, 109 women for PCR and for serologic tests). At entry, hypertension was present in 46% of men and 31% of women, diabetes in 19% and 13%, and cigarette smoking in 26% and 13%, respectively. In the 70 male cases, mean ± SD age was 49 ± 11, BMI 29.5 ± 4.2, LDLC 102 ± 45, HDLC 41 ± 15, and TG 204 ± 229 mg/dL. In 16 female cases, mean ± SD age was 43 ± 7, BMI 28.1 ± 5.5, LDLC 103 ± 32, HDLC 45 ± 11, and TG 191 ± 170 mg/dL. Male cases were more likely than male controls to have factor V Leiden (6 of 60, 10% vs 4 of 149, 3%, $p = .035$), high Lp(a) (≥ 35 mg/dL) (25 of 69, 36% vs 7 of 40, 18%, $p = .039$), high PAI-Fx (> 21.1 U/mL) (15 of 61, 25% vs 3 of 39, 8%, $p = .036$), high (> 150%) factor VIII (15 of 57, 26% vs 1 of 38, 3%, $p = .003$), and high (> 150%) factor XI (9 of 55, 16% vs 0 of 38, 0%, $p = .0096$). Female cases were more likely then female controls to have high factor VIII (5 of 14, 36% vs 13 of 109, 12%, $p = .033$) and were more likely to have low free protein S (3 of 14, 21% vs 3 of 107, 3%, $p = .02$). In patients sustaining MI-angioplasty-CABG events ≤ age 45, we speculate that hereditary thrombophilias (factor V Leiden, factor VIII, factor XI, low free protein S) and hypofibrinolysis (PAI-Fx, Lp(a)) promote arterial thrombosis that may be synergistic with atherosclerotic endothelial injury. In patients with MI-angioplasty-CABG events ≤ age 45 and concurrent hereditary thrombophilia, we speculate that thromboprophylaxis may have value in secondary prevention of subsequent ATCVD.

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UNEXPLAINED SPORADIC FIRST-TRIMESTER MISCARRIAGE: FACTOR V LEIDEN GENE MUTATION. J. Munjal, L. Rovner, S. Gogineni, T. Tracy, P. Wang, C.J. Glueck, The Jewish Hospital, Cincinnati, OH.

Background: The propensity to form thrombi is physiologically increased in normal pregnancy secondary to reduction in naturally occurring anticoagulants, an increase in coagulation factors, and a reduction in fibrinolysis. We hypothesized that when the physiologic hypercoagulability of pregnancy is superimposed on the thrombophilic G1691A factor V Leiden mutation, sporadic first-trimester miscarriage and repetitive pregnancy loss are promoted. We hypothesized that the thrombophilic G1691A factor V Leiden gene mutation was a common, significant, treatable cause of sporadic first-trimester miscarriage. **Methods:** We compared the frequency of the G1691A factor V Leiden mutation in women with ≥ 1 pregnancy and 1 miscarriage with women having ≥ 1 pregnancy and 0 miscarriages. In 848 Caucasian women with consecutive measures of the factor V Leiden mutation, we compared the frequency of the V Leiden mutation in 136 women with ≥ 1 pregnancy and 1 miscarriage (260 live births, 136 miscarriages), 50 women with ≥ 1 pregnancy and 2 miscarriages (83 live births, 100 miscarriages), 53 women with ≥ 1 pregnancy and ≥ 3 miscarriages (recurrent pregnancy loss) (109 live births, 227 miscarriages), and 609 women with ≥ 1 pregnancy and 0 miscarriages (1,473 live births). We used PCR techniques to characterize the thrombophilic G1691A V Leiden [FV] gene mutation. **Results:** Of the 609 controls, 41 (6.7%) had FV heterozygosity versus 15 heterozygous and 2 homozygous FV cases (17 of 136, 12.5%) with 1 sporadic miscarriage, $\chi^3 = 5.2$, $p = .023$, vs 8 of 53 (15%, 7 heterozygous and 1 homozygous) with ≥ 3 miscarriages, Fisher's $p = .048$, vs 2 of 50 (4%) with 2 miscarriages. The V Leiden frequency in cases with 1 sporadic miscarriage (17 of 136, 12.5%) did not differ from recurrent pregnancy loss cases with ≥ 3 miscarriages (8 of 53, 15%), $\chi^2 = 0.22$, $p = .64$. **Conclusions:** After unexplained sporadic first-trimester miscarriage, as well as after recurrent pregnancy loss, to provide the option to prospectively optimize subsequent live birth outcomes with low molecular weight heparin throughout pregnancy, we suggest that measurements be done of the FV mutation, a treatable etiology for sporadic miscarriage, as well as for recurrent pregnancy loss.

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PROLACTIN ALTERS BCR-MEDIATED APOPTOSIS AND RECEPTOR EDITING. E. Peeva, G.E. Rosenfeld, J. Gonzalez, S. Saha, Albert Einstein College of Medicine, Bronx, NY.

Background: Autoreactive lymphocytes are constantly generated and eliminated to maintain tolerance. Two major mechanisms for tolerance induction are deletion and receptor editing. In the spleen, the majority of negative selection occurs in transitional B cells, mainly in the T1 B-cell subset, resulting in a T1:T2 ratio > 1. We have shown that prolactin-altered negative selection of autoreactive specificities allows for survival and activation of DNA-reactive B cells and development of lupus in mice that do not develop lupus spontaneously. In BALB/c mice, treatment with prolactin decreases the number of T1 B cells and increases the number of T2 B cells leading to a T1/T2 ratio < 1, which indicates that prolactin impairs negative selection. **Purpose:** The aim of this study is to characterize the mechanisms by which prolactin breaks B-cell tolerance. Deletion and receptor editing as means of negative selection were examined using a combination of flow cytometry and real-time PCR experiments. **Methods:** Eight- to 10-week-old female BALB/c mice were treated with prolactin (100 μg/d) or placebo (normal saline) for 1 month. Splenocytes were isolated, and RBC lysis was performed. Flow cytometric measurement of anti-IgM Ab-induced apoptosis was used to determine the effect of prolactin on BCR-mediated deletion in transitional T1 ($CD19^+AA4.1^+CD21^-CD23^-$) and T2 ($CD19^+AA4.1^+CD21^+CD23^+$) B-cell subsets. The effect of prolactin on receptor editing was evaluated by RAG-1 and RAG-2 mRNA expression and the presence of kappa/lambda-positive B cells. RNA was isolated by RNAeasy kit from B cells purified with Dynabeads. RAG-2 mRNA expression was determined by real-time PCR. Flow cytometry was used to determine the number of B cells coexpressing kappa/lambda light chains in the transitional T1 and T2 and mature marginal zone (MZ) ($CD19^+AA4.1^-CD21^+CD23^-$) and follicular (Fo) ($CD19^+AA4.1^-CD21intermediateCD23^+$) B-cell subsets. The real-time data were obtained by a Light Cycler real-time PCR machine. Flow cytometry data were acquired by an LSRII flow cytometer, and data analysis was done by *FlowJo* software. **Results:** After BCR stimulation with anti-IgM antibody (10 μg/mL) as a surrogate antigen, T1 B-cell subset from prolactin-treated mice showed significantly less annexin V–positive B cells than the T1 subset from placebo-treated mice ($p = .023$). As per our microarray data, this effect of prolactin on B-cell deletion may be mediated by prolactin-induced overexpression of the antiapoptotic molecules Bcl-2, Birc-1, and/or IFNalfaR. In addition, treatment with prolactin induced a two- and threefold increase in RAG-1 and RAG-2 mRNA expression in B cells ($p = .012$), as well as an elevated number of kappa/lambda-expressing B cells with T2 ($p = .028$) and Fo ($p = .001$) phenotype, an indication of continued receptor editing. **Conclusion:** Treatment with prolactin increases the resistance to anti-IgM-mediated apoptosis of T1 B-cell subsets, which, under normal circumstances, is a commonplace for negative selection of the autoreactive specificities. The failed ability to delete by apoptosis may explain the increased survival of self-reactive B cells destined for deletion. In addition, increased serum prolactin levels lead to increased receptor editing in splenic B cells, and the V(D)J recombination in the peripheral lymphoid organs has been associated with generation of pathogenic autoantibodies and autoimmunity. Thus, alterations of both BCR-mediated deletion and receptor editing may be implicated as mechanisms in prolactin-induced breakdown of B-cell tolerance.

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NRF2 REGULATES HYPEROXIA-MEDIATED NOX4 EXPRESSION AND REACTIVE OXYGEN SPECIES PRODUCTION. S. Pendyala, I.A. Gorshkova, D. He, H. Cho, S.R. Kleeberger, V. Natarajan, University of Chicago, Chicago, IL; Research Triangle Park, NC.

Rationale: We have demonstrated earlier that Nox4, a homologue of Nox2 (gp91phox), is highly expressed in human pulmonary artery endothelial cells (HPAECs) and involved in hyperoxia-induced reactive oxygen species (ROS) production and signal transduction. Nrf2 is a transcriptional factor that is activated in hyperoxia and is known to regulate a number of genes involved in antioxidant defense mechanisms in the lung. Here we have investigated the role of Nrf2 in regulating hyperoxia-induced Nox4 expression and ROS generation in HPAECs. **Methods/Results:** In HPAECs, mRNA expression of Nox4 is several-folds higher compared with Nox2 (gp91phox), and exposure of cells to hyperoxia (95% O2) resulted in up-regulation of expression of Nox4 and p22phox but not Nox1 or Nox3. Nrf2 is up-regulated in short-term (3 hours) hyperoxia as much as twofold. Down-regulation of Nrf2 mRNA with siRNA attenuated Nox4 expression in normoxic HPAECs; however, enhanced ROS generation under both normoxia and hyperoxia (3 hours). Exposure of Nrf2 wild-type mice to hyperoxia (100% O_2) for 24 and 48 hours resulted in enhanced Nox4 expression in the lung compared with normoxia. Further, $Nrf2^{-/-}$ mice exposed to hyperoxia (24 and 48 hours) showed decreased Nox4 expression in the lung

compared with normoxia. However, the expression of Nox2 was increased in Nrf2$^{-/-}$ mice exposed to hyperoxia. **Conclusion:** These results demonstrate that hyperoxia-induced Nox4 expression and ROS production is regulated by Nrf2 in lung endothelium.

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FLAGELLIN IS THE MAJOR STIMULUS FOR THE INDUCTION OF CYTOPROTECTIVE HEAT SHOCK PROTEIN HSP25 IN GUT EPITHELIUM. E. Petrof, M. Musch, M. Ciancio, J. Sun, M. Hobert, E. Claud, A. Gerwirtz, E. Chang, University of Chicago, Chicago, IL; Atlanta, GA.

Flagellin is a bacterial protein responsible for activation of toll receptor 5 (TLR5), which we hypothesize is involved in the induction of cytoprotective heat shock proteins in intestinal epithelial cells. Using flagellin derived from the bacterial pathogen, *Salmonella typhimurium*, our studies confirm that flagellin induces Hsp25 in different intestinal epithelial cell lines in a time- and dose-dependent manner. In addition, Hsp25 induction is observed only when flagellin is added to the basolateral side of polarized intestinal epithelial cells, consistent with the known location of TLR5. The ability of flagellin to induce Hsp25 expression is likely transcriptional as it is abolished by treatment with actinomycin D. It also requires p38 MAP kinase activation, and Western blot analysis indicates that treatment with the p38 MAP kinase inhibitor blocks Hsp25 production. Flagellin-mediated Hsp25 induction is associated with increased protective effects against oxidant stress, an effect that is at least partially mediated by p38 MAP kinase. Use of siRNA against Hsp25 demonstrates that flagellin-mediated protection against oxidant stress is at least partially mediated through Hsp25 induction. In a mouse model of *S. typhimurium* infection, not only does infection with wild-type and a flagellin-deletion mutant strain of *S. typhimurium* show that flagellin induces Hsp25 in vivo, it also demonstrates that the major stimulus for Hsp induction is actually flagellin and not LPS. These data provide evidence that flagellin is required for *Salmonella*-mediated induction of Hsp25 expression in intestinal epithelium. This may explain, in part, why more intestinal damage is seen in mice infected with the aflagellate strain, as opposed to the wild-type strain, of *S. typhimurium*. Flagellin-induced expression of Hsp25 may be one strategy used by the host to protect itself against the deleterious effects of *Salmonella* infection.

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ATORVASTATIN ATTENUATES LRP5/WNT-MEDIATED ATHEROSCLEROSIS AND OSTEOPOROSIS IN A MOUSE MODEL OF EXPERIMENTAL HYPERCHOLESTEROLEMIA. N.M. Rajamannan, M. Subramaniam, F.C. Caira, S. Stock, T. Hefferan, A. Flores, T.C. Spelsberg, Northwestern University, Chicago, IL.

Atherosclerosis and osteoprorosis are the leading causes of morbidity and mortality in the aging population in the United States. Evidence indicates that hyperlipidemia plays a paradoxical role in these disease processes. However, the hyperlipidemic mechanisms of atherosclerotic calcification and decrease bone mass are not well understood. We have previously shown that cardiovascular calcification expresses an Lrp5/Wnt-mediated osteoblast phenotype in humans. We hypothesize that hyperlipidemia plays a role in cardiovascular calcification and osteoporosis via reagulation of the Lrp5/Wnt pathway. We propose to test this hypothesis in an experimental hypercholesterolemia model and further test if statins play a protective role in this process LDLR$^{-/-}$ mice ($n = 60$). Group I ($n = 20$) normal diet, group II ($n = 20$) 0.2% chol diet (w/w), and group III ($n = 20$) 0.2% (w/w) chol diet + atorv for the development of calcification. The aortic valves and aortas (AVAs) were examined for proliferation, calcification, Lrp5/Wnt, and bone matrix markers. Bone formation was assessed by micro–computed tomography (microCT), calcein injection, osteocalcin, and cbfa-1 and osteopontin expression. **Results:** The cholesterol diet induced complex bone formations by microCT in the calcified aortic valves and aortas with an increase in cellular proliferation, osteopontin, osteocalcin, Lrp5, and cbfa-1 expression. Atorvastatin reduced bone formation, cellular proliferation Lrp5, and cbfa-1 levels in the AVAs. Ex vivo analysis of calcein label demonstrated an increase in calcein in the hypercholesterolemia AVAs and bones with attenuation of the label with atorvastatin in these tissues. The cholesterol-treated femurs demonstrated an increase in bone resorption, with a decrease in Lrp5/Cbfa1. The cholesterol-treated femurs demonstrated an increase in calcein incorporation. **Conclusion:** Hypercholesterolemic AVA calcification and bone turnover are attenuated by atorvastatin and are mediated in part by an Lrp5/Wnt pathway. This model may have future implications in the treatment of cardiovascular calcification and osteoporosis with statin therapy.

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EFFECTS OF INSULIN AND FREE FATTY ACIDS ON ASYMMETRIC DIMETHYL ARGININE, A NOVEL CARDIOVASCULAR RISK FACTOR. S.S. Shankar, R. Shankar, R.V. Considine, H.O. Steinberg, Indiana University, Indianapolis, IN.

Background: Asymmetric dimethyl L-arginine (ADMA) has emerged as a novel cardiovascular risk factor. ADMA has been shown to induce endothelial dysfunction. Further, plasma ADMA levels are tightly regulated in healthy individuals and have been found to be increased in type 2 diabetes as well as other insulin-resistant states. However, the mechanisms of regulation of ADMA levels are still unknown. Blunting of insulin's ability to induce glucose uptake and a concomitant elevation of free fatty acids are metabolic hallmarks of insulin resistance and type 2 diabetes. **Hypothesis:** We hypothesized that insulin and free fatty acids play a role in regulating ADMA levels. **Methods:** Toward this end, we performed two sets of independent experiments in two groups of healthy, normotensive subjects with normal glucose tolerance. (A) Group A: We studied 8 healthy subjects (age 31 ± 4 years, BMI 24 ± 1, body fat 20.4 ± 4.1%, LDL cholesterol 82 ± 10 mg/dL, HDL cholesterol 50 ± 13 mg/dL, mean arterial pressure 93 ± 3 mm Hg). Subjects underwent a 240-minute hyperinsulinemic euglycemic clamp (120 mU/m^2/min). (B) Group B: We studied 6 healthy subjects (age 33 ± 3 years, BMI 23.7 ± 0.5, LDL cholesterol 125 ± 14 mg/dl, HDL cholesterol 48 ± 4 mg/dL, mean arterial pressure 91 ± 2 mm Hg). Subjects underwent a 360-minute infusion of Intralipid 20% with heparin 0.2 units/kg/min. Plasma ADMA concentrations were measured at baseline in both groups, at steady-state hyperinsulinemia in group A, and at the end of 360 minutes of infusion of free fatty acids in group B. ADMA concentrations were determined by reverse-phase HPLC. **Results:** Group A: Subjects had normal insulin sensitivity, with glucose disposal rates of 8.1 ± 1.1 mg/kg/min. Plasma ADMA levels were 0.42 ± 0.05 μM at baseline and decreased significantly in response to insulin to 0.30 ± 0.05 μM at steady state of the clamp ($p < .05$). The fall in ADMA levels correlated significantly with the steady-state glucose disposal rates ($r = .7$). Group B: Plasma ADMA levels were 0.39 ± 0.12 μM at baseline and rose significantly to 0.58 ± 0.14 μM at the end of the 360-minute infusion of free fatty acids ($p < .05$). **Summary:** Our results demonstrate for the first time that (a) insulin acutely lowers ADMA levels, (b) the decrease in ADMA in response to insulin appears to be related to the magnitude of insulin-mediated glucose uptake, and (c) free fatty acids acutely increase ADMA levels. **Conclusions:** We conclude that insulin and free fatty acids appear to regulate ADMA; further, blunting of the effect of insulin to lower ADMA and a concomitant exaggeration of the effect of free fatty acids to increase ADMA may, in part, explain the elevated ADMA levels and, in turn, both the endothelial dysfunction and the heightened cardiovascular risk in insulin resistance and type 2 diabetes.

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SUBVERSION OF TYPE I INTERFERON ANTIVIRAL DEFENSE IN HUMAN AIRWAY EPITHELIAL CELLS BY ADENOVIRUS INFECTION. L. Shi, M. Ramaswamy, L.J. Manzel, D.C. Look, University of Iowa Carver College of Medicine, Iowa City, IA; San Diego, CA.

A prerequisite for successful viral invasion and replication in host cells is a mechanism for avoiding antiviral defense, particularly those regulated by interferons. Therefore, viral evolution has generated mechanisms to resist host cell antiviral systems, but the biochemical basis for evasion of interferon (IFN) effects in the airway by adenoviruses is incompletely understood. To identify the molecular mechanisms for adenoviral inhibition of IFN-dependent antiviral immunity, we examined adenovirus type 5 (AdV) effects on expression of type I IFN-dependent genes and on levels and activation of Janus family kinase-signal transducer and activator of transcription (JAK-STAT) signaling components in primary cultures of human airway epithelial cells. We found that wild-type AdV infection inhibited IFN-α-induced expression of the antiviral proteins MxA, Stat1, and PKR using immunoblot analysis of cell lysates from uninfected and virus-infected airway epithelial cells. Replication-deficient AdV-d312 did not have this capacity. Similarly, using real-time RT-PCR analysis of RNA levels, we found that IFN-α-induced mRNA expression for these antiviral genes was also inhibited by AdV, suggesting that the virus caused a global blockade in type I IFN-dependent gene expression. This conclusion was confirmed when we found that infection with AdV for 12 hours or greater inhibited subsequent IFN-α-dependent phosphorylation and nuclear translocation of the Stat1 and Stat2 transcription factors that are required for activation of type I IFN-dependent genes. There was no evidence of epithelial cell injury, generation of a soluble extracellular inhibitor, or altered type I IFN receptor chain 1 or 2 cell surface expression, suggesting that viral effects were mediated inside epithelial cells. Further examination of the type I IFN-activated JAK-STAT pathway revealed that AdV infection also caused a loss of IFN-α-induced phosphorylation of the receptor-associated Jak1 and Tyk2 tyrosine kinases. This global blockade of signaling pathway activation appeared to occur through modulation of Jak1 expression by adenoviral down-regulation of Jak1 mRNA levels in epithelial cells. AdV infection inhibited other Jak1-dependent signaling cascades in airway epithelial cells, including interleukin (IL)-6-dependent phosphorylation of Stat3. In contrast, IL-4- and IL-13-dependent phosphorylation of Stat6 was not affected during AdV infection, and these pathways can function independently of Jak1, indicating that the virus modulates specific signaling pathways. Based on these and previous results, AdV inhibits type I IFN-induced gene expression through at least two mechanisms: (1) expression of the E1A oncoprotein that inhibits IFN-induced transcription factors within the first few hours of infection and (2) down-regulation of the expression of Jak1 after 12 hours of infection. These findings suggest that AdV uses multiple strategies to ensure inhibition of antiviral effects of IFN throughout the viral replication cycle to subvert the airway immune response and establish a productive infection.

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COVERT VISUAL ATTENTION AND MOVEMENT PREPARATION IMPAIRMENTS IN EPILEPSY. E.K. St. Louis, J. Dennhardt, S.J. Luck, University of Iowa, Iowa City, IA; Davis, CA.

Background/Rationale: Identification of subtle cognitive impairments that may adversely affect quality of life in epilepsy is often difficult. The N2pc and lateralized readiness potential (LRP) event-related potential (ERP) paradigms are capable of detecting and quantifying covert, real-time neural processing with exquisite temporal sensitivity. We hypothesized that the N2pc and LRP are delayed in epilepsy. **Methods:** Ten epilepsy subjects (4 mesial temporal, 1 extratemporal, 3 idiopathic generalized, and 2 new onset, each receiving one to three AEDs) and 20 controls underwent ERP recording from standard 10-20 electrode sites while performing a feature-conjunction visual-search task. Data analysis compared ERP waves from equivalent channels ipsilateral and contralateral to the target using Student's *t*-test, ANOVA, and jackknifing data resampling procedures. **Results:** N2pc peak latency was significantly delayed in epilepsy subjects by approximately 35 msec ($p < .016$; data shown in the Table and Figure). LRP peak latency was also significantly delayed in epilepsy subjects. P1, N2, and P3 latencies did not differ between epilepsy and control subjects. **Conclusions:** There is a significant delay in visual attentional processing in epilepsy subjects and additional impairment of movement preparation but not categorization. Although subtle and covert to bedside detection, these additive delays in the first half-second of neural processing could underlie subjectively impaired cognitive functioning in epilepsy patients and substantially impact their performance during demanding psychomotor tasks requiring rapid reaction time, such as driving. Future research will focus on determining the causes of these delays in cognitive processing, including epilepsy syndrome, brain lesions, and antiepileptic drugs.

Delayed N2pc and LRP ERP Responses in Epilepsy

ERP Component	*EEG Channels*	*Control Avg (msec)*	*Epilepsy Avg (msec)*	*Difference (msec)*	p *Value*
P1	O2	82.8	87.2	4.4	NS
N2pc	OL/R	260.0	293.6	33.6	.016
N2pc	T5/6	268.0	306.4	38.4	NS
P3	P3/4	428.0	449.3	21.3	NS
LRP	C3/4	580.4	627.6	47.1	.001

Statistically significant prolongations of mean N2pc and LRP fractional peak latencies were seen in epilepsy subjects in occipital and central derivations. No delay occurred during categorization (P3).

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MITOCHONDRIAL BINDING AND GLUCOSE PHOSPHORYLATION ARE BOTH NEEDED FOR THE PROTECTIVE EFFECTS OF HEXOKINASE I AND II. L. Sun, S. Shukair, F. Moazed, T. Naik, H. Ardehali, Northwestern University, Chicago, IL.

Alterations in glucose metabolism have been demonstrated in diverse disorders, ranging from heart disease to cancer. The first step in glucose metabolism is carried out by the hexokinase (HK) family of enzymes. Overexpression of HKI and HKII in tissue culture protects against oxidant-induced cell death. The protective effects of these enzymes are thought to be due to either an increase in glucose phosphorylation or closure of the mitochondrial permeability transition pore (mPTP) as a result of HK binding to the voltage-dependent anion channel (VDAC) on the mitochondria. VDAC is believed to form part of mPTP, the opening of which causes cellular injury. The relative contribution of HK binding to the mitochondria and the increase in glucose phosphorylation to the overall protective effects of HKs are not clear. Furthermore, there is no clear evidence supporting the hypothesis that HK