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