desaturase 1 (SCD1), and fatty acid synthase (FAS) at the protein and mRNA levels, as well as the incorporation of palmitate into adipose triglycerides. This coincides with the rapid suppression of sterol regulatory element binding protein 1c (SREBP-1c) and peroxisome proliferator–activated receptor (PPARγ) mRNA in adipose tissue, independent of circulating insulin and glucose levels. In a series of studies in which we selectively obliterated the STAT3 or PI3K pathway of the leptin receptor in the hypothalamus using either a cell-permeable peptide inhibitor of STAT3 or the PI3K inhibitor LY294002, we found that the effects of MBH leptin on adipose tissue lipogenesis are dependent on the central activation of PI3K but not STAT3. We further analyzed the body composition in a genetic model (s/s mice) in which the leptin receptor carries a S1138A mutation that renders it unable to signal through STAT3 while leaving its other proximal signaling pathways intact and compared it with db/db mice (complete inactivation of all leptin receptor signaling). Interestingly, after 2 months of pairfeeding, the s/s mice have lower body fat but conserved lean body mass, further supporting the hypothesis that STAT3-independent signaling pathways regulate adipocyte lipogenesis. These findings contrast with our recent work that demonstrated that the control of hepatic glucose fluxes, food intake, and gonadotropin secretion by central leptin critically depends on intact STAT3 signaling. Thus, this work unveils a crosstalk between STAT3-independent signaling in the MBH and adipose tissue lipid metabolism that occurs independently of food intake or circulating insulin and glucose levels. Furthermore, these results demonstrate that central pathways can rapidly modulate the expression of transcription factors such as SREBP1c and PPARγ by as yet unidentified mechanisms.

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ERK, P38, AND JNK SIGNALING PATHWAYS ARE IMPORTANT IN CHEMOKINE AND CYTOKINE INDUCTION BY *BACILLUS ANTHRACIS* SPORES IN A HUMAN LUNG SLICE MODEL. K. Chakrabarty, J. Booth, E.S. Duggan, K. Coggeshall, J.P. Metcalf, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Bacillus anthracis, the causative agent of inhalational anthrax, enters a host through the pulmonary system before disseminating throughout the body. Our previous work has shown that human alveolar macrophages play a critical role in the initial innate immune response to *B. anthracis* spores through cell signal–mediated cytokine release. We propose that the lung epithelia also play an important role in the innate immune response to pathogens, and we have developed a human lung slice model to study this process. Exposure of our lung slice model to *B. anthracis* (Sterne) spores caused rapid activation of the mitogen-activated protein kinase signaling pathways ERK, P38, and JNK. This was followed by an increase in mRNA of several cytokines and chemokines. This was reflected on a translational level with a peak fold increase of TNF-α, IL-6, IL-8, MIP-1α, and the MCP-1 protein of 25, 3, 9, 34, and 5, respectively, as determined by ELISA. Inhibition of individual pathways by the signaling inhibitors UO126, SP 600125, and SB 0203580 was not sufficient to block induction of chemokines and cytokines to background levels. When the three inhibitors were combined, induction of IL-6 and IL-8 was completely blocked and of MCP-1 and MIP-1α was partially blocked. Taken together, these data show activation of pulmonary epithelium in response to *B. anthracis* spore exposure. Thus, the lung epithelia actively participate in the innate immune response to *B. anthracis* infection through cell signal–mediated elaboration of cytokines and chemokines.

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ENDOTHELIAL BARRIER REGULATION BY SIMVASTATIN: ROLE OF RHO, RAC, AND NADPH OXIDASE. W. Chen, J.R. Jacobson, J.N. Garcia, University of Chicago, Chicago, IL.

The statins are a class of HMG CoA-reductase inhibitors used clinically for their ability to lower serum cholesterol; however, not all of their clinical benefits, including enhanced endothelial cell (EC) barrier function, 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, including Rho and Rac, although we have previously reported the paradoxical activation of cytosolic Rac by simvastatin. While the inhibition of Rho attenuates actin stress fiber formation, promoting EC barrier function, the inhibition of Rac at the cell membrane prevents activation of NADPH oxidase and subsequent superoxide generation, known to be EC barrier disruptive. We sought to determine the relative regulatory effects of simvastatin on Rac and NADPH oxidase activities in the context of EC barrier protection. Human pulmonary artery ECs treated with simvastatin (5 μM, 16 hours) were found to have a significant decrease in membrane Rac (38% decrease), consistent with the inhibition of geranylgeranylation. Using a FITC-dextran transwell permeability assay, concomitant treatment of EC with xanthine (200 μM, 1 hour) and xanthine oxidase (30 mU/mL, 1 hour) to generate superoxide resulted in barrier disruption that was attenuated by simvastatin (5 μM, 16 hours, 49% decrease), consistent with the inhibition of NADPH oxidase. Moreover, LPS-induced (1 μg/mL) superoxide production measured by DHE fluorescence was also significantly reduced by simvastatin (50% decrease). Finally, compared with simvastatin treatment (5 μM, 16 hours), thrombin-induced permeability (1 U/mL, 5 minutes) was only modestly attenuated by the inhibition of Rac via siRNA (20% as effective as simvastatin), whereas the use of the Rho inhibitor Y-27632 (10 μM, 30 minutes) affected a more pronounced attenuation (70% as effective as simvastatin). These data suggest that EC barrier protection by simvastatin, although largely due to Rho inhibition, is also attributable to the inhibition of Rac at the cell membrane and the subsequent attenuation of superoxide generation by NADPH oxidase. Our findings contribute to defining mechanisms by which simvastatin modulates EC barrier properties, which may lead to new clinical applications.

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ESOPHAGEAL DYSMOTILITY IN EOSINOPHILIC ESOPHAGITIS: ANALYSIS USING HIGH-RESOLUTION ESOPHAGEAL MANOMETRY. J. Chen, S.K. Ghosh, J. Pandolfino, P.J. Kahrilas, I. Hirano, Northwestern University, Chicago, IL.

Background: Eosinophilic esophagitis (EE) is an increasingly recognized cause of dysphagia and food impaction. In many cases, strictures are not apparent on endoscopy, raising questions as to the mechanism of impaired deglutition. Prior reports have documented eosinophilic infiltration of the muscularis propria and myenteric plexus that could induce esophageal dysmotility. **Aim:** Characterize esophageal motor function in EE using high-resolution esophageal manometry (HRM) and newly described manometric parameters. **Methods:** Twenty-four patients with EE were studied with a 36-channel solid-state HRM assembly and analyzed using *ManoView* software (Sierra Scientific). Analysis was based on 10 5 mL water swallows per patient. Esophageal peristalsis was quantified by distal esophageal body peristaltic point velocity and pressurization front velocity (PFV), which was the propagation rate of an intact 30 mm Hg pressure wave. In the absence of a continuous propagation wave, a swallow was classified as a null PFV. A patient was classified as null, normal, or elevated PFV based on the dominant pattern (6) of 10 swallows. Esophagogastric junction (EGJ) relaxation was quantified using the lowest mean residual pressure over a 3-second interval (E-sleeve relaxation) and integrated relaxation resistance (IRR). A higher IRR signifies impaired EGJ relaxation and consequently higher resistance to esophageal emptying. All abnormal HRM values were referenced to the 95% upper limit of normal values derived from 75 controls. **Results:** The median patient age was 42 years (range 14–80 years). The most common presenting symptoms were dysphagia (83%) and heartburn (12.5%). Endoscopic findings included rings (50%), furrows (58%), and exudates (33%). On HRM, 14 patients (58%) had increased distal segment contraction velocity (median 8.4 cm/s), whereas 3 patients (12%) had an elevated PFV (median 4.2 cm/s). Two patients had elevated intrabolus pressures as evidenced by an elevated PFV but normally propagated peristaltic contraction. Nine patients were classified as having a null PFV and only one had significantly elevated esophageal contractile pressures (295 mm Hg). Seven patients had an elevated IRR (median 3.3 mm Hg/s), and of this group, 5 patients also had increased E-sleeve relaxation pressures (median 18 mm Hg). **Conclusions:** (1) Manometric manifestations of EE are heterogeneous. (2) An elevation in esophageal peristaltic velocity was the most common abnormality. (3) Subsets of EE patients demonstrated failed esophageal peristalsis (null PFV) and impaired EGJ relaxation (elevated IRR). (4) Functional abnormalities on the basis of neuromuscular involvement could contribute to dysphagia in EE.

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CHRONIC FATIGUE IN THE GENERAL POPULATION: HIGHER LEVELS OF NEUROVASCULAR TONE. A. Cho, R. Barndt, N. Mina, Bethel Public Service Clinic, Downey, CA; ; Drexel University College of Medicine, Philadelphia, PA; Detroit, MI.

Our pilot studies (PSs) show that chronic fatigue (CF) is a problem in the general population and is associated with higher adrenergic neurovascular tone (ANVT). ANVT is measured by systolic time intervals (STI = PEP/LVET × 100%). Baseline (low stress level) ANVT is predicted by temperance analysis testing (r = .98, p < .01). ANVT can be increased by pain, stress, certain foods, and sympathomimetic drugs, which were avoided during the study. In our prospective studies, as with PSs, a random sample of the general population was acquired using patients with a normal distribution of STI values (25–56%). Patients were 3/1 women/men, age range 30 to 65 years. Exclusions were patients with elevated C-reactive protein, HbA_{1c} > 6.0, depression, fibromyalgia, and smokers. PS criterion of STI at 25 to 36% was used to identify CF patients with significantly higher symptom levels (SL = 1–100 scale of fatigue scored by patient) versus normal age-matched controls (C). Blind correlations were made by serial measurements of systolic blood pressure (SBP), SL, cardiac output (CO), and systemic vascular resistance (SVR) by two-dimensional echocardiography. This was done at baseline (time 1 = T1, without significant external stress) and during treatment (Rx) (T2). T2 was 1 year for group (G)1 and G2 and at 6 months for G3. All data were placed into a blind matrix for later analysis. Patients were grouped by STI ranges (G1 25–30%, G2 31–36%, G3 a random sample of patients 25–36%, and C 50–56%) using PS guidelines. Rx of G1 and G2 CF patients consisted of amitriptyline (10–50 mg/d) and diltiazem CD (240–360 mg/d) and 500 mg calcium/d. G3 patients received 1,500 mg of calcium/d without other medications. Prospective results: Group means are shown. See Table. Where * = significantly different from C at p < .01 by t-test. ** = Significant (**sig) change from T1 to T2 at p < .01 by t-test. CF was found in 28% of the random sample of the general population. G1, G2, and G3 had significantly lower STI and CO with significantly higher SL at T1 versus C. Rx significantly reduced SL and SVR and increased STI and CO in G1 and G2. G3 patients had sig** increase in SL with conventional calcium therapy. This was associated with sig** increases in SVR, with sig** decreases in STI and SBP. G3 patients had sig** increases in CF and classic symptoms of fibromyalgia according to the criteria of the American College of Rheumatology. These studies show the importance of measurement of CO and SVR in addition to BP measurement using the standard formula for SVR. CF occurs in the general population owing to higher ANVT without inflammatory disorders. Thus, reduction in ANVT and increased CO significantly reduces SL in CF.

G	*#*	*STI T1/2*	*SBP T1/2*	*CO T1/2*	*SVR T1/2*	*SL T1/2*
1	100	33*/44**	105/111	3.5*/4.5**	1,646*/1,387**	30*/4**
2	100	27*/43**	100/112**	2.8*/4.4**	1,914*/1,436**	60*/6**
3	50	31*/22**	103/91**	3.1*/2.2**	1,935*/2,109**	46*/92**
C	100	53/54	110/109	5.5/5.7	1,125/1,071	0/0

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ENDOTHELIN 1 DECREASES LUNG EDEMA CLEARANCE IN ALVEOLAR EPITHELIAL CELLS VIA ENDOTHELIAL ET-B RECEPTOR ACTIVATION AND NITRIC OXIDE GENERATION. A. Comellas, A. Briva, M. Butti, J. Chen, J. Litvan, Z. Azzam, E. Lecuona, L. Pesce, M. Yanagisawa, J.I. Sznajder, Northwestern University, Chicago, IL; Dallas, TX.

Rationale: In models of acute lung injury, high levels of endothelin 1 (ET-1) are linked with a rapid increase in edema formation. It has been shown that decreased alveolar fluid clearance is associated with increase hospital mortality in patients with acute lung injury. We hypothesized that ET-1 via ET-B receptor activation and nitric oxide (NO) generation impairs the ability of the lung to reabsorb fluid from the alveolar space by inhibiting the alveolar epithelial Na,K-ATPase. **Methods:** (A) Isolated-perfused rat lung model: Alveolar fluid clearance was measured using an isolated-perfused rat lung model by determining the changes in concentration of Evans blue–tagged albumin in the instillate as a function of time. (B) Alveolar epithelial cells (AECs) were isolated from pathogen-free male Sprague-Dawley rats, treated with ET-1 to assess Na,K-ATPase activity by an ouabain-sensitive 86Rb+ uptake and protein analysis by Western blotting. (C) Human microvascular endothelial cells cocultured in six-well plates with AECs in the presence and absence of endothelin. (D) Immunocytochemistry performed in AECs and rat lung tissue. **Results:** Isolated rat lungs perfused for 60 minutes with different concentrations of ET-1 (10–10 M to 10–6 M) had a decrease in alveolar fluid reabsorption in a dose-dependent fashion. A nonselective ET-A/B receptor antagonist blocked the endothelin decrease in lung edema clearance. An ET-B receptor agonist decreased alveolar fluid clearance to a similar degree compared with ET-1 (≈50%). When ET-1 was perfused in vascular endothelin B receptor–deficient rats, the decrease in alveolar fluid clearance was prevented. ET-1 decrease in alveolar fluid clearance was also blocked by a nitric oxide antagonist (L-NAME) and cGMP antagonist (ODQ). Neither the Na,K-ATPase activity nor its plasma membrane expression was affected in vitro when AECs were directly incubated with endothelin. However, coculture with endothelial cells in the presence of endothelin caused a decrease in Na,K-ATPase activity in AECs. **Summary:** We provide for the first time evidence that the endothelium regulates alveolar fluid clearance; specifically, ET-1 impairs the ability of the lung to clear edema via the endothelial ET-B receptor activation, nitric oxide generation, and cGMP signaling in AECs.

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