G	#	AC 1/2	AS 1/2	ASBP 1/2	PP 1/2	RPPHG 1/2	%SCA 1/2
C	100	16/6	6/6	110/110	40/40	9/9	1/1
L1	50	16/25	6/14*	111*131*	40/51*	23*/24	0/21*
L2	50	16/16	6/6	110/111	40/40	23*/8	1/1
M	50	24*/16	15*/6	126*/110	50*/40	21*/9	20*/1
Md	50	32*/16	26*/7	136*/114	56*/40	22*/8	35*/1
MdS	50	45*/17	42*/7	147*/116	67*/40	21*/9	58*/2

23

CALCIUM THERAPY CAUSES PROGRESSION OF CORONARY STENOSIS IN TYPE A WOMEN. R. Barndt, S. Jagtap, N. Mina, A. Stavrakis, A.Y. Cho, M. Castandi, Bethel Public Service Clinic, Downey, CA; Drexel University College of Medicine, Philadelphia, PA; Detroit, MI; Los Angeles, CA.
Our pilot study (PS) shows calcium therapy (Rx) causes systolic hypertension (SBP > 120 mm Hg) and coronary stenosis (CS) in type A women (TA) on calcium hormonal replacement therapy (CHRRx). Low systolic time intervals (STI < 40) and TA behavior were predicted in general population screening by a significant (Sig* at $p < .01$ by t-test = TT) rise in pulse pressure (PP) to handgrip (RPPHG > 15 mm Hg at 5 PSI, 3 minutes). Both identify high adrenergic neurovascular tone (ANVT with STI = PEP/LVET × 100%). STI predicted TA behavior test results ($r = .98$, $p < .001$). In prospective studies, normal TA women were randomized into two groups (G1, G2) with type B (TB) women as controls (C). G1, G2, and C had normal systolic blood pressures (SBP = 110 ± 10), with no significant coronary stenosis by ultrasonography using methods previously reported by our clinic (see Table below) at time 1 (T1 = start). General population screening revealed 40% of the population (500/1250) on CHRRx had systolic hypertension and Sig* PP increase predictive of Sig* %CS by previous PS equations. G3 was randomly selected (200 of 500) TA women on CHRRx for 10 years versus TB behavioral C, also on therapy for 10 years. All study patients were age 55 ± 5 years old and had LDL < 130, HbA_{1c} < 6.1, Hgb > 13, normal serum tryglycerides (< 145 mg%), and normal C-reactive protein levels and were nonsmokers. All Gs were treated each day (qd) with 1,500 mg calcium, 0.625 mg estrogen, and 2.5 mg progesterone with informed consent. G2 and G3 also had Rx (Rx2) qd of amitriptyline 10 to 50 mg, Tenormin 13 to 100 mg, and diltiazem CD 240 to 360 mg to reduce ANVT, SBP, STI, and systemic vascular resistance (SVR) to CG levels. Serial measurements were made at T1 and T2 (1/2) (T2 = 6 years) in all Gs (G3 = 10 years). Serial measurements were made of SBP, PP, RPPHG, STI, SVR, AS (aortic stiffness), AC (aortic collagen = %AC/area), and maximum %CS with ultrasonic measurements by methods previously reported by our clinic in standard units. In PS, the greatest degree of stenosis and change was found in the left anterior descending (LAD). Quality of life (QL 1–100) was assessed. Data were placed into a blind matrix for analysis later. Prospective results by G analysis: G means shown. See Table. Where * = Sig difference from CG at $p < .01$. ** = Sig change from T1 to T2 at $p < .02$ both by TT. G1 reveals **Sig progression of AC and maximum %CS in the LAD. Multiple regression analysis revealed SBP and SVR (reflected by STI) as the Sig** risk factors predictive of CS (mR = 0.97, $p < .001$ in G1 at T2 and G3 at T1). G2 (a matched G to G1) on Rx2 demonstrates *Sig prevention of progression of AC and %CS compared with G1. G3 at T2 shows Sig** regression of AC and %CS due to SBP, SVR, and STI reduction to C levels. This study demonstrates that systolic hypertension (SBP > 120) and high SVR are significant vascular risk factors during CHRRx in TA women. Thus, control of SBP (< 121 mm Hg), SVR (< 1,600 standard units), and STI (> 45) is necessary for the prevention or regression of AC and %CS during calcium Rx.

G	#	SBP 1/2	RPP 1/2	STI 1/2	SVR 1/2	AS 1/2	PP 1/2	AC 1/2	%CS 1/2	QL 1/2
1	56	110/142**	15*/22**	33*/24**	1540*/1700**	6/17**	40/62**	17/37**	0/32**	60*/65**
2	75	108/110	15*/9**	34*/50**	1500*/1232**	6/6	40/40	17/17	1/0	96/94
3	200	151*/113**	23*/10**	24*/46**	2225*/1280**	30*/8**	71*/43**	48*/20**	62*/2**	62*/92**
C	100	111/110	10/9	53/52	994/1009	6/6	40/40	17/17	0/1	98/97

24

PARADOXICAL CHANGES IN INSULIN TOLERANCE IN MICE WITH NONALCOHOLIC FATTY LIVER DISEASE INDUCED BY SEDENTARY ACTIVITY AND AN AMERICAN FAST FOOD–TYPE DIET. M. Basaranoglu, L.H. Tetri, E.M. Brunt, B.A. Neuschwander-Tetri, Saint Louis University, St. Louis, MO.
Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent forms of chronic liver disease in the United States. Insulin resistance plays a central role in both the development and progression of NAFLD. Contributing factors to this may include the increasingly sedentary lifestyle of the population and increased consumption of a high-fat diet and high-fructose corn syrup (HFCS). The aim of this study was to characterize the glucose and insulin tolerance of sedentary mice fed a diet similar in composition to commonly consumed fast food (FFD). **Methods:** Male C57/BL6 mice ($n = 10$ in each treatment group) were fed ad libitum a FFD diet containing trans-fats (Harlan-Teklad, 43% of calories from fat) and water containing HFCS equivalent (6 g/kg/d) or standard chow and water. To promote sedentariness in the FFD group, the cages' wire racks were removed. The insulin tolerance tests were performed after 6 hours of food deprivation at 4, 8, and 12 weeks of feeding; regular human insulin (1 U/kg) was injected intraperitoneally, and blood glucose was measured at 0, 20, 40, and 60 minutes. Glucose tolerance test was performed at 8 weeks by the administration of glucose 1 g/kg intraperitoneally after 12 hours of food deprivation. Blood glucose was measured at 0, 20, 40, 60, and 150 minutes. **Results:** Hepatic steatosis increased progressively over 8 weeks in a distinctly zone 1 to zone 3 distribution pattern, similar to pediatric NAFLD. At 8 weeks, the triglyceride content of the FFD livers was 24 μg/mg (SD 8.0) and the control triglyceride content was 8.2 μg/mg (SD 1.6) ($p < .01$). Baseline fasting glucose levels were higher in the FFD mice than controls throughout the study period (at weeks 4, 8, and 12; $p < .05$). As shown in the Table below, the blood glucose levels after insulin injection were paradoxically lower in FFD mice than controls in mice fed for 4 and 8 weeks; in contrast, the blood glucose levels after insulin injection in mice fed the FFD for 12 weeks were higher than controls, suggesting impaired glucose tolerance developed by this later time point in sedentary mice fed the FFD. Glucose tolerance testing showed substantially higher glucose levels in the FFD mice after 8 weeks of feeding, indicating earlier onset of impaired glucose tolerance than insulin resistance. The differences were significant at 20, 40, 60, and 150 minutes ($p < .01$). **Conclusions:** The increased insulin responsiveness following 4 or 8 weeks but not 12 weeks of sedentary activity and feeding FFD might indicate initially increased insulin sensitivity or, alternatively, impaired insulin clearance or impaired counterregulatory mechanisms against low glucose levels. After 12 weeks, impaired insulin responsiveness was found. These findings might be explained by the following: (1) the unique metabolism of fructose in the liver because fructose is a precursor for triglyceride synthesis and only a small amount of fructose enters into the systemic circulation, (2) impaired insulin clearance, (3) increased oxidant stress in the liver, (4) impaired islet cell function in the pancreas, or (5) insulin resistance develops in the liver and adipose tissue earlier than in muscle. Because insulin tolerance testing is a measure of muscle glucose uptake, it is also possible that increasing fat content in muscle over time might cause peripheral insulin resistance by week 12 in this model.

Insulin Tolerance Test Glucose, % of Time 0 (± SD)

	20 min	40 min	60 min
FFD-4 wk	42 ± 15*	36 ± 15*	42 ± 12*
Ctrl-4 wk	62 ± 14	69 ± 13	87 ± 27
FFD-8 wk	54 ± 23*	42 ± 22*	46 ± 19
Ctrl-8 wk	78 ± 34	64 ± 25	78 ± 44
FFD-12 wk	55 ± 5	50 ± 11	56 ± 12*
Ctrl-12 wk	56 ± 18	41 ± 19	33 ± 10

*$p < .05$.

25

EFFECT OF ACETALDEHYDE UPON CATHEPSIN G AND MAST CELL CHYMASE: NON–RENIN-ANGIOTENSIN SYSTEM IMPLICATIONS. A.S. Brecher, R.C. Dubord, Bowling Green State University, Bowling Green, OH.
Hypertension is commonly observed in alcoholics. Both the renin-angiotensin system and the non–renin-angiotensin system (NRAS) have been implicated in the dynamics for the maintenance of blood pressure. Acetaldehyde has earlier been reported to enhance the generation of the rate-limiting angiotensin I (Ang I) in bilaterally nephrectomized rat plasma and to inhibit the activity of several angiotensinases (A, B, and M) in human serum, thereby promoting a hypertensive set of reactions. In the current study, the effect of acetaldehyde upon cathepsin G and mast cell chymase has been investigated. Acetaldehyde at 223.5 down to 11.2 mM concentrations enhanced cathepsin G activity at all levels employed in a statistically significant manner. Since cathepsin G is one of several enzymes transforming Ang I into Ang II and is also capable of cleaving Ang II directly from angiotensinogen, it is suggested that alcoholism, which will generate exogenous acetaldehyde from ingested alcohol, may be a contributory factor for an elevated cathepsin G activity and, consequently, hypertension via the NRAS. Mast cell chymase activity also is elevated upon exposure to 440 mM acetaldehyde and is diminished with 27 mM acetaldehyde. Since both enzymes also degrade Ang II, degradative effects may be partially neutralized.

26

EFFECT OF POLYAMINES UPON BLOOD COAGULATION: POSSIBLE IMPLICATIONS IN ALCOHOLICS. A.S. Brecher, G.E. Reeves, J.N. Poulimenos, K.D. Gray, Bowling Green State University, Bowling Green, OH.
Polyamines such as protamine sulfate have been widely used clinically to neutralize the anticoagulant effect of excessive heparin. Protamine itself exhibits concentration-dependent anticoagulant and procoagulant effects. This laboratory has earlier reported that acetaldehyde exerts synergistic prolongation of the anticoagulant effect of heparin upon prothrombin time (PT). In the current investigation, it is seen that acetaldehyde, the primary metabolite of ethanol metabolism, reacts synergistically with protamine to effect a prolongation of PT beyond the individual effects of acetaldehyde and protamine on the PT. In an analogous study, the effect of polylysine (1-4K) and acetaldehyde upon activated partial thromboplastin time (APTT) was studied. It was observed that the polylysine (PL) prolonged APTT. When PL was preincubated with plasma at RT for 15 minutes, followed by a further 15 minutes with acetaldehyde, an additional prolongation time was observed. When acetaldehyde was preincubated with plasma prior to the addition of PL, a synergistic APTT was noted. When a PL-acetaldehyde mixture was preincubated prior to the addition to plasma, a drastic reduction in the prolongation of APTT was seen, suggesting that PL and acetaldehyde may detoxify one another by a Schiff base reaction under highly specific conditions.

27

LEPTIN REGULATES ADIPOSE TISSUE LIPOGENESIS THROUGH HYPOTHALAMIC PATHWAYS THAT REQUIRE PI3K BUT ARE INDEPENDENT OF STAT3 SIGNALING. C. Buettner, E.D. Muse, A. Pocai, M. Myers, L. Rossetti, Mount Sinai Medical Center, New York and Bronx, NY.
Adipose tissue metabolism is a major factor in the control of body fat mass. In the long term, the size and the metabolism of our adipose depots have a pivotal impact on glucose fluxes and insulin resistance. A better understanding of the regulatory pathways that control body adiposity will improve our understanding of the association between obesity and insulin resistance, with implications for the pathophysiology and treatment of diabetes. Leptin regulates fuel partitioning by promoting lipid oxidation and protein synthesis and by curtailing lipogenesis, resulting in a selective loss of adiposity while preserving lean body mass. Here we examined whether the central administration of leptin modulates the expression of key lipogenic enzymes in visceral fat pads. Because insulin and glucose can also alter the expression of these genes and central leptin is known to affect both, all rats received a 6-hour infusion of leptin or vehicle into the mediobasal hypothalamus (MBH) while the circulating glucose and insulin levels were kept constant at basal levels in all groups (pancreatic basal insulin clamp). Central administration of leptin to conscious rats markedly down-regulated the adipose tissue expression of several key lipogenic enzymes, including acetyl-CoA carboxylase (ACC), stearoyl

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.

28

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.

29

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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30

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.

31

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

32

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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