LDLR$^{-/-}$ mice fed high-fat diabetogenic diets (HFD)—a model of T2DM and aortic vascular calcification. Four-week-old LDLR$^{-/-}$ mice were fed either normal mouse chow (CHOW; $n = 5$), HFD with twice-weekly sc vehicle injection (HFD + VEH; $n = 10$), or HFD with twice-weekly sc infliximab injection (HFD + INX at 10 mg/kg; $n = 10$). Animals were sacrificed after 4 weeks; serum, aorta, and heart were harvested for analysis. Animals fed an HFD exhibited hyperglycemia, hyperlipidemia, and hyperleptinemia, with significantly increased levels of TNF-α; moreover, markers of inflammation and oxidative stress, viz., haptoglobin and 8-F isoprostane (IsoP), were concomitantly up-regulated. Injection with infliximab did not prevent HFD-induced hyperglycemia, hyperlipidemia, or hyperleptinemia; however, both haptoglobin and IsoP induction were inhibited by infliximab treatment. HFD-induced T2DM up-regulated osteogenic BMP2-Msx2-Wnt signaling in the aorta. Compared with the HFD + VEH group, aortic BMP2-Msx2-Wnt signaling was significantly attenuated in the HFD + INX group, to a level that approximated that of the CHOW animals; aortic BMP2, Msx2, Wnt3a, and Wnt7a were down-regulated by infliximab. Next, we generated transgenic mice that overexpress TNF-α in the vascular smooth muscle of large arteries (SM22-TNF-α transgenics). Compared with wild-type siblings, SM22-TNF-α transgenic mice had marked up-regulation of the ostegonic BMP2, Msx2, Wnt3a, and Wnt7a signals in the aorta. Finally, HFD-induced aortic calcium content was significantly reduced by infliximab treatment (1.44 0.19 vs 1.00 0.07 g calcium/mg dry aortic weight; $p = .03$, two-tailed t-test). Thus, in this model of T2DM, diet-induced inflammation, oxidative stress, aortic BMP2-Msx2-Wnt signaling, and vascular calcification are regulated by TNF-α. TNF-α-dependent low-grade vascular inflammation may represent the mechanistic link for the remarkable similarities in phenotype and histopathology of MAC in T2DM and CKD5.

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BCR SERINE/THREONINE KINASE ENHANCES ANGIOTENSIN II–MEDIATED NUCLEAR FACTOR KB TRANSCRIPTIONAL ACTIVATION IN VASCULAR SMOOTH MUSCLE CELLS VIA INHIBITION OF PEROXISOME PROLIFERATOR–ACTIVATED RECEPTOR γ. J. Alexis, W. Che, B. Ding, S. Ito, N. Wang, C. McClain, V. Korshunov, B.C. Berk, C. Yan, J. Abe, University of Rochester, Rochester, NY.

Bcr is a serine/threonine kinase and is highly expressed in the neointima after vascular injury. Because angiotensin II (Ang II) and inflammation play important roles in the development of intimal proliferation, we hypothesized that Bcr is an important regulator of Ang II–mediated nuclear factor (NF)-κB activation. By transfecting vascular smooth muscle cells (VSMCs) with dominant negative Bcr (DN-Bcr), we demonstrated that DN-Bcr inhibited Ang II–mediated NF-κB activation in VSMCs (82 ± 10% inhibition, $n = 3$, $p < .01$). Similarly, we demonstrated that Bcr siRNA inhibited Ang II–mediated NF-κB activation in VSMCs (79 ± 7%, $n = 3$, $p < .01$). In a survey of nuclear factors that might regulate NF-κB activation, we found that wild-type (WT) Bcr decreased ligand-mediated peroxisome proliferator–activated γ (PPARγ) activity (86 ± 12% inhibition, $p < .01$), whereas DN-Bcr significantly enhanced ligand-mediated PPARγ activity (2.4-fold, $p < .01$). Assessment by in vitro kinase assay demonstrated that Bcr phosphorylates PPARγ. Overexpression of WT-Bcr kinase did not inhibit ligand-mediated PPARγ1 S82A or S82D mutant transcriptional activity, suggesting that Bcr regulates PPARγ activity via S82 phosphorylation. To assess the physiologic role of Bcr, we examined the effect of Ang II on Bcr expression and transcriptional activation. Using VSMCs, we demonstrated that Ang II only weakly increases Bcr kinase activity but significantly increased its expression after 6 hours of Ang II stimulation (4.3 ± 0.8-fold increase, $p < .01$). In addition, we found that whereas Ang II inhibits PPARγ activation, Bcr siRNA reverses Ang II inhibition of PPARγ. To determine the role of Bcr/PPARγ on Ang II–mediated NF-κB activation, we cotransfected VSMCs with DN-Bcr and DN-PPARγ. We found that DN-PPARγ reversed DN-Bcr-mediated inhibition of NF-κB activation. In addition, WT-Bcr-induced NF-κB activation was inhibited by PPARγ1 S82A mutant but not WT-PPARγ1, strongly suggesting that Bcr-induced inhibition of PPARγ activity reciprocally increases NF-κB activation and regulates Ang II–mediated NF-κB activation. Finally, our preliminary data suggest a decrease in intima to media ratio (0.31 vs 0.73) in Bcr knockout mice compared with controls in a partial ligation model of the left carotid artery. Given our findings that Bcr kinase inhibits PPARγ activation and enhances Ang II–mediated NF-κB transcription, we believe that Bcr acts as an important regulator of the sensitivity of VSMCs to inflammatory stimuli.

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UNIQUE PARASYMPATHETIC PROFILE OF THE PULMONARY VEINS AND POSTERIOR LEFT ATRIUM. R. Arora, J. Ng, J. Ulphani, R. Belin, J. Goldberger, A. Kadish, Northwestern University, Feinberg School of Medicine, Chicago, IL.

Background: The pulmonary veins (PVs) have recently been shown to play a major role in the genesis of atrial fibrillation (AF). Focal "triggers" and "drivers" in the PV appear to be significantly modified or regulated by the parasympathetic nervous system. Nonetheless, the detailed autonomic profile of the PVs—especially as it compares with the posterior left atrium (PLA) and the rest of the left atrium—has not been well characterized. We hypothesized that the left atrium exhibits a heterogeneous electrophysiologic response to autonomic maneuvers, with the activation and repolarization characteristics of the PVs being different from the rest of the left atrium. Since IKAch is the ion channel that is primarily responsible for vagal effects on left atrial refractoriness, we further hypothesized that interregional variation in repolarization (in response to vagal maneuvers) is due to differences in the expression and spatial distribution of IKAch within the PVs, PLA, and left atrial appendage (LAA). **Methods:** In 14 dogs, high-density plaques were sutured onto the left inferior PV (8 × 5 electrodes), the PLA (7 × 3 electrodes), and LAA (7 × 3 electrodes) for bipolar electrogram recording and pacing. Epicardial mapping was performed in the PVs, PLA, and LAA under the following conditions: baseline, 20 Hz cervical vagal stimulation (VS), propranolol (P), P + VS, and P + atropine. Effective refractory periods (ERPs) were measured and conduction vectors were computed at multiple sites. Western blotting and immunostaining were performed for IKAch (Kir3.1/3.4). **Results:** In response to VS, there was a significant decrease in ERP (9%) in the PV compared with baseline ($p = .001$). In contrast, ERP changes in the PLA and LAA in response to VS were not as pronounced. In the presence of beta-blockade, vagal stimulation (P + VS) resulted in a more pronounced decrease in ERP in the PV (17.7%), PLA (19.9%), and LAA (11.6%) (P + VS vs P − PV: $p < .001$, PLA: $p = .003$, LAA: $p = .036$) compared with VS alone. ERP decrease in the PV and PLA was significantly greater than in the LAA ($p < .05$). Cumulative vagal effect (ERP difference between P + VS and P + ATR) was found to be greatest in the LAA (ERP change: LAA > PV, $p = .04$; LAA > PLA, $p = .12$). The cumulative vagal effect in the PV, PLA, and LAA corresponded to the relative expression of the IKAch subunits Kir3.1 and Kir3.4 in these regions (for both Kir3.1 and 3.4, LAA > PLA > PV). However, vagal-induced ERP shortening was significantly more heterogeneous in the PLA compared with the LAA (variance of ERP shortening: PLA > LAA, $p = .04$). Heterogeneity of ERP shortening corresponded to the heterogeneity of IKAch distribution in each region (variance of the number of Kir3.1-positive cells counted under six high-power (40×) fields: PV = PLA > > LAA, $p < .01$). With VS and/or P, there was evidence of regional conduction delay in the PVs with a significant change in activation direction. Similar activation changes were not seen in the PLA and LAA. **Conclusions:** The PVs and PLA demonstrate unique activation and repolarization characteristics in response to autonomic manipulation. The heterogeneity of vagal responses correlates with the pattern of IKAch distribution in the left atrium. The peculiar autonomic characteristics of the PVs and PLA may create substrate for reentry and atrial fibrillation.

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FIBROMYALGIA: PATHOPHYSIOLOGY AND THERAPY OF A REVERSIBLE ISCHEMIC SYSTEMIC NEUROVASCULAR DISORDER. R. Barndt, A. Cho, N. Mina, Bethel Public Service Clinic, Downey, CA; Drexel University College of Medicine, Philadelphia, PA; Detroit, MI.

Our pilot studies (PSs) show that chronic low blood flow in fibromyalgia is associated with muscle pain, headaches, increased left ventricular muscle collagen (LVMC), and coronary artery stenosis (CAS), suggesting hypoxic injury. A predictive relationship was found by blind correlation between high adrenergic neurovascular tone (ANVT)/duration of illness (years) and the increase in LVMC and CAS in pilot studies, both at $r = .97$, $p < .001$. ANVT is measured by systolic time intervals (STI = PEP/LVET × 100%). Therapy resulting in reduction of ANVT with increased systemic flow (cardiac output [CO]) resulted in significantly reduced symptom levels (SL) in PSs. Prospective patients were selected by criteria of the America College of Rheumatology with normal C-reactive protein, systolic blood pressure (SBP) < 121, LDL < 103, HbA_{1c} < 6.0, Hgb > 12, and nonsmokers. These were age 16 to 65 years, 7/1 female/male, with the mean duration of disease at 6 years for group (G)1 (32 patients) and 12 years for G2 (31 patients). G1 and G2 were compared with age-/sex-/race-matched normal controls (C = 40 patients). Serial measurements were made of SBP, STI, CO, systemic vascular resistance (SVR), LV end diastolic volume (EDV = cc/m^2), LVMC, right (R) CAS, and left anterior descending (LAD) CAS by our previously reported ultrasonic methods. Patients' SLs were recorded at 0 to 100 (fatigue + pain). Data were placed into a blind matrix for analysis later. Treatment (Rx) (as in PS) with diltiazem CD 240 to 360 mg q/d and amitriptyline 10 to 125 mg q/d resulted in significant improvement in SL ($p < .01$ by t-test [TT]) as shown below from time (T)1 (start) to T2 (duration of Rx) (1/2). G1 was treated for 6 years and G2 for 12 years. **Prospective Results:** Group means shown. See Table. Where: * = Significantly different from C at $p < .01$ by TT. G1 and G2 were found to be significantly different from C by high ANVT (low STIs), high SVR, low CO, and higher LVMC at T1. G2 was shown to be significantly different than G1 at T1 by the following: EDV, LVMC, RCAS, and LADCAS, revealing the significance of the duration of illness ($p < .01$ by TT). Regression analysis of G1 + G2 demonstrated the percentage of CAS and the percentage of LVMC were predicted by ANVT (STI)/duration of illness at T1 (both at $r = .97$, $p < .001$), using PS equations. This multivariant analysis reveals that STI and duration of illness were the only significant parameters explaining 94% ($R^2 = .94$) of the variability of the relationships at T1. Rx significantly reduced ANVT and significantly increased CO. This resulted in significantly regressed LVMC and %CAS. Reduction in LVMC resulted in significantly increased LVED volume/M2 at T2, reversing the restrictive cardiomyopathy, which was present at T1. The CAS at T1 was significantly greater in the larger RCA (> LAD) ($p < .01$ by TT) in all cases, as in PS, suggesting a chronic wall tension-hypoxic injury. The changes in CAS and LVMC paralleled each other, as shown by the regression analyses supporting systemic hypoxia as the injury at T1 since critical %CAS (> 70%) was not present. Thus, reduction in ANVT and increase in CO resulted in reversal of this ischemic systemic ANVT disorder.

G	*SBP 1/2*	*STI 1/2*	*CO 1/2*	*SVR 1/2*	*EDV 1/2*	*LVMC 1/2*	*RCAS 1/2*	*LADCAS 1/2*	*SL 1/2*
1	91*/110	22*/42	2.3*/4.6	2110*/1345	55*/87	40*/2	28*/0	17*/0	95*/2
2	90*/109	21*/41	2.2*/4.5	2084*/1351	32*/86	58*/3	61*/1	39*/1	98*/3
C	110/108	53/54	5.5/5.7	1125/1053	88/90	3/2	0/0	0/0	0/0

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NEW GOALS IN SYSTOLIC HYPERTENSION THERAPY ARE REVEALED BY VASCULAR COLLAGEN. R. Barndt, N. Mina, A.Y. Cho, S. Jagtap, A. Stavrakis, Bethel Public Service Clinic, Downey, CA; Detroit, MI; Drexel University College of Medicine, Philadelphia, PA; Los Angeles, CA.

Ultrasonic measurement (UM) of % aortic collagen (AC) and % coronary stenosis (CS) reveals that systolic hypertension (SH) begins at systolic blood pressure (SBP) of 121 mm Hg by group analysis (mean 126 ± 6). Our pilot studies (PSs) show that UM of AC is predictive of AC assay in separate in vitro tissue studies ($r = .97$, $p < .001$). AC predicts average SBP (ASBP), pulse pressure (PP), aortic stiffness (AS), and maximum %CS in coronary arteries (%SCA in left anterior discending [LAD]), all at $r = .97$, $p < .001$ in PS. UM of %SCA predicted %SCA by angiography in clinical PS and separate postmortem angiographic studies, all at $r = .97$, $p < .001$. In PS, PP at normal systemic vascular resistance (SVR) predicted % AC ($r = .97$, $p < .001$). Normal SVR was predicted by a rise in PP < 11 mm Hg during sustained isometric handgrip (RPPHG = 5 psi for 3 minutes) with 0 to 20 mg hydralazine ($p < .01$ by t-test). Prospective patients (ProsGroup [G]) were randomly selected and grouped by PS %AC levels (mean AC ± range values) to predict different hypertension (H) levels: control (C) G = 16 ± 2, mild (M) = 24 ± 3, moderate (Md) = 32 ± 4, and Md severe (MdS) = 45 ± 8. Labile hypertension (LH) Gs (L1 and L2) were randomly selected and had AC at 16 ± 2 with significant RPPHG (RPPHG > 15 mm Hg). All groups were matched by age (mean 50), sex (M/F = 1/1), and race. Exclusions: Smoking, diastolic H (> 85 mm Hg), LVH, diabetes, hyperlipidemia, and chronic diseases. Serial measurements were made of AC, AS (PP/aortic diameter distention in mm), %SCA, PP, ASBP by AC, and baseline cuff SBP at normal SVR. Data were analyzed by blind matrix. BP was regulated every 6 weeks for 10 years in all with AC > 21 or RPPHG > 10 mm Hg. All groups except for C and L1 were treated with Tenormin (12–100 mg/d) and Cardura (1–16 mg/d) (Rx) to maintain SBP < 121 and RPPHG < 11 mm Hg. ProsG results: G means at start/end (1/2). See Table. Where * = significant (Sig*) difference from CG at $p < .01$ by t-test. This study shows that Sig* SH begins at ASBP 121 mm Hg, AC 21%, and AS 11, causing Sig* %SCA (maximum SCA in LAD). Regression analysis revealed PS formulas predict ProsG: AS, PP, ASBP, and %SCA by AC (all at $r = .96$, $p < .001$). Sig* AC and %SCA regression occurred with Rx at SBP < 121 and RPPHG < 11, without sig* changes in serum lipids. Sig* regression of AC correlated with functional improvement of the aorta (AS, $p < .01$ by t-test). Rx of LH in G L2 prevented progression of AC and SCA that was seen in matched G L1. There were no adverse vascular events. This prospective study shows that AC and SCA can be reversed by Rx of SBP to C levels. Thus, UM of AC and SCA proves that SH begins to cause vascular damage at SBP > 120 mm Hg and RPPHG > 10 mm Hg and should be treated to reduce vascular risk.

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

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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

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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

*$p < .05$.

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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.

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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.

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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